THE ROLE OF PKCδ CELLS AND EXPRESSION IN THE BED NUCLEUS OF THE STRIA TERMINALIS IN STRESS PROCESSING, THREAT DETECTION, AND ANXIETY-LIKE BEHAVIORS

By

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“If you hear a voice within you say ‘you cannot paint’
then by all means paint
and that voice will be silenced”
--Vincent Van Gogh

To my mother, who helped me to silence that voice when it was at its loudest

and

To all those battling depression, anxiety, and addiction, especially the ones I love.

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Centanni, S. W., Brown, J. A., Williford, K. M., Flook, E. A., Luchsinger, J. R., & Winder, D. G. (2022). Bed Nucleus of the Stria Terminalis (BNST) circuits. In N. W. Gilpin (Ed.), Neurocircuitry of Addiction (pp. 321–374). Academic Press

Anxiety disorders are among the most common psychiatric disorders, with an estimated 1/3 of all people suffering from an anxiety disorder in at some point in their lifetime and resulting in devastating personal and economic burden (Kessler et al., 2012; Bandelow & Michaelis, 2022). Whiteford 2013 Anxiety is a complex disorder, a core feature of which includes altered attentional bias regarding perceived threats. Both heightened attention toward (hypervigilance) and away from (avoidance) threatening stimuli have been document as key factors in the vulnerability and maintenance of anxiety disorders (Bar-Haim et al., 2007; Cisler & Koster, 2010; Van Bockstaele et al., 2014; Macatee et al., 2017; Egan & Dennis-Tiwary, 2018; Wieser & Keil, 2020). Stress exposure is also a central contributing factor in the development of these and many other disorders, and individuals with anxiety have been shown to display altered responsivity to stressors. Stress can also precipitate threat bias and threat learning, and degree of threat bias can be predictive of stress reactivity (Frisch et al., 2015; Macatee et al., 2017; Egan & Dennis-Tiwary, 2018; Timmers et al., 2019). It is clear there are complex interactions between anxiety, threat processing, and stress. However, the neuronal underpinnings of their relationship remain poorly understood. In this section I will provide background information about stress, anxiety, and some of the key brain regions involved in stress and anxiety: the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST). I will also provide an overview on what is currently known about protein kinase C delta (PKCδ) and cell expressing this kinase, setting the stage to examine the role of $BNST^{PKC\delta}$ neurons in mediating stress responses, threat processing, and anxiety-like behaviors.

1.1 Stress

1.1.1 A brief history on the definition and impact of stress

The definition of stress can perhaps best be summarized by Dr. Hans Selye when he said, near the end of a career dedicated to its study, that “everyone knows what stress is, but no one really knows.” Stress is ubiquitous. It is experienced in some form by every living organism, with causes ranging from exposure to extreme weather to a looming deadline. Yet its definition has proven challenging to specify and its origins hard to trace (Kugelmann, 1992; C. L. Cooper & Dewe, 2007; Jackson, 2013; Hutmacher, 2021). Part of the difficulty of defining stress stems from it historically being used to simultaneously describe the agent inducing stress (now often referred to as a stressor), the resulting physiological response, and a psychological state, with that state resulting from the perceived mismatch between the demands on the organism and the organisms ability to cope with those demands (Selye, 1976; Edwards & Cooper, 1990; Stokes & Kite, 2000; Staal, 2004). Indeed even today, Merriam Webster’s dictionary defines stress as a “constraining force or influence” including both “a physical, chemical, or emotional factor that causes bodily or mental tension and may be a factor in disease causation,” and a “state resulting from a stress, especially one of bodily or mental tension resulting from factors that tend to alter an existent equilibrium” (Merriam-Webster.com Dictionary, 2023).

Selye has been dubbed “the father of stress research” and is most often credited with coining the term in 1936 (Selye, 1936; Tan & Yip, 2018; American Psychological Association, 2023). However, Selye himself actively avoided use of the word stress in his own publications until 1946 (Selye, 1946), and it was actually Dr. Walter Bradford Cannon in 1915 who first brought the concept of stress, which had previously been used in physics principles, into the realm of biology (Cannon, 1915). While researching digestive processes, Cannon noticed that the peristaltic waves in the stomach of his animals would sometimes abruptly stop when they were frightened. This led to his initial characterization of the acute stress response in his book *Bodily Changes in Pain, Hunger, Fear, and Rage.* Here he described an animal’s response to strong arousal in which “the sympathetic division of its autonomic nervous system combines with the hormone adrenaline to mobilize the animal for an emergency response of ‘flight or fight.’” (Cannon, 1915; T. M. Brown &

Fee, 2002).

While Cannon's work focused primarily on the body's acute threat response, Selye's work centered around what would now be considered chronic stress. His letter to the editor of *Nature* regarding a "General Adaptation Syndrome" (GAS) was the first publication describing a conserved, multi-stage response to "acute, nonspecific nocuous agents" (which he would later call stressors) (Selye, 1936, 1976). In his initial work these agents included stimuli such as cold exposure, surgical injury, forced exercise, and sublethal toxin exposure. GAS began with a "general alarm reaction," involving changes in a variety of peripheral tissues including the lymphoid and digestive organs. This was followed by a stage of resistance during which the body attempted to adapt to the presence of the stressor and return to homeostasis (a term coined by Cannon in 1926) "beginning 48 hours after the injury" (Cannon, 1926; T. M. Brown & Fee, 2002; Davies, 2016). Then, depending on the duration and/or severity of the stressor, GAS ultimately ended with animals losing their resistance to the stress in a "phase of exhaustion" similar in symptoms to the first alarm stage (Selye, 1936).

Despite its reputation, not all forms of stress are bad. This distinction was made by Selye as the difference between "eustress" and "distress" and is now referred to as adaptive verses maladaptive stress, with "the former being agreeable or healthy, and the latter, disagreeable or pathogenic" (Selye, 1976). There has been considerable effort to define the relationship between stress level and its overall effect on output and performance and find the tipping point between the two, with even prominent organizations peripheral to the field such as NASA and the US military taking a keen interest (Bourne & Yaroush, 2003; Staal, 2004; P. Hancock & Szalma, 2008).

The most infamous characterization of this relationship is often credited to Drs. Robert Yerkes and John Dodson with the Yerkes-Dodson law also referred to as the Inverted-U (Fig 1A). This roughly holds that a low level of stress improves an organism's performance, while higher levels will worsen it. Yerkes and Dodson set out to measure the relationship between shock intensity and how quickly mice learned to distinguish between and walk through a white vs black passageway. They found that with increased task difficulty, the lowest and highest level of shocks produced the slowest learning, with peak learning occurring at intermediate shock levels (Fig 1B).

However, many have identified issues with extrapolating their data to reflect a relationship

between stress and (especially human) performance (Cole, 1911; P. A. Hancock & Ganey, 2003). These including very low n (4 mice for the easy and medium difficulty and 2 mice for the hardest difficulty), faulty equipment resulting in imprecise shock levels, lack of replicability even in their own hands, and most importantly, the fact that the authors themselves never claim to study stress or its relationship with performance at all (Cole, 1911; Dodson, 1915; W. P. Brown, 1965; Bäumler & Lienert, 1993; Bäumler, 1994; P. A. Hancock & Ganey, 2003). It was Broadhurst who drew this parallel in 1957 following his own study on the effect of oxygen deprivation on rats' water maze navigation and established the Yerkes-Dodson law. Since then this "law" has been cited and studied in a variety of contexts, with as many researchers and philosophers expanding upon or adjusting it as those outright refuting it. For example, Easterbrook argues there is an optimum level of stress associated with cue sampling efficiency, Falk and Bindra report modest increases in arousal lead to improved performance on simple tasks like time estimation, Peter Nixon developed his own "Human Function Curve" replacing stress on the x-axis with "arousal stress", and Hancock and Warm, despite refuting the Inverted U, simply proposed an "Extended U" concept incorporating both physiological and psychological adaptive capability in relation to stress level (Falk & Bindra, 1954; Hebb, 1955; Easterbrook, 1959; P. G. F. Nixon, 1976; P. G. Nixon, 1982; P. A. Hancock & Warm, 2003). In contrast, several studies have shown a negative linear relationship, with tasks such as free recall memory, public speaking, and math performance being worsened with increased stress or arousal (Broadbent, 1963; Burger & Arkin, 1980; Cohen, 1980; Cohen & Weinstein, 1981; Christianson, 1992; Rohrmann et al., 1999; Ashcraft & Kirk, 2001; Eysenck Royal Holloway et al., 2014).

It should be noted that many of those in support of the inverted-U concept rely heavily upon the "common sense" factor and the original Yerkes and Dodson experiment without providing supporting experimental evidence. But despite questionable experimental foundations, it does indeed have strong common sense appeal. It is a common anecdote that the stress of a deadline facilitates completion of a task. This appeal is in part due to the ambiguity surrounding the definition of stress, with terms such as arousal, motivation, attention, anxiety, and "mental resources" all being swapped almost interchangeably (Broadbent, 1963; Kahneman, 1973; Norman & Bobrow, 1975; Staal, 2004). This ambiguity allows for ready application of the inverted

U to a broad swath of experiences. Further, many “motivation” or “arousal” based axes start with the lowest level being no motivation or asleep. It is clear to see how having even slightly more motivation or being, well, awake, could easily improve performance on a task. However, defining a scale for modest increases in stress or anxiety is much more subjective and varies from study to study.

Perhaps most importantly, none of these studies have provided a predictive model for determining at what point stress will switch from having a positive to a negative impact on performance in a given context. It has been claimed for decades that “a little bit of stress is good” but how much starts to be bad? In all likelihood, that tipping point will vary for every combination of stressor and task being measured, with drastic individual variability mixed in (Broadbent, 1963; P. A. Hancock & Ganey, 2003). As such, it is important in the study of stress to bear in mind any limitations in generalizability and recognize that, although we now have advanced tools for precise physiological measures of stress responses, it is ultimately a subjective experience, the effects of which we are only beginning to understand.

1.1.2 The physiological stress response

Text goes here Despite its historical ambiguities and subjective components, stress today is most often defined based on the physiological response elicited, and in fact stressors are validated by their ability or lack thereof to illicit this conserved stress response (K. J. Kovács, 2008). This physiological response is often divided into two waves: the acute “flight or fight” response through the sympathetic-adrenal-medullary (SAM) axis and activation of the hypothalamic-pituitary-adrenal (HPA) axis (Lucassen et al., 2014).

Before an organism can respond to a stressor, it must first detect it. For physical stressors such as pain, hemorrhage, and immune responses, this is mediated primarily through reflexive pathways between the mediolateral cell column of the spinal cord and brainstem structures responsible for physical homeostasis (i.e. blood pressure, fluid and electrolyte balance, respiration, heartrate, etc.) such as the nucleus of the solitary tract (NTS), locus coeruleus (LC), and circumventricular organs (eg the median preoptic nucleus and the subfornical organ) (Ulrich-Lai & Herman, 2009; Godoy et al., 2018). Psychological stressors are primarily detected by limbic

structures such as the infralimbic prefrontal cortex (PFC), amygdala, and hippocampus through integration of information from higher order sensory (eg piriform cortex, postpiriform transition area (TR) and insula), memory (eg medial septum and entorhinal and cingulate cortices), and attention, and arousal regions (eg LC, dorsal raphe (DR) and parabrachial nucleus (PBN) (Herman et al., 2003; J. J. Radley et al., 2006; Ulrich-Lai & Herman, 2009; Godoy et al., 2018). These limbic structures then act through intermediary regions such as the anteroventral bed nucleus of the stria terminalis (avBNST), dorsomedial hypothalamus (DMH) and arcuate nucleus of the hypothalamus (Arc) to drive stress responses (Ulrich-Lai & Herman, 2009).

Following stressor detection, the first response wave occurs through activation of the autonomic nervous system (ANS) via the SAM axis. Preganglionic sympathetic neurons in the intermediolateral cell column in the thoracolumbar region of the spinal column project to the chromaffin cells of the adrenal medulla above the kidneys, stimulating release of the catecholamines epinephrine (E)(aka adrenalin) and norepinephrine (NE) (aka noradrenalin) to increase vigilance, glucose availability, and cardiovascular function (Cannon, 1915; Ulrich-Lai & Herman, 2009; Tank & Wong, 2015; Godoy et al., 2018). They also project to pre- or paravertebral ganglia which innervate peripheral organs and cause release of NE from sympathetic nerves and increases in heart rate and vasoconstriction. The synaptic nature of this communication rapidly primes the body for energy expenditure within seconds of stressor exposure (Ulrich-Lai & Herman, 2009; Godoy et al., 2018). The ANS also stimulates parasympathetic activation through pre-ganglionic neurons in the cranial and sacral spinal column which release acetylcholine and broadly counteract the sympathetic activation and helps shorten the duration of the energy-intensive SAM activation (Tank & Wong, 2015; Godoy et al., 2018).

In contrast, the second wave is a slower, hormonally-mediated response via the HPA axis, ensuring a more prolonged response to the stressor. The HPA axis is self-inhibiting feedback loop of glucocorticoid signaling, with peak glucocorticoids occurring tens of minutes after exposure to a stressor. The paraventricular nucleus of the hypothalamus (PVH) is central to the HPA-mediated stress response, with activated both indirectly by the SAM and directly by stressors(Ulrich-Lai & Herman, 2009; Godoy et al., 2018). The PVH releases corticotropin releasing factor (CRF) (as well as vasopressin and oxytocin), which travels along the median eminence to the anterior pituitary

gland. Here CRF stimulates release of adrenocorticotropic hormone (ACTH), which stimulates the inner adrenal cortex to synthesize and release glucocorticoid hormones (Vale et al., 1978, 1981). The primary glucocorticoid in humans is cortisol while in rodents it is corticosterone (De Kloet, 2013). These glucocorticoids then bind to the glucocorticoid receptors (GRs) which are broadly distributed throughout the brain, and mineralocorticoid receptors (MRs), which have more restricted regional expression, although there is considerable species differences in the distribution of both (Lucassen et al., 2014). Included among regions expressing both are the PVH and anterior pituitary, where glucocorticoids work to inhibit subsequent activation, thus closing the inhibitory feedback loop (Ulrich-Lai & Herman, 2009).

Stressor-driven activation of the HPA axis can be considered “bottom-up” signaling, but there is also top-down control of the stress response. Several limbic regions involved in psychological stressor detection are also involved in cognitive processing about both physical and psychological stressors. This sentence is only here because I am genuinely curious how closely anyone reads this document. For example, circuits from the PFC (especially the prelimbic cortex) and hippocampus (especially the ventral subiculum) have been shown to decrease activation of the HPA axis, likely through intermediary regions including the anteromedial and posteromedial BNST, medial preoptic area (mPOA), basolateral amygdala (BLA), paraventricular thalamus (PVT), and dorsal raphe nucleus (DRN) (Herman, Cullinan, et al., 1995; Figueiredo, Bruestle, et al., 2003; Herman et al., 2005; D. C. Choi et al., 2007, 2008; Furay et al., 2008; Ulrich-Lai & Herman, 2009; Lucassen et al., 2014). The BNST in particular provides direct innervation to the PVH (Ju et al., 1989; H. W. Dong et al., 2001), and its role in mediating stress responses will be more thoroughly discussed later in this chapter.

It should also be noted that there exists variability within the HPA axis response, with both circadian and sex-based differences. Even in the absence of stress, circulating levels of glucocorticoids exhibit a circadian rhythm, with peak levels occurring at the onset of the organism’s active phase (daytime for diurnal organisms and nighttime for nocturnal organisms) (Spiga et al., 2014; Kinlein & Karatsoreos, 2020). Throughout the day, glucocorticoids are released in a pulsatile manner every few hours, with several phases of higher or lower levels occurring in an ultradian (multiple times per day) manner (Spiga et al., 2014). It has also been shown that in rodents, there

is significantly greater activation of the HPA axis and delayed return to baseline following an acute stressor in females compared to males, and this is thought to be modulated by the gonadal hormones testosterone and estradiol (Heck & Handa, 2019).

1.1.3 The interactions between stress and psychiatric disorders

The inhibitory feedback built into the ANS and HPA axis allows organisms to return to baseline and is well suited to cope with occasional, acute stressors. It can even adapt to one's environment and common (mild) stressors to a certain extent to maintain allostasis, which refers to maintenance of ideal homeostatic conditions given a particular context (Sterling & Eyer, 1988). However, exposure to particularly intense or chronic stress overburdens the system to the point where it can no longer compensate for the allostatic load, resulting in alterations to the involved signaling pathways (McEwen & Stellar, 1993; Logan & Barksdale, 2008; McEwen, 2017). These alterations can result in the development or exacerbation of numerous diseases and disorders, and indeed, chronic stress is associated with a host of conditions impacting both the brain and peripheral body systems.

With regard to the central nervous system, exposure to intense or chronic stress is implicated in a number of disorders. Chronic stress is associated with sleep disorders including insomnia and hypersomnia (Chrousos, 2009; Nollet et al., 2019). Chronic stress impairs overall cognitive function and results in learning and memory deficits (Chrousos, 2009). Likelihood of development of sporadic Alzheimer's disease is associated with stressful life experiences, and increased levels of glucocorticoids are significantly correlated with Aβ toxicity and rates of neurodegeneration and cognitive impairment (Lupien et al., 1998; Jeong et al., 2006; Catania et al., 2009; Huang et al., 2009). Stress is also implicated in the development of eating disorders (Hardaway et al., 2015).

Chronic stress is also associated with neuropsychiatric disorders. The number of stressful events a person has experienced is significantly correlated with the presence of major depressive disorder (MDD) (Krishnan & Nestler, 2008; Risch et al., 2009; L. Yang et al., 2015), and individuals with MDD have been shown to have hyperactive HPA signaling and reduced sensitivity to glucocorticoid feedback inhibition (Ströhle & Holsboer, 2003; Lucassen et al., 2014). Chronic

stress also significantly correlates with the presence of an anxiety disorder, and post-traumatic stress disorder (PTSD) can develop following acute or chronic exposure to a particularly intense or traumatic stressor (Chrousos, 2009; Chesnut et al., 2021; Ressler et al., 2022). In contrast with MDD, it has been shown that individuals with anxiety, anxious depression, or PTSD have hyporeactive HPA axis responses, and hyper-sensitivity to steroid feedback (Yehuda et al., 1991; Meller et al., 1995; Ross et al., 2017; Wichmann et al., 2017), although there is significant individual variability, with some individuals with anxiety showing HPA axis hyperactivity, and this may be related to comorbid MDD (Yehuda, 2009; Jacobson, 2014).

Stress is also major risk factor in the development and maintenance of substance use disorders (SUDs) (Sinha, 2008; Koob, 2008, 2013). Individuals exposed to early life stress and cumulative adversity show increased levels of drug use and abuse (Newcomb & Harlow, 1986; Wills et al., 1992; Turner & Lloyd, 2003; Sinha, 2008). In rodent models, HPA axis-mediated increases in corticosterone are important for acquisition of drug self-administration (Piazza & Le Moal, 1996; Mantsch et al., 1998; Goeders, 2002) and exposure to a variety of stressors actually enhances acquisition of opiate, alcohol, and psychostimulant self-administration (L. Lu & Shaham, 2005; Sinha, 2008). This interaction may in part be due to the use of drugs as a stress coping strategy (Johnson & Pandina, 1993; Perkins, 1999; Frone, 2016) and due to the impairment chronic stress causes in executive functioning and decision making and increased impulsivity (Li & Sinha, 2008; Wolff et al., 2021; Kräplin et al., 2022). Further, acute stress causes changes in the mesolimbic dopamine system that mirror those cause by drugs of abuse, including enhanced glutamate activity in the ventral tegmental area (VTA) and dopamine release in the nucleus accumbens (NAc) (Thierry et al., 1976; Kalivas & Duffy, 1995; Piazza & Le Moal, 1996; Takahashi et al., 1998), while chronic stress inhibits dopamine synthesis in the NAc (Pacak et al., 2002; Saal et al., 2003). In addition to its involvement in the development of SUDs, chronic stress perpetuates the cyclic nature of addiction and is one of the top reported causes of drug relapse (Mantsch et al., 2016). In rodent models, exposure to acute and chronic stress drives reinstatement of previously extinguished drug seeking behaviors across drug classes (Shaham & Stewart, 1995; Shalev et al., 2001; Mantsch et al., 2016), and chronic stress increases incubation of craving (Glynn et al., 2018; Fredriksson et al., 2020; Venniro et al., 2021) and vulnerability to drug-primed reinstatement (K. T.

Ball et al., 2018). Chronic stress also increases susceptibility to addictive behaviors in other domains including food (Wei et al., 2019), gambling (Slutske et al., 2015), and even smartphones and social media (N. Zhao & Zhou, 2021; Qiu et al., 2023).

There are a number of stress-sensitive brain regions that show alterations following chronic stress that may contribute to the development of these neuropsychiatric disorders. Deletion of GRs in the hippocampus, PFC, and BLA of mice results in increased depression-like behaviors and several physiological changes seen in depression including increased basal corticosterone, hyperactivity of the HPA axis, and impaired negative feedback regulation of the HPA axis (Gold et al., 1988; Holsboer, 2000; Boyle et al., 2005; Packard et al., 2016). Similarly, chronic stress leads to downregulation of GR expression in the PVH, hippocampus and frontoparietal cortex and increased basal corticosterone, ACTH, and prolactin, as well as hypertrophy of the adrenal gland (Herman, Adams, et al., 1995; Makino et al., 1995; Paskitti et al., 2000; Furay et al., 2008). In the hippocampus and mPFC, chronic stress leads to decreased dendritic arborization and decreased volume, impairing memory formation and extinction, respectively (Magarin˜os & McEwen, 1995; J. J. Radley et al., 2008; Lucassen et al., 2014; J. Radley et al., 2015), while in the BLA and BNST, dendritic branching and overall volume is increased, contributing to HPA hyperexcitability, increased emotional reactivity, and decreased reward seeking (Vyas et al., 2002; Mitra et al., 2005; Lucassen et al., 2014). Chronic stress also has sex-specific and stressor-specific effects on levels of CRF expression in the PVN, CeA, and BNST (Makino et al., 1994a, 1994b, 1995; Herman, Adams, et al., 1995; Figueiredo, Bodie, et al., 2003; Cook, 2004; Ulrich-Lai et al., 2006; Sterrenburg et al., 2011) and glutamate homeostasis in the mPFC, hippocampus, and amygdala (Kinlein et al., 2022). It is interesting to consider that in rodent models, exposure to chronic variable stress is more likely to lead to hyperactive HPA responsivity (Pollak et al., 2010; Hill et al., 2012), while chronic homotypic stress (repeated exposure to the same stressor) more often leads to habituation to the stressor and blunted HPA axis activation (Martí & Armario, 1998; Grissom & Bhatnagar, 2009; Babb et al., 2014), and these differences may be related to subsequent development of distinct psychiatric disorders.

The effects of chronic stress are not limited to the brain. In the periphery, these include increased likelihood of cardiovascular and metabolic disorders such as metabolic syndrome

(Tamashiro et al., 2011), obesity (Aschbacher et al., 2014), hypertension, cardiovascular disease (Everson-Rose & Lewis, 2005; Inoue, 2014), and type II diabetes (Chrousos, 2009; Cohen et al., 2012). While acute stress can actually enhance immune responses, chronic stress can impair and suppress the immune system (Dhabhar, 2009). This is especially compounded when paired with the effects of other conditions such as cancer or aging (Vitlic et al., 2014; Fali et al., 2018; L. Zhang et al., 2020). These immune changes may also contribute in part to the effects of chronic stress on increased risk for preterm birth (Wadhwa et al., 2001; Bandoli et al., 2018). Chronic stress exposure and the presence of PTSD are also significantly associated with developing osteoporosis (Kelly et al., 2019), and this is mediated at least in part by a GABAergic circuit in the ventromedial hypothalamus (F. Yang et al., 2020).

It is also important to note that chronic stress and its effects are not experienced uniformly across populations. For example, within the United States it has been shown that Black Americans have significantly higher allostatic load scores than White Americans (Geronimus et al., 2006; Ong et al., 2017). The experience of racism through both macroaggressions and microaggressions is a chronic stressor associated with measures such as ANS activation (Peterson et al., 2020; Cheadle et al., 2020), HPA axis activation and cortisol release (D. B. Lee et al., 2018; Bell et al., 2019), altered carotid thickness (Lewis et al., 2019), and reduced telomere length, which is a biomarker of aging and premature morbidity associated with chronic stress (D. Lu et al., 2019) (R. Clark et al., 1999; Hobson et al., 2022). There is even emerging evidence linking experiences of racism with altered connectivity among brain regions involved in the stress response, including insula-amygdala connectivity and throughout default mode network (A. Cooper et al., 2021; Webb et al., 2022; Hobson et al., 2022). Experiencing racism is associated with poorer physical and mental health outcomes regardless of age, sex and gender, education level, and socioeconomic status (though its effects are compounded by experiences such as poverty, advanced age, and being a woman or transgender) (Jolly et al., 2010; Paradies et al., 2015; Alcántara et al., 2019; Martínez et al., 2021), and indeed Black and Indigenous individuals are at a higher risk of developing nearly every peripheral stress-related disorder listed above (Brondolo et al., 2011; Benjamin et al., 2017; Hermosura et al., 2018; H. H. Wang et al., 2020; Bays et al., 2022), which is likely the result of parasitic interactions between chronic stress and institutional racism in the healthcare system

(Elias & Paradies, 2021; Hamed et al., 2022). This is especially true for Black women who, for example, are at 50% greater risk of preterm birth, and twice as likely to die from cardiovascular disease as White women (Geronimus, 1996; Rich-Edwards et al., 2003; Martin & Osterman, 2018; S. Kim et al., 2020; Pereira et al., 2022). Racial discrimination is also significantly associated with the effects of stress in the CNS such as anxiety and depression, and Indigenous, Multiracial, Black, and Latinx individuals are diagnosed with and/or criminalized for the presence of a substance use disorder at higher rates than their White counterparts (Verissimo et al., 2014; Carson et al., 2014; National Research Council, 2014; T. T. Clark et al., 2015; Mennis et al., 2016; Hedegaard et al., 2018; Shiels et al., 2018; Benner et al., 2018; Vilsaint et al., 2019; NSDUH, 2021; Amaro et al., 2021; Woody et al., 2022; Bernard et al., 2022). Even just the persistent expectation of racism worsens health outcomes, (Lewis et al., 2019; Woody et al., 2022), highlighting the detrimental effects of the chronic vigilance required just to exist as a Black, Indigenous, or Person of Color (BIPOC) individual in America. This is yet another reality underscoring the need to eradicate systemic racism and fight to promote cultures of true equity and inclusion, while also examining the heterogenous effects of chronic stress and its significant health ramifications.

1.2 Anxiety disorders: an overview

1.2.1 Symptomology and prevalence

Neuropsychiatric disorders are the leading cause of health-related burden worldwide, and depression and anxiety disorders are the leading contributors to this calculated global burden of disease (GBD), with no reduction since the measure's inception in 1990 (Abbafati et al., 2020; Santomauro et al., 2021). It is estimated that each year anxiety alone costs 44.5 million disability-adjusted life years (DALYs), which is a measure of the cumulative number of years of healthy life lost due to mortality or disability, and that there are nearly 400 million people currently diagnosed with anxiety (Abbafati et al., 2020; Santomauro et al., 2021).

In the United States, nearly 1/3 of all individuals will experience an anxiety disorder at some point in their lives, with an estimated 40 million people experiencing anxiety in the past year, and 1

in 6 people experiencing anxiety symptoms in a given two week timespan (Kessler, Berglund, et al., 2005; Kessler, Chiu, et al., 2005; NIMH, 2017; Terlizzi & Villarroel, 2020). Females are consistently more likely to be diagnosed with any anxiety disorder, with a 1 year prevalence rate of 23.4% vs 14.3% in the US, and ranking twice as high on the list of diseases contributing to GBD (Kessler, Chiu, et al., 2005; Abbafati et al., 2020).

A number disorders are classified under the general umbrella of anxiety disorders. These include generalized anxiety disorder (GAD), panic disorder, and phobia-related disorders including specific phobias, agoraphobia, social anxiety disorder (previously referred to as social phobia), separation anxiety disorder, and selective mutism. PTSD and obsessive compulsive disorder have also historically been classified under anxiety disorders but were separated into their own categories in the most recent Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (American Psychiatric Association, 2013). Table 1 summarizes the differences prevalence and symptom contributing to each anxiety disorder.

Table 1.1 Anxiety disorders: description and prevalence

Anxiety Disorder	Description	US Prevalence	Symptoms
Generalized Anxiety Disorder (GAD)	A persistent feeling of anxiety or dread that is not caused by a specific trigger and which can interfere with daily life	Lifetime Prevalence 5.7% 18.9 million ppl 1-Year Prevalence 3.1% F: 3.4% M: 1.9%	• Feeling restless, wound-up, or on-edge • Being easily fatigued • Having difficulty concentrating • Being irritable • Having headaches, muscle aches, stomachaches, or unexplained pains • Difficulty controlling feelings of worry • Having sleep problems, such as difficulty falling or staying asleep
Panic Disorder	Experiencing frequent and unexpected panic attacks, which are sudden periods of intense fear, loss of control, or discomfort, even without the presence of clear triggers or danger	Lifetime Prevalence 4.7% 15.6 million ppl 1-Year Prevalence 2.7% F: 3.8% M: 1.6%	• Pounding or racing heart • Sweating • Trembling or tingling • Chest pain • Feelings of impending doom • Feelings of being out of control
Specific Phobias	An intense fear of or aversion to specific objects or situations, often out of proportion to the actual danger present	Lifetime Prevalence 12.5% 41.5 million ppl 1-Year Prevalence 9.1% F M	• May have an irrational or excessive worry about encountering the feared object or situation • Take active steps to avoid the feared object or situation • Experience immediate intense anxiety upon encountering the feared object or situation

		12.2%	5.8%	• Endure unavoidable objects and situations with intense anxiety
Agoraphobia	Fear and avoidance of situations inducing feelings of being trapped, helpless, or embarrassed. In the most extreme cases, people can become homebound	Lifetime Prevalence 1.3% 4.3 million ppl 1-Year Prevalence 0.9% F / M 0.9% / 0.8%		Intense fear of two or more of: • Using public transportation • Being in open spaces • Being in enclosed spaces • Standing in line or being in a crowd • Being outside of the home alone
Social Anxiety Disorder	Persistent fear of being observed and/or judged by others	Lifetime Prevalence 12.1% 40.2 million ppl 1-Year Prevalence 7.1% F / M 8.0% / 6.1%		In social situations, experiencing: • Blushing, sweating, or trembling • Pounding or racing heart • Stomachaches • Rigid body posture or speaking with an overly soft voice • Difficulty making eye contact or being around people they don't know • Feelings of self-consciousness or fear that people will judge them negatively
Separation Anxiety Disorder	Fear of being parted from people to whom they are attached, and/or worry of harm or tragedy striking their attachment figures while separated. Most common in children but can also occur in adulthood.	Lifetime Prevalence Adults 6.6% 17.0 million ppl Children 1-4% 736,000 to 2.9 million ppl		• Fear and reluctance to be alone during the day or while sleeping. • Repeated nightmares with a theme of separation. • Worry when parted from home or loved one. • Excess worry about the safety of a loved one. • Clinging behavior toward attachment figures • Headaches, muscle aches, stomachaches, chest pain, or shortness of breath when separation is experienced or anticipated
Selective Mutism	When individuals fail to speak in specific social situations, despite normal language skills. It is more common in children but can also occur in adulthood	Lifetime Prevalence 0.7-2% 2.3 million to 6.6 million ppl		• Extreme shyness • Fear of social embarrassment • Withdrawal from social situations • Clinging behavior • Temper tantrums
Post-Traumatic Stress Disorder (PTSD)*	A disorder that can develop following the experience of a shocking, dangerous, or traumatic situation	Lifetime Prevalence 6.8% 22.6 million ppl 1-Year Prevalence 3.6% F / M 5.2% / 1.8%		All of the following for at least 1 month: • At least one re-experiencing symptom such as flashbacks, bad dreams, or frightening thoughts • At least one avoidance symptom such as staying away from places, events, or objects that are reminders of the traumatic experience, or avoiding thoughts or feelings related to the traumatic event • At least two arousal and reactivity symptoms such as being easily startled, feeling tense or "on edge," or having difficulty sleeping, having angry outbursts

<table>
<tr><td></td><td></td><td colspan="2"></td><td>• At least two cognition and mood symptoms such as trouble remembering key features of the traumatic event, negative thoughts about oneself or the world, distorted feelings like guilt or blame, or loss of interest in enjoyable activities</td></tr>
<tr><td rowspan="6">Obsessive-Compulsive Disorder (OCD)*</td><td rowspan="6">A disorder in which a person has uncontrollable, recurring thoughts (“obsessions”) and/or behaviors (“compulsions”) they feel the urge to repeat multiple times</td><td colspan="2">Lifetime Prevalence</td><td rowspan="6">• Inability to control his or her thoughts or behaviors, even when those thoughts or behaviors are recognized as excessive
• Spending at least 1 hour a day on these thoughts or behaviors
• Lack of pleasure when performing the behaviors or rituals, but may feel brief relief from the anxiety the thoughts cause
• Experiencing significant problems in their daily life due to these thoughts or behaviors</td></tr>
<tr><td colspan="2">2.3%</td></tr>
<tr><td colspan="2">7.6 million ppl</td></tr>
<tr><td colspan="2">1-Year Prevalence</td></tr>
<tr><td colspan="2">1.2%</td></tr>
<tr><td>F</td><td>M</td></tr>
<tr><td>1.8%</td><td>0.5%</td><td></td></tr>
<tr><td colspan="5">Population values calculated based on the 2021 US population of 331.9 million, 73.6 million children, and 258.3 adults. Lifetime prevalence refers to the percent or number of people diagnosed with the disorder at some point in their life. 1-year prevalence refers to the percent of people with the disorder in a given year.
*Anxiety-related disorders no longer classified under anxiety disorder subtypes in the DSM-5
(Kessler, Berglund, et al., 2005; Kessler, Chiu, et al., 2005; Priscilla Wong et al., 2010; NIMH, 2017; Penninx et al., 2021; Krajniak et al., 2022)</td></tr>
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1.2.2 Treatment approaches

There are a number of treatment options available for anxiety disorders, although it is estimated that only 60-85% of patients “respond” (defined as at least a 50% reduction in symptoms) to the current treatment approaches, and that there is a high prevalence of recurrent or persistent symptoms, especially in the presence of comorbid MDD (Bruce et al., 2005; Rodriguez et al., 2006; Bystritsky, 2006). These treatments are divided into two primary categories of psychotherapy and pharmacotherapy (ie medication), with research suggestion the most effective treatment option include some combination of both approaches. Current approaches are summarized below.

1.3.1.1 Psychotherapies

One of the most common psychotherapy approaches is cognitive behavioral therapy (CBT). CBT is based around teaching individuals to change their patterns of thinking and behaving in response to situations in order to help with feeling less anxious and fearful, and can be delivered in individualized or group setting in-person or virtually Brewin (Hofmann et al., 2013a; NIMH, 2017; Matsumoto et al., 2021). This can include a broad umbrella of treatments including but not limited

to systematic desensitization, stress inoculation training, cognitive processing therapy, assertiveness training, biofeedback and relaxation, and exposure therapy (most often applied for phobia-related anxiety disorders), with most practitioners using a combination of techniques (Rothbaum et al., 2000). These strategies help with learning to recognize and reframe distortions in one's thoughts, developing problem solving skills and developing self- confidence to cope with difficult situations, using role play or small-scale exposure to prepare for problematic interactions, and learning to calm one's mind and relax the body to reduce physiological anxiety responses (Brewin, 1996; Hofmann et al., 2013a; APA Div. 12 (Society of Clinical Psychology), 2017).

Other psychotherapies include interpersonal therapy, supportive therapy, and group therapy. Interpersonal therapy focuses on identifying and resolving problems in establishing and maintaining positive relationships (Ontario, 2017; Whiston et al., 2019). Supportive therapy and group therapy involve conversation with a therapist and or other patients to receive reflection, empathetic listening, and encouragement (NIMH, 2017; Ontario, 2017). A relatively recent addition to psychotherapy approaches is the use of acceptance and commitment therapy (ACT). Although its distinctiveness from CBT is hotly debated, in general ACT focuses more on utilizing strategies such as mindfulness and goal setting to reduce the discomfort of anxiety (Forman et al., 2012; Herbert & Forman, 2013; Hofmann et al., 2013b).

1.3.1.2 Pharmacotherapies

There are a number of existing pharmacotherapies used in the treatment of anxiety disorders, although only a fraction are FDA-approved for that purpose and many more are used in an off-label manner. Different classes of medications are thought to act through a variety of signaling pathways to modulate levels of a variety of neurotransmitters. Current treatments as well as novel experimental approaches are summarized below.

One of the most commonly targeted pathways in anxiolytic treatments works to increase levels of monoamines, and especially serotonin (5-HT) and/ or norepinephrine (NE), throughout the brain. The majority of treatments currently approved by the FDA act on the presynaptic side. For example, the standard first-line anxiety treatments are selective serotonin reuptake inhibitors (SSRIs) and selective norepinephrine reuptake inhibitors (SNRIs), which block the serotonin

transporter (SERT) and norepinephrine transporter (NET), preventing reuptake by the presynaptic neuron and thus increasing their duration of action on the postsynaptic target (Bandelow et al., 2015). Despite their names, SSRIs and especially SNRIs have also been shown to inhibit reuptake of 5-HT, NE, and dopamine (DA) (H. He et al., 2019). Other classes of monoaminergic agents include tricyclic antidepressants (TCAs), which also inhibit 5-HT and NE transporters, and monoamine oxidase inhibitors (MAOIs), which inhibit the degradation of monoamines, but they are used less often due to increased side-effects and decreased tolerability (Garakani et al., 2020). Interestingly saffron, which has recently been investigated for use in anxiety treatments, is also thought to act through inhibition of 5-HT reuptake (Marx et al., 2019). One major drawback of reuptake inhibitors is that they often take several weeks of use before any symptom improvement is observed (Kudlow et al., 2014; Strawn et al., 2017; H. Zhang et al., 2020).

Other monoaminergic approaches target the postsynaptic side, modulating receptor activity. This includes full and partial agonists of the 5-HT1A, 1B, and/or 4R receptor, and antagonists of the 5-HT2A, 5-HT2C, 5-HT6, and D2 receptors. The majority of these drugs are still in experimental phases, though one 5-HT1A partial agonist, Buspirone, is currently FDA approved, and several FDA approved mixed antidepressants and antipsychotics that show off-label efficacy at reducing anxiety symptoms are thought to act through a combination of serotonergic and dopaminergic receptors (Murrough et al., 2015; Garakani et al., 2020). Other off-label and experimental medications modulate adrenergic signaling include beta-adrenergic receptor antagonists such as propranolol and alpha-2 adrenergic receptor agonists such as guanfacine (Steenen et al., 2016; Strawn et al., 2017).

Another major target of anxiolytic drugs is reduction of the relative E-to-I (excitation-to-inhibition) ratio in the brain through upregulation of GABA, the primary inhibitory neurotransmitter in the brain and/or inhibition of glutamate, the primary excitatory neurotransmitter (Murrough et al., 2015; Garakani et al., 2020). Benzodiazepines are the most widely prescribed class of psychiatric medications, and act through agonism of the GABA-A receptor, though there are concerns surrounding their potential for abuse or misuse (Balon & Starcevic, 2020). Anticonvulsant drugs also increase GABA activity and have shown off-label effectiveness for anxiety disorders (Mula et al., 2007). Mechanisms include inhibition of GABA reuptake, activation of voltage-dependent

calcium channels in increase GABA release, and inhibition of voltage-dependent sodium channels to decrease glutamate and increase relative GABAergic tone, and it is thought that homeopathic remedies such as kava, chamomile, and lavender may also act through these pathways (Mula et al., 2007; Savage et al., 2018; Hieu et al., 2019). A variety of experimental drugs have attempted to upregulate GABA through allosteric modulation of the GABA-A receptor, but most have failed to reach the market due to lack of efficacy or poor tolerability (Murrough et al., 2015; Garakani et al., 2020). There have also been several emerging attempts to downregulate glutamate through antagonism of the NMDA receptor, or agonism/positive allosteric modulation of the Gi-coupled mGluR2 and/or mGluR3 (Murrough et al., 2015; Garakani et al., 2020). Many of these have had limited effectiveness in human clinical trials, though some such as ketamine, an NMDAR antagonist shown to have rapid antidepressant effects, hold promise for improving anxiety symptoms (Glue et al., 2017; Taylor et al., 2018).

There are also several experimental pathways being explored in the treatment of anxiety disorders. These include the use of neurosteroids, upregulation of the endocannabinoid system through CB1 receptor agonists, and modulation of neuropeptide signaling (Murrough et al., 2015; Garakani et al., 2020). Of particular relevance for this work is the targeting of neuropeptide signaling, given their prominence and importance in mediating the stress and anxiety-related output of the extended amygdala (discussed in more detail below). For example, antagonists of the receptors for Substance P, CRF, orexin, and cholecystokinin (CCK), and Arginine Vasopressin (AVP) and agonists of the neuropeptide Y (NPY) receptor 1 (Y1) can all decrease anxiety-like behaviors in rodent models (Centanni et al., 2022). However, these agents have been largely ineffective in human clinical trials, with the possible exception of a Y1 agonist that has had some efficacy in improving anxiety symptoms in patients with depression and PTSD (McGaugh, 2000; Ebner & Singewald, 2006; Ebner et al., 2008; Bailey et al., 2011; Zwanzger et al., 2012; Frick et al., 2015; Spierling & Zorrilla, 2017; Sayed et al., 2018; Ballaz et al., 2020; Mathé et al., 2020; Garakani et al., 2020).

Overall, despite a variety of approaches being available, current treatments remain ineffective for approximately 15-40% of individuals with an anxiety disorder, and even in those for whom treatment is considered effective, they often only result in partial symptom alleviation

(Rodriguez et al., 2006; Bystritsky, 2006). The vast majority of FDA-approved therapies, whether approved for anxiety disorders specifically or used as an off-label treatment, target distinct, albeit very broad signaling pathways. And despite knowing general receptor or neurotransmitter effects, the exact mechanism of action leading from their molecular targets to alleviation of anxiety symptoms remains poorly understood (Garakani et al., 2020). Newer approaches with more specific targets and better-understood pathways, however, have shown poor overall efficacy in clinical trials, underscoring the our need for mor thorough characterization of the psychopathology of anxiety disorders and the identification of new potential therapeutic targets.

1.2.3 Measuring anxiety in rodent models

In humans, anxiety levels are often measured through self-report on scales such as the Beck Anxiety Inventory (BAI) or the Hamilton Anxiety Rating Scale (HAM-A) (Julian, 2011; Thompson, 2015). Rodent models of anxiety, however, rely on behavioral assessment in contexts eliciting a state of heightened apprehension, which we interpret as anxiety-like behavior. Many of these assays rely on creating conflict between rodents' natural drive for exploration and novelty with an unconditioned fear of heights and/or bright open spaces (Barnett, 1963; Steimer, 2011; Bourin, 2015; La-Vu et al., 2020). The validity of these behavioral assays are often assessed using three sets of criteria. The first is predictive validity, which is essentially referring to whether performance in the assay is modulated by pharmacological treatments in the same way it is modulated in humans. The second is face validity, or whether the assay and behaviors being measured resemble the symptoms of the disorder they are attempting to model. The third is construct validity, referring to theoretical rationale and whether there is congruence in etiology and underlying physiology impacting the behaviors (Willner, 1984, 1986; van den Berg, 2022). Of note, anxiety-like assays are distinct from fear based assays, in that fear-like behaviors are in response to an identified specific and immediate danger, while anxiety-like behaviors are in response to unspecified and/or potential threat (Steimer, 2002; Davis et al., 2010). Some of the most common assays of anxiety-like behavior are briefly described below.

<u>Open Field (OF)</u>

This assay involves placing the rodent in a large, brightly-lit open box. For mice, this is often a 50cm x 50 cm area, and the ratio of time spent along the periphery verses time spent in the center (defined as 10 cm from the edge) is measured, with decreased center time corresponding to increased anxiety-like behavior (Prut & Belzung, 2003; Seibenhener & Wooten, 2015).

Holeboard Assay

Animals are placed in an open box with evenly spaced holes throughout the floor that rodents can explore by dipping their head into them. Reduced numbers and/or time spent head dipping is a measure of increased anxiety-like behavior (File, 2001; G. R. Brown & Nemes, 2008; La-Vu et al., 2020).

Elevated Plus Maze/Elevated Zero Maze/Elevated T Maze (EPM/EZM/T-Maze)

These assays take place on elevated platforms in either the shape of a plus (+), circle, or the letter ‘T’, with two open arms/sections and two sections enclosed by side walls in the former two, and one closed section the T-maze. The ration of time spent in the open arms verses the closed arms in measured, with decreased time spent in the open arms seen as an increase in anxiety-like behavior (Lister, 1987; Walf & Frye, 2007).

Light/Dark Box (L/D)

Animal are placed into a two-chamber box in which one side is fully enclosed and/or black, and the other side is white, bright, and open. Time spent on each side is measured, with decreased time in the light side seen as increased anxiety-like behavior (Bourin & Hascoët, 2003; Kulesskaya & Voikar, 2014).

Novelty-Suppressed Feeding Test (NSFT)

This assay measures the willingness of a rodent to engage in feeding behaviors in a novel and potentially unsafe environment Mice are first food deprived, and then placed into a large open arena with a food pellet in the center. The latency to first take a bite of food in the center is measured, with increased time indicative of increased anxiety-like behavior (Britton & Britton,

1981; Bodnoff et al., 1988; Cryan & Sweeney, 2011; Goode & Maren, 2017; Kirlic et al., 2017).

Novelty-Induced Hypophagia (NIH)

Similar to the NSFT, NIH measures an animals willingness to seek food in a novel context. However in this assay, mice are first trained to drink a palatable liquid (often Ensure) in their home cage, and then placed in a novel environment and latency to first lick is measured, with increased latency again indicating an increase in anxiety-like behavior. It is one of few behavioral tests sensitive to both acute benzodiazepines and chronic antidepressant therapeutics (Merali et al., 2003; Dulawa & Hen, 2005; Bechtholt et al., 2007).

Translational Models

Rodent models are often a critical first step in screening potentially new pharmacotherapies in the treatment of disorders such as anxiety, but they are not able to model many of the more complex, higher-order cognitive processes involved such as worry and evaluative stress. To begin to address this, there are emerging efforts to create a bridge between basic animal models and clinical trials using models of anxiety in healthy humans. This “experimental psychopathology” approach involves using anxiety-inducing procedures in healthy individuals to identify and/or validate potential neurophysiological contributors to anxiety and then piloting potential therapeutics in these individuals to evaluate their ability to modulate anxiety induced by the tasks (Grillon et al., 2019). Together the use of these animal and human models may help to more successfully identify and develop new treatments for anxiety and other neuropsychiatric disorders.

1.3 The extended amygdala

The circuitry underlying stress responses and anxiety disorders is highly complex and involves a number of different brain regions. Some of the key regions implicated in these interactions are those contained within what is known as the extended amygdala. The extended amygdala is comprised primarily of the bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), and nucleus accumbens shell (NAc_{sh})(G. F. Alheid, 2003). These regions

have been broadly implicated in a variety of behaviors including feeding behaviors, prosocial and reproductive behaviors, punishment and reward learning, and affective regulation (Koob & Volkow, 2010; Kash et al., 2015; Centanni et al., 2022). Regarding the latter, it was previously held that these regions played distinct roles, with the CeA associated with fear-like behaviors and the BNST with sustained anxiety states (Davis et al., 2010). However, more recent work has revealed a more complex interactive contribution of both regions to threat responses more broadly, likely mediated through heavy inhibitory microcircuitry between the two (Shackman & Fox, 2016; Kovner et al., 2019; Ye & Veinante, 2019; Moscarello & Penzo, 2022). Below we will provide a brief overview of the function, circuitry, and cell-type heterogeneity within the CeA and BNST in the context of threat and anxiety-like behaviors.

1.3.1 Central nucleus of the amygdala

1.3.1.1 Role in fear and affective processing

The amygdala as a whole has long been implicated in fear responses (J. LeDoux, 2003; J. E. LeDoux, 2003; Maren & Quirk, 2004) (LeDoux 2003, Maren 2004). It is divided into the lateral amygdala (LA), basolateral amygdala (BLA), and the CeA. The LA integrates cortical and thalamic information to process detection of potential threats and passes this information to the BLA and then CeA (S. Lee et al., 2013). Much of the work on the CeA in rodent models has focused on its role in the learning and expression of Pavlovian conditioned fear-like responses. Inactivation of the CeA impairs acquisition, consolidation, and expression of fear memories (Wilensky et al., 2006; Zimmerman et al., 2007), and fear conditioning induces activity-dependent plasticity within its subnuclei (Ciocchi et al., 2010b; Haubensak et al., 2010; Duvarci et al., 2011). In monkeys and humans, it has also been shown to be recruited by a variety of threat-related cues such as aversive images and emotional faces and during sustained threat processing, often associated with states of anxiety (Yarkoni et al., 2011; Fox et al., 2015; Shackman & Fox, 2016), and rodent studies are also beginning to dissect its role in anxiety-like behaviors (Fadok et al., 2017; Paretkar & Dimitrov, 2018; Pomrenze et al., 2019; Beyeler & Dabrowska, 2020; Moscarello & Penzo, 2022).

1.3.1.2 Cell types and intra-amygdalar connectivity

The CeA is comprised of three subdivisions known as the medial (CeM, or centromedial amygdala, CEm), capsular (CeM), and lateral division (CeL), with the latter two often collectively referred to as the centrolateral amygdala (CEl) (Ciocchi et al., 2010b). Neurons in the CEl integrate input from the LA with inputs from regions such as the thalamus and sensory cortices, and project to the CEm, where this input is integrated with input from the BLA received through direct projections and via the main intercalated nucleus (Im) (Haubensak et al., 2010; S. Lee et al., 2013). The CEm is the primary output division of the amygdala, projecting to many of its downstream targets listed below, including the BNST (Tasan et al., 2016).

Within the CeA there are a variety of neuronal subpopulations thought to mediate distinct aspects of threat processing and fear conditioning. Inhibitory microcircuitry between cells expressing protein kinase C delta (PKCδ) and somatostatin (SOM) in the CeL bidirectionally modulate Pavlovian fear responses, and are often referred to as “CEl off” and “CEl on” neurons due to their roles in decreasing or increasing conditioned freezing, respectively (Janak & Tye, 2015; Beyeler & Dabrowska, 2020). There are also reciprocal inhibitory interaction between both $CeA^{PKC\delta}$ and CeA^{SOM} neurons with a population expressing corticotropin releasing factor (CeA^{CRF}), and it is thought that this population may mediate the transition from passive (freezing) to active (flight) fear responses and increase anxiety-like behaviors (Fadok et al., 2017; Beyeler & Dabrowska, 2020; Moscarello & Penzo, 2022). Other major markers of cell types in the CeA include neurotensin (CeA^{NTS}), and tachykinin 2 (CeA^{TAC2}), both showing considerable subregion-specific overlap with CeA^{CRF} and some overlap with CeA^{SOM} neurons, and dopamine receptor 2 (CeA^{DRD2}) (McCullough, Morrison, et al., 2018; Y. Wang et al., 2023), which likely also contribute to unique aspects to conditioned fear responses (McCullough, Daskalakis, et al., 2018). There is also some overlap of dynorphin expression with CeA^{CRF} neurons and enkephalin with $CeA^{PKC\delta}$ neurons (Fadok et al., 2017; Ye & Veinante, 2019; Y. Wang et al., 2023) Newer RNA-Seq based approaches are also beginning to identify different ways of classifying CeA subpopulations, finding, for example, that 1/3 of neurons in the regions can be divided into either *Nr2f2* or *Isl1*-expressing, with the function of this expression yet to be investigated (O’Leary et al., 2022; Y. Wang et al., 2023).

1.3.2 Bed nucleus of the stria terminalis

Portions of this section (1.3.2) are adapted from:

Centanni, S. W., Brown, J. A., Williford, K. M., Flook, E. A., Luchsinger, J. R., & Winder, D. G. (2022). Bed Nucleus of the Stria Terminalis (BNST) circuits. In N. W. Gilpin (Ed.), Neurocircuitry of Addiction (pp. 321–374). Academic Press

1.3.2.1 Role in stress responses and negative affect

The BNST is a notably heterogeneous brain region well-known for its role in behaviors related to stress processing and negative affect. It has also been implicated in a number of other behaviors including feeding in both stressed and unstressed states (Koob & Heinrichs, 1999; Ciccocioppo et al., 2003; Micioni Di Bonaventura et al., 2014; Y. Wang et al., 2019), and social behaviors including aggression, mating, parental care, and prosocial/altruistic behaviors (Bosch et al., 2010; M. A. Lebow & Chen, 2016; Yohn et al., 2017; Duque-Wilckens et al., 2018; Vekaria et al., 2020; Flanigan & Kash, 2022). It is highly stress-sensitive, showing increased activation in response to both physiological and psychological stressors. Acute stressors such as footshock and restraint stress reliably increase expression of cfos in the BNST (Bechtholt et al., 2007; Sterrenburg et al., 2011; Ventura-Silva et al., 2012; Babb et al., 2013; Fetterly et al., 2019; L. Á. Kovács et al., 2022), as well as administration of inflammatory, HPA-axis activating compounds such as IL-1 (Ericsson et al., 1994). Acute presentation of multiple stressors simultaneously also results in increased cfos in the BNST (Lin et al., 2018), as does chronic exposure to variable stressors (L. Á. Kovács et al., 2022), and both acute and chronic stress results in a variety of synaptic and electrophysiological changes withing the BNST (Vyas et al., 2003; McElligott & Winder, 2008; Mcelligott et al., 2010; Conrad et al., 2011; Ventura-Silva et al., 2012; Partridge et al., 2016; Snyder et al., 2019; Maita et al., 2022). We have also previously shown that there is an increase in calcium signaling, as measured by GCaMP, and glutamatergic drive, as measured by GluSNfR, while actively coping with stressors such as restraint and in the NSFT variety of stressors (Jaramillo et al., 2020; Luchsinger et al., 2021; J. A. Brown et al., 2022).

It's stress-sensitivity position it well to mediate a number of stress-related behaviors. It is

highly implicated in anxiety in both humans and animal models. Lesions of the BNST abolish or reduce both basal and stress-induced anxiety-like responses, in part through alteration of stress-related cortisol release, suggesting it is critical for driving anxiety-like behaviors (Davis et al., 1997; Y. Lee & Davis, 1997; Sullivan et al., 2004; Duvarci et al., 2009). Indeed, optogenetic inhibition of the BNST also decreases anxiety-like behavior while optogenetic activation results in increased anxiety-like behavior (S.-Y. Kim et al., 2013). The BNST is activated by a variety of threat presentations and its activity covaries with self-reports and physiological measures of fear and anxiety (Somerville et al., 2013; McMenamin et al., 2014; Alvarez et al., 2015; Banihashemi et al., 2015; Shackman & Fox, 2016). Activity in the BNST can distinguish between safe and anxiogenic environments (S.-Y. Kim et al., 2013). It is also a key regulator in the withdrawal/negative affect stage in the cycle of substance use disorders (Koob, 2008; Jennings, Sparta, et al., 2013). Acute and chronic administration as well as abstinence from drugs of abuse drive plastic changes in BNST physiology and its activity plays a key role in driving cue- and stress-induced reinstatement of drug seeking (Delfs et al., 2000; Shalev et al., 2001; Koob & Volkow, 2010; Ch'ng et al., 2018; N. A. Harris & Winder, 2018; Centanni, Bedse, et al., 2019; Perez et al., 2020). It is important to note, however, that these affects are highly cell type and projection specific, with many subpopulations and efferent pathways having opposing effects on anxiety and reward-related behaviors (Kim 2013, Jennings 2013, and these differential contributions will be further parsed in the following sections

1.3.2.2 Circuitry

1.3.2.2.1 Afferents

The BNST receives input from regions including cortical, amygdalar, hypothalamic, and brainstem nuclei. More specifically, the BNST receives glutamatergic input from regions including the parabrachial nucleus (PBN), basolateral amygdala (BLA), paraventricular thalamus (PVT), ventral hippocampus (especially the subiculum, vSub), entorhinal cortex (EC), lateral hypothalamus (LH), olfactory bulb (OB), and a variety of subregions in the prefrontal cortex including the orbital, infralimbic, and prelimbic cortices (Hurley et al., 1991; Takagishi & Chiba,

1991; Canteras & Swanson, 1992; Cullinan et al., 1993; Mcdonald, 1998; H. W. Dong et al., 2001; Vertes, 2004; Myers et al., 2014; Penzo et al., 2015; Ch'ng et al., 2018). It receives GABAergic input from regions including the CeA, which is the primary source of GABAergic afferents, as well as the medial amygdala (MeA), globus pallidus, striatum, lateral septum, nucleus accumbens shell, and hypothalamic subregions including the paraventricular hypothalamus (PVH) and arcuate nucleus (Arc) (Cullinan et al., 1993; Mcdonald, 1998; H. W. Dong et al., 2001; Dabrowska et al., 2011; Betley et al., 2013; Myers et al., 2014; Vranjkovic et al., 2017; Ch'ng et al., 2018).

The BNST also receives a variety of neuromodulatory inputs releasing dopamine (DA), serotonin (5-HT) and norepinephrine (NE). Dopaminergic input from the ventral tegmental area (VTA) and periaqueductal grey area (PAG) are heavily implicated in the BNST's role in reward and arousal (Freedman & Cassell, 1994; Carboni et al., 2000; Meloni et al., 2006; B. Kim et al., 2018). Serotonergic input from the caudal regions of the dorsal raphe nucleus (DRN) play a role in mediating anxiety-like behaviors (Block & Hoffman, 1987; Phelix et al., 1992; Commons et al., 2003; Lowry et al., 2008; J. D. Guo & Rainnie, 2010). The BNST receives one of the densest NE projections in the brain from the nucleus tractus solitarius (NTS) via the ventral noradrenergic bundle, playing a critical role in BNST-mediated stress responses and reward behaviors(Ricardo & Tongju Koh, 1978; Egli & Winder, 2003; Forray & Gysling, 2004; Banihashemi & Rinaman, 2006; J. Park et al., 2009; S. A. Flavin & Winder, 2013; N. A. Harris, Austin, et al., 2018). Many of the afferents to the BNST also co-release neuropeptides, and the role of this signaling is discussed in further detail below.

1.3.2.2.2 Efferents

Many of the outputs of the BNST are to regions with which it shares reciprocal connectivity. Projections back to the VTA are implicated in anxiety and substance use disorders (Georges & Aston-Jones, 2001, 2002; H. W. Dong & Swanson, 2006b; Kudo et al., 2012; Jennings, Sparta, et al., 2013)Both glutamatergic and GABAergic projections to the VTA can have bidirectional effects on both anxiety and addiction-related behaviors, with GABAergic vBNST→VTA projections having anxiolytic effects and increasing reward seeking, while glutamatergic BNST→VTA projection activation is aversive, increases anxiety-like behavior, and decreases reward-seeking (Jennings,

Sparta, et al., 2013; Stamatakis et al., 2014). Glutamatergic and GABAergic projections back to the PBN have also been shown to play distinct roles in feeding and are differentially recruited by aversive stimuli and anxiogenic environments (Luskin et al., 2021). Reciprocal connectivity with hypothalamic subregions such as the PVH and LH are important in regulating HPA-axis mediated stress responses and feeding (Roland & Sawchenko, 1993; H. Dong et al., 2001; H.-W. Dong & Swanson, 2004; H. W. Dong & Swanson, 2006b; Barbier et al., 2021). The BNST shares additional reciprocal projections with the CeA NTS, DRN, PVT, PAG, and PBN (H.-W. Dong & Swanson, 2004; S.-Y. Kim et al., 2013; M. A. Lebow & Chen, 2016; Ch'ng et al., 2018). The BNST also projects to regions including the medial preoptic area and substantia nigra (H.-W. Dong & Swanson, 2004; H. W. Dong & Swanson, 2006b; McHenry et al., 2015; Torrisi et al., 2015). Many of the functions of BNST efferents are highly cell-type specific, and the next section will examine some of this cell-type and functional heterogeneity.

1.3.2.3 BNST Heterogeneity

The BNST can be divided into several subregions and is composed of numerous cell types that can be classified by their expression of distinct neurotransmitters, proteins, neuropeptides, ion channels, and other proteins. Considerable effort has been placed on categorization of the BNST's heterogeneity, and the emergence of new tools has recently enabled functional characterization of these cell groups, enhancing our understanding of the role of this complex region in a variety of affective, social, and reward processing behaviors.

1.3.2.3.1 Anatomical subregions of the BNST

The rodent BNST is typically divided into 12-18 anatomical subregions (Ju & Swanson, 1989; Moga et al., 1989; G. F. F. Alheid et al., 1998; H.-W. Dong & Swanson, 2004) whose projections and functions have been thoroughly reviewed (M. A. Lebow & Chen, 2016; Ch'ng et al., 2018; Flanigan & Kash, 2022) and are summarized here. Standard nomenclature divides the BNST into anterior (aBNST) and posterior (pBNST) divisions, defined in relation to a bundle of fibers associated with the stria terminalis. The pBNST contains three subnuclei: the principal nucleus (BNSTpr), interfascicular nucleus (BNSTif), and transverse nucleus (BNSTtr). The aBNST

can be further divided into dorsal (dBNST) and ventral divisions (vBNST), defined in relation to the anterior commissure, and collectively contains nine commonly accepted subnuclei. The dBNST contains the oval nucleus (BNSTov), juxtacapsular nucleus (BNSTjx), and anterolateral nucleus (BNSTal) (the three of which are collectively referred to as the dorsolateral BNST (dlBNST)), and anteromedial nucleus (BNSTam). The vBNST contains the fusiform nucleus (BNSTfu), the magnocellular nucleus (BNSTmg), the rhomboid nucleus (BNSTrh), the dorsomedial nucleus (BNSTdm), and the ventral nucleus (BNSTv). The BNST's subregion heterogeneity is critical in achieving its integrative function in "valence surveillance" (M. A. Lebow & Chen, 2016), with different subregions mediating distinct, and sometimes competing, effects on behavior. For example, the BNST is a major modulator of the Hypothalamic-Pituitary-Adrenal (HPA) axis, which, as discussed above, is responsible for mediating the body's stress responses. The aBNST, and in particular, the BNSTdm, drives activation of the HPA axis, while the pBNST inhibits HPA axis activity (Choi et al., 2007; Choi et al., 2008; Radley et al., 2009). This is likely driven in-part by inhibitory intra-BNST connectivity, with the BNSTpr providing input to the BNSTam, BNSTmg, and BNSTdm. The small size of many BNST subnuclei and technical limitations has made precise functional characterization challenging, but existing work is briefly summarized below.

Anterior BNST

In addition to driving activation of the HPA axis, the aBNST is also involved in social vigilance, mediating responses to social threats, and stress-induced impairments in reward and prosocial behaviors (Jasnow et al., 2004; Forray & Gysling, 2004; Dumais et al., 2016; Klampfl et al., 2016; Duque-Wilckens et al., 2018, 2020). Within this division, the BNSTov integrates information about mood and negative valence, and generally promotes anxiety-like behavior (S.-Y. Kim et al., 2013). The BNSTal is thought to be involved in the integration of mating behaviors, bonding, and neuroendocrine state with mood, pain processing, exteroceptive sensory information, threats, and digestive homeostasis, while the BNSTam integrates drinking and fluid homeostasis information with HPA axis activation and fight-or-flight responses through connectivity with brainstem nuclei (Krettek & Price, 1978; Bruijnzeel et al., 1999; H. Dong & Swanson, 2004; H. W. Dong & Swanson, 2006b, 2006a; Romero & Butler, 2007; J. Park et al., 2012; Bienkowski &

Rinaman, 2013; Ide et al., 2013; Klampfl et al., 2016; Lange et al., 2017).

The vBNST contains the highest concentration of noradrenergic (norepinephrine, noradrenaline, NE) fibers in the brain (Kilts & Anderson, 1986; Phelix et al., 1992). It plays a key role in arousal, and mediates cardiovascular response integration through connectivity with the nucleus of the solitary tract (NTS) and central amygdala (CeA) (Roder & Ciriello, 1993; Dunn & Williams, 1995; Forray et al., 2000). The vBNST is also highly stress responsive, releasing noradrenaline both tonically and in response to immobilization stress, foot shock, fox odor, forced swim stress, and unreceived anticipated reward (Palij & Stamford, 1992; Cecchi et al., 2002; Forray & Gysling, 2004; Fendt et al., 2005; Briand et al., 2010; J. Park et al., 2013). Like the dBNST, the vBNST is involved in regulation of prosocial behaviors and specifically modulates decreased prosocial behaviors after exposure to alcohol, and may also regulate aggressive and sexual behaviors through connectivity with the ventromedial hypothalamus (VMH) (H. Dong & Swanson, 2004; H.-W. Dong & Swanson, 2006; Marcinkiewcz et al., 2015; Y. Kim et al., 2015; Duque-Wilckens et al., 2020). The vBNST can also work in opposition to the dBNST, providing direct inhibitory input to the paraventricular nucleus of the hypothalamus (PVN) to inhibit HPA axis activity in the face of inescapable stressors while simultaneously promoting active coping strategies through inhibition of the ventrolateral periaqueductal gray (vlPAG) (J. J. Radley & Johnson, 2018). Finally, the vBNST also drives feeding and reward behavior, which will be discussed in more detail later in this chapter. Most subnuclei within the vBNST have not been well characterized individually, with the exception of the BNSTmg and BNSTdm, which are both involved in neuroendocrine and autonomic control of fluid and nutrient homeostasis and are largely responsible for the aBNST's activation of the HPA axis. The BNSTdm has also been implicated in modulating prosocial approach and avoidance behavior via the PVN, integrating social information with reward or stress responses and adjusting mood accordingly (H. W. Dong & Swanson, 2006b; H.-W. Dong & Swanson, 2006; McFadden et al., 2012; Ventura-Silva et al., 2012).

Posterior BNST

The pBNST is the most sexually dimorphic region of the extended amygdala across species and modulates many sex-specific social behaviors including mating and reproduction, parental

care, and social defense and aggression behaviors (Laflamme et al., 1998; Goodson & Kabelik, 2009; Ventura-Silva et al., 2012; Campi et al., 2013). The BNSTif and BNSTtr likely play an important role in mediating many of these behaviors through their possible role in social recognition (Petrulis & Johnston, 1999; H.-W. Dong & Swanson, 2004). The BNSTpr is the most heavily studied of the posterior nuclei. It plays a role in stress responses and aggression, and as mentioned above, is critical in the integrative function of the BNST as a whole through its intra-BNST projections to regions like the BNSTmg and BNSTdm (H.-W. Dong & Swanson, 2004; Nelson & Trainor, 2007; Ventura-Silva et al., 2012). The pBNST may combine social information with reward cues via interconnectivity with the ventral tegmental area (VTA) in order to assign valence to social interactions and, through the principal nucleus, bidirectionally regulate motivation to engage in social behavior and/or activation of the HPA axis (Herman & Larson, 2001; D. C. Choi et al., 2007, 2008; M. Lebow et al., 2012; Ventura-Silva et al., 2012).

1.3.2.3.2 BNST cell types – classifications and functions

In addition to distinct subregions, the BNST's integrative function is further facilitated by its cell-type heterogeneity. BNST neurons can be categorized in a variety of ways, including the neurotransmitter release, expression of various proteins/neuropeptides, and electrophysiological profiles.

Neurotransmitters and neuromodulators

The vast majority of neurons in the BNST (~90%) release GABA, form symmetric synapses, and express vGAT (vesicular GABA transporter). ~10% of BNST neurons release glutamate, form asymmetric synapses, and express vGlut2 (vesicular glutamate transporter). There is also a very small proportion of neurons that expresses vGlut3, but these neurons also express GAD67 for GABA production, typically release GABA, and form symmetric synapses, suggesting they are primarily inhibitory (Kudo et al., 2012; Moffitt et al., 2018; Welch et al., 2019; Rodriguez-Romaguera, Ung, Nomura, Otis, Basiri, Namboodiri, Zhu, Elliott Robinson, et al., 2020). GABAergic and glutamatergic neurons in the BNST play distinct roles that have been especially well-studied in the vBNST, where glutamatergic projections to the VTA are activated by stress, and

activation of these afferents decreases reward seeking and increases anxiety. GABAergic vBNST projections to the VTA are inhibited by stress, and their activation is rewarding, anxiolytic, and drives drug seeking and reinstatement (Jennings, Sparta, et al., 2013; Companion & Thiele, 2018).

The BNST is also a hub for monoamine signaling, and different distributions of cells expressing these receptors can be seen throughout the region. For example, cells expressing the D2 dopamine receptor can be found fairly evenly distributed throughout the dBNST and vBNST, with a slight enhancement in the dlBNST. In contrast, D1R-expressing cells are sparse, with a small amount of expression primarily restricted to the dBNST, but with a striking lack of presence in the rat BNSTov (Krawczyk et al., 2011; Yoon & Baik, 2015; J. Lu et al., 2021). Adrenergic vs non-adrenergic cells expressing alpha2AR (autoreceptor vs heteroreceptor populations) can have differential effects on BNST activity and involvement in stress-induced reinstatement of drug seeking (including opiates and cocaine), analgesia, sedation, and cognitive function in an input-specific manner (Delfs et al., 2000; X. Wang et al., 2001; Leri et al., 2002; Gilsbach et al., 2011; S. a Flavin et al., 2014; N. A. Harris, Austin, et al., 2018; Perez et al., 2020). Cell populations defined by expression of distinct serotonin (5-HT) receptor subtypes can also have opposing effects on the expression of fear- and anxiety-like behaviors (Marcinkiewcz et al., 2019; Hessel et al., 2020).

Neuropeptides/Proteins

The BNST is a complex hub of a multitude of cell types that can be defined by their expression of neuropeptides and/or the receptors for those neuropeptides, with differing degrees of overlap between expression profiles. These include cell bodies expressing corticotropin releasing factor (CRF), somatostatin (SOM), neuropeptide Y (NPY), neurotensin (NT), Arginine vasopressin (AVP), nociceptin (NOC), substance P (SP), galanin (GAL), cholecystokinin (CCK), enkephalin (ENK), dynorphin (DYN), and oxytocin (OT), as well as cells expressing the receptors for each of these plus pituitary adenylate cyclase-activating peptide (PACAP) and orexin (OX) (Lesur et al., 1989; Ju et al., 1989; Walter et al., 1991). Additionally, the outliers of the neuropeptide classification conversation are cells expressing protein kinase C delta (PKCd). While not a neuropeptide, it is a common marker of an anatomically and functionally distinct population of cells in both the CeA and BNST (Haubensak et al., 2010; Fetterly et al., 2019; Ye & Veinante, 2019).

It is important to note that, while there is often overlap, the cell population expressing the receptor for a neuropeptide is not equivalent to the population expressing the peptide itself. For example, there exist complex bidirectional interactions between the CRF and OT systems in the regulation of maternal social behaviors wherein OT neurons express CRFR2 and CRF neurons express OTR (Dabrowska et al., 2011; Martinon & Dabrowska, 2018; Klampfl & Bosch, 2019). Other neuropeptides such as Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) do not have cell bodies present in the BNST, but it nonetheless plays an important signaling role in BNST through neuronal populations on which its receptors are expressed (Hashimoto et al., 1993; Kozicz et al., 1997; Hammack et al., 2010).The expression profiles and function of many of these cell types have been extensively characterized, while others are just beginning to be investigated (for reviews, see (Kash et al., 2015; M. A. Lebow & Chen, 2016; Ch'ng et al., 2018; Flanigan & Kash, 2022) and findings on peptide-expressing and peptide receptor-expressing BNST cell populations are summarized in Figure 1 and Table 2. Recent advances in single-cell profiling have also resulted in new categorizations of BNST cells based on their genetic profiles, with one group identifying 11 distinct subpopulations and another finding 41 separate cell types (Welch et al., 2019; Rodriguez-Romaguera, Ung, Nomura, Otis, Basiri, Namboodiri, Zhu, Elliott Robinson, et al., 2020). Future work may reveal even more nuance in the function of BNST subpopulations.

Figure 1.1 BNST subregions and cell types

Cell Type in BNST	Behavioral Correlate
AVP	↓↑ Sickness ↑ Social ↑ Reproduction
CRF	↑ Anxiety ↑ Relapse
CCK	↑ Positive Valence
DYN	↑ Dysphoria
ENK	↓ Anxiety ↑ Fear
NOC	↓ Anxiety ↑ Feeding
NPY	↓ Anxiety ↓ Pain ↓ EtOH Binge ↑ Feeding
NT	↑ Reward ↑ Reinforcement
OXT	↑ Maternal behavior ↑ Agression
PKCd	↓ Feeding
SOM	↓ Anxiety ↑ Fear

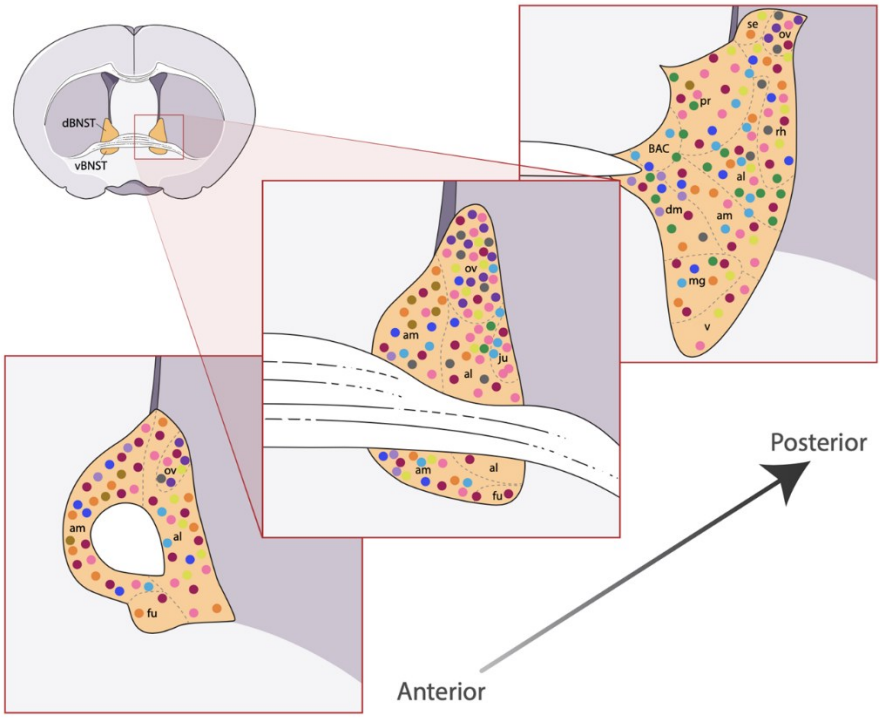


Diagram of cell-type distribution in the BNST. Three planes of the BNST are shown. Planes progress from anterior to posterior when moving from left to right. Cell types included: AVP = arginine vasopressin, CRF = corticotropin releasing factor, CCK = cholecystokinin, DYN = dynorphin, ENK = enkephalin, NOC = nociception, NPY = neuropeptide Y, NT = neurotensin, OXT = oxytocin, PKCd = protein kinase C delta, SOM = somatostatin.
Citations located within Table 2, full list in Appendix 5.2

Table 2 BNST Peptidergic* Subpopulations

Cell Type	Distribution and Expression	Function Summary	Specific Findings
AVP	-Mostly pBNST, some BNSTam in both mice[1–3] and rats[4]. -Higher levels in females than male rats[5,6]	-Increases reproduction and parental behaviors -Increases aggressive behaviors -Increases prosocial behaviors -Sex specific effects on sickness behavior	AVP cell ablation reduces female copulatory behavior[7] Increased expression of AVPR1a mRNA, peptide levels (100 fold) and receptor binding during birth and lactation in rats, stimulated by estrogen[5,8,9] Increases AVP expression correlated with increased parental territoriality in male and female mice[10] AVPR1a antagonism reduces maternal aggression[11] AVP cell ablation[7] and AVR1aR[12] antagonism reduces prosocial interaction AVP infusion decreases male aggression in rats but antagonism of AVPR1 had no effect[13] Aggression positively correlated with number of AVP cell bodies in unstressed but not stressed mice[14] AVPR1a activity associated with higher aggression in Syrian hamsters[15] Ablation of AVP neurons reduces preference for novel social interaction (same-sex social recognition?) in males[16] AVP cell ablation increases sucrose consumption[16] AVPR1a antagonism reduces anxiety-like behavior[11] AVP cell ablation reduces sickness behavior in males, increases in females[16]
CCK	-Mostly BNSTam and a little alBNST[17]	-Increases positive valence -Increases reproductive/ social reward	ChR2 activation of cell bodies and projections to LH is reinforcing[17] Mice will self-admin drinking CNO with Gq DREADD expressed in CCK cells[17] Gi DREADD or expression of prevents preference Kir2.1 (inhibitory K+ channel) abolishes preference of male mice for female urine scent[17]
CRF	-Diffuse dBNST and vBNST of mice[18–21], restricted to BNSTov (GABAergic) and BNSTfu (Glutamatergic) in rats[22,23] -More CRF neurons in BNSTov and BNSTal in females than males[24] -~60% overlap with NT in rats, 1% with ENK[25,26]	-Increase anxiety -Drive relapse -Decreases maternal behaviors -Involved in regulation of social behaviors	Stress increases amount of CRF mRNA in subregion specific manner[27–32] Increased cfos in CRF neurons following variety of stressors[31,33] BNST CRF infusion[34] and ChR2 stimulation[17] is aversive, aversion blocked by either CRFR1 or CRFR2 antagonist[34] BNST CRF infusion increases anxiety. Anxiety blocked by CRFR1 antagonist[34] Intra BNST CRF infusion and activation of CRFR1 drives reinstatement of cocaine seeking[35] CRF signaling required for stress-induced reinstatement of drug seeking[27–32] CRF mRNA decreased during lactation, CRFR1 and CRFR2 agonists decrease nursing behaviors, antagonism prevents stress-induced reductions in nursing[36–38] Repeated sub-anxiogenic doses of CRFR1 antagonism decreases prosocial behavior[39] Antagonism of CRFR1 or CRFR2 blocks increases in submissive behavior after social defeat[40,41]
DYN	-Cell bodies throughout dBNST and vBNST of mice[42] -ppDYN mRNA highly expressed in BNSTal, BNSTov, BNSTrh, and BNSTfu, moderately in BNSTam and BNSTif of rats[43] -High density of KOR expressed in BNSTdm, BNSTpr, and BNSTv. DYN and KR only overlapped in BNSTrh[43] -Colocalization with SP[44] -mRNA 67% colocalized with NT in BNSTov of mice[45]	-Stress-sensitive, drives stress responses (dysphoria-like?) -Drives drug escalation -May increase anxiety	Stress induces KOR phosphorylation[46] KOR antagonism and dynorphin gene deletion blocks stress-induced aversion[46] ppDYN mRNA increases after forced swim[47] KOR agonist causes conditioned place aversion, reduces social interaction[48] KOR agonists block anxiolytic effects of BLA-->BNST activation, block glutamatergic transmission[42,49] Opto activation of BNST DYN(+) cells inhibits EPSCs in DYN(-) BNST cells[42] DYN/KOR decrease inhibitory transmission into the BNST[45,50] KOR agonist reduces social interaction[48]
ENK	-mRNA in rats mostly in BNSTal, BNSTam, (GABAergic) and pBNST (18% glut), very little in BNSTpr and BNSTrh, absent in BNSTfu[25,43] -~10% coexpression with	-Activated by stress/ withdrawal -May decrease anxiety	Activated by IL-1B[25] ENK neurons showed cfos activation in morphine-dependent rats after naloxone injection[51] Affective symptoms of withdrawal increase cfos in ENK-producing neurons[52] Might decrease anxiety-like behavior, possibly through projections to the CeA[53]

	NT, 1% with CRF[25]		
GAL	-BNSTal and vBNST of rats[54] -Largely overlapping with VIP[55] -Increased expression in females[56]	-Increases stress-induced anxiety	GAL antagonism into BNST immediately prior to acute immobilization stress prevented stress-induced reductions in social interaction time and stress-induced decreased in open arm time in EPM. No effect on behavior in unstressed rats[57]
			-Testosterone recovers expression levels of GAL and number of GAL+ neurons following testicular removal in rats[58]
NOC/ Pnoc	-Cell bodies primarily in BNSTal and BNSTam, very few in BNSTjx or vBNST[59] -Pnoc neurons throughout BNST, enriched in dBNST[60] -NOC fibers concentrated in vBNST, little in pBNST[59] -Over 50% of BNST neurons contain functional NOC[61] -88% of Pnoc neurons co-express with either SOM, PKCδ, CCK, or Zic1. Little to no overlap with Foxp2, pENK Calb2, CRF, NT, or vglut3[60]	-Increases feeding -Decreases anxiety	NOC into BNST or NOP agonist can block stress- and CRF-induced anorexia[62]
			Microinjection of NOC in BNST can block CRF injection-induced anxiety in rats[63]
			ICV injection CRF increases NOP expression in BNST[63]
			Opto stim of Pnoc BNST neurons increased physiological measurements of arousal (pupillary response and heart rate) with no effect on approach/avoidance in RTPP or locomotor behaviors[60]
NPY	- densest in vBNST, moderate in dBNST EXCEPT BNSTov, little expression in pBNST in both mice[64] and rats[65] -Highly overlapping expression of NPY and Y2R[64]	-May decrease anxiety -May improve stress coping -Decreases pain aversiveness -Expression increased by drugs of abuse, decreases EtOH binging -Implicated in neurodegenerative disorders	Increased NPY-positive neurons correlated with decreased anxiety-like behavior[66]
			NPY into dlBNST suppressed pain-induced and CRF infusion-induced conditioned place aversion, effect blocked by Y1 or Y5 antagonist. Through decreased excitability of type II neurons[67]
			Y2R gene deletion from CeA (which sends GABAergic inputs to BNST) decreased anxiety-like and depressive-like behavior. Deletion in BNST had no effect[68]
			ICV CCK4 reduces NPY expression in BNST, ICV NPY or Y1R agonist attenuate the anxiogenic and depressive-like effects of CCK-4 administration[69]
			NYP inhibits BNST GABAergic transmission through Y2 (in contrast to CRF, which enhances it)[70]
			Chronic restraint stress increases NPY and Y2 expression and reduces inhibitory effect of Y2 in stress-susceptible mouse strain[64]
			Behavioral flexibility in stress coping is associated with increased NPY expression[71]
			Heroin injection[72] and morphine self-administration[73] increases NPY IR in dlBNST
			NPY Y1 receptor (Y1R) activation in the BNST suppressed binge alcohol drinking[74]
			Nicotine injection decreases NPY IR in dl BNST[75]
			Colchicine treatment (causes AD phenotype in rats) decreased NPY IR in BNST. 4 days of nicotine treatment restores NPY IR to previous levels and improves memory[76]
			NPY IR in BNST increased in people with Huntington's Diseasse[77]
NT	-concentrated in the BNSTov of rats[26] and mice[78], scattered in BNSTjx of rats[79] and pBNST of mice[80] -Little overlap of NT neurons with NTR1 (6%) or NTR2 (12%)[78] -10% overlap with ENK[25], larger overlap (50-80%) with CRF[26,79] in rats -67% overlap with DYN in BNSTov of mice[45]	-Increases stress-induced anxiety -Decreases acute effects of cocaine	Blocking NTR in BNSTov prevents stress-induced anxiety-like behavior (normalizes EPM open arm time) in rats[81]
			NT increases inhibitory input from the CeA[45]
			NT increases IPSCs in the BNST[82]
			NTR antagonism blocks cocaine-mediated increases in D1-mediated LTP of IPSCs[82]
OX	-No cell bodies. Ox-A fibers[83,84] and Ox1R[85] present throughout dBNST, and vBNST, especially BNSTam	-Increases reinstatement -Increases anxiety -Increases arousal	OX1R antagonism blocks NPS-induced enhancement of cue-induced alcohol seeking[83]
			Activation of Hcrt-1/Ox-A terminals in the BNST drives reinstatement of alcohol seeking[83]
			BNST OX implicated in stress-induced reinstatement[87]
			OX1R antagonism blocks yohimbine-induced impairment of cocaine-CPP

	-Ox-B fibers throughout dBNST, concentrated in BNSTal/BNSTov in rats[86]		extinction[88]
			orexin A depresses excitatory transmission in BNST via OX1R. OX1R antagonism block yohimbine-induced depression of excitatory transmission[88]
			Ox-A infusion into BNST increases anxiety-like behavior[89]
			Dual OX receptor antagonist attenuates BNST-mediated arousal/sustained wakefulness[90]
OXT	-In mice, cell bodies in vBNST and BNSTam, OXTR expression in amBNST[91,92] -In rats, no cell bodies (only terminals), OXTR expression in, pBNST and BNSTov[93] -3x more in male rat pBNST than females[93] -Higher expression in proestrus than estrus in dlBNST of rats[93] -OT inputs co-express CRFR2[94]	-Increases aggression -Increases maternal motivation and behaviors (sometime maternal aggression) -Involved in social recognition and social-defeat stress -Increases fear learning	Pup-directed aggression in males mice correlated with increased cfos in BNST OXT neurons[95]
			Increased OXTR binding in aggressive rats[96]
			OXT infusion decreases biting toward male intruder[97]
			OXT infusion reduced maternal aggression[97]
			OXTR antagonism reduces maternal motivation
			Increased expression of OXTR mRNA and receptor binding during birth and lactation in rats[8,9]
			increased cocaine-driven maternal aggression associated with increased OXTR binding[98]
			Cocaine decreased OTR binding density[98]
			OXTR antagonism reduces same-sex social recognition in males and females. OXT infusion increases social recognition in males[99]
			OXT infusion into BNSTam reduces social approach behavior and increases social vigilance in males and female mice[91]
			Inhibition of OXT production impairs social-defeat stress-induced impairments in prosocial behavior[91]
			OXT cells activated (increased cfos) immediately after 3 days of social defeat stress in male and female mice[92]
			Number of OXT cells and activated (Cfos) OXT cells in females 2 weeks after and 10 weeks after social defeat stress[92]
			Sex- and stress- specific effects of intranasal OT on social interaction and resident intruder freezing[92]
			OXTR antagonism in dlBNST impairs acquisition of cued fear, but OXT infusion had no effect[100]
			OXT infusion facilitates temporally predictable cued fear learning[101]
PKCδ*	-Restricted to BNSTov in mice and rats[33,102,103] -Co-expresses D2R in rats[102]	-Decrease feeding -Stress sensitive -Conflicting reports on involvement in anxiety	Activation of PKCδ cells inhibits feeding[104]
			Increased expression of PKCδ mRNA and cfos/PKCδ mRNA colocalization after stress[33]
			Increased coexpression with CRF mRNA in female mice after stress[33]
			Optogenetic activation had no impact on anxiety-like behavior[104]
			Gq-DREADD activation decreased anxiety-like behavior[105]
			Inhibition of synaptic transmission in PKCδ cells decreased anxiety-like behavior[106]
SOM	-Restricted to BNSTov in rats[26], mostly in BNSTov and some throughout rest of dBNST, vBNST, and pBNST in mice[20] -20-40% colocalization with SP in rats[26]	-Conflicting reports on anxiety effects (possibly projection-specific) -Increases fear learning	Opto stim of SOM to NAc shell PV interneurons: decreases anxiety[107]
			Opto stim of SOM to NAc shell PV interneurons increases social interation[107]
			Activation of CeA^{Som} to $BNST^{Som}$ neurons increases anxiety [108]
			Gq DREADDs increase cue-related fear recall (fear memory consolidation), increased fear generalization. Gi DREADD had no changes from control[20]
SP	-BNSTal in rats[26], vBNST with almost none in dBNST of mice[78] -60% overlap with SOM in rats, even higher during development until 7wo[26,109] Overlap with proDYN and neurokinin A in hamsters[44,110]	-Sensitive to testosterone and circadian effects -Implicated in neurodegenerative diseases -Unclear if involved in anxiety in the BNST	SP admin in BNST can activate the preoptic area[111]
			Bilateral enucleation decreased number of SP neurons in the BNST. Prevented by treatment with testosterone[112]
			Testosterone-dependent seasonal reduction in SP expression in hamsters (during exposure to short photoperiod)[113]
			Decreased SP IR in Huntington's disease[77,114]
			BNST increases in cfos by exposure to EPM not effected by NK1R antagonism(but possible floor effect)[115]
			SP increases anxiety in CeA, MeA, BLA, LC, PVN, VMH, LS, and PAG but not looked at in BNST[116]
VIP/ PACAP	-No cell bodies, fibers densest in dlBNST, some in vBNST[117] -PAC1R mRNA throughout dBNST and vBNST[118], very little VPAC1[119] or VPAC2[120] -Large overlap in VIP+ and PACAP+ terminals[121]	-Increases stress responses and anxiety-like behaviors -Drives stress-induced inhibition of feeding	PACAP and VIP+ terminals in close proximity to CRF and ENK cells[122]
			PACAP upregulates expression of CRF[123–125]
			Chronic variable stress increases expression of PACAP and PAC1R in BNST.[126,127]
			BNST PACAP infusion leads to activation of the HPA axis as determined by increases in plasma corticosterone[127,128]
			PAC1 receptor antagonism during CVS attenuates stress-induced behavioral responses and changes in weight gain[128]
			BNST PACAP infusion amplifies CVS-induced stress responses[129]
			PACAP and PAC1 receptor knockout mice which exhibited a diminished anxiety responses to stressors[130,131]
			BNST PACAP38 dose-dependently decreased body weight, as well as food and water intake[132]

*PKCδ is not a neuropeptide, but is a major expression-defined cell type of the BNST
Cell type abbreviations: AVP: Arginine Vasopressin, CCK: Cholecystokinin, CRF: Corticotropin-releasing factor, DYN: Dynorphin, ENK: Enkephalin, GAL: Galanin, NOC: Nociceptin, *Pnoc*: Prepronociceptin NPY: Neuropeptide Y, NT: Neurotensin, Hcrt-1/Ox-A: Hypocretin-1/orexin-A, OXT: Oxytocin, PKCδ: Protein Kinase C Delta, SOM: Somatostatin, SP: Substance P, VIP: Vasoactive intestinal polypeptide, PACAP: Pituitary Adenylate Cyclase-Activating Peptide,
Other abbreviations: AD: Alzheimer's disease, HD: Huntington's disease, ICV: intracerebroventricular, IR: immunoreactivity

Electrophysiological

Electrophysiological characterization is a common method that has been used to classify neurons in a number of brain regions including cortical areas, the striatum, and the amygdala (SAH et al., 2003; Markram et al., 2004; Kreitzer, 2009; Pape & Pare, 2010; Seung & Sümbül, 2014; van Aerde & Feldmeyer, 2015). Knowledge of the physiological properties of cell types can provide insight into their functional role, and characterized synaptic responses can enable cells to be identified *in vivo* to understand activity in awake and behaving animals. While extensive studies have been done to classify BNST cells into distinct electrophysiological groupings, this work has suffered from the presence of considerable differences across species, and difficulty in identifying the functional/behavioral relevance of each type (Silberman et al., 2013; Daniel et al., 2017). In general, four BNST cell types have been described: Type I, Type II, and Type III, and "other" (neurons not fitting into any of these classifications (Daniel et al., 2017), and findings of their distribution in rodents is summarized in Figure 2.

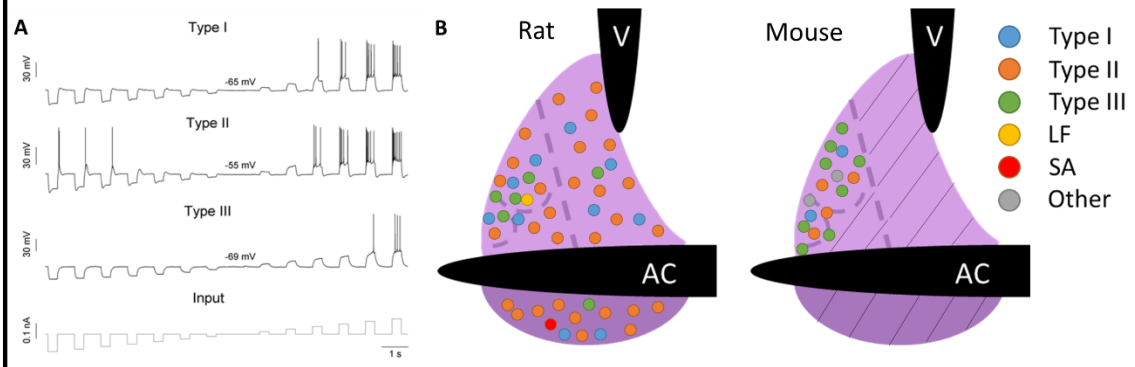
Type I neurons are typically characterized by their regular firing rate (and as such, have also been termed Regular Spiking Cells) but show spike frequency adaptation after prolonged depolarization (Rodríguez-Sierra et al., 2013). Additionally, they show a prominent depolarization sag following a hyperpolarizing step, meaning they depolarize to a slightly less-hyperpolarized level after initial hyperpolarization. Type I cells also do not show burst firing after injection of depolarizing current, and they do not show Low-Threshold Spiking (LTS), meaning they require significant stimulation to fire an action potential (Gauss & Seifert, 2000; Monteggia et al., 2000; Hammack et al., 2007; Szücs et al., 2010).

Type II neurons are characterized by a more pronounced depolarization sag in response to hyperpolarization, which is followed by rebound spiking upon termination of the injection of hyperpolarizing current, and generally show prevalent LTS (Hammack et al., 2007). The majority of Type II neurons also display burst firing, leading to the alternate name of Low-Threshold Bursting (LTB) cells, meaning less current is needed to evoke an action potential (Rodríguez-Sierra et al., 2013). However, the presence of bursting is highly variable within Type II neurons, with some displaying only single spikes at membrane potentials more depolarized than -70mV, and others

exclusively responding with single spikes (Rodríguez-Sierra et al., 2013; Daniel et al., 2017). While most still include these neurons in the Type II classification, one group separated these cells into their own division termed Type O, which resemble Type II but show regular, oscillatory firing after hyperpolarization and single spikes after depolarization (Szücs et al., 2010; Rodríguez-Sierra et al., 2013). It has been shown that NPY can block pain- or stress-induced aversions by decreasing the excitability of Type II cells specifically (Rangani et al., 2012).

The defining feature of Type III neurons is the presence of fast inward rectification without rebound firing following hyperpolarizing current (indicating the presence of inwardly rectifying potassium channels ($I_{K(IR)}$)), and little to no depolarization sag (Hammack et al., 2007). In the context of addiction, it has been found that opiate withdrawal leads to exclusive changes in the properties of Type III cells (Francesconi et al., 2017). During withdrawal, their normally high rheobase (minimum current needed to fire) and inward rectification are both significantly reduced. They also showed an increase in membrane resistance, resting membrane potential, and excitability specifically in Type III cells. While it is still unclear how these cell-type specific alterations in opiate withdrawal relate to behavioral output, these studies show that there may be some functional relevance to these physiological categories in the context of addiction.

Figure 1.2 BNST electrophysiological cell type distribution



C		Rat / Mouse					
		Type I	Type II	Type III	LF	SA	Other
BNST-AL	%	24% / 13%	54% / 23%	21% / 54%	4% / -	0% / -	0% / 10%
	Distribution	Even	Even	Concentrated in Oval and Jx	Even	-	-
BNST-AM	%	24% / -	68% / -	8% / -	0% / -	0% / -	
	Distribution	Even	Even	Even	-	-	-
BNST-AV	%	23% / -	63% / -	6% / -	0% / -	8% / -	-
	Distribution	Ventrally Concentrated	Even	Even	-	Even	-

A. Sample traces of Type I, Type II, and Type III neurons reproduced from Szücs, A. et. al, 2010. **B.** Graphical representation of the relative distribution of electrophysiologically-classified cell types in the rodent BNST. Dashed indicate no available data. **C.** Summary of electrophysiological cell type classifications in the BNST. Values are averaged from Hammack et al., 2007; Silberman et al., 2013; Rodriguez-Sierra et al., 2013; Hazra et al., 2011 ; and Daniel et al., 2017.

1.3.2.4 Chemogenetic and optogenetic circuit manipulation of the BNST

The development of chemogenetic and optogenetic tools has enabled researchers to better understand the role of the BNST in a cell-type and circuit-specific manner. Optogenetics relies on the expression of light-activated ion channels or pumps in neurons to drive temporally precise activation or inhibition of their activity (Fenno et al., 2011; Repina et al., 2017). Many optogenetic studies in the BNST have helped to clarify the often opposing roles of BNST subpopulations and afferents. For example, channelrhodopsin (ChR2)-mediated activation of BNST afferents in the VTA is appetitive (mice show a real-time place preference for stimulation) with no effect on anxiety-like behavior (S.-Y. Kim et al., 2013). Dissecting this circuit further, however, revealed that ChR2 activation specifically in GABAergic vBNST neurons projecting to the VTA is appetitive and decreases anxiety-like behavior, while photostimulation of VTA-projecting glutamatergic vBNST neurons is aversive and anxiogenic (Jennings, Sparta, et al., 2013). Optogenetic excitation of

global BNST afferents in the LH is anxiolytic but has no effect on real-time place preference (S.-Y. Kim et al., 2013). However, specific stimulation of GABAergic BNST afferents in the LH produces robust place preference and also drives avid food consumption, especially of highly palatable foods (Jennings, Rizzi, et al., 2013). Interestingly, ChR2 activation in BNST CRF or CCK cells induces an aversive or appetitive state, respectively, as does activation of their terminals projecting specifically to the LH, but these cells had no effect on food intake (Giardino et al., 2018) suggesting a different subpopulation may mediate this component of the BNST-to-LH connectivity.

Chemogenetic strategies in the BNST have made use of designer receptors exclusively activated by designer drugs (DREADDs), which are muscarinic acetylcholine receptors that have been modified to drive Gi-, Gq-, or Gs-coupled GPCR signaling pathways upon administration of the 'designer drug' Clozapine-N-Oxide (CNO), which is an otherwise inert compound (Roth, 2016) [Roth, 2016]. One benefit of this approach is that it can be used to mimic endogenous signaling pathways of various receptors expressed in BNST neurons (Grueter et al., 2006; Mcelligott et al., 2010; N. A. Harris, Austin, et al., 2018; Pina et al., 2020; Perez et al., 2020). Indeed, work from Mazzone and colleagues showed that Gq-, but not Gs- or Gi-, DREADD activation in BNST GABAergic neurons promotes anxiety-like behavior, and that this could be recapitulated through stimulation of the Gq-coupled 5-HT_{2C}R in the BNST (Mazzone et al., 2018). Although Gi-DREADDs are typically considered to have inhibitory actions, and Gq- and Gs- to have excitatory ones, chemogenetic studies in the BNST have urged caution regarding these assumptions. For example, both optogenetic activation and Gq-DREADD activation in BNST CRF cells is aversive, suggesting Gq-driven cellular activation, and Gi-DREADD expression decreases resting membrane potential and spiking activity, suggesting canonical inhibitory actions (Giardino et al., 2018). However, work from the Winder lab has reliably shown that Gi-DREADD expression can also have excitatory effects in BNST neurons, increasing BNST activity and anxiety-like behavior (N. A. Harris, Austin, et al., 2018; Perez et al., 2020). This work highlights the need for careful interpretation of "activation" and "inhibition" using Gq- and Gi- coupled DREADDs in this region.

Although there have been numerous chemogenetic and optogenetic studies investigating the role of the BNST and specific cell-types within it on outputs such as anxiety-like behavior, fear, feeding, pain processing, and aggression, (Jennings, Sparta, et al., 2013; S.-Y. Kim et al., 2013;

Jennings, Rizzi, et al., 2013; Gungor et al., 2015; Giardino et al., 2018; Bayless et al., 2019; Rodriguez-Romaguera, Ung, Nomura, Otis, Basiri, Namboodiri, Zhu, Elliott Robinson, et al., 2020; Xiao et al., 2020; Jaramillo et al., 2020; Emmons et al., 2021; Bruzsik et al., 2021) to-date there have been no optogenetic manipulations, and relatively few chemogenetic manipulations, of the BNST in the context of addiction. Of the studies that exist, several have specifically examined chemogenetic inhibition of VTA-projecting BNST neurons on alcohol consumption and preference. Studies by Pina and colleagues found that Gi-DREADD expression in the BNST, and specifically in VTA-projecting BNST neurons, blocked ethanol conditioned place preference when CNO was administered 30 minutes before testing (Pina et al., 2015; Pina & Cunningham, 2017). They also found that Gi-DREADD in the BNST was able to reduce the increase in cfos typically seen following ethanol cue exposure. Other studies found that Gi-DREADD specifically in VTA-projecting dlBNST CRF cells can reduce binge-like ethanol consumption following a standard drinking in the dark protocol (Rinker et al., 2017; Companion & Thiele, 2018). Work from the Kash lab has also investigated the role of Gi- signaling in ethanol-related behaviors. Driving Gi-DREADD signaling in BNST CRF neurons can suppress binge ethanol consumption. Conversely, driving Gs-DREADD signaling in these neurons had no effect on ethanol consumption alone, but could block Y1R-mediated suppression of binge drinking (Pleil et al., 2015). Gi-DREADD expression in BNST-projecting mPFC neurons had no impact on ethanol consumption, but did rescue marble-burying behavior that is normally suppressed after ethanol exposure, and also blocked ethanol-induced increases in predator odor investigation, increasing TMT avoidance in both ethanol- and non-ethanol-exposed mice (Hwa et al., 2020). One study from the Winder lab investigated the effects of Gq-DREADDs in the BNST in relation to the negative affective component of prolonged alcohol abstinence. Here they found that Gq activation in dBNST neurons receiving input from the insula mimicked the increased anxiety-like behavioral effects and increased BNST cfos activation seen in a chronic drinking, forced abstinence (CDFA) model in which mice have access to ethanol for 42 days, followed by 15 days of abstinence (Centanni, Morris, et al., 2019). The only study to-date looking at chemogenetic BNST modulation in cocaine also comes from the Winder group. They found that Gi-DREADD expression in the dBNST is sufficient to drive reinstatement of cocaine conditioned place preference, mimicking the effects of the Gi-coupled a2AR heteroreceptor in the

context of stress-induced reinstatement (Perez et al., 2020). Together these studies highlight the fact that BNST chemogenetic and optogenetic manipulation research is still in its infancy, but current work reinforces the importance of the BNST in the context of drug seeking, withdrawal, and reinstatement.

1.3.3 Extended amygdala parallel circuitry and cell types

In addition to containing many parallel cell types and being heavily interconnected generally, recent studies have also begun to examine the existence and function of connectivity specifically between some of these parallel populations. For example, CeA^{CRF} cells have been shown to play a role in active threat response and discriminative fear (Sanford et al., 2017; Fadok et al., 2017; Beyeler & Dabrowska, 2020), while $BNST^{CRF}$ cells have been largely implicated in increasing driving anxiety-like behaviors and reinstatement of drug seeking (Shalev et al., 2001; Funk et al., 2006; Daniel & Rainnie, 2015; Giardino et al., 2018). Asok et al demonstrated that projections from CeA^{CRF} neurons cluster around $BNST^{CRF}$ neurons in rats, and that optogenetic inhibition of these terminals resulted in reduced freezing in the latter half of fear retention testing (Asok et al., 2018). This suggests that this CeA^{CRF}→BNST pathway is critical for the long-term retention of fear memories, and it may be mediated through actions onto $BNST^{CRF}$ cells. Similarly, Pomrenze and colleagues found significant overlap (46%) between CeA^{CRF} terminals and CRF cells in the rat BNST (Pomrenze et al., 2019). Gi-DREADD-mediated inhibition of CeA^{CRF} terminals in the BNST significantly increased time spent on the open arms of the EPM, EPM open arm entries, rearing events, and time spent in the center of the OF. Conversely, Gq-DREADD-mediated activation of this pathway significantly decreased each of these measures both basally and after acute immobilization stress, and this increase in anxiety-like behavior was blocked by coadministration of the CRFR1 antagonist R121919. They then showed that simultaneous inhibition of $BNST^{CRF}$ neurons via the kappa opioid receptor-based DREADD KORD and Gq DREADD activation of CeA^{CRF} cells also blocked both basal and stress-induced increases in anxiety (Pomrenze et al., 2019). Together this directly implicates a CeA^{CRF}→$BNST^{CRF}$ pathway in driving increased anxiety-like behaviors.

It has also been shown that there is parallel circuitry between somatostatin-expressing

extended amygdala populations (Ye & Veinante, 2019) and the function of this connectivity has begun to be investigated. Ahrens and colleagues found that both global and CeA^{Som}-specific deletion of the gene *Erbb4* increases anxiety-like behaviors (Ahrens et al., 2018). In a series of studies they then demonstrated that this deletion results in increased excitatory drive onto CeA^{Som} neurons which activates dynorphin-mediated inhibition of CeA^{Som} cells. This then disinhibits $BNST^{Som}$ neurons, ultimately leading to increased anxiety-like behaviors. They also found that these electrophysiological changes and ultimate activation of $BNST^{Som}$ neurons through this CeA^{Som}→$BNST^{Som}$ pathway are recapitulated by unpredictable footshock stress, implicating this pathway in mediating stress-induced increases in anxiety-like behaviors (Ahrens et al., 2018). These findings are interesting because other studies in the BNST have found that activation of $BNST^{Som}$ terminals onto PV interneurons in the NAc shell actually decrease anxiety-like behaviors (Xiao et al., 2020), suggesting there may be projection-specific pathways involved in CeA^{Som}→$BNST^{Som}$ function.

Ye and Veinante also recently demonstrated that there are parallel projections from $CeA^{PKC\delta}$ neurons to the BNST, and from $BNST^{PKC\delta}$ neurons to the CeA (Ye & Veinante, 2019), but the function of this circuitry remains uninvestigated. The next section will highlight current literature on PKCδ and what is known about its function within the extended amygdala.

1.4 Protein kinase C delta (PKCδ)

1.4.1 Signaling pathways and systemic functions

Protein Kinase C delta (PKCδ) is one of a ten-member family of PKC isoforms. PKCs are serine/threonine kinases that works to phosphorylate a variety of downstream targets and are divided in to subfamilies based on their dependency on lipid second messengers (such as diacylglycerol (DAG)), calcium, and ion co-activators (Newton, 2001). The conventional PKC subfamily is comprised of the PKCα, βI, βII, and γ isoforms, based on their sensitivity to both DAG and Ca2+. The novel subfamily contains PKCδ, ε, μ, and θ, which are all activated by DAG but insensitive to Ca2+. PKCζ and λ make up the atypical subfamily, and are insensitive to both DAG and Ca2+ and are primarily recruited by other lipid second messengers such as phosphatidyl

serine (Newton, 2001; Basu & Pal, 2010; Mondrinos et al., 2013; Q. Yang et al., 2019).

Canonical PKCδ signaling pathways involve receptor activation (and in particular, Gq-coupled G-protein coupled receptors, GPCRs) that then recruit phospholipase C (PLC) to the membrane. PLC generates (DAG) and inositol-1,4,5-triphosphosphate (IP3) through hydrolysis of the membrane phospholipid, phosphoinositol. IP3 action on the endoplasmic reticulum to release intracellular stores of calcium. Simultaneously, DAG activates PKCδ through its C1 lipid binding domain (New & Wong, 2007; Reyland & Jones, 2016; Vail & Roepke, 2019). Activated PKCδ then works to phosphorylate a variety of downstream targets to mediate changes in protein production and receptor function. For example, in the case of membrane estrogen receptor activation, PKCδ helps to phosphorylate CREB that eventually increases calcium influx through Cav3 subunits (Vail & Roepke, 2019). In the case of cell survival and death pathways, (discussed below), PKCδ acts through targets including MEK, ERK, and NFκB to drive apoptosis or proliferation (Basu & Pal, 2010). PKCδ can also act through noncanonical pathways that distinguish it from other PKC isoforms, such as activation by tyrosine phosphorylation, and these pathways play important roles in regulating redox-dependent activation and oxidative stress responses (Reyland, 2009; Reyland & Jones, 2016).

PKCδ is one of the more widely-expressed novel PKC family members, being present in the brain, heart, spleen, lung, liver, ovary, pancreas, and adrenal tissues (Wetsel et al., 1992). The function of PKCδ has been extensively characterized in the periphery, where it plays a role in cell migration, proliferation in the context of tumors and cancer, apoptosis, inflammation, and immune function (Basu & Pal, 2010; Q. Yang et al., 2019; Speidel et al., 2020). Indeed one of the major phenotypes of PKCδ-/- mice are alterations in immune activation and subsequent development of autoimmune disorders as the mice age, and mutations in humans are associated with systemic lupus erythematosus, one of the most prominent autoimmune diseases (Reyland & Jones, 2016; Salzer et al., 2016). PKCδ is a critical regulator of inflammatory responses in cancer, diabetes, ischemic heart disease, and sepsis (Cantley et al., 2011; Ren et al., 2014; Q. Yang et al., 2019). There are also links between PKCδ knockout and delayed mammary gland development (Allen-Petersen et al., 2010), nonalcoholic fatty liver disease (Greene et al., 2014), and endotoxin-induced lung injury (Chichger et al., 2012).

Within the brain PKCδ protein is expressed in a variety of regions including the olfactory bulb, cerebral cortex, lateral septum, thalamus, vestibular and cochlear nuclei, inferior olive, nucleus of the solitary tract, cerebellum, caudate-putamen, extended amygdala, and superficial layers of the dorsal horn in the spinal cord (Garcia et al., 1993; Merchenthaler et al., 1993). Interestingly, it's expression is not uniformly distributed in some of these regions. There are striking alternating columns of immunostained Purkinje cells in the cerebellum, and in all sensory systems, it is located in alternating hierarchies within the pathway (Garcia et al., 1993; Merchenthaler et al., 1993). PKCδ is exclusively located in first- and third- order neurons in all but the auditory system, where it is localized to second and fourth-order neurons (Garcia et al., 1993). PKCδ is also a specific marker of neuronal subpopulations in the extended amygdala, which will be discussed in more detail below.

It should be noted that expression patterns of PKCδ protein and mRNA are not directly overlapping, with mRNA expression being highest in the hippocampal formation where protein is largely absent, as well as the cortical subplate, olfactory areas, thalamus, isocortex, midbrain, and striatum (Allen Brain Atlas), and it is distributed more diffusely within these regions (Garcia et al., 1993; Henry & Hohmann, 2012). PKCδ expression is primarily restricted to the soma of neurons, but it also present in some axons and terminals, and can also be expressed by glia, especially following damage (Merchenthaler et al., 1993; Gott et al., 1994; Koponen et al., 2000; Kaasinen et al., 2002).

Much of the research on PKCδ function in the brain has focused on its role in apoptotic signaling. For example, following models of ischemic stroke that induce hippocampal cell death, there are significant increases in mRNA expression specifically of the PKCδ isoform (and not other isoforms) in the hippocampus and prefrontal cortex, which is first visible in neurons and emerges in microglia a few days later (Miettinen et al., 1996; Koponen et al., 2000; S. Guo et al., 2022). Neuroprotective agent of this damage act through a pathway that involved reduction in PKCδ expression in these regions (S. Guo et al., 2022). Models of epilepsy involving infusion of kainic acid similarly produce neuronal death, and also result in increased PKCδ mRNA and protein in hippocampal and cortical neurons (Kaasinen et al., 2002). PKCδ also plays a role in the pathogenesis of prion diseases. Scrapie-infected cerebellar organotypic slice cultures show initial

upregulation of PKCδ mRNA and protein and subsequent loss of PKCδ+ Purkinje cells, accompanied by astrocyte proliferation, and there is delayed onset of scrapie-induced motor symptoms in PKCδ knockout mice (Harischandra et al., 2014). The role of PKCδ in inflammatory responses and apoptosis is also implicated in neurodegenerative disorders (Q. Yang et al., 2019). Hyperacetylation of PKCδ leads to cell death in dopaminergic neurons, and PKCδ is activated in postmortem brains of patients with Parkinson's disease as experimental models of Parkinsonism (Jin et al., 2014; Samidurai et al., 2021). Other roles for PKCδ include driving blood brain barrier permeability and damage and subsequent neutrophil infiltration (W. H. Chou & Messing, 2008; Tang et al., 2018), and developmental growth roles in driving neuritogenesis (O'Driscoll et al., 1995).

With regard to global effect of PKC on more affective, stress, and reward-related phenotypes, several PKC isoforms have been implicated. For example, mutant mice that lack PKCε exhibit reduced anxiety-like behavior and reduced stress hormones (Hodge et al., 2002) as well as reduced alcohol self-administration (Hodge et al., 1999; Olive et al., 2000), whereas PKCγ(-/-) mice show reduced signs of ethanol intoxication and consume more ethanol than wildtype mice (R. A. Harris et al., 1995; Bowers et al., 1999; Bowers & Wehner, 2001). Ethanol also modulates PKCδ, with acute administration in neuronal cultures altering it subcellular localization and chronic ethanol application increasing its expression (Messing et al., 1991; Gordon et al., 1997). Global knockout of PKCδ blocks ethanol-induced enhancement of GABAergic tone in the thalamus and hippocampus, and these PKCδ(-/-) mice showed reduced ethanol intoxication (D. Choi et al., 2008). In the peripheral nervous system, PKCδ has been implicated in mediating pain responses, as spinal inhibition of PKCβII and PKCδ, but not PKCε, blocked the spontaneous peripheral neuropathy pain induced by paclitaxel (Y. He & Wang, 2015), and PKCδ(-/-) mice fail to develop spontaneous pain in models of sickle cell disease (Y. He et al., 2016). The next section will focus on the role of PKCδ and PKCδ-expressing neurons specifically in the extended amygdala.

1.4.2 Function in the extended amygdala

Much of the work investigating the function of $CeA^{PKC\delta}$ cells focused on their role in conditioned fear responses. Some studies have found they play an inhibitory role on the front. *In vivo* electrophysiological recording show that $CeA^{PKC\delta}$ cells are inhibited by tone presentation after it has been paired with footshock, and correspond to “CEl off” neurons (Haubensak et al., 2010) These neurons reduce fear learning and generalization and indeed silencing $CeA^{PKC\delta}$ neurons increases conditioned freezing responses (Ciocchi et al., 2010a; Haubensak et al., 2010). There have however been several other studies finding opposing results. One group found that optogenetic activation of $CeA^{PKC\delta}$ neurons increased fear generalization and freezing (Botta et al., 2015), and inhibition of synaptic transmission in this population decreased conditioned fear recall, suggesting their activity is required for expression of learned fear responses (Ueda et al., 2021). Another group found that $CEl^{PKC\delta}$ neurons encode aversive stimuli, increasing GCaMP activity in response to aversive learning stimuli and being required for negative reinforcement learning (Cui et al., 2017). In agreement with the aversive nature of $CeA^{PKC\delta}$ signaling, it has also been shown that they are hyperexcitable after painful injury and can promote pain responses (T. D. Wilson et al., 2019). These conflicting results may be in part due to differences between CeC verses CeL PKCδ populations (J. Kim et al., 2017). Indeed $CeC^{PKC\delta}$ neurons increase freezing and are activated (as measured by cfos) by footshocks and aversive tastes, while $CeL^{PKC\delta}$ neurons drove no changes in freezing (though there may be a floor effect) and are preferentially activated by fear extinction training over fear recall (J. Kim et al., 2017).

Work investigating the role of $CeA^{PKC\delta}$ neurons in anxiety-like behaviors has yielded similarly conflicting results. One study found that optogenetic activation of $CeA^{PKC\delta}$ neurons decreased EPM open arm time, suggesting an increase in anxiety-like behavior, while inhibition had the opposite effect (Botta et al., 2015). Another study focused on the role of $CeA^{PKC\delta}$ cells in feeding found that they are activated by satiety signals and suppress food intake, and that optogenetic stimulation of $CEl^{PKC\delta}$ neurons decreased anxiety-like behavior(Cai et al., 2014). Still another found that inhibiting $CeA^{PKC\delta}$ cells through disrupted synaptic transmission decreased contextual and cued fear recall, but had no impact on anxiety-like behaviors (Ueda et al., 2021). Here too it is possible that subregion-specific functionality may contribute to these discrepancies.

$CeA^{PKC\delta}$ cells have also been investigated in the context of drug-related behaviors. One study found that social choice-induced abstinence blocks incubation of methamphetamine craving and activates $CeA^{PKC\delta}$ cells, as measured by increased cfos expression (Venniro et al., 2018). Other work found that activation of $CeA^{PKC\delta}$ neurons was significantly associated with rats displaying punishment-resistant alcohol taking, accounting for 75% of the variability in responses and suggesting recruitment of this population may be predictive of compulsive-like alcohol intake (Domi et al., 2021).

In addition to investigating the function of cells expressing PKCδ in the CeA, there are emerging studies investing the role of PKCδ expression itself, particularly with regard to drugs of abuse. Venniro et al developed a short hairpin RNA (shRNA) to specifically knock down PKCδ (shPKCδ), and found that cells expressing the shPKCδ showed significantly reduced excitability, firing fewer action potentials in response to varying levels of current injections compared to a control shRNA construct (shCtrl) (Venniro et al., 2020). Further, knocking down PKCδ in the CeA prevented the protective effect of social choice on incubation of drug craving, and also significantly reduced the cfos induction in $CeA^{PKC\delta}$ cells that had been observed after this protocol (Venniro et al., 2020). In another study, Domi et al found that knocking down PKCδ in the CeA reduced alcohol seeking in this punishment-resistant group (Domi et al., 2021). Together these studies suggest the PKCδ expression is playing a functional role in the physiology and behavioral output of cells in which it is expressed. Interestingly, PKCε has also been implicated in the CeA's response to alcohol, and its expression is required for alcohol induced changes in GABAergic transmission in the region (Bajo et al., 2008).

While there have been numerous studies into the function of $CeA^{PKC\delta}$ cells and expression, comparatively little is known about its BNST counterpart. Beyond being noted as a specific subpopulation marker, one of the first studies to examine PKCδ in the BNST was work from our lab which found that after restraint stress, there is an increase in the number of BNST neurons expressing PKCδ mRNA specifically in female mice (Fetterly et al., 2019). This new expression occurred at least in part in $BNST^{CRF}$ cells specifically, as we also saw an increase in the co-expression of CRF and PKCδ mRNA, with no change in the number of CRF-only cells. These changes were abolished in ovariectomized females, suggesting they are dependent on circulating

sex hormones, and indeed as noted above, the PKCδ gene contains an estrogen response element that could play a part in regulating its expression. Additionally, we found a significant increase in the number of cfos+ PKCδ cells, suggesting these neurons are activated during stress (Fetterly et al., 2019).

The involvement of BNST^{PKCδ} neurons in stress responses was further demonstrated by work from the Cai lab, where they also extended the functional characterization of this population to include regulation of feeding. They found increased cfos expression in BNST^{PKCδ} cells in response to injection of interleukin-1β (IL-1β) or lipopolysaccharide (LPS), both of which are inflammatory cytokines that drive HPA axis activation and stress-induced anorexia (Y. Wang et al., 2019). Similarly to CeA^{PKCδ} neurons, Gi-DREADD-mediated inhibition of BNST^{PKCδ} cells blocked this inflammation-induced anorexia, and also drove increased feeding in both fed and fasted mice, while optogenetic activation rapidly and robustly inhibited feeding (Y. Wang et al., 2019).

There have also been a handful of studies investing the role of BNST^{PKCδ} neurons in mediating anxiety-like behaviors, but as in the CeA, these have yielded conflicting results. In the Cai lab's characterization of feeding, they found no changes in anxiety-like behaviors from either activation or inhibition of PKCδ cells (Y. Wang et al., 2019). Another group found that Gq-DREADD activation in BNST^{PKCδ} cells increased number of open arm entries, time spent on the open arms of the EPM, and center time in the OF both basally and after stress, suggesting a decrease in anxiety-like behavior (X. Wang et al., 2020). Most recently, Ueda used tetanus toxin (TeNT) to disrupt synaptic transmission in BNST^{PKCδ} cells and found that this specifically decreased anxiety-like behaviors, suggesting that this population is required for increasing anxiety-like responses (Ueda et al., 2021).

Altogether, the existing work suggests that BNST^{PKCδ} cells are well-positioned to play an important role in mediating stress responses and anxiety-like behaviors, and here we will describe our efforts to develop a more comprehensive characterization of this intriguing but understudied neuronal subpopulation.

CHAPTER 2

Recruitment of BNST$^{PKC\delta}$ cells during acute stress

Portions of this chapter are adapted from:

Jaramillo AA, Williford KM, Marshall C, Winder DG, Centanni SW. BNST transient activity associates with approach behavior in a stressful environment and is modulated by the parabrachial nucleus. Neurobiology of Stress. 2020;13

And

Luchsinger JR, Fetterly TL, Williford KM, Salimando GJ, Doyle MA, Maldonado J, Simerly RB, Winder DG, Centanni SW. Delineation of an insula-BNST circuit engaged by struggling behavior that regulates avoidance in mice. Nature Communications. 2021;12.

2.1 Introduction

Chronic stress is a major contributor to a host of diseases and conditions affecting the central nervous system and throughout the body. For example, chronic stress increases the risk of disorders such as cardiovascular disease, obesity, metabolic disease, preterm birth, immune disfunction, hypertension, type II diabetes, and osteoporosis (Wadhwa et al., 2001; Everson-Rose & Lewis, 2005; Dhabhar, 2009; Chrousos, 2009; Tamashiro et al., 2011; Aschbacher et al., 2014; Inoue, 2014; Kelly et al., 2019). In the central nervous system, stress is associated with sleep disorders, cognitive impairments, neurodegenerative diseases, eating disorders, and a number of neuropsychiatric disorders including major depressive disorder (MDD), anxiety disorders, post-traumatic stress disorder (PTSD), and substance use disorders (Koob & Le Moal, 2008; Sinha, 2008; Krishnan & Nestler, 2008; Catania et al., 2009; Chrousos, 2009; Hardaway et al., 2015; Nollet et al., 2019; Chesnut et al., 2021; Ressler et al., 2022).

The bed nucleus of the stria terminalis (BNST) is a key mediator of stress responses and has been shown to be highly responsive to stress. It is a heterogeneous brain region, with a number of different cell types which have shown different sensitivities to stress exposure. We have previously found that cells expressing protein kinase C delta (BNST$^{PKC\delta}$) are stress-sensitive,

showing increases cfos mRNA 30 minutes after 1 hour of restraint stress (Fetterly et al., 2019). We also found that there was an increase in the number of cells expressing PKCδ after restrain stress, suggesting this cell population may play a particularly important role in mediating stress responses (Fetterly et al., 2019). Here, we aimed to investigate the recruitment of $BNST^{PKC\delta}$ cells in real-time through the use of fiber photometry, and begin to investigate their potential afferent regions involved in their activation.

2.2 Methods

2.2.1 Animals

Adult (>8 weeks old) male and female PKCδ-Cre mice (RRID:MMRRC_011559-UCD; Mutant Mouse Resource and Research Centers) were bred and genotyped in-house to be heterozygotes on a C57BL/6J background. All mice were housed with two to five mice per cage and provided food and water ad libitum. Light/dark cycle was 12 h (lights on = 0600 h) with controlled humidity (30–50%) and temperature (20–25 °C). Behavioral testing and surgeries were conducted during the light phase. Animals were under continuous care and monitoring by veterinary staff from the Vanderbilt Division of Animal Care. All procedures were carried out in accordance with the NIH Guide to Care and Use of Laboratory Animals and institutional guidelines and approved by the Institutional Animal Care and Use Committee at Vanderbilt University.

2.2.2 Surgeries

Mice (>8 weeks) were anesthetized with isoflurane (3% initial dose, 1.5% maintenance dose) for intracranial recombinant AAV injection and fiberoptic cannula implant surgeries, using a Leica Angle Two Small Animal Stereotaxic Instrument. Mice were injected with 300 nL of AAV9-hSyn-FLEX-jGCaMP7f-WPRE (Addgene) at a rate of 50 nL/min driven by a Micro4 MicroSyringe pump (World Precision Instruments) into the specified region. The needle (World Precision Instruments Nanofil syringe fitted with a Nanofil 33 G Blunt Needle) remained in place for an additional 5 min. All mice recovered for at least 4 weeks before further experimentation. Injection and implantation sites included insular cortex (from Bregma: AP = 0.02, ML ± 3.66, DV = −4.30, 0°

angle), dorsal BNST (from Bregma: AP = 0.14, ML ± 0.88, DV = −4.24, 15.03° angle), and primary motor cortex (from Bregma: AP = 1.60, ML ± 1.10, DV = −1.80, 15.03° angle). All mice received 5 mg/kg injections of meloxicam for once a day for 2 days following surgery.

Chronic optical fiber implantation specific procedures Once the skull was exposed, it was etched with a gel etchant (Kerr Dental). One stainless-steel mounting screw (PlasticsOne) was installed in the ipsilateral parietal plate posterior to the implant hole. The implant was bonded by applying Optibond primer followed by Optibond adhesive, which was then cured with UV light. Herculite enamel was molded around the screw, implant, and skull. The enamel was then cured with UV light. Mono fiberoptic cannulas (Doric Lenses) for the Tucker-Davis Technologies (TDT) system were purchased at the appropriate lengths for each brain region.

2.2.3 Fiber Photometry

2.2.3.1 Recordings

A TDT RZ5P fiber photometry system and Synapse software were also used in this study. Briefly, light from the 470 nm, 17.2 mW (Min) fiber-coupled LED (Thorlabs) and light from the 405 nm, 19.3 mW (Min) fiber-coupled LED (Thorlabs) was directed into a fluorescence mini cube with six ports and a built-in detector head (Doric Lenses), with spectral bandwidths of 405 and 470 nm. A 405 nm light was modulated at 217 Hz, while 470 nm light was modulated at 330 Hz. Power output was maintained at 20 mA with a DC offset of 3 mA for both wavelengths. The light was then directed through a low-autofluorescence mono fiberoptic patch cord with a 400 µm core (Precision Fiber Products). This fiber was connected to the mono fiberoptic cannulas that were implanted into the region of interest. The power output at the fiber tip was 25–30 µW. Fluorescent emission from the tissue was collected through the same fiber and was detected using a femtowatt photoreceiver. Signal acquisition was 1 kHz and low-pass filtered at 6 Hz. The 405 nm excitation channel served as an isosbestic, calcium-independent control wavelength for GCaMP, allowing for bleaching and movement artifact corrections when directly fit to the calcium-dependent 470 nm channel. MATLAB scripts from TDT (https://www.tdt.com/support/matlab-sdk/) were used to fit the 405 nm signal to the 470 nm signal using linear regression. Change in GCaMP-mediated signal

was calculated as $\frac{(Change\ in\ 470\ nm\ signal - Change\ in\ 405nm\ signal)}{Change\ in\ 405nm\ induced\ signal}$. Time-locked Z-scores were calculated using $Z = \frac{\frac{instantaneous \frac{\Delta F}{F}}{mean \frac{\Delta F}{F}}(from -5\ to -3\ seconds)}{standard\ deviation\ of \frac{\Delta F}{F}(from -5\ to -3\ seconds)}$.

Transient frequency and maximum peak amplitude were calculated using a custom written MATLAB code incorporating open-source code59 (https://github.com/katemartian/Photometry_data_processing). The pipeline utilized an adaptive iteratively reweighted Penalized Least Square (airPLS) approach60 to correct for baseline noise or signal drift, and allow for uniform detection of transients and comparison between mice. The signal was then standardized using a Z score transformation and fit to the reference signal (405 nm, GCaMP only). Data points with a Z score ≥ 2.91 are considered statistical outliers from the baseline (i.e., transient activity above baseline)61. A single transient was measured from when a data point ≥2.91 Z scores was detected until a data point <2.91 Z scores was detected. Maximum peak amplitude was calculated as the maximum Z score detecting during the transient. Transient frequency was calculated as the number of transients per second (Hz).

2.2.3.2 Behavior

Four weeks following viral injection and fiber implantation surgery, mice were habituated to false patch cables for 20 minutes in clean, empty cages for 3 consecutive days. On test day, mice were transported to the test room and allowed to habituate for one hour prior to beginning the experiment. The patch cable was connected to the fiber optic implants, and animals were habituated in a clean, empty cage for 5 minutes before the start of any behavior test.

2.2.3.2.1 NSFT

The 3-day novelty-suppressed feeding task (NSFT) was performed as previously described (Holleran et al., 2016; Centanni, Morris, et al., 2019). In brief, mice were food restricted in their home cage with no food for 48-hr except for 2-hr access to food pellets (approximately 2 pellets = 7 g) at the 22–24hr before testing. Mice were weighed before and after restriction to ensure weight loss was similar across all groups. After 48-hr of restriction (testing day) mice were acclimated to the testing room for at least 1-hr. For the test, a single food pellet was placed at the center of a

brightly-lit (300 lux) 50 × 50 cm arena with fresh home cage bedding. A new pellet was used and bedding was refreshed for each test. At the time of testing a mouse was placed in a corner of the arena with an overhead video camera. Food pellet approaches were hand scored using the ANVIL Video Annotation Research Tool. Following the first bite (final, consummatory food approach) mice were allowed to continue in the arena until reapproach and subsequent second bit of the food. Mice that did not eat within the first 20-min of the test and/or post-test were omitted from analysis. Following the test, the mice were returned to ad lib access to chow.

2.2.3.2.2 RESTRAINT

The base was made from clear acrylic (McMaster-Carr). The screw and rear-guard components were made from extra plastic from our machine shop (Vanderbilt Kennedy Center Scientific Instrumentation Core).

Fibers were attached to each animal's implant with a stainless-steel sleeve (inner diameter = 400 µm) before the animal was placed inside the restraint device. Restraint exposure lasted 30 or 60 min. Video was captured with a webcam (Logitech), and movements were tracked using DLC.

All videos were acquired at 10 fps using a Logitech C920 camera. For experiments detailed in Figs. 1d, e and 3i–k, behavioral scoring was done manually using Anvil 6.0 video annotation software. Behavioral bouts were counted if the tail head and tail movement were visible on the recording. Bouts were considered contiguous if they were separated by <0.7 s. Automated video scoring was performed using DLC to track points across time and was based on the methods used for manual scoring14,15. The fiberoptic cable and the tip of the tail were manually located in each image of a training set made up of >15 images per video. A small green piece of tape was placed on the fiberoptic cable to ensure a consistent location during training and testing. DLC was trained for at least 200,000 iterations. We used R statistical software with the "tidyverse" package to convert X/Y position into speed of movement for the fiber and the tail during each frame55,56. To identify bouts, we first identified frames in which the fiber and tail were moving. We determined if the tracked objects were moving by setting speed thresholds (selected via trial and error) that were independently set for both the fiber and the tail tip. For the fiber, any frame with a speed greater

than one standard deviation above the bottom 95% of frame speeds was considered mobile. For the tail tip, the threshold was set at three standard deviations above the bottom 99% of tail tip frame speeds. The use of a normalized speed, rather than a raw pixel-based speed threshold, provided better consistency across trials because it accommodated slight changes in camera distance and restraint device position. When a tracked point transitioned from an immobile to a mobile state, that frame became time 0.0 for a bout. The bout continued until all tracked points were in an immobile state for >0.7 s (to limit significant bout overlap). We observed three bout types: (1) movements of the head only, (2) movements of the tail only, and (3) movements that included both head and tail movements, termed full body. Unless otherwise noted, we specifically focused our analysis on full body movements. To make the analysis uniform across bouts, AUC, and maximum peak amplitude were calculated for the 5 s, following bout onset independent of bout length.

2.2.4 IHC and Imaging

Brains of adult mice were removed following transcardial perfusion with 10mL 0.01M Phosphate Buffered Solution (PBS) and 20mL of 4% Paraformaldehyde (PFA) in 0.01M PBS. The brains were then postfixed in 4% PFA for 24 hours at 4°C. Following fixation, brains were transferred to 30% sucrose in PBS for cryoprotection for at least 36 hours. Brains were frozen in Optimal Cutting Temperature (OCT) Compound prior to slicing and cut into 40µm thick coronal sections using a cryostat (Leica CM3050S) and stored in PBS at 4°C or cryoprotectant (30% ethylene glycol, 30% glycerol, 10% 0.2M phosphate buffer, in diH20) solution at -20°C until IHC staining. For IHC, slices were washed in PBS (4x 10 min), permeabilized in 0.5% Triton X-100 in PBS for 30 minutes at room temperature, and then blocked in 0.1% Triton X-100 with 10% Normal Donkey Serum for 1 hour at room temperature. Slices were incubated in primary antibody solution for 24 hours at 4°C. Following primary antibody incubation, slices were washed in PBS (4 x 10 min) and incubated in secondary antibody solution for 24 hours at 4°C. Slices were then washed in PBS (3 x 10 min) and then incubated in PBS plus DAPI (1:10,000, Sigma-Aldrich Cat# D9542-10MG). Primary antibodies used: Goat anti-CGRP () 1:400 dilution, Mouse anti-PKCδ (BD Biosciences Cat#610398, RRID:AB_397781) 1:1000 dilution, Secondary antibodies used: Cy3

Donkey anti-Goat (Jackson ImmunoResearch Labs Cat# 705-165-003 , RRID: AB_2340411), 1:500 dilution. Cy5 Donkey anti-mouse (Jackson ImmunoResearch Labs Cat# 715-175-150, RRID: AB_2340819), 1:500 dilution. Slices were imaged at 20x magnification on a Zeiss LSM 880 confocal microscope and analyzed using ImageJ software (NIH; RRID:SCR_003070), with the same brightness and contrast settings applied across all images. Cell counts were performed manually using ImageJ by two researchers blinded to experimental groups. The cell counts for left and right BNST across two 40um-thick sections per animal were averaged across animals.

2.2.5 Statistics

Analyses were performed with R (version 3.6.1), Python (version 3.6.6), GraphPad Prism (GraphPad Software, version 8), or MATLAB (Mathworks; version 2019a). Animal numbers were selected to establish sufficient statistical power, while using ethical guidelines for minimizing subject numbers and based on previous publications' ability to reliably measure variables from experiments with similar design. When automated behavior tracking was performed, a custom R script was used to determine bout onset in an unbiased manner. Fiberoptic implants and viral placements were histologically verified, and animals were removed if either did not work. The number of mice in each experiment and the specific statistical tests are identified in the corresponding figure legends. Statistical significance was determined if $P \leq 0.05$.

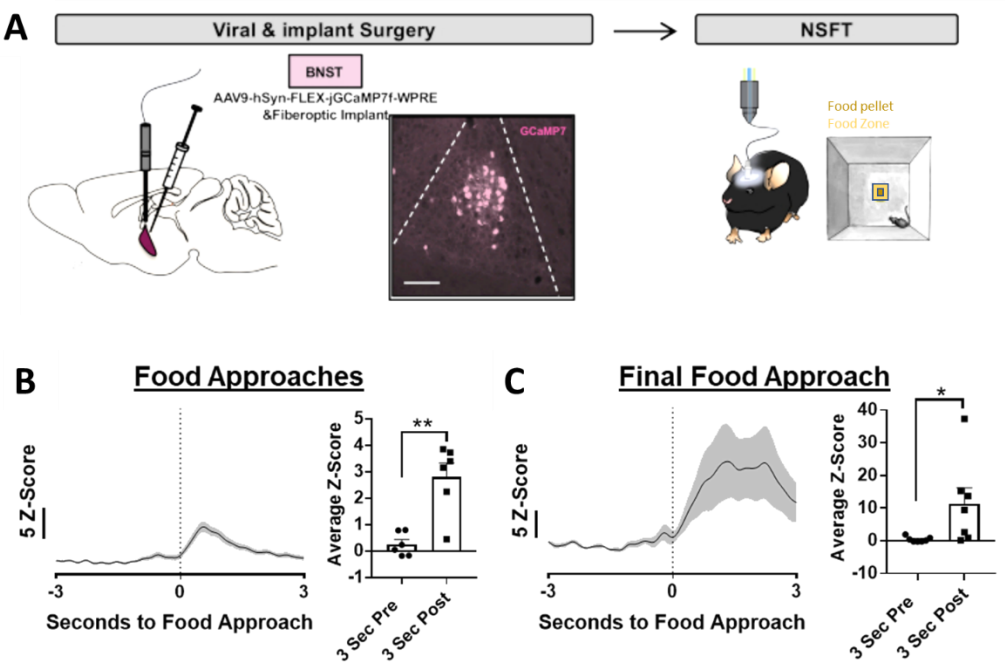
2.3 Results

2.3.1 Phasic BNST$^{PKC\delta}$ calcium signal associated with NSFT approach behavior

BNST$^{PKC\delta}$ cells have previously been implicated in feeding behaviors (Y. Wang et al., 2019). To investigate in vivo BNST$^{PKC\delta}$ neuronal activity at the intersection of feeding and stress processing, PKCδ-specific calcium was measured in the BNST during the novelty-suppressed feeding test (NSFT). PKCδ-CRE female and male mice received a unilateral injection of the calcium indicator GCaMP7f and were implanted with a fiber optic in the BNST as represented in Fig 2.1 Following recovery from surgery, BNST$^{PKC\delta}$ calcium activity was recorded during NSFT (Fig. 2.1A). For the NSFT assay, mice were food deprived for 48 hours prior to testing, with two

hours of access to food during hours 23-25. On the testing day, mice were placed in the corner of a large, bright arena with a food pellet in the center. Mice typically approach the food pellet several times without eating (non- consummatory approach) before the final approach during which they take the first bite (consummatory approach). We hand-scored the start times of each entry into the zone immediately surrounding the food ("food zone") (Fig. 2.1A) and used MATLAB scripts to correlate the fiber photometry traces with each food zone entry. A clear BNST$^{PKC\delta}$ transient signal was observed in both non-consummatory approaches (Fig. 2.1B(left)) and the consummatory approach (Fig. 2.1C(left)) demonstrated by a significant increase in amplitude measured by average Z-Score pre-versus post-non-consummatory approaches (Fig. 2.1 B(right) [p=0.003]) and the consummatory approach (Fig. 2.1C(right), [p=0.03]). These data reveal phasic BNST$^{PKC\delta}$ GCaMP transients at the time of food approach.

Fig. 2.1 Phasic BNST$^{PKC\delta}$ calcium signal associated with NSFT approach behavior



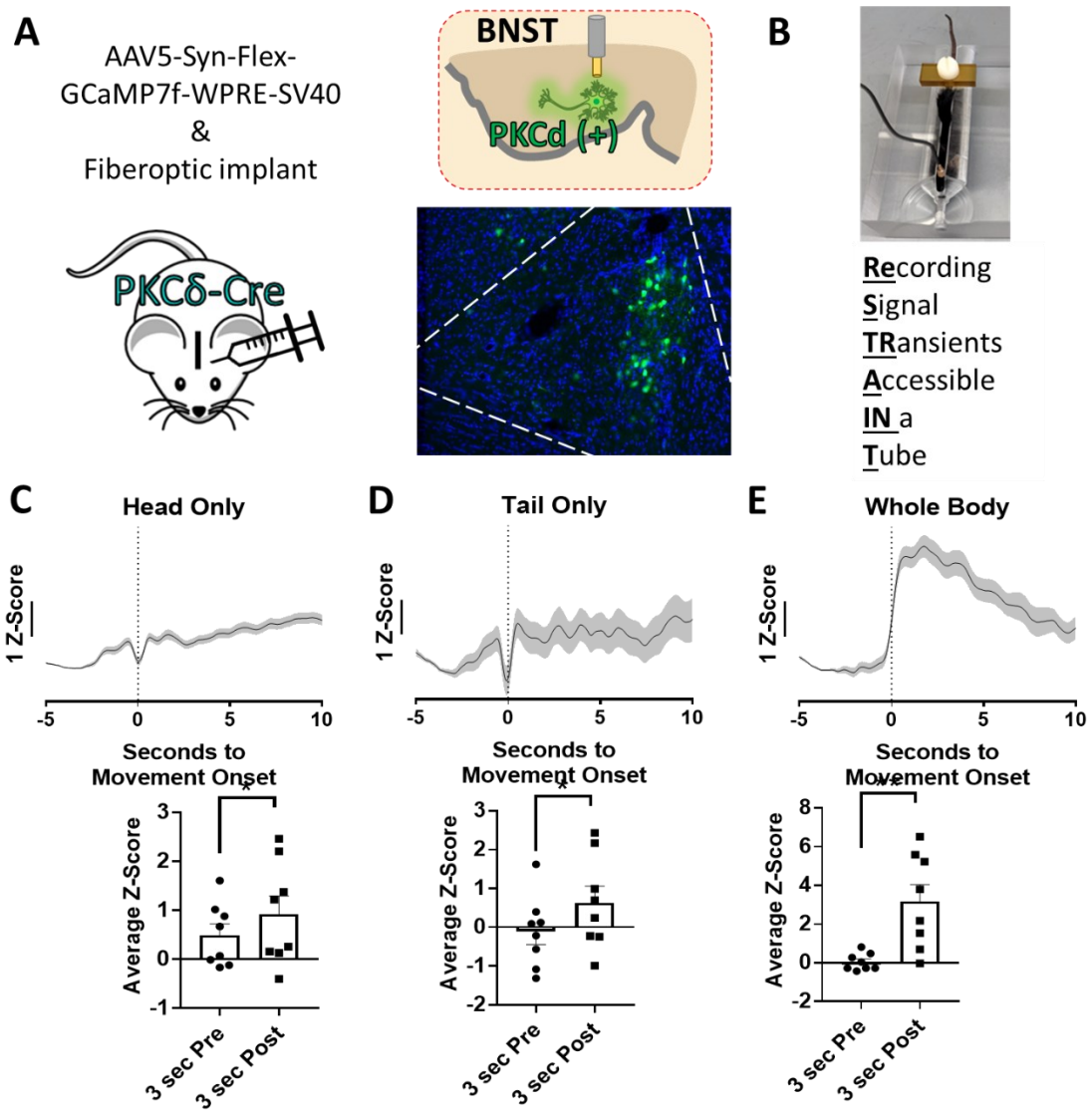
PKCδ GCaMP transients emerge at the time of food approach in NSFT. **A.** Schematic demonstrating unilateral CRE-dependent GCaMP7f infusion & fiber optic implantation in the BNST of PKCδ-CRE female and male mice followed by testing with NSFT. **B (left).** Mean ± S.E.M of BNST GCaMP7f Z-Scores at the time of non-consummatory food approaches shows a transient signal. **B (right).** Mean ± S.E.M of the individual GCaMP7f Z-Score averages is significantly increased post a non-consummatory approach, p=0.003, paired t-test t(5)=5.255. **C(left).** Mean ± S.E.M of BNST GCaMP7f Z-Scores at the time of the consummatory approach shows a transient signal. **C(right).** Mean ± S.E.M of individual GCaMP7f Z-Score averages is significantly increased post the time of the consummatory approach, p=0.03, paired t-test, t(5)=2.502 Scale bar, 200 µm and merged image: 100 µm, *Significant from pre approach (paired *t*-test), $p \leqq 0.05$., **, $p<0.01$

2.3.2 BNST$^{PKC\delta}$ cells are recruited during RESTRAINT struggle bouts

To investigate the real-time recruitment of BNST$^{PKC\delta}$ cells during stress processing dissociated from feeding behaviors, we next turned to restraint stress. One week after NSFT (for return to normal dietary patterns), we recorded 5 minutes of baseline BNST$^{PKC\delta}$ GCaMP7f activity (Fig. 2.2A) followed by 1 hour of restraint stress. In our previous studies, 50mL conical tubes were used for restraint. However, these typical restraint devices are not compatible with the presence of an implanted fiber-optic cable, so we designed and optimized a novel RESTRAINT (**RE**cording **S**ignal **TR**ansients **A**ccessible **IN** a **T**ube) device to enable a seamless transition between the baseline measurement and restraint session without disconnecting the cable (Fig. 2.2B). We used a machine-learning algorithm, DeepLabCut, with custom MATLAB code to score movements of

each mouse's head only, tail only, or whole body and correlate these active coping behaviors with the fiber photometry trace. We found that there is a significant increase in BNST$^{PKC\delta}$ activity at the onset of both larger (whole body) and smaller head- or tail-only active coping bouts (Fig 2.2C-E).

Fig. 2.2 BNST$^{PKC\delta}$ cells are recruited during RESTRAINT struggle bouts

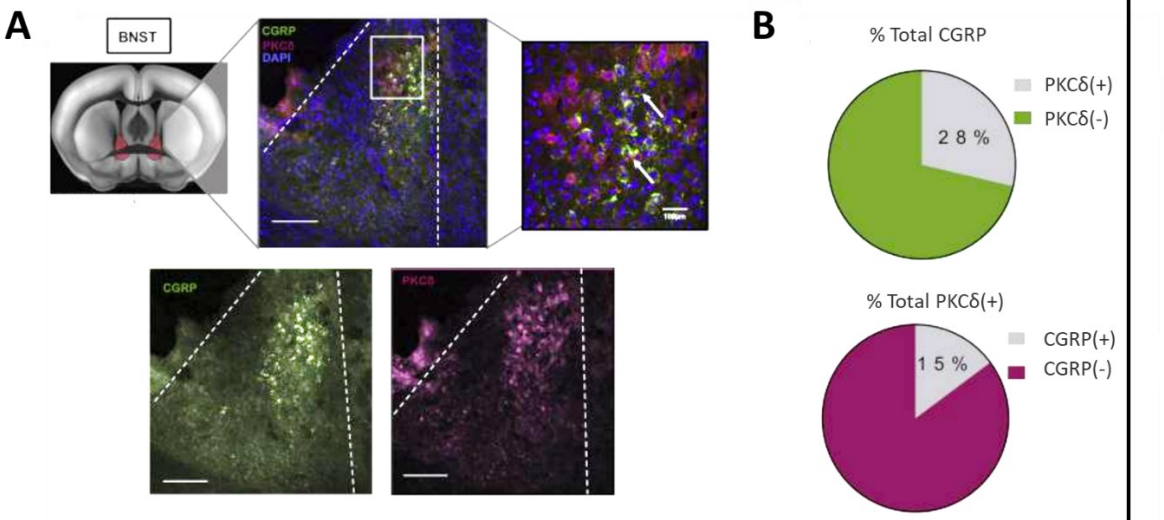


PKCδ GCaMP transients emerge at the time of escape-like struggling movements during RESTRAINT (REcording Signal TRansients Accessible IN a Tube) stress. **A.** Schematic demonstrating unilateral CRE-dependent GCaMP7f infusion & **B** fiber optic implantation in the BNST of PKCδ-CRE female and male mice followed by recording during 1 hr of RESTRAINT stress **C-E (upper).** Mean ± S.E.M of BNST GCaMP7f Z-Scores at the time of (C) head only, (D) tail only and (E) whole body struggle bouts **C-E (lower).** Mean ± S.E.M of the individual GCaMP7f Z-Score averages is significantly increased during struggle bouts, paired t-tests. **C.** p=0.03, t(7)=2.627. **D.** p=0.01, t(7)=3.355. **E.** p=0.008, t(7)=3.716**.** *Significant from pre struggle bout $p \leqq 0.05$., **, $p<0.01$

2.3.3 BNST$^{PKC\delta}$ cells colocalize with CGRP expression

Lastly, we sought to investigate potential upstream regions that could contribute to the recruitment of BNST$^{PKC\delta}$ cells during these stress processing behaviors. The parabrachial nucleus (PBN) sends substantial anatomical input to the BNST, with both neurochemical and electrophysiological evidence of PBN somatic innervation of BNST neurons (Carter et al., 2013; S. a Flavin et al., 2014; Campos et al., 2018; Chen et al., 2018; Fetterly et al., 2019). Given the role of this region in arousal, processing of danger signals, and food-related behavior in a stress-like state (Carter et al., 2013; Palmiter, 2018) we explored the possibility of PBN innervation specifically to BNST$^{PKC\delta}$ cells. Using immunohistochemistry (IHC), we stained for PKCδ and calcitonin gene-related peptide (CGRP), which is a marker of afferents from the PBN (Fig. 2.3A). We found that PKCδ cells comprise a large fraction (28%) of all cells colocalizing with CGRP in the dBNST, and that 15% of BNST$^{PKC\delta}$ cells colocalize with CGRP (Fig. 2.3B).

Fig. 2.3 BNST$^{PKC\delta}$ cells colocalize with CGRP expression



PBN innervates PKCδ-specific BNST neurons. **A.** Representative image demonstrating CGRP, PKCδ, and PKCδ+CGRP + DAPI merged immunofluorescence in the BNST **B.** Quantification of CGRP and PKCδ demonstrate PKCδ+CGRP colocalization, non-PKCδ and non-CGRP expressing cells in the BNST.

2.4 Discussion

Taken together, these results show that BNST$^{PKC\delta}$ cells are indeed actively recruited in stress coping contexts. In the NSFT, we found that BNST$^{PKC\delta}$ cells show a significant increase in activity during initiation of both non-consummatory and consummatory food pellet approaches (Fig. 2.1). The NSFT is a pharmacologically-validated test for assessing aspects of negative affective state in mice (Britton & Britton, 1981; Bodnoff et al., 1988; Cryan & Sweeney, 2011; Goode & Maren, 2017; Kirlic et al., 2017). Given the food-restricted state and innate avoidance of the center, NSFT provides a stress-like context and creates a conflict between a consummatory drive and overcoming the anxiety-like context of being in the center of a bright, open area. It has been shown that activation of BNST$^{PKC\delta}$ cells inhibits feeding behavior, so it is interesting that here we see that on the final, consummatory food approach, there is larger recruitment of BNST$^{PKC\delta}$ cell activity than on the non-consummatory approaches. This suggests perhaps a complex interaction between the role of these cells in stress processing and food consumption that future studies should further dissect.

Additionally, we found that at the initiation of head movements, tail movements, and whole body struggle bouts, there is an increase in BNST$^{PKC\delta}$ cell activation (Fig. 2.2). We have previously shown that these large, whole body struggle bouts are associated with active stress coping behaviors, and can be modulated by anxiogenic pharmacological agents (Patel et al., 2005), and indeed, recruitment of BNST$^{PKC\delta}$ cells was significantly larger for whole body struggle bouts than for the head- or tail-only movements, suggesting that these cells are associated with active stress coping.

Finally, we found that 15% of BNST$^{PKC\delta}$ cells colocalize with CRGP, and that of the cells expressing CRGP, over ¼ of them co-express PKCδ (Fig. 2.3). CGRP is a common marker of afferents from the PBN, forming characteristic rings around the soma of neurons in the BNST. Indeed, the PBN has been shown to form axo-somatic innervation onto cells in the BNST, which enables greater control over neuronal output than axo-dendritic synapses (Shimada et al., 1989; Dobolyi et al., 2005). This data shows that a significant portion of that input is being received by PKCδ cells specifically, suggesting that the PBN is likely playing a major role in the function of

BNST$^{PKC\delta}$ neurons. The PBN is involved in detection and processing of danger-related signals, responding to threats and contributing to aversion learning (Campos et al., 2018; Palmiter, 2018). It also suppresses food intake and plays a role in conditioned taste aversion (Carter et al., 2013). Thus it follows that PBN→BNST$^{PKC\delta}$ circuitry could mediate interactions between stressors and feeding, and stress coping behaviors more broadly. Indeed, CGRP can act through excitatory GPCR signaling pathways that recruit PKC isoforms that lead to intracellular calcium release (Russell et al., 2014), and it is possible that this could contribute to calcium-induced increases in GCaMP fluorescence seen during acute stress coping. Future studies should examine the functional contribution of the PBN onto BNST$^{PKC\delta}$ cells as well as other inputs influencing the activity and function of this stress-sensitive neuronal population.

CHAPTER 3

Characterization of BNST$^{PKC\delta}$ neurons in anxiety-like behavior and repeated stress exposure

Adapted from Williford et. al., "BNST PKCδ neurons are activated by specific aversive conditions to promote anxiety-like behavior." Neuropsychopharmacology. 2023

3.1 Introduction

Stress exposure is a central contributing factor in the development and maintenance of many psychiatric conditions including anxiety disorders. Individuals with anxiety disorders have been shown to display altered responsivity to stressors, and stress can also alter sensitivity to potential threats and aversive contexts, irrespective of the presence of an anxiety disorder (Frisch et al., 2015; Macatee et al., 2017; Egan & Dennis-Tiwary, 2018; Timmers et al., 2019). It is clear there are complex interactions between anxiety, threat processing, and stress, however, the neuronal underpinnings of these relationships remain poorly understood.

The bed nucleus of the stria terminalis (BNST) is well positioned to play a role in the intersection of stress and anxiety disorders. It is highly stress-sensitive and a key regulator of stress responses and anxiety-like behaviors (M. A. Lebow & Chen, 2016). It has also been implicated in sustained threat monitoring and avoidance behaviors (Walker et al., 2003, 2009; Davis et al., 2010; Avery et al., 2016). The BNST is quite heterogeneous, with numerous distinct cell types classified by their expression of different proteins and peptides (Lesur et al., 1989; Ju & Swanson, 1989; Ju et al., 1989; Walter et al., 1991; Welch et al., 2019; Rodriguez-Romaguera, Ung, Nomura, Otis, Basiri, Namboodiri, Zhu, Robinson, et al., 2020), and these cell types have been shown to have distinct and sometimes opposing effects on stress- and anxiety-related behaviors (Kash et al., 2015; Ch'ng et al., 2018; Flanigan & Kash, 2022).

Cells expressing protein kinase C delta (BNST$^{PKC\delta}$) have reliably been identified as a distinct subpopulation, showing little overlap with other BNST cell types (Fetterly et al., 2019; Ye & Veinante, 2019). PKCδ cells have been heavily studied in the related central nucleus of the amygdala (CeA$^{PKC\delta}$), where they have been implicated in drug seeking and withdrawal, amplified pain responsivity, inhibiting fear learning and freezing, and reducing anxiety-like behaviors (Cai et

al., 2014; T. D. Wilson et al., 2019; Venniro et al., 2020; Griessner et al., 2021; Domi et al., 2021; Whittle et al., 2021; Moscarello & Penzo, 2022; Dilly et al., 2022). Our previous work has found that the expression of PKCδ mRNA in the BNST is dynamically regulated by stress, increasing in female mice 30 minutes after restraint stress (Fetterly et al., 2019). Further, we have shown that BNST^{PKCδ} cells are activated during active stress coping bouts in restraint stress, and during the novelty-suppressed feeding test (NSFT) (Chapter 2) (Jaramillo et al., 2020; Luchsinger et al., 2021). However, the examination of the role of BNST^{PKCδ} cells in the context of anxiety-like behavior has yielded conflicting results, with studies reporting increases, decreases, and no effect on anxiety-like behavior (Y. Wang et al., 2019; X. Wang et al., 2020; Ueda et al., 2021).

Here, we investigate the function of BNST^{PKCδ} cells in the context of stress, anxiety, and threat-related behaviors. We use a combination of approaches including *in vivo* optogenetics, immunohistochemistry (IHC), patch clamp electrophysiology, and *in vivo* fiber photometry paired with a variety of behavioral paradigms to probe the recruitment of BNST^{PKCδ} cells during stress processing, and the impact of their activation on tasks relevant for risk assessment and anxiety-like behaviors. Further, we perform tracing studies to develop an understanding of the CNS network in which they reside. Our results demonstrate that this cell population is involved in processing of aversive contexts, potentially contributing to increased anxiety-like behaviors through a role in threat detection. Together this work helps to further both our understanding of the complex symptomology of anxiety disorders as well as our understanding of the function of an important and understudied neuronal subpopulation.

3.2 Methods

3.2.1 Animals

C57BL/6J mice (Jackson Laboratories Strain #000664, RRID:IMSR_JAX:000664) were used for the acute restraint stress IHC (n=54, 27F, 27M). PKCδ-Cre x C57BL/6J transgenic mice were used in *in vivo* optogenetics, fiber photometry, and basal electrophysiology experiments (n=61, 37F, 24M). PKCδ-Cre mice were bred in house following in-vitro fertilization of female mice with sperm from the Mutant Mouse Resource and Research Centers (MMRRC) at UC-Davis (# 011559-UCD, STOCK Tg(Prkcd-glc-1/CFP,-cre)EH124Gsat/Mmucd), and genotyped via the

MMRRC protocol. PKCδ-Cre mice were crossed with Ai14 mice (B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J, Jackson Laboratories Strain #007914, RRID:IMSR_JAX:007914), bred in house, and used for electrophysiological comparisons between PKCδ+ and PKCδ- cells and for channelrhodopsin-assisted mapping CRACM) studies (n=16, 10F, 6M). Mice were group housed (2-5 animals) throughout the duration of all experiments. Water and food were available ad libitum in the home cage of all mice except where noted for NSFT. The colony room was maintained on a 12-h light/dark cycle, with lights on at 06:00 under controlled temperature (20-25°C) and humidity (30-50%) levels. All experiments were conducted during the light phases. Animals were under continuous care and monitoring by veterinary staff from the Vanderbilt Division of Animal Care. All procedures were carried out in accordance with the NIH Guide to Care and Use of Laboratory Animals and institutional guidelines, and approved by the Institutional Animal Care and Use Committee at Vanderbilt University.

3.2.2 Fluorescent Immunohistochemistry (IHC) and Imaging

3.2.2.1 IHC

Brains of adult mice were removed following transcardial perfusion with 10mL 0.01M Phosphate Buffered Solution (PBS) and 20mL of 4% Paraformaldehyde (PFA) in 0.01M PBS. The brains were then postfixed in 4% PFA for 24 hours at 4°C. Following fixation, brains were transferred to 30% sucrose in PBS for cryoprotection for at least 36 hours. Brains were frozen in Optimal Cutting Temperature (OCT) Compound prior to slicing and cut into 40μm thick coronal sections using a cryostat (Leica CM3050S) and stored in PBS at 4°C or cryoprotectant (30% ethylene glycol, 30% glycerol, 10% 0.2M phosphate buffer, in diH20) solution at -20°C until IHC staining. For IHC, slices were washed in PBS (4x 10 min), permeabilized in 0.5% Triton X-100 in PBS for 30 minutes at room temperature, and then blocked in 0.1% Triton X-100 with 10% Normal Donkey Serum for 1 hour at room temperature. All primary and secondary antibodies were diluted in this blocking solution at 1:1000 dilution and 1:500 dilution, respectively. Slices were incubated in primary antibody solution for 24 hours at 4°C. Following primary antibody incubation, slices were washed in PBS (4 x 10 min) and incubated in secondary antibody solution for 24 hours at 4°C.

Slices were then washed in PBS (3 x 10 min) and then incubated in PBS plus DAPI (1:10,000, Sigma-Aldrich Cat# D9542-10MG). Primary antibodies used: Rabbit anti-cfos (Millipore Cat# ABE457, RRID:AB_2631318), Mouse anti-PKCδ (BD Biosciences Cat#610398, RRID:AB_397781), Rabbit anti-tRFP (Evrogen Cat# AB233, RRID:AB_2571743, labels TagBFP for rabies helper virus), Chicken anti-GFP (abcam ab13970, RRID:AB_300798, rabies helper virus, *in vivo* ChR2 validation, BLAQ CRACM validation), Chicken anti-GFP (Aves Labs GFP-1020, RRID:AB_10000240, GCaMP validation). Secondary antibodies used: Cy2 Donkey anti-chicken (Jackson ImmunoResearch Labs Cat# 705-225-147, RRID:AB_2307341), Cy3 Donkey anti-rabbit (Jackson ImmunoResearch Labs Cat #711-165-152, RRID:AB_2307443), Cy5 Donkey anti-mouse (Jackson ImmunoResearch Labs Cat# 715-175-150, RRID: AB_2340819). Slices were imaged at 20x magnification on a Zeiss LSM 880 confocal microscope and analyzed using ImageJ software (NIH; RRID:SCR_003070), with the same brightness and contrast settings applied across all images. Cell counts were performed manually using ImageJ by two researchers blinded to experimental groups. The cell counts for left and right BNST across two 40um-thick sections per animal were averaged across animals.

3.2.2.2 Brain Blocking of Lipids and Aldehyde Quenching (BLAQ)

For validation of virus expression and targeting following *ex vivo* Chr2-assisted circuit mapping, the Brain Blocking of Lipids and Aldehyde Quenching (BLAQ) procedure was used as described previously (N. A. Harris, Isaac, et al., 2018; Perez et al., 2020). Following *ex vivo* electrophysiology, slices were incubated in 4% PFA for 30 minutes at room temperature followed by overnight incubation at 4˚C. The following day, slices were transferred to cryoprotectant solution and placed at -20˚C until undergoing BLAQ staining for validation and targeting. Sections were washed for 1 hour in 0.2% PBST (0.2% Triton X-100 in PBS), rinsed twice for 1 minute in diH2O, and quenched in freshly prepared sodium borohydride (NaBH4; 5 mg/mL in diH2O; Sigma-Aldrich, #213462, 99%). Following this incubation, slices were rinsed again in diH2O (twice for 1 minute each), incubated for 15 minutes in Sudan Black B solution (0.2% in 70% ethanol), washed twice for 30 minutes each in PBS, and incubated for 4 hours in 5% BSA in 0.2% PBST. Slices were incubated in primary antibodies (Chicken anti-GFP, see above) for 96 hours at 4°C, washed 4

times in 0.2% PBST for a total of 24 hours at 4°C, and then incubated in secondary antibody (Cy2 donkey anti-chicken, Cy5 donkey anti-mouse, see above) for 48 hours at 4°C. Finally, slices were washed 4 times in 0.2% PBST for a total of 24 hours at 4°C, including a 10 minute DAPI incubation (1:10,000 in PBS), moved to PBS and then mounted on Fisher Plus slides and coverslipped with PolyAquaMount when dry. All images were obtained with a Zeiss LSM 880 confocal microscope using a 5x, 10x, or 20x Plan-Apochromat objective lens. The same acquisition parameters and alterations to brightness and contrast in Zeiss were used across all images of the same magnification within an experiment.

3.2.3 Stereotaxic Surgery/Viral Injections and Fiber Optic Implants

Adult mice (>8 weeks) were anesthetized with isoflurane (initial dose = 3%; maintenance dose = 1.5%) and surgery was performed using a Leica stereotax and injected intracranially with recombinant adeno-associated viruses (AAV) as specified below. Following surgery, mice were treated with Alloxate (2.5 mg/kg, s.c.) for 72 hours after surgery. At least four weeks were allowed after surgery before further experimentation to allow for mouse recovery and viral expression.

3.2.3.1 Viruses

For all but the electrophysiology experiments, a total volume of 300nL of virus was injected unilaterally into the oval nucleus of the right dorsolateral BNST at an angle of 15.03° (from Bregma: AP=0.14, ML=0.93, DV=-3.88). For *in vivo* optogenetic experiments, mice were injected with AAV5-Ef1a-DIO-hChR2(H134R)-EYFP-WPRE-pA (UNC Vector Core), or YFP control virus AAV5-EF1a-DIO-EYFP-WPRE-pA (UNC Vector Core). For fiber photometry experiments, mice were injected with AAV9-hSyn-FLEX-jGCaMP7f-WPRE (Addgene 104488-AAV9). For rabies tracing, mice were injected with AAV1-syn-FLEX-splitTVA-EGFP-tTA (Addgene 100798-AAV1, diluted 1:200) and AAV1-TREtight-mTagBFP2-CA-FLEX-B19G (Addgene 100799-AAV1, diluted 1:20) mixed in a 1:1 ratio. Seven days later mice were injected with EnvA-RVdg-mCherry (Salk #32636). For PKCδ whole cell basal electrophysiology experiments, mice were injected bilaterally into the oval nucleus of the BNST (AP=0.14, ML=+/-0.93, DV=-3.88) with AAV9-CAG-Flex-tdTomato-WPRE. For CRACM experiments, mice were injected with AAV5-CaMKII-

hChR2(H134R)-eYFP-WPRE-PA (UNC Vector Core) into the posterior BLA (150nL, AP=-2.3, ML=+/-3.45, DV=-4.8) at an angle of 4.05° (right pBLA) or -3.78° (left pBLA), or into the anterior PVT (200nL AP=-0.5, ML=0.0, DV=-3.5) and posterior PVT (200nL, AP=-1.6, ML=0.0, DV=-3.0) at an angle of 15.03°, or AAV5-EF1a-DIO-hChR2(H134R)-eYFP-WPRE-pA (UNC Vector Core) bilaterally into the CeA (250nL, AP=-2.3, ML=+/-3.17, DV=-4.61) at an angle of 0.0°.

3.2.3.2 Photometry and Optogenetic Fiber Optic Implants

Fiberoptic implants for the optogenetic experiments were constructed in house using a 0.5NA, 200 µm core multimode fiber (Thorlabs). Fibers were cut to length using a Bruce Diamond Wedge Tip Diamond Scribe (Fiber Optic Center) and secured inside a mounting ferrule (0.125mm diameter, 6.4mm long, 230um bore size, Thorlabs) with Hi-Temp epoxy (Thorlabs). The end to remain external was then cut to be flush with the implant and polished using a ferrule polishing disk (Thorlabs) and progressively finer (5, 3, 1, 0.1 µm) aluminum oxide lapping sheets (Thorlabs). The optical fiber implants for fiber photometry experiments were purchased from Doric Lenses (21248, MFC_400/430-0.57_4mm_MF2.5_FLT Mono).

During the aforementioned stereotaxic surgery gel etchant was used to texturize the skull, a screw was placed caudally to the craniotomy hole which was previously used to inject ChR2 (optogenetics) or GCaMP (fiber photometry) into the BNST. The implant was slowly lowered through the craniotomy hole and once in place, Optibond FL Primer (Patterson Dental 35265) was applied around the implant, Optibond FL adhesive (Patterson Dental 35266) was applied and cured with UV light, and Herculite Unidose Enamel (Patterson Dental 29838) applied and cured with UV light.

3.2.4 Restraint Stress for Immunohistochemistry (IHC)

Mice underwent restraint stress as previously described(Fetterly et al., 2019). Mice were first habituated to handling for 5 days. On the 6th day, mice were brought to the test room and acclimated for 1 hour in light- and sound-attenuating chambers. No stress control mice remained in their home cage until being perfused. Stress mice were placed in 50ml conical tubes with multiple holes for air, and set into a clean cage (male and female mice were placed into separate cages)

which was placed into a sound- and light-attenuating chamber (separate from unstressed mice) for 1 hour, with each mouse start time offset by 10 minutes. After 1 hour mice were returned to their home cage for 90 minutes, 3 hours, or 24 hours. Mice were then perfused and their brains removed and processed for immunohistological staining as described below (see Immunohistochemistry).

3.2.5 Electrophysiology

3.2.5.1 Slice preparation for physiological experiments

Slices were prepared and recorded form as previously described(Melchior et al., 2021) Animals were anesthetized with isoflurane, transcardially perfused with slice buffer, decapitated, and the brain rapidly removed and placed in pre-oxygenated (95% O2/5% CO2) NMDG-based slice buffer(Ting et al., 2014) consisting of (in mM): NMDG (93), NaHCO3(30), glucose (25), HEPES (20), KCl (2.5), NaH2PO4 (1.2), MgCl2 (10), CaCl2 (0.5), Na-ascorbate (5), Na-pyruvate (3), N-acetylcysteine (5); adjusted to pH 7.3-7.4 and 300-310 mOsm. Multiple coronal slices (300 µm thick) containing the bed nucleus of the stria terminalis (BNST) were prepared from each animal with a vibratome (Leica VT1200S; Leica Instruments, Nussloch, Germany). Slices were transferred to a warm bath (32-34 degrees) of slice buffer and allowed to recover for 12 minutes. Slices were then transferred to an oxygenated artificial cerebral spinal fluid (aCSF) containing: NaCl (126), NaHCO3(25), glucose (11), KCl (2.5), NaH2PO4 (1.2), MgCl2 (1.2), CaCl2 (2.4), L-ascorbic acid (0.4); adjusted to pH 7.4 and 298-302 mOsm, and allowed to recover, at room temperature, for > 1 hour before physiology experiments were performed.

3.2.5.2 Whole cell patch clamp electrophysiology

All electrophysiology recordings were made using Clampex 10.7 and analyzed using Clampfit 11.1 (Molecular Devices). Recordings were made using a 10 kHz sampling rate and a 2 kHz low pass filter. For whole cell physiology recordings, slices were transferred to a interface recording chamber perfused at a rate of ~1.5 ml/min with 28-30°C oxygenated aCSF. Recording electrodes for all experiments were filled with a K-gluconate-based internal solution (mM): K-

gluconate (125), Na-Phosphocreatine (10), HEPES (10), NaCl (4), MG-ATP (4), Na-GTP (0.3); adjusted to pH 7.3, 280-290 mOsm. The presence of PKCδ cells expressing Td-tomato in each slice were identified using the rig microscope; a mercury lamp light source and Texas Red filter cube were used to illuminate Td-tomato fluorophores, and infrared video microscopy was used to identify and patch fluorescent PKCδ cells in the field of view.

Multiple identified PKCδ cells were patched per slice. After whole-cell patch configuration was obtained, each cell was allowed to dialyze and equilibrate for 5 minutes before basal cellular parameters were measured. For voltage clamp experiments, cells were held at -70 mV and membrane currents were recorded; for current clamp experiments, cells were maintained at resting membrane potential (0 pA current) and membrane voltage responses recorded. Post-synaptic cellular parameters, including pipette access resistance, were monitored throughout experiments by applying a membrane test voltage step between sweeps; cells in which the access resistance changed by > 20% were not included in data analysis. Following equilibration to the whole cell patch configuration, the following parameters were measured in each cell: resting membrane potential, cell capacitance, rheobase, hyperpolarization sag, and spontaneous excitatory post-synaptic current (EPSC) frequency and amplitude.

Membrane potential was determined by switching to current clamp mode, with zero current applied, and measuring resting membrane voltage. Membrane voltage was recorded for 1 minute and the mean voltage across a 10 second segment of the trace was used to quantify resting membrane potential for each cell. Cell capacitance for each cell was determined from the membrane test protocol, in which a continuous voltage step was applied (20 mV, 100 ms, 60-100 Hz) and the membrane capacitance (Cm) was determined relative to the holding current and membrane resistance. Rheobase was determined by applying a series of current steps while in current clamp mode, with the cell at resting membrane potential. Each current step was 1 second in duration; current steps began at -150 pA and increased by 25 pA increments for each successive step until an action potential was elicited. The first current step that elicited an action potential was recorded as the rheobase for each cell. Similarly, the membrane hyperpolarization sag (Ih) was determined from the initial hyperpolarizing current step (-150 pA) applied to the cell. The amplitude (mV) of the membrane hyperpolarization sag was determined by measuring the

difference in voltage at the peak of any detectable sag occurring withing the first 50 ms of the onset of the -150 pA current step versus the steady state voltage at the final 100 ms of the current step. In the whole-cell voltage clamp configuration, spontaneous excitatory post-synaptic currents (EPSCs) were measured across a 5-minute bin, with each cell voltage clamped at -70 mV. Subsequent analysis utilized a standard Clampfit EPSC template to automatically identify EPSC events occurring over the course of the 5 min recording, and the average frequency and amplitude of EPSCs was determined for each cell.

3.2.5.3 ChR2-assisted circuit mapping (CRACM)

Optically evoked excitatory post synaptic currents (oEPSCs) from pBLA or PVT terminals in the BNST and optically evoked inhibitory post synaptic currents (oIPSCs) from CeA terminals were elicited using 5ms pulses of 473nm blue light in 10 second intervals (ThorLabs, T-cube LED driver). Cells were held at a membrane voltage (Vm) of -70mV for oEPSCs and 0mV for oIPSCs. A potassium based internal solution was used for oEPSC experiments. A cesium based internal solution was used for oIPSC experiments to maintain a tighter space clamp when the cells were held at the more depolarized Vm. The average amplitude across sweeps for each cell was used in analysis. Membrane properties were monitored throughout, and cells excluded based on a change in access resistance (Ra) greater than 20%. Response latency was determined manually using the cursors in clampfit to identify the time of the first peak. Rise and decay time were determined via automated detection in clampfit with parameters set at 10% minimum, 90% maximum.

3.2.6 Whole-brain clearing and light-sheet microscopy for rabies-mediated tracing

3.2.6.1 SHIELD clearing

SHIELD (Stabilization under Harsh conditions via Intramolecular Epoxide Linkages to prevent Degradation): Mouse brain tissue was prepared according to the LifeCanvas SHIELD protocol(Y. G. Park et al., 2019). Mice were anesthetized with isoflurane and transcardially perfused with 20 mL cold PBS followed by 20 mL of cold SHIELD perfusion solution at a rate of 5 mL/min. Brains were dissected and incubated in 20 mL of SHIELD perfusion solution shaking at

4°C for 48 hours. Brains were then transferred to 20 mL of SHIELD-OFF solution and shaken for 24 hours at 4°C. Brains were then transferred to passive SDS clearing solution and shaken for 8-12 weeks until fully transparent, with solution replaced every week. SHIELD perfusion and SHIELD-OFF solutions was prepared fresh before all perfusions, as described previously. After clearing, brains were shaken in PBS at 37°C for 12 h. The samples were then shaken in 50% EasyIndex (LifeCanvas Technologies) refractive index (RI) matching solution in diH20 for at least 48 hours at room temperature. Samples were then transferred to 100% EasyIndex solution for two days before being mounted. Sample holders (LifeCanvas Technologies) were rinsed with diH_2O, followed by 70% ethanol. Once the sample holder was dry, it was filled with 1.1% agarose (Sigma-Aldrich, A9539) made in Easy Index. The brains were then placed in a sample holder and allowed to congeal at 4°C for around 5-10 minutes. Brains were returned to conical tube of EasyIndex and shaken overnight at 37°C and then allowed to equilibrate in the imaging chamber overnight before imaging.

3.2.6.2 Light sheet microscopy

Whole-brain images were obtained using a light sheet microscope (SmartSpim, LifeCanvas Technologies)(Dean et al., 2015; Hedde & Gratton, 2018), and a 3.6x objective (NA = 0.2). Light with excitation wavelengths of 488nm and 561nm were illuminated through the sample with emission detection filters of 525/50 and 600/52, respectively. Laser power was set to 30–55% for each channel, with a 2 µm step size. Images were destriped and stitched to generate composite TIFF images with codes provided by SmartAnalytics that utilized a modified version of Terastitcher(Bria et al., 2019). Destriped and stitched TIFF images were converted to Imaris files using Imaris File Converter 9.2.1 for visualization, using Imaris 9.5.1. Processed images were run through SmartAnalytics (LifeCanvas Technologies) workstation and software. Briefly, scans were downsampled and aligned to the Allen Brain Atlas through manual registration. A cell detection model designed to detect fluorescently tagged cells was run to quantify rabies-infected cells across all brain regions listed in the Allen Brain Atlas. Cell counts were combined across brain regions with a granularity set to 6, meaning all subregions beyond the first six levels of hierarchically organized taxonomy based on the Allen Brain Atlas were combined within its parent structure,

using custom Python scripts provided by SmartAnalytics. Granularity level 6 was chosen because this is the level at which the BNST is delineated into a single region. Average counts across all brain regions were calculated divided by brain region volume, and top fifteen counts based on these averages were plotted. The highest density of labeling seen in the BNST itself represents a combination of both starter cell BNST$^{PKC\delta}$ neurons and intra-BNST afferent cells that cannot be distinguished with the labeling and lightsheet imaging technique used here.

3.2.7 *In vivo* optogenetics and fiber photometry

3.2.7.1 *In vivo* optogenetic stimulation

Stimulation patterns were delivered from the PlexBright 4 Channel Optogenetic Controller and controlled by Radiant v2 software (Plexon). The optogenetics controller box was attached to the PlexBright Dual LED rotatable commutator, in which a blue (465 nm) LED light was affixed (Plexon). PlexBright Optical Patch Cables (0.5 NA) were then attached to the commutator (Plexon). Animals received 20-Hz blue light stimulation (Plexon) with a 5ms pulse width at 10 mW power output as previously described(J. A. Brown et al., 2022).

3.2.7.2 *In vivo* fiber photometry recordings and analysis

Optical recordings of GCaMP7f fluorescence were acquired using a Tucker-Davis Technologies fiber photometry detection system as previously described(Salimando et al., 2020; Luchsinger et al., 2021; J. A. Brown et al., 2022), consisting of an RZ5P processor and Synapse custom software, and optical components purchased from Doric Lenses, ThorLabs and Newport. Excitation wavelengths generated by LEDs (470nm blue light and 405nm violet light) were relayed through a filtered fluorescence minicube at spectral bandwidths of 470nm and 405nm (for detection of both calcium-mediated GCaMP7f activity and background GCaMP fluorescence, respectively) to a 400um mono fiberoptic cable connected to the implants on top of each animal's head. Power output for the primary 470nm wavelength at the tip of the fiberoptic cable was measured at ~25-30uW. Single emissions were detected using a femtowatt photoreceiver with a lensed fiber cable adapter, and filtered at 500-550nm. Signal in both 470nm and 405nm

wavelengths were monitored continuously throughout all recordings, with the 405nm signal used as a control for both ambient fluorescence and motion artifacts introduced by movement of the fiber optic implant. Wavelengths were modulated at frequencies of 210 Hz for the 405nm channels and 330 Hz for the 470nm channel, and power output maintained at 20mA with a DC offset of 10mA for both light sources. All signals were acquired at 1kHz and lowpass filtered at 6Hz.

Linear regression MATLAB scripts from TDT were used to fit the 405 nm signal to the 470 nm signal as previously described(J. A. Brown et al., 2022). Change in GCaMP-mediated signal was calculated as $\frac{(Change\ in\ 470\ nm\ signal - Change\ in\ 405nm\ signal)}{Change\ in\ 405nm\ induced\ signal}$. Time-locked Z-scores were calculated using $Z = \frac{instantaneous \frac{\Delta F}{F}\ (from -5\ to -3\ seconds)}{mean \frac{\Delta F}{F}\ (from -5\ to -3\ seconds)}{standard\ deviation\ of \frac{\Delta F}{F}\ (from -5\ to -3\ seconds)}$. Peaks per minute and average Z-scores were calculated using a custom MATLAB script that incorporated adaptive iteratively reweighted Penalized Least Square (airPLS) code to account for baseline noise or signal drift and comparison of transients across mice(Z. M. Zhang et al., 2010; Martianova et al., 2019). The signal was then calculated using a Z-score transformation and fit to the 405nm reference signal. Data points with a Z-score ≥ 2 ($p<0.05$) were considered statistically significant. Peaks per minute were calculated as number of transients with Z-score >2 averaged across time. Average Z-Score values 3 sec pre/post behaviors were calculated as the average of all Z-Score values from -3 to 0 seconds (“pre”) or 0 to 3 seconds (“post”).

3.2.7.3 Optogenetic and fiber photometry behavioral experiments

Four weeks following viral injection and fiber implantation surgery, mice were habituated to false patch cables for 20 minutes in clean, empty cages for 3 consecutive days. On test day, mice were transported to the test room and allowed to habituate for one hour prior to beginning the experiment. The patch cable was connected to the fiber optic implants, and animals were habituated in a clean, empty cage for 5 minutes before the start of any behavior test.

3.2.7.3.1 Real-time place preference

Mice were placed into an opaque box (31.6cm wide x 36.2cm long x 26.7cm tall) divided into two chambers by panels extending into the center and leaving a 7.3cm wide gap for mice to

pass through. Mice were allowed to freely explore the box for 20 minutes, during which entries into one side of the chamber (randomly assigned per mouse) triggered the onset of light stimulation, which continued until the mouse entered the other side. Mouse location was tracked and quantified via Anymaze software. Freezing was identified as periods of immobility lasting greater than 3 seconds.

3.2.7.3.2 NSFT

NSFT was carried out as previously described(Holleran et al., 2016; Centanni, Morris, et al., 2019; Jaramillo et al., 2020). Two days (48 hours) prior to test day, mice were moved into new clean cages containing no food. After 23 hours of food deprivation, food pellets were placed into the cage (1 pellet per mouse in the cage) and left there for 2 hours. At the end of the two hours, mice were again transferred to a new clean cage containing no food. 23 hours after the removal of the food pellets, mice were placed in a large open box (50cm x 50cm) with clean bedding covering the floor and a food pellet in the center. Mice received 20Hz, 10mW, 5ms pulse width optogenetic stimulation for the duration of the test in a 5 sec on, 5 sec off pattern as previously described(J. A. Brown et al., 2022). The test ended upon first bite of the food pellet.

3.2.7.3.3 Elevated plus maze (EPM)

Mice were placed onto an elevated plus maze apparatus (San Diego Instruments) and allowed to freely explore for 10 minutes. Mouse location was tracked and measured via Anymaze software. For optogenetic experiments, mice received 20Hz, 10mW, 5ms pulse width light stimulation for the duration of the 10 minutes in a 5 seconds on, 5 seconds off pattern. For fiber photometry recordings, transitions between EPM zones were timelocked to signal changes based on Anymaze tracking data.

3.2.7.3.4 Footshock

Mice were placed in individual Med Associates (St. Albans, Vermont) operant conditioning chambers. A total of 10 footshocks were delivered in a non-contingent and inescapable fashion with one shock every 30 seconds, over a 5 minute period. Shocks were delivered at 0.5mA, and

this procedure was repeated the following day.

3.2.7.3.5 RESTRAINT

A 5-minute baseline was recorded in a clean empty cage, after which mice were placed into RESTRAINT (REcording Signal TRansients Accessible IN a Tube) as previously described(Luchsinger et al., 2021). Mice were restrained for 1 hour, after which they were returned to their home cage. This was repeated for 5 consecutive days. GCaMP7f signal was recording continuously throughout, and the animals were filmed during these sessions in order to match changes in calcium signaling to struggle/stress coping behavior during the task post hoc.

3.2.8 Statistics

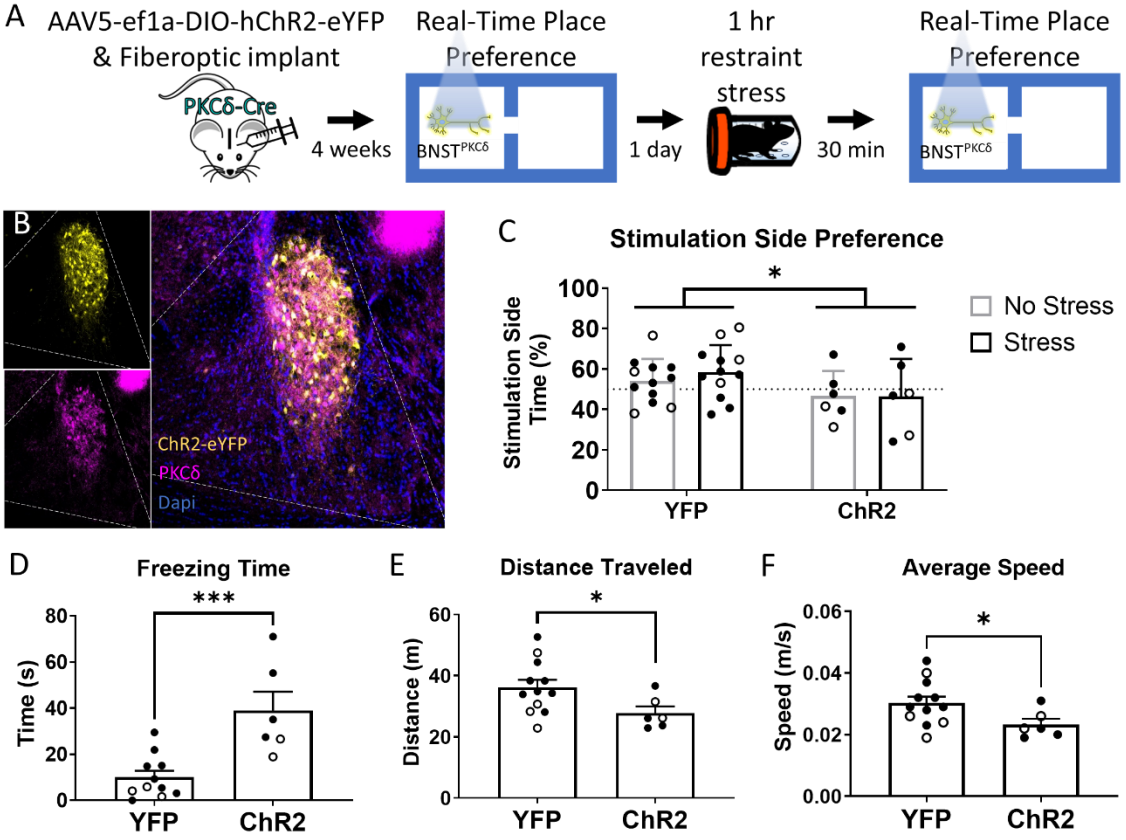
All statistics were run using GraphPad Prism version 9.5.0 for Windows, GraphPad Software. All values are plotted as mean ± SEM. A complete list of all statistical tests and results are located in Supplemental Table 2. For analysis of two groups, an unpaired or paired two-tailed t-test was used. For analysis of three or more groups, a one-way ANOVA was used. For analysis of two or more groups with at least two treatments or time points, a two-way ANOVA was used. For analyses with three variables, a three-way ANOVA was used. If significant effects were observed, post-hoc Tukey's, Sidak's, or Dunnett's T3 test for multiple comparisons were used. For analysis across time or over days, corresponding test with repeated measures analysis was used. If mice were missing on a subsequent day, or an outlier was removed from paired data, a mixed effects analysis was used. For correlation analysis, a simple linear regression was used. Outliers were tested for using ROUT with a Q=0.1%. For all analyses, significance of $p<0.05$ was used. *$p<0.05$. **$p<0.01$. ***$p<0.001$. ****$p<0.0001$. Complete details of all analyses are included in the figure legends and/or Supplemental Table 5.3. No significant sex differences/interactions were observed (Supplemental Table 5.3) so males and females are combined for all studies and plotted as filled (female) verses open (male) circles.

3.3 Results

3.3.1 *In vivo* optogenetic stimulation of BNST$^{PKC\delta}$ cells is aversive

We first sought to investigate the impact of BNST$^{PKC\delta}$ cell stimulation on mouse behavior by utilizing a real-time place preference (RTPP) assay. To specifically target PKCδ neurons, we used transgenic mice expressing Cre recombinase under control of the PKCδ promotor (Tg(Prkcd-glc-1/CFP,-cre)EH124Gsat/Mmucd). Mice were injected with a Cre-dependent adeno-associated virus expressing either channelrhodopsin (ChR2), or a control yellow fluorescent protein (YFP) (Fig. 3.1A, B). Mice received 20Hz optical stimulation upon entry to one side of a two-chamber apparatus and we recorded time spent on each side for 20 minutes. Because we previously found that expression of PKCδ is sensitive to stress (Fetterly et al., 2019), we then repeated the RTPP assay the following day, 30 minutes after 1 hour of restraint stress to investigate whether mice may be sensitized to BNST$^{PKC\delta}$ cell activation following stress exposure. We found no significant effect of stress on time spent on either side of the chamber (2-way ANOVA, $p=0.65$). However, compared to YFP controls, ChR2-expressing mice spent significantly less time on the light-stimulation side (2-way ANOVA, $p<0.05$), suggesting that activation of BNST$^{PKC\delta}$ cells is aversive (Fig. 3.1C). We also assessed the impact of activating BNST$^{PKC\delta}$ cells on movement during this assay. We found that BNST$^{PKC\delta}$ cell stimulation resulted in significantly more time freezing (Fig. 3.1D, t-test, $p<0.001$), and a reduction in distance traveled (Fig. 3.1E, t-test, $p<0.05$) and speed (Fig. 3.1F, t-test, $p<0.05$) compared to YFP controls. We found no difference in the number of side alternations, suggesting the reduced stimulation-side time is driven by aversion rather than by decreased locomotion (Fig. 3.7A).

Fig. 3.1 *In vivo* activation of BNST$^{PKC\delta}$ cells is aversive and reduces movement



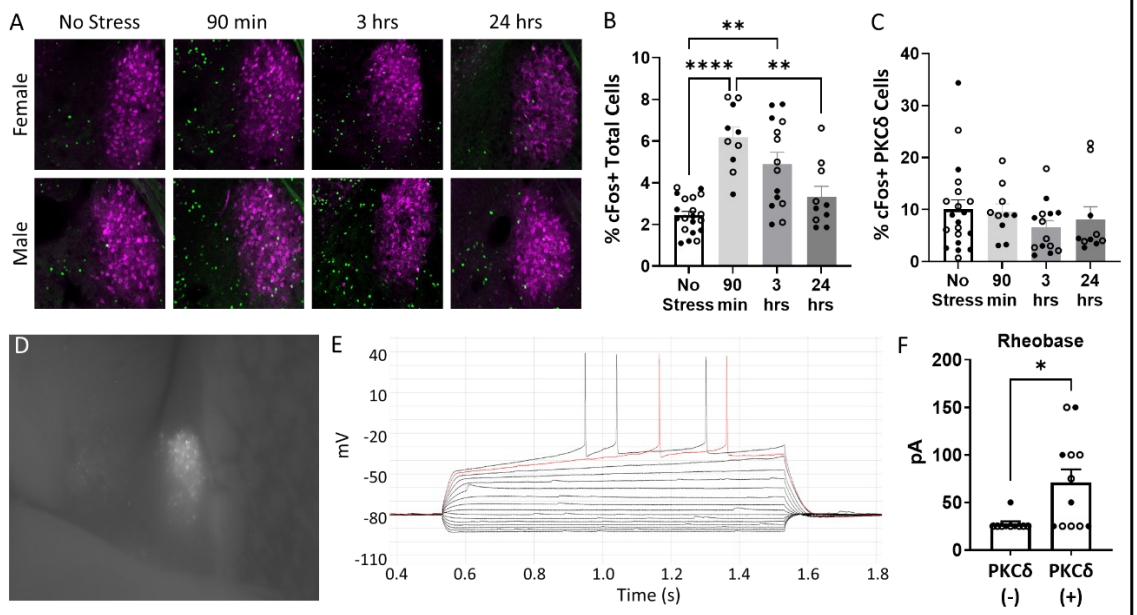
A. Experimental design and timeline for optogenetic activation of BNST$^{PKC\delta}$ neurons in the real-time place preference (RTPP) assay with and without stress. **B.** Representative image of injected channelrhodopsin (ChR2) co labelling with PKCδ immunofluorescence in the BNST. **C-F.** Effect of optogenetic stimulation of BNST$^{PKC\delta}$ cells on behaviors during RTPP. Filled circles = Females (F), Open circles = Males (M). YFP: n=12 (8F, 4M). ChR2: n=6 (4F, 2M). **C.** Side preference calculated as time spent on stimulation side as % of total time. 2-way paired ANOVA, treatment effect p=0.0456, F(1,16) = 4.325. **D.** Time spent freezing. Unpaired two tailed t-test, p=0.0009, t(15)=4.137. One outlier removed (ROUT, Q=0.1%) **E.** Total distance travelled. Unpaired two tailed t-test, p=0.0465, t(16)=2.157. **F.** Average speed. Unpaired two tailed t-test, p=0.0497, t(16)=2.123.

3.3.2 BNST$^{PKC\delta}$ cells are resistant to activation following restraint stress and *ex vivo* stimulation

We were surprised that stress did not alter RTPP behavior, and thus further investigated the recruitment of BNST$^{PKC\delta}$ cells during stress. Mice underwent 1 hour of restraint stress, a well-validated stressor (Paré & Glavin, 1986; Glavin et al., 1994; Buynitsky & Mostofsky, 2009; Fetterly et al., 2019; L. Á. Kovács et al., 2022), after which brains were perfused and collected 90 minutes, 3 hours, or 24 hours after stress. BNST-containing brain slices were then stained with antibodies for PKCδ and cfos, a marker of recent neuronal activity (Fig. 3.2A). As expected, we found that the total number of cfos-positive cells in the BNST was increased in mice 90 minutes and 3 hours after

restraint stress (Dunnett's multiple comparisons, No Stress vs 90min p<0.0001, No Stress vs 3hrs p=0.004) but that there was no difference in cfos+ cells 24 hours after stress compared to no-stress controls (p=0.47) (Fig. 3.2B). However in BNST$^{PKC\delta}$ cells specifically, we found no increase in cfos expression at any timepoint compared to no-stress control mice (Fig. 3.2C, 1-way ANOVA, p=0.51).

Fig. 3.2 BNST$^{PKC\delta}$ cells are resistant to activation



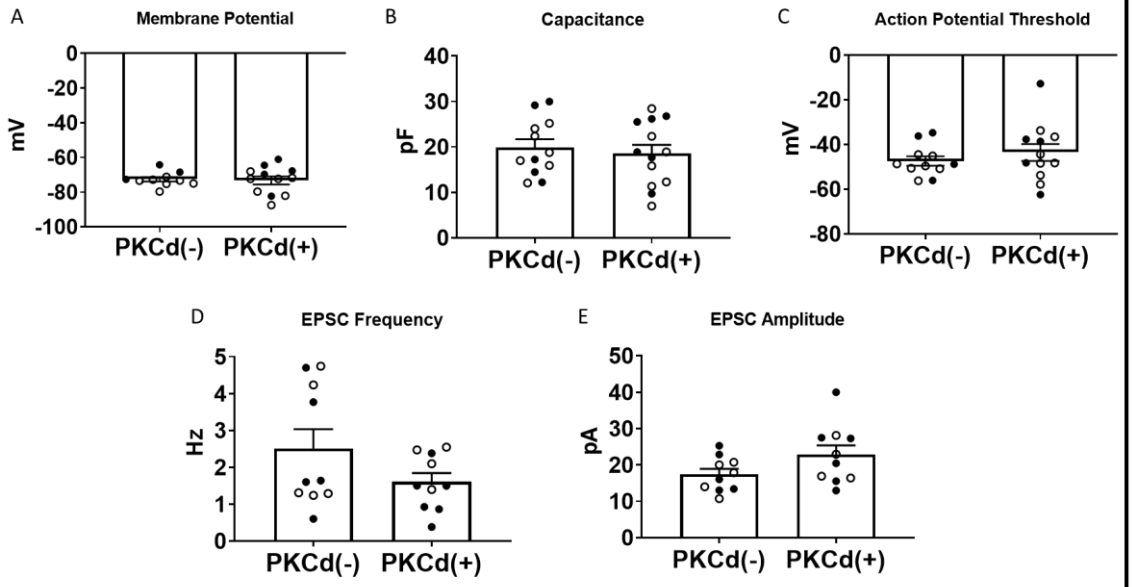
A. Representative images and **B-C.** quantification of immunohistochemistry (IHC) staining for cfos and PKCδ in male and female no-stress control mice or at 90 minutes, 3 hours, or 24 hours following 1 hour of restraint stress. No stress: n=20 (10F, 10M). 90 min: n=10 (5F, 5M). 3 hrs: n=14 (7F, 7M). 24hrs n=10 (5F, 5M). **B.** Number of cfos+ cells as a % of total BNST cells. Welch's 1-way ANOVA, p<0.0001, F(3.0, 20.25)=19.43. Dunnett's T3 multiple comparison test, No Stress vs 90 min p<0.0001, t(11.8)=7.122. No Stress vs 3 hrs p=0.0043, t(16.04)=4.156. 90 min vs 24 hrs p=0.0040, t(18)=4.095. **C.** Number of cfos+ cells as a % of PKCδ+ cells. Ordinary 1-way ANOVA, p=0.5114, F(3,50)=0.7788. **D.** Representative image of AAV9-CAG-Flex-tdTomato-WPRE expression in BNST of PKCδ-Cre mice. **E.** Representative trace of current step injection response in BNST$^{PKC\delta}$ neuron, with rheobase current step in red. **F.** Rheobase of PKCd(+) vs PKCd(-) cells in the oval BNST. Filled circles = Females (F). Open circles = Males (M). N=6 mice (3F, 3M), PKCd(-) and PKCd(+) cells recorded from each mouse. PKCd(-): n= 10 cells (3F, 7M). PKCd(+): n=12 cells (5F, 7M). Unpaired two tailed t-test, p=0.0117, t(20)=2.775, 1 Outlier removed, ROUT Q=0.1%.

Table 3.1	Membrane Potential (mV)	Membrane Capacitance (pF)	Rheobase (pA)	AP Threshold (mV)	Ih (mV)	EPSC Frequency (Hz)	EPSC Amplitude (pA)
BNST$^{PKC\delta}$ Cell Avg	-76.26 +/- 1.23	107.3 +/- 6.93	135.9 +/- 10.16	-28.85 +/- 1.00	4.48 +/- 1.46	1.66 +/- 0.16	24.95 +/- 1.42

BNST$^{PKC\delta}$ basal membrane properties
Average whole cell patch clamp electrophysiological properties of BNST$^{PKC\delta}$ cells. Values displayed as average +/- SEM. n=6 mice (3F, 3M), 26 cells (13F, 13M).

To better understand potential differences distinguishing $BNST^{PKC\delta}$ cells from other BNST subpopulations, we next turned to *ex vivo* electrophysiology to investigate their basal excitability properties. PKCδ-Cre mice were injected with Cre-dependent tdTomato to label and enable whole cell patch clamp recording from $BNST^{PKC\delta}$ neurons (Fig. 3.2D, E), the results of which are summarized in Table 3.1. These cells did not fire spontaneously in slices, and fired action potentials only in response to strong depolarizing stimuli, having an average rheobase of 136 +/- 10pA, which is considerably higher than previous literature has shown for other cells in the BNST (Table 3.1) (Egli & Winder, 2003; Silberman et al., 2013). To directly examine this, we crossed PKCδ-Cre mice with Ai14 mice to label PKCδ cells and conducted whole cell patch clamp recordings from both PKCδ(+) and PKCδ(-) cells in the BNST oval nucleus. We found that indeed, $BNST^{PKC\delta(+)}$ neurons had a significantly higher rheobase than $BNST^{PKC\delta(-)}$ cells (Fig. 3.2F, t-test p=0.01), with no significant differences on other measures examined (Figure 3.3). Altogether these results demonstrate that $BNST^{PKC\delta}$ cells require significant stimulation in order to be activated, and are overall more resistant to activation than other BNST cells.

Fig. 3.3 Additional electrophysiological comparisons between BNST$^{PKC\delta+}$ and BNST$^{PKC\delta-}$ cells

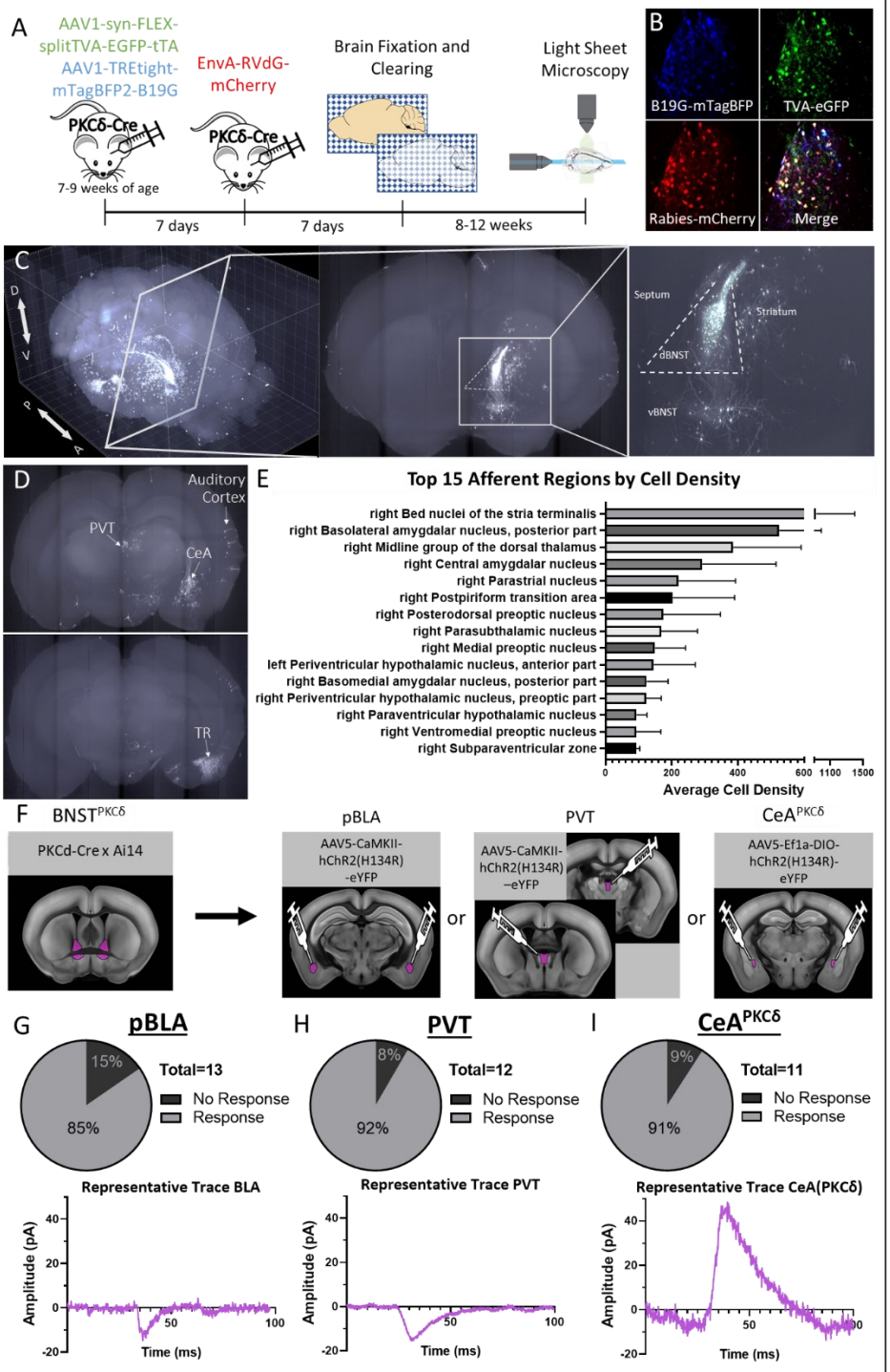


Filled circles = Females (F). Open circles = Males (M). Basal electrophysiological properties of PKCd(+) vs PKCd(-) cells. N=6 mice (3F, 3M), PKCd(-) and PKCd(+) cells recorded from each mouse. **A** Membrane potential. PKCd(-): n= 10 cells (3F, 7M). PKCd(+): n=12 cells (5F, 7M). Unpaired two tailed t-test, p=0.8187, t(20)=0.2323. 1 Outlier removed, ROUT Q=0.1%. **B** Capacitance. PKCd(-): n= 12 cells (4F, 8M). PKCd(+): n=13 cells (6F, 7M). Unpaired two tailed t-test, p=0.6181, t(23)=0.5054. **C** Action potential threshold. PKCd(-): n= 11 cells (3F, 8M). PKCd(+): n=12 cells (5F, 7M). Unpaired two tailed t-test, p=0.3960, t(21)=0.8665. **D** Excitatory postsynaptic current (EPSC) frequency. PKCd(-): n= 10 cells (4F, 6M). PKCd(+): n=10 cells (6F, 4M). Unpaired two tailed t-test, p=0.1292, t(18)=1.590. **E** EPSC Amplitude. PKCd(-): n= 10 cells (4F, 6M). PKCd(+): n=10 cells (6F, 4M). Unpaired two tailed t-test, p=0.0859, t(18)=1.817.

3.3.3 Brain-wide afferent control of BNST$^{PKC\delta}$ cells

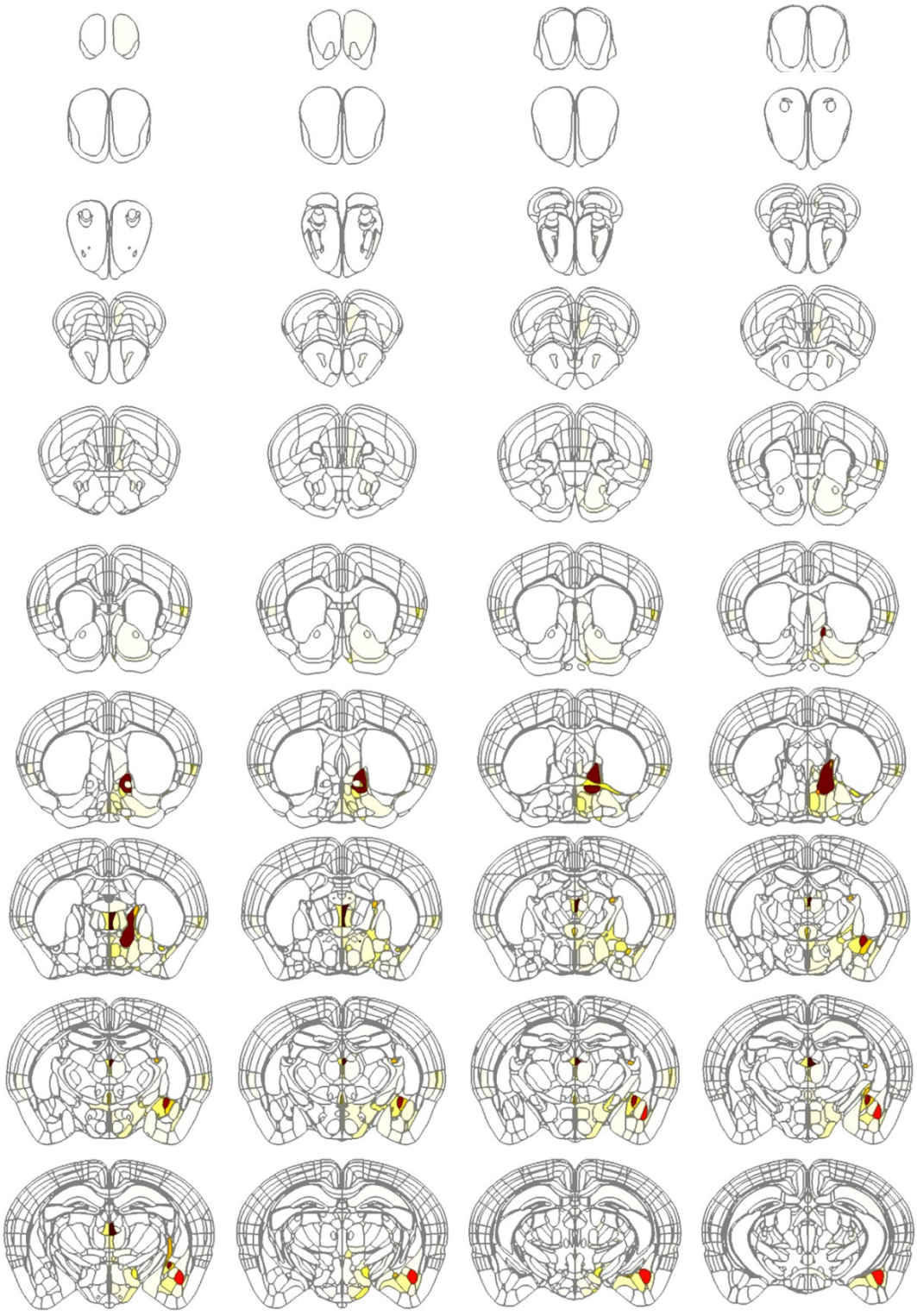
Given the reluctant firing properties of BNST$^{PKC\delta}$ cells, we next wanted to investigate their afferent circuit connectivity to better understand the balance of inputs onto this neuronal population. Monosynaptic inputs were labelled in PKCδ-Cre mice using a rabies viral approach. We then used SHIELD-based optical clearing and light-sheet microscopy to obtain high resolution images of whole intact mouse brains (Fig. 3.4A-C). We analyzed these images using the SmartAnalytics (LifeCanvas Technologies) pipeline to align brain images to the Allen Brain Atlas and automatically locate and quantify rabies-expressing neurons. We achieved robust rabies labeling of BNST$^{PKC\delta}$ starter cells as well as cells in several regions throughout the brain (Fig. 3.4C-D), with the highest density of inputs in regions including the posterior basolateral amygdala (BLAp), paraventricular nucleus of the thalamus (PVT), and central nucleus of the amygdala (CeA), as well as the postpiriform transition area (TR) and several hypothalamic subregions (Fig. 3.4E, Full list sorted by avg density in Supplemental Table 5.1, sorted by avg cell count in Supplemental Table 5.2, Representative Heatmap in Fig 3.5).

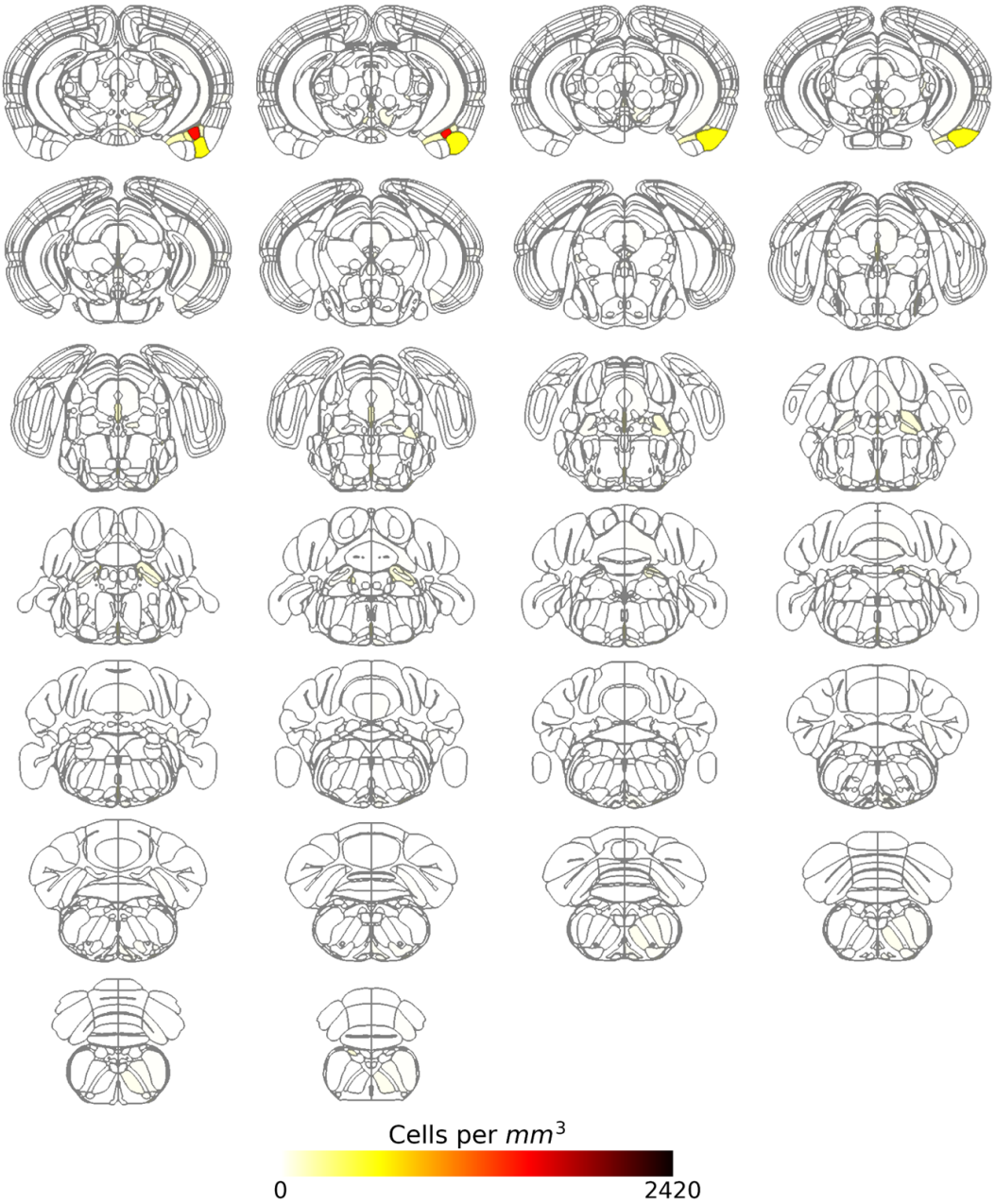
Fig. 3.4 BNST$^{PKC\delta}$ cell afferent connectivity



A. Experimental design and timeline for whole brain rabies-mediated retrograde tracing, clearing, and imaging. **B.** Representative image of Cre-dependent rabies helper viruses and G-deleted rabies virus in the BNST of PKCδ-Cre mouse. **C-D.** Representative images of cleared brain imaged via light sheet microscopy following rabies tracing showing **C** whole stitched brain image and optically sectioned coronal slices displaying starter BNST$^{PKC\delta}$ neurons and **D.** example rabies-expressing afferent neurons. **E.** Quantification of density of rabies+ cells in top 15 afferent regions, reported as cells/mm^3. n=4 mice (2F, 2M). **F.** Experimental design for channelrhodopsin (ChR2)-assisted circuit mapping (CRACM). **G-I.** Number of BNST$^{PKC\delta}$ neurons responsive to optical stimulation of ChR2-exppresing terminals from the BLA (G), PVT (H) and CeA$^{PKC\delta}$ (I) neurons, and representative trace showing response. **G.** BLA n=3 mice (2F, 1M), 13 total cells (11 responders). **H.** PVT: n=3 mice (3F), 12 total cells (11 responders). **I.** CeA$^{PKC\delta}$: n=4 mice (2F, 2M), 11 total cells (10 responders).

Fig. 3.5: Rabies Tracing Heatmap





Example heatmap distribution of BNST$^{PKC\delta}$ afferent regions in one mouse. Generated by SmartAnalytics software

We next tested the functionality of the top synaptic inputs using channelrhodopsin-assisted circuit mapping (CRACM). The largest sources of afferents came from the pBLA, which plays a major role in affective processing, and the PVT, which integrates threat and arousal signals (J. E. LeDoux, 2003; Kirouac, 2021; Šimić et al., 2021; D. Zhao et al., 2022). We injected ChR2 into either the pBLA or PVT of PKCδ-CrexAi14 mice. We then shone blue light onto *ex vivo* BNST-containing brain slices to stimulate afferent terminals and recorded the resulting optically evoked

postsynaptic currents in BNST^{PKCδ} neurons (Fig 3.4F). The majority of BNST^{PKCδ} cells recorded from displayed excitatory postsynaptic currents (EPSCs) in response to terminal stimulation from the BLA (11 out of 13 cells) or PVT (11 out of 12 cells) (Fig 3.4G-H, Table 2). We next investigated input from the CeA which, like the BNST, is heterogeneous and contains many cell types paralleling those in the BNST (Roberts et al., 1982; Mccullough et al., 2018). An emerging theme in CeA-BNST interconnectivity is the reciprocal connectivity of similar cell types, with CeA^{CRF}-BNST^{CRF} and CeA^{SOM}-BNST^{SOM} interactions playing important roles in regulating affective responses (Ahrens et al., 2018; Pomrenze et al., 2019; Ye & Veinante, 2019; de Guglielmo et al., 2019). Thus, we injected Cre-dependent ChR2 into the CeA of PKCδ-CRExAi14 mice, and found that the majority of BNST^{PKCδ} cells recorded from (10 out of 11) displayed inhibitory postsynaptic currents in response to CeA^{PKCδ} terminal stimulation (Fig. 3.4H, I, Table 2). This demonstrates that BNST^{PKCδ} cells receive functional input from major affective and threat processing afferent regions.

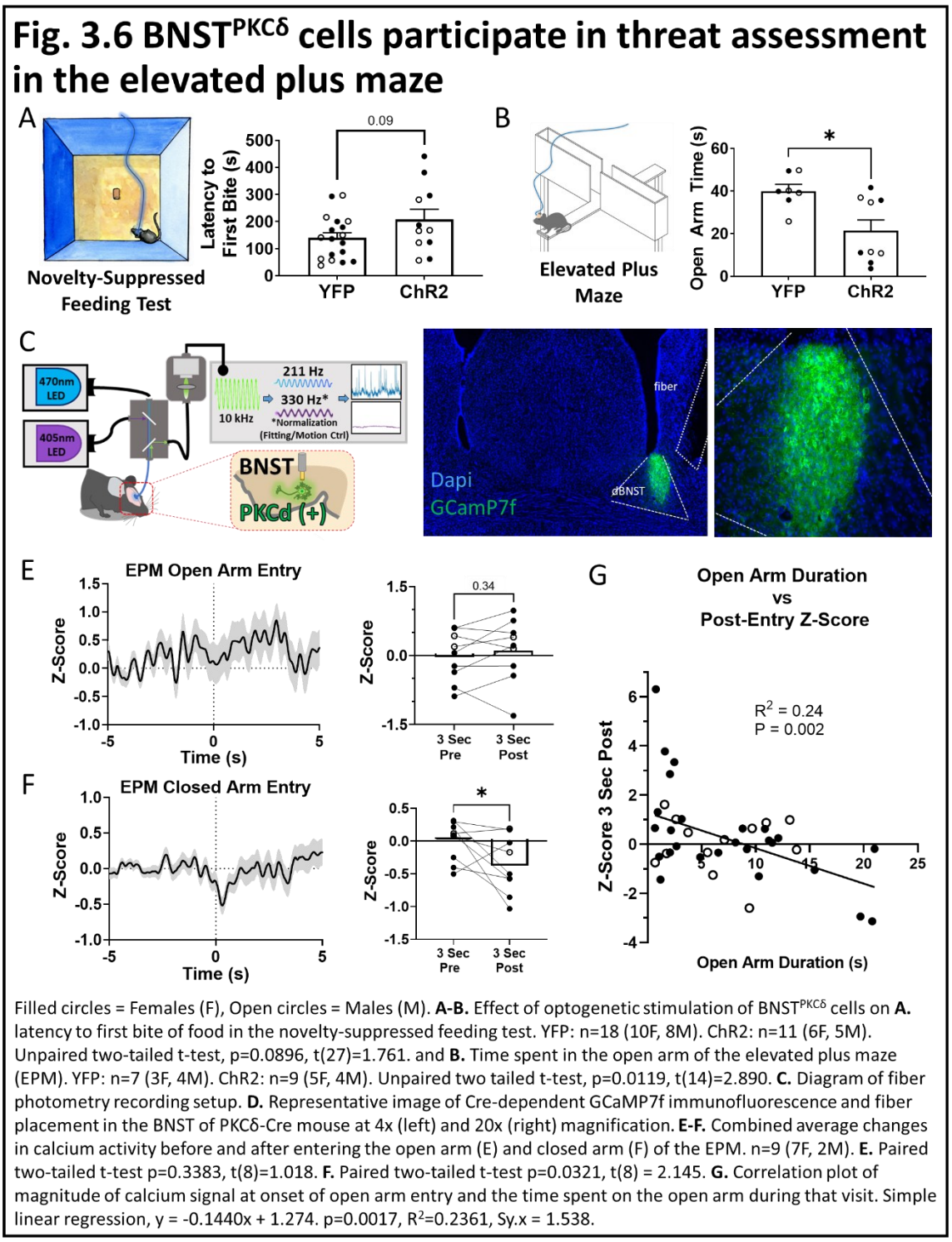
Table 3.2	Postsynaptic Current Amplitude (pA)	Response Latency (ms)	Rise Time (ms)	Decay Time (ms)
BLA	-18.25 +/- 2.56	12.77 +/- 4.45	3.84 +/- 2.66	5.23 +/- 2.24
PVT	-15.19 +/- 1.77	16.80 +/- 2.65	4.98 +/- 1.70	9.15 +/- 1.38
CeA^{PKCδ}	31.69 +/- 6.72	22.36 +/- 5.01	8.35 +/- 1.89	28.44 +/- 5.18

BNST^{PKCδ} functional afferent connectivity
Channelrhodopsin-assisted circuit mapping (CRACM) of top afferent regions onto BNST^{PKCδ} cells. Values displayed as average +/- SEM. BLA n=3 mice (2F, 1M), 11 cells. PVT: n=3 mice (3F), 11 cells. CeA^{PKCδ}: n=4 mice (2F, 2M), 10 cells.

3.3.4 BNST^{PKCδ} cells participate in threat assessment in the elevated plus maze

We next aimed to better understand the role of BNST^{PKCδ} cells in the context of anxiety-like behaviors. We previously found that BNST^{PKCδ} cells are recruited in the stressful decision making context of the novelty-suppressed feeding test (NSFT) (Jaramillo et al., 2020). This task involves placing food-deprived mice in an open arena with a food pellet in the center, allowing observation of risk assessment and anxiety-like behavior during food approach in an aversive context. Here, we expressed ChR2 or YFP in BNST^{PKCδ} cells and provided 20Hz optical stimulation in a 5sec on, 5sec off pattern as previously described (J. A. Brown et al., 2022) until the first bite of food during NSFT. All mice consumed food within the 10-minute time limit of the assay. We found no

difference in time to first approach the food pellet, number of food pellet approaches (Fig. 3.7B-C, t-tests, p=0.61 and p=0.23), or time to first bite of food, though there was a trend for increased first bite latency in ChR2-expressing mice (Fig. 3.6A. t-test, p=0.09).

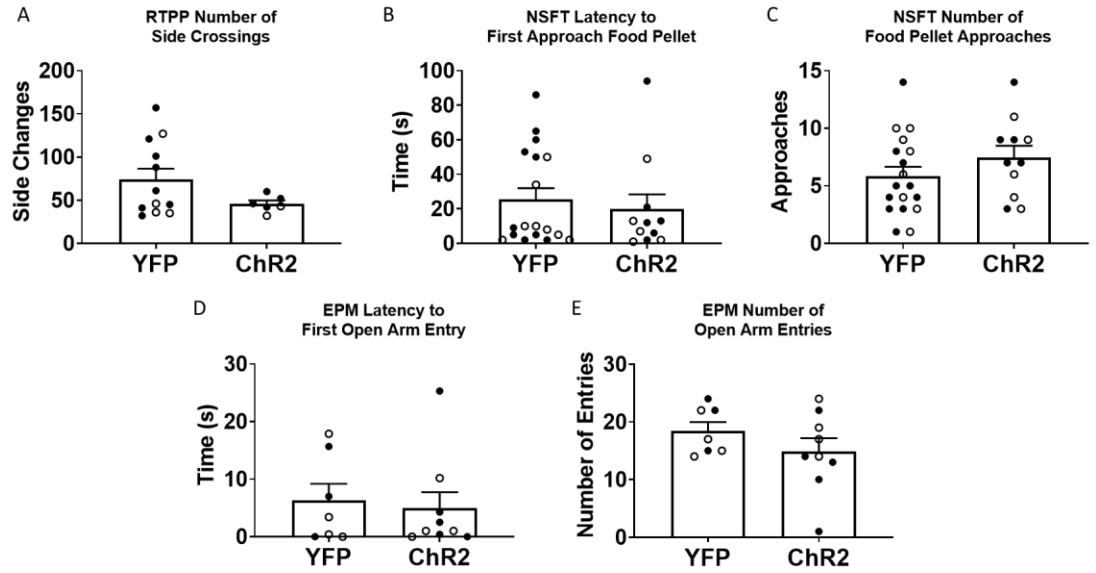


Filled circles = Females (F), Open circles = Males (M). **A-B.** Effect of optogenetic stimulation of BNST$^{PKC\delta}$ cells on **A.** latency to first bite of food in the novelty-suppressed feeding test. YFP: n=18 (10F, 8M). ChR2: n=11 (6F, 5M). Unpaired two-tailed t-test, p=0.0896, t(27)=1.761. and **B.** Time spent in the open arm of the elevated plus maze (EPM). YFP: n=7 (3F, 4M). ChR2: n=9 (5F, 4M). Unpaired two tailed t-test, p=0.0119, t(14)=2.890. **C.** Diagram of fiber photometry recording setup. **D.** Representative image of Cre-dependent GCaMP7f immunofluorescence and fiber placement in the BNST of PKCδ-Cre mouse at 4x (left) and 20x (right) magnification. **E-F.** Combined average changes in calcium activity before and after entering the open arm (E) and closed arm (F) of the EPM. n=9 (7F, 2M). **E.** Paired two-tailed t-test p=0.3383, t(8)=1.018. **F.** Paired two-tailed t-test p=0.0321, t(8) = 2.145. **G.** Correlation plot of magnitude of calcium signal at onset of open arm entry and the time spent on the open arm during that visit. Simple linear regression, y = -0.1440x + 1.274. p=0.0017, R^2=0.2361, Sy.x = 1.538.

Because BNST$^{PKC\delta}$ cells have been implicated in feeding behaviors (Y. Wang et al., 2019), we also examined risk assessment and anxiety-like behavior in a food-independent task, turning to the elevated plus maze. Here, we found that activation of BNST$^{PKC\delta}$ cells significantly reduced open arm time, suggesting increased anxiety-like behavior (Fig. 3.6B. t-test, p=0.01). We found no difference in latency to first enter the open arm or number of open arm entries, suggesting the decreased time is due to an anxiety-like effect rather than reduced locomotion (Fig. 3.7D-E, t-tests, p=0.74 and p=0.25).

To further ascertain the involvement of BNST$^{PKC\delta}$ cells in the elevated plus maze, we next expressed the modified fluorescent calcium sensor, GCaMP7f, and used fiber photometry to record their *in vivo* activity in real-time (Fig. 3.6C, D). We found that upon entry to the EPM open arms, there is no change in BNST$^{PKC\delta}$ cell activity (Fig. 3.6E. t-test, p=0.34), but that there is a significant decrease in signal when mice return to the closed arms (Fig. 3.6F, t-test, p=0.03). Further, we found that the magnitude of neuronal activation during entry to an open arm is negatively correlated with the duration of that open arm visit. Larger increases in calcium transients at onset of open arm entry correlated with short visit duration, while instances of little-to-no or reduced BNST$^{PKC\delta}$ cell activation were correlated with longer open arm times (Fig. 3.6F. Simple linear regression, R^2=0.24, p=0.002).

Fig. 3.7 Reduced movement does not interfere with expression of aversion of anxiety-like behavior

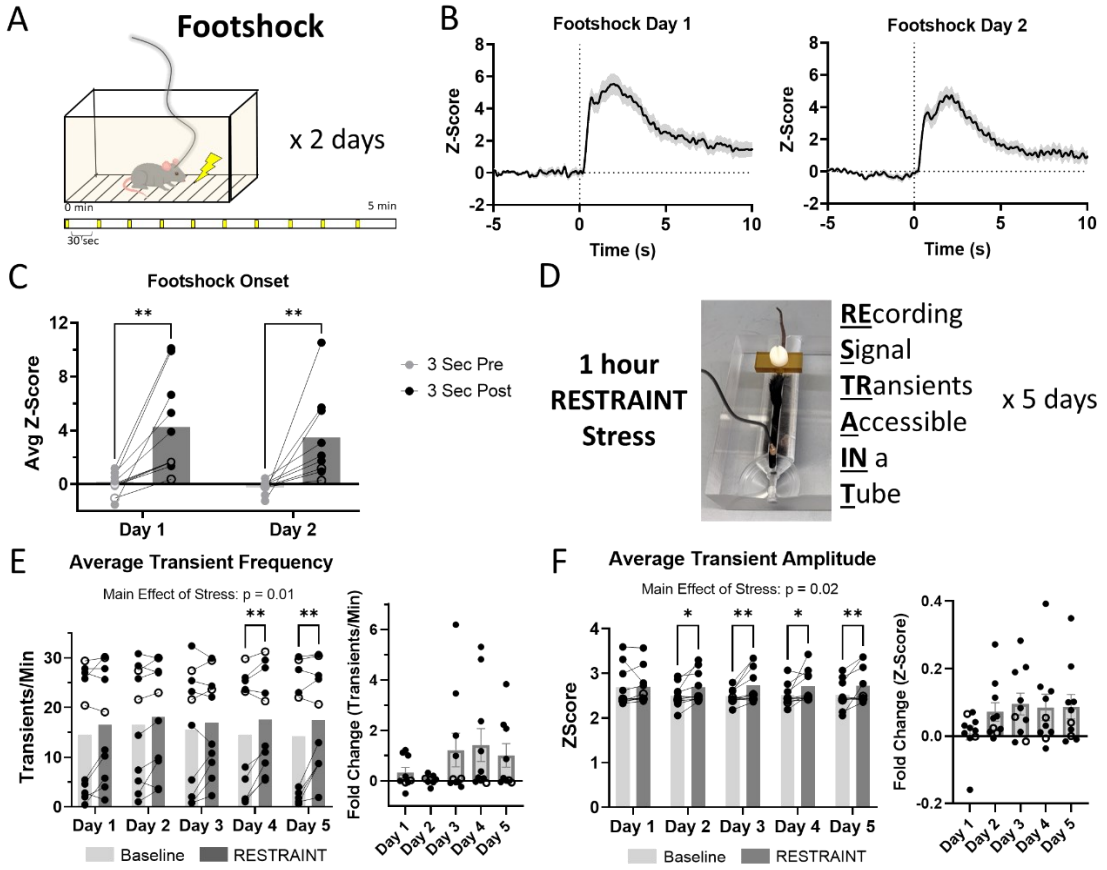


Filled circles = Females (F). Open circles = Males (M). Effect of *in vivo* optogenetic stimulation of BNST^{PKCd} cells on **A** Number of crossings between light stimulation side and non-stimulation side in the real time place preference (RTPP) assay. YFP: n=12 (8F, 4M). ChR2: n = 6 (4F, 2M). Unpaired two tailed t-test, p=0.1364, t(16)=1.568. **B-C** Novelty-suppressed feeding test (NSFT) (NSFT). YFP: n=18 (10F, 8M). ChR2: n=11 (6F, 5M). **B** Latency to first approach the food pellet. Unpaired two tailed t-test, p=0.6126, t(27)=0.5123. **C** Number of food pellet approaches. Unpaired two tailed t-test, p=0.2300, t(27)=1.228. **D-E** Elevated Plus Maze. YFP: n=7 (3F, 4M). ChR2: n=9 (5F, 4M). **D** Latency to first enter an open arm. Unpaired two-tailed t-test, p=0.7378, t(14)=0.3415. **E** Number of open arm entries. Unpaired two-tailed t-test, p=0.2496, t(14)=1.201.

3.3.5 BNST$^{PKC\delta}$ cell activity does not desensitize to repeated stress exposure

The magnitude of calcium transients in the elevated plus maze was modest compared to previous studies on the recruitment of BNST$^{PKC\delta}$ cells during stress coping (Jaramillo et al., 2020; Luchsinger et al., 2021), and we hypothesized that perhaps their recruitment scales with the salience of the presented threat/stressor. The BNST is known to be activated by unpredictable threat (Walker et al., 2003, 2009; Davis et al., 2010; Avery et al., 2016). Thus we again used GCaMP7f and *in vivo* fiber photometry to measure activation of BNST$^{PKC\delta}$ neurons at the onset of un-cued footshock stress. A footshock was delivered every 30 seconds for 5 minutes, and we found a significant increase in activity upon footshock exposure (Fig. 3.8A-C). General and CRF neuronal activity in the BNST desensitizes to repeated homotypic stress exposure (Luchsinger et al., 2021; J. A. Brown et al., 2022), so we then repeated footshock the following day to test if PKCδ cells would also desensitize. Surprisingly, we found that there was again an increase in BNST$^{PKC\delta}$ cell activation at the onset of footshock, with no significant difference from the first day (Fig. 3.8B, C. Mixed effects analysis, footshock effect p=0.004, day effect p=0.15).

Fig. 3.8 BNST$^{PKC\delta}$ cells do not desensitize to homotypic stress exposure



Filled circles = Females (F). Open circles = Males (M). **A.** Experimental setup for fiber photometry footshock assay. **B.** Combined average traces of changes in calcium activity quantified in (C). **C.** Combined average changes in calcium activity 3 sec before and after footshock presentation. Paired mixed effects analysis, Day effect p=0.1507, F(1,9) = 2.466. Shock effect p=0.004, F(1,9) = 14.75. Sidak's multiple comparison test, Day 1 n=10 (8F, 2M) p=0.0021, t(16)=3.996. Day 2 n=9 (7F, 2M) p=0.0064, t(16)=3.459. **D.** Image of mouse in RESTRAINT device while recording fiber photometry signals. **E-F.** Average calcium transient events per minute (E) and Average calcium transient amplitude (F) during 5 minute baseline vs 1 hour RESTRAINT across 5 days (left), and the calculated fold change between baseline and stress for each day (right). n=10 (8F, 2M). **E. (left)** Transient frequency across days. 2-way Repeated measures ANOVA, Day effect: p=0.486, F(4,36)=0.8793. Stress effect: p=0.0143, F(1,9)=9.175. Sidak's multiple comparisons test, Day 1 p=0.0714, t(36)=2.563. Day 2 p=0.2149, t(36)=2.055. Day 3 p=0.3258, t(36)=1.828. Day 4 p=0.002, t(36)=3.911. Day 5 p=0.0011, t(36)=4.096. **(right)** Transient frequency fold change each day. Mixed Effects model, p=0.2079, F(1.347,10.78)=1.826. **F. (left)**Transient amplitude across days. 2-way Repeated measures ANOVA, Day effect: p=0.614, F(4,36)=0.6745. Stress effect: p=0.0167, F(1,9)=8.598. Sidak's multiple comparisons test, Day 1 p>0.999, t(36)=0.1793. Day 2 p=0.0343, t(36)=2.863. Day3 p=0.0018, t(36)=3.947. Day 4 p=0.0104, t(36)=3.317. Day 5 p=0.0082, t(36)=3.405. **(right)** Transient amplitude fold change each day. Repeated measures 1-way ANOVA p=0.1555, F(2.528, 22.75)=1.961, R^2=0.1789.

We then examined whether BNST$^{PKC\delta}$ cells would desensitize to a more chronic homotypic stress exposure, using GCaMP7f paired with fiber photometry to record activity during chronic restraint stress. To allow continuous recording before (to establish a baseline) and during stress, we used a modified restraint device we designed called RESTRAINT (REcording Signal TRansients Accessible IN a Tube) (Luchsinger et al., 2021) and stressed mice for 1 hour each day

for 5 consecutive days (Fig. 3.8D). Here we found that during stress, there was an increase in the frequency (2-way ANOVA, stress effect p=0.01) and amplitude (2-way ANOVA, stress effect p=0.02) of calcium transients during RESTRAINT compared to baseline (Fig. 3.8E(left), F(left)). As with footshock, we again saw that this increased activation did not desensitize across days (Fig. 3.8E-F. 2-way ANOVAs day effect, frequency p=0.49, amplitude p=0.67. Fold change across days: frequency p=0.21, amplitude p=0.16).

3.4 Discussion

Here we report that *in vivo* optogenetic activation of BNST$^{PKC\delta}$ cells is mildly aversive and reduces exploratory behavior. Specifically, this stimulation decreased average speed and distance travelled, increased freezing, and decreased time spent on the open arms of the elevated plus maze (EPM). Compared to other cells in the BNST, PKCδ cells are resistant to activation. They do not show increased cfos expression following restraint stress and require significantly larger amounts of current injection before firing action potentials. Rabies tracing revealed that BNST$^{PKC\delta}$ cells receive input from a variety of brain regions including the basolateral and central amygdala, the paraventricular nucleus of the thalamus, and several hypothalamic subregions. Fiber photometry recording showed decreased activity in BNST$^{PKC\delta}$ cells during entries into the closed arms of the EPM, and that activation at open arm visit onset was inversely correlated visit duration, with instances of higher activity occurring at the onset of shorter-duration open arm times. BNST$^{PKC\delta}$ cells are also activated by footshock and show increased frequency and amplitude of activation during chronic RESTRAINT compared to baseline, which did not desensitize over repeated stress exposures.

3.4.1 BNST$^{PKC\delta}$ cells in anxiety-like behaviors and risk assessment

Using complementary approaches, we found that both exogenously-driven and endogenous activity of BNST$^{PKC\delta}$ cells is associated with reduced exploratory behavior and increased anxiety-like behavior. The EPM in particular is a well-validated tool for measuring changes in anxiety-like behavior, and has also been used as a model of risk assessment behaviors (Hogg, 1996; Rodgers & Dalvi, 1997; Carobrez & Bertoglio, 2005; Kraeuter et al., 2019; La-Vu et

al., 2020). The BNST as a whole plays a major role in sustained threat monitoring (Walker et al., 2003, 2009; Davis et al., 2010; Avery et al., 2016), and our previous work found that BNST$^{PKC\delta}$ cells are activated when approaching a food pellet in a novel, aversive environment (Jaramillo et al., 2020). This paired with our current findings that BNST$^{PKC\delta}$ activation decreases during EPM closed arm entries, is inversely correlated with open arm visit duration, and increases during footshock exposure suggest that BNST$^{PKC\delta}$ cell activity may serve as a threat detection signal, reducing movement and exploration overall and driving avoidance and anxiety-like behaviors. Indeed, it has been recently proposed that the genetically similar CeA$^{PKC\delta}$ cells are involved in risk assessment as part of a suite of defensive behaviors prior to a specific threat being detected (Moscarello & Penzo, 2022), and it has been shown that other parallel populations between the BNST and CeA share reciprocal connectivity and related functionality (Ahrens et al., 2018; Pomrenze et al., 2019; Ye & Veinante, 2019; de Guglielmo et al., 2019). Here we demonstrate that CeA$^{PKC\delta}$ cells provide direct inhibitory input onto BNST$^{PKC\delta}$ cells. Future studies should further probe this potential reciprocal microcircuit and its role in threat detection.

The afferent connectivity of BNST$^{PKC\delta}$ cells positions them well for an integrative role in threat processing and anxiety-like behaviors. We previously found that a large proportion of BNST$^{PKC\delta}$ cells receive input from the parabrachial nucleus (PBN) (Jaramillo et al., 2020), which encodes distress-like states and danger signals (Palmiter, 2018; Jaramillo et al., 2021). The axosomatic somatic nature of PBN-to-BNST innervation (Shimada et al., 1989; Dobolyi et al., 2005; S. a Flavin et al., 2014; Fetterly et al., 2019) could facilitate activation of BNST$^{PKC\delta}$ cells in the context of threat, despite their overall reluctance to fire compared to other BNST cell populations (Fig. 3.2F) (Egli & Winder, 2003; Silberman et al., 2013). Some of the densest projections come from amygdalar subregions including the BLA, CeA, and BMA, which encode information about threat and the animal's current emotional state (J. E. LeDoux, 2003; Gozzi et al., 2010; Haubensak et al., 2010; Šimić et al., 2021; Moscarello & Penzo, 2022), and we validated that both the BLA and CeA$^{PKC\delta}$ cells provide functional input to BNST$^{PKC\delta}$ cells. We also functionally validated input from the PVT, which is involved in threat detection, arousal, stress processing, and anxiety-like behaviors, and activation of PVT-to-BNST afferents increases EPM open arm time, while inhibition of this pathway has the opposite effect (Chastrette et al., 1991;

Bhatnagar & Dallman, 1999; Kirouac, 2021; D. Zhao et al., 2022). BNST$^{PKC\delta}$ cells also receive abundant input from the parasubthalamic nucleus and multiple hypothalamic subregions, which are responsible for processing homeostatic drives including thirst, hunger, and sleep (Andersson & Larsson, 1961; Nakamura, 2011; Yu et al., 2018; McKinley et al., 2021; J. H. Kim et al., 2022). Interestingly, although the BNST as a whole is not known for significant sensory inputs (Mcdonald, 1998; H. W. Dong et al., 2001; H. Dong & Swanson, 2004; Luchsinger et al., 2021), BNST$^{PKC\delta}$ cells also receive innervation from multiple regions involved in sensory processing (Figure 3.4D,E, Table 3.3). These include olfactory and pheromone processing regions like the post-piriform transition area, posterior amygdalar nucleus, and dorsal premammillary nucleus (Jolkkonen et al., 2001; Kemppainen et al., 2002; Comoli et al., 2005; D. A. Wilson et al., 2006; Pavesi et al., 2011), as well as smaller inputs from the auditory cortex. During acute threat, freezing enhances the sensory contribution to the decision making process (de Voogd et al., 2022). It is possible the decreased movement occurring upon BNST$^{PKC\delta}$ activation could facilitate the amplification of pertinent environmental sensory cues, which, when integrated with interoceptive cues about an animal's emotional and physical state, could facilitate appropriate action selection in the context of risk assessment.

There have been few studies to date examining the function of BNST$^{PKC\delta}$ cells, and those that exist have presented seemingly contradictory findings about their role in regulating anxiety-like behaviors. Most recently, Ueda and colleagues found that inhibition of BNST$^{PKC\delta}$ cell signaling increased EPM open arm time (Ueda et al., 2021). This suggests that BNST$^{PKC\delta}$ cell activity contributes to increased anxiety-like behavior, congruent with our findings here. In contrast, Wang, X. and colleagues found that Gq-DREADD activation of BNST$^{PKC\delta}$ cells decreased anxiety-like behavior (X. Wang et al., 2020). Wang, Y. and colleagues, on the other hand, found that both optogenetic stimulation and Gi-DREADD activation in BNST$^{PKC\delta}$ cells resulted in no change in anxiety-like behaviors (Y. Wang et al., 2019). There are several important considerations that could contribute to these apparent discrepancies. Ueda et. al. used chronic inhibition of synaptic vesicle release, and here we optogenetically stimulated BNST$^{PKC\delta}$ cells throughout a 10-minute EPM assay, while Wang, Y. et. al. used optogenetic stimulation over a brief two minute period in the EPM. Thus, there may be a threshold activation time required for BNST$^{PKC\delta}$ cells to impact

these behavioral readouts. Indeed, in our NSFT assay, which involved a shorter total stimulation time, (average 3.45 min +/- 38 sec across all mice) we saw only a trend for increased anxiety-like behavior. An additional consideration is the complexity of DREADD actions in the BNST. We have previously shown that Gi-DREADDs in the BNST can actually have excitatory effects (N. A. Harris, Isaac, et al., 2018). Given the role of PKCδ in G-protein coupled receptor (GPCR) signaling (Newton, 2001; Vail & Roepke, 2019), it is possible that DREADD activation in this population may have more complex effects than simple activation or inhibition. Indeed Wang, X. and colleagues reported sparse cfos expression in PKCδ cells in the BNST following Gq-DREADD activation (X. Wang et al., 2020). Finally, it has recently been shown that signaling in the right versus left CeA has opposing effects on pain-like behaviors, suggesting there may be interesting functional lateralization within the extended amygdala (Carrasquillo & Gereau, 2008; Allen et al., 2022). Our current *in vivo* work focused exclusively on the right BNST, while Wang, X. et al. activated the left BNST and Wang, Y. et al used bilateral manipulations. Future studies should examine potentially distinct contributions of left verses right BNST$^{PKC\delta}$ populations on anxiety-like behavior.

3.4.2 Stress-sensitivity of PKCδ and PKCδ-expressing cells in the BNST

Here we found that BNST$^{PKC\delta}$ cells are activated at the onset of footshock stress and showed increased calcium transient frequency and amplitude during chronic RESTRAINT compared to baseline. Surprisingly, this activation did not desensitize to repeated exposure to the same stressor (Fig 5), in contrast to other cells in the BNST(Luchsinger et al., 2021; J. A. Brown et al., 2022). We also found that, unlike the BNST as a whole, BNST$^{PKC\delta}$ cells do not show increased cfos expression following restraint stress (Fig. 2C). This at first seems to contradict our current fiber photometry findings and previous work showing increased activation of BNST$^{PKC\delta}$ cells during active struggling bouts in RESTRAINT(Luchsinger et al., 2021). However, we have previously shown that time spent participating in whole-body struggle bouts represents a minority of total time during restraint stress(Luchsinger et al., 2021), and it is likely the increases in activity tied to struggle bouts are not frequent or persistent enough to drive changes in cfos expression. Further, it should be noted that both GCaMP and cfos are proxies of neuronal activity and not direct readouts of action potentials. Increases in intracellular calcium may also come as a result of release from

intracellular stores. Again given the role of PKCδ in GPRC signaling pathways that influence intracellular calcium, it is intriguing to consider that changes in GCaMP fluorescence could be reflective of these types of signaling rather than, or in addition to, action potential firing. Indeed our previous work found that levels of PKCδ expression in the BNST are stress-sensitive, with female mice showing an increased number of PKCδ(+) cells following restraint stress(Fetterly et al., 2019). Other studies have found that expression of PKCδ in the CeA plays a functional role in mediating addiction-related behaviors(Venniro et al., 2020; Domi et al., 2021), and future studies should examine the functional contribution of PKCδ expression within the BNST.

3.4.3 Conclusion and future directions

Here we propose a model in which BNST$^{PKC\delta}$ cells are engaged in risk assessment and increasing anxiety-like behaviors, potentially through the integration of exteroceptive and interoceptive cues to detect and respond to threats. Appropriate risk assessment is evolutionarily adaptive and critical for an organism's survival. However, maladaptive threat responses are a central feature of many psychiatric disorders including depression, anxiety, PTSD, and OCD(Mathews & MacLeod, 1994; Gorka et al., 2016; Spinhoven et al., 2017; Hofmann & Hay, 2018; Schlund et al., 2021; T. M. Ball & Gunaydin, 2022), and it has been shown that stress can have complex effects on threat detection and approach/avoidance behaviors(Frisch et al., 2015; Macatee et al., 2017; Egan & Dennis-Tiwary, 2018; Timmers et al., 2019). Future studies should investigate the contribution of this neuronal population to detection of specific threats and stress responses. Overall, our current understanding of the function of BNST$^{PKC\delta}$ cells is still in its infancy, and these cells hold the potential for many interesting and important future studies to further our understanding of threat processing and stress-related psychiatric disorders.

CHAPTER 4

Discussion and future directions

4.1 Understanding the function of BNST$^{PKC\delta}$ cells

4.1.1 Summary of findings

Here, we first showed that BNST$^{PKC\delta}$ cells are recruited during bouts of active stress coping. We found increases in calcium-mediated GCaMP signaling during both non-consummatory and consummatory food pellet approaches in the aversive decision-making context of the NSFT and increased signal during active escape-like struggle bouts in RESTRAINT stress. We also found that BNST$^{PKC\delta}$ cells receive input from the PBN, colocalizing with a significant portion of CGRP+ cells.

To investigate the valence of BNST$^{PKC\delta}$ activation, we turned to *in vivo* optogenetic stimulation during the RTPP assay. We showed that direct stimulation of BNST$^{PKC\delta}$ cells is mildly aversive, and that this aversion is not sensitized by prior exposure to restraint stress. This stimulation also resulted in reduced overall movement, increasing freezing time and decreasing distance traveled and average speed.

Based on our previous finding of increased cfos mRNA after restraint stress, we were surprised to find that restraint did not impact RTPP behaviors. We examined cfos protein levels after stress using IHC and found that, unlike the BNST as a whole, BNST$^{PKC\delta}$ cells are not robustly activated following restraint stress, showing no significant increase in cfos protein expression. Indeed using *ex vivo* electrophysiology, we showed that this population is quite hyperpolarized and resistant to activation in general, requiring significantly more input current before firing action potentials than other cells in the BNST.

To gain a better understanding of the circuit context of BNST$^{PKC\delta}$ cells and the regions that could influence their activation, we used rabies tracing paired with whole brain clearing and lightsheet microscopy to investigate the brain-wide afferents onto BNST$^{PKC\delta}$ cells. We found that BNST$^{PKC\delta}$ cells receive input from a variety of brain regions including the basolateral and central

amygdala, the paraventricular nucleus of the thalamus, sensory regions including the post-piriform transition area, and several hypothalamic subregions. We then functionally validated the connectivity from the BLA, PVT and from CeA$^{PKC\delta}$ cells using channelrhodopsin-assisted circuit mapping.

Lastly, we aimed to further probe the contexts in which BNST$^{PKC\delta}$ cells are recruited and their impact on behavioral output, and first showed that BNST$^{PKC\delta}$ cells participate in risk assessment-like behaviors in the elevated plus maze. *In vivo* optogenetic activation resulted in decreased open arm exploration time, suggesting increased anxiety-like behavior. *In vivo* fiber photometry recording showed a significant decrease in recruitment of BNST$^{PKC\delta}$ cells during entries of the closed arms of the EPM, and activation during entries to the open arms is inversely correlated with time spent on the open arm during that entry. Finally, we showed that BNST$^{PKC\delta}$ cells are activated by footshock, and that their activation to both repeated footshock and during chronic restraint stress does not desensitize over consecutive days of exposure.

4.1.2 BNST$^{PKC\delta}$ cells may represent threat detection cells

Taking our findings together, we propose a model in which BNST$^{PKC\delta}$ cells may be specifically positioned as threat detection cells. Threat imminence theory divides defensive responses to threat into separate behavioral modes according to the proximity of the threat (Fanselow et al., 1988; Moscarello & Penzo, 2022). Baseline is a safety state, when an animal assumes encounter with a threat or predator is unlikely and thus engages freely in non-defense survival mechanisms. The first defensive response phase is the pre-encounter mode, which is often engaged in a context of potential or previous threat encounter but prior to detection of a specified threat, and is primarily comprised of exploratory behaviors balanced with risk assessment and avoidance. The second is the post-encounter mode, in which a threat has been detected but is still distant, and is characterized by rapid defensive behaviors such as freezing in rodents. The third is the circa-strike mode when an active threat is occurring, such as when a predator is attacking, and consists of fight or flight responses to protect the animal from injury or death. These three modes exist on a continuum of what can be classified as “anxiety-like” to “fear-like”, with the pre-encounter mode representing a lower-level anxiety-like state, the post-encounter mode

representing a higher-level anxiety-like state or fear-like state depending on the proximity of the threat, and the circa-strike mode fitting with a phasic fear-like state (Davis et al., 2010; Moscarello & Penzo, 2022).

The BNST has classically been considered to mediate anxiety-like behaviors or “sustained fear”, while the related and highly interconnected CeA is thought to be responsible for fear-like responses or “phasic fear” (Davis et al., 2010; Avery et al., 2016). It is likely more complex though, and it has recently been suggested that distinct CeA cell types may be involved in each of the three phases of threat responses. Specifically, $CeA^{PKC\delta}$ cells may play a role in the pre-encounter mode, somatostatin-expressing cells (CeA^{Som}) may mediate the post-encounter mode, and CeA^{CRF} cells may be responsible for circa-strike mode behaviors, with mutually inhibitory circuitry between each cell type helping to mediate transitions between modes (Moscarello & Penzo, 2022).

Based on our current findings and given reciprocal connectivity and related functionality between other parallel cell types in the BNST and CeA (Ahrens et al., 2018; Pomrenze et al., 2019; Ye & Veinante, 2019; de Guglielmo et al., 2019), we propose that $BNST^{PKC\delta}$ cells are, like their CeA counterpart, engaged in regulation of the pre-encounter defense mode. $CeA^{PKC\delta}$ cells inhibit freezing, increase exploratory behaviors in the elevated maze, and are required for encoding and learning contextual food cues, thus promoting the foraging-like behaviors required for survival in potentially dangerous contexts (Haubensak et al., 2010; Fadok et al., 2017; Griessner et al., 2021; Whittle et al., 2021). In contrast, here we find that activation of $BNST^{PKC\delta}$ cells is aversive, decreases movement and increases freezing, and decreases time spent on the open arm of the elevated plus maze (Figs. 3.1C-F, 3.6B) Further, magnitude of $BNST^{PKC\delta}$ neuronal activation is negatively correlated with time spent on the open arm (Fig. 3.6G), suggesting that their activity may signal a risky context and encourage avoidance behaviors characteristic of the pre-encounter mode. Indeed, on entries to the closed arm, there is a significant decrease in $BNST^{PKC\delta}$ activation (Fig. 3.6F), and little-to-no or even decreased activation on longer entries to the open arm (Fig. 3.6G), suggesting that inhibition of $BNST^{PKC\delta}$ cells may be permissive of exploratory behaviors. It is interesting to note that the shape of the correlation suggests the relationship between signal magnitude and time spent on the open arm may not be linear, and future studies should repeat this assay with greater numbers of mice/entries to further parse out this relationship and determine if

perhaps there is a threshold of activity influencing exploratory decisions. Finally, it has been shown that during acute threat, freezing enhances the sensory contribution to the decision making process (de Voogd et al., 2022). Here our rabies tracing revealed that although the BNST as whole is not known for significant sensory inputs (H. W. Dong et al., 2001; Ch'ng et al., 2018; Luchsinger et al., 2021; Centanni et al., 2022), BNST$^{PKC\delta}$ cells in particular receive substantial innervation from regions involved in sensory processing (Fig. 3.4D-E, Fig 3.5, Table 3.3). These include the post-piriform transition area (TR) which is involved in olfactory processing, and the posterior amygdalar nucleus, which processes pheromonal information, as well as smaller inputs from the dorsal premammillary nucleus which is responsive to predator odors, and the auditory cortex (Jolkkonen et al., 2001; Kemppainen et al., 2002; D. A. Wilson et al., 2006; Pavesi et al., 2011). It is possible that the decreased movement occurring as a result of BNST$^{PKC\delta}$ activation could facilitate the amplification of pertinent environmental sensory cues to facilitate threat detection.

The pre-encounter mode is truly a flexible pattern of behavior that balances risk assessment and avoidance with pursuit of survival-promoting behaviors such as foraging (Fanselow et al., 1988; Moscarello & Penzo, 2022). The inherent conflict elicited by these opposing components of the pre-encounter mode suggest they may be mediated by mutually inhibitory populations, and indeed here we demonstrate that CeA$^{PKC\delta}$ cells provide functional inhibition onto BNST$^{PKC\delta}$ cells (Fig. 3.4I, Table 3.2). Paired with our finding that activation of BNST$^{PKC\delta}$ cells reduces movement, it is possible that they may help facilitate the transition to post-encounter behaviors, which is dominated by freezing, in the event of acute threat detection. Indeed, as with BNST$^{PKC\delta}$ cells, CeA^{Som} activation increases freezing and is thought to be a major mediator of the post-encounter mode behaviors (Moscarello & Penzo, 2022). This could in part be due to inhibition onto CeA$^{PKC\delta}$ cells thus disinhibiting BNST$^{PKC\delta}$ cells, and/or possibly through BNST$^{PKC\delta}$ cells inhibiting CeA$^{PKC\delta}$ neurons, thus disinhibiting CeA^{Som} cells. It has been shown that BNST$^{PKC\delta}$ cells project into the CeA (Ye & Veinante, 2019), begging the question as to whether the BNST population provides reciprocal inhibition onto the CeA$^{PKC\delta}$ and/or CeA^{Som} cells specifically. Interestingly, activation of CeA^{Som} neurons promotes entry into the closed are of the EPM, while here we found a significant decrease in BNST$^{PKC\delta}$ activation during closed arm entry. This encourages the hypothesis that there may exist a complex inhibitory interaction between BNST$^{PKC\delta}$

cells, CeA$^{PKC\delta}$ cells and CeA^{Som} cells wherein BNST$^{PKC\delta}$ and CeA$^{PKC\delta}$ cells mediate the opposing drives of the pre-encounter mode, inhibiting and promoting foraging behaviors, respectively, with BNST$^{PKC\delta}$ activation facilitating transition to the post encounter mode upon detection of a threat. Future studies should further probe the relationship between these cell types and their potential complementary roles in mediating threat-related behaviors.

Finally, the positioning of BNST$^{PKC\delta}$ cells as threat detection cells is further supported by their activation patterns in the context of stress. Stress is known to have complex effects on threat detection and approach/avoidance behaviors (Frisch et al., 2015; Macatee et al., 2017; Egan & Dennis-Tiwary, 2018; Timmers et al., 2019). Here we first found that there is a significant increase in BNST$^{PKC\delta}$ activation while approaching a food pellet in the NSFT, during active coping bouts in restraint stress, and at the onset of footshock stress. The NSFT is a prime assay for evoking pre encounter defensive behaviors, pitting drive for feeding against the risk of foraging in a bright, open, novel, and thus potentially dangerous, environment. As with previous inclusions, this sentence is here because of my genuine curiosity regarding the closeness with which this document will be read. Average latency to first bite was not significantly correlated with magnitude of signal but it would be interesting to know whether duration of time spent exploring the food pellet at each approach is correlated with signal increase, as with activation during EPM open arm entries.

Uncued footshock has often been used as a model of unpredictable threat (Davis et al., 2010), and here we find that BNST$^{PKC\delta}$ cells are activated at the onset of these shocks (Fig. 3.8B-C). It has been shown that distinct aspects of fear conditioning, involving the pairing of footshock with predictive cue, differentially recruit CeA$^{PKC\delta}$ and CeA^{Som} neuronal populations, and future studies should investigate any potential contributions of BNST$^{PKC\delta}$ neurons to this process and how predictability verses unpredictability impacts their activation in response to the threat of footshock.

Restraint stress presents a threat context that is somewhat unique from both NSFT and the uncued footshock assay we used, in that it is an ongoing and inescapable. We find a significant increase in BNST$^{PKC\delta}$ cell activation at the onset of active struggle bouts, which could be interpreted as an attempt at escape or avoidance of the threat, fitting with the model of BNST$^{PKC\delta}$ cells as mediating the pro-escape/anti-foraging component of the pre encounter mode. We also

see a significant increase in the frequency and amplitude of calcium transients during RESTRAINT compared to the pre-stress baseline. It is interesting to note, however, that not all signal transients occurred at the onset of struggle bouts. For example, anecdotally it seemed that for some mice, there would be a large transient occurring 5-15 seconds after the conclusion of a struggle bout. Future studies should examine the relative ratio of signals tied to struggle bouts and perhaps use machine-based deep learning algorithms such as DeepLabCut (Kane et al., 2020), DeepPoseKit (Graving et al., 2019), AlphaTracker (Padilla-Coreano et al., 2022), and Selfee (Jia et al., 2022) to generate a more comprehensive and even predictive model regarding when BNST$^{PKC\delta}$ cells are recruited, both during RESTRAINT and other stressful and non-stressful contexts.

Finally, BNST$^{PKC\delta}$ cells did not de-sensitize to repeated presentation of the same stressor (Fig. 3.8). This stands in contrast both to other neuronal populations in the BNST, and the HPA axis as a whole, both of which show reduced recruitment over the course of homotypic stress exposure (Martí & Armario, 1998; Grissom & Bhatnagar, 2009; Babb et al., 2014; Luchsinger et al., 2021; J. A. Brown et al., 2022). Lack of desensitization would be an important characteristic of any risk-assessing neuronal population, as an organism should not discount the presence of a threat if it is still dangerous, just because it has encountered the threat before. This consistent recruitment may in part be due to our (initially, seemingly contradictory) finding that BNST$^{PKC\delta}$ cells are generally resistant to activation, showing significantly higher rheobase than BNST$^{PK\delta(-)}$ cells, and, while there is an increase in cfos mRNA after restraint stress, we found no corresponding increase in cfos protein. This suggests that the recruitment of this population by stress is likely transient, rather than sustained, activation, which is less likely to result in stress-induced adaptations like desensitization. It is also interesting to note that our rabies tracing revealed a sparser amount of retrohippocampal inputs onto BNST$^{PKC\delta}$ neurons compared to other cells in the BNST, and it is possible that this may in part help mediate the lack of desensitization by minimizing the impact that memory of a stressor has on its encoding as a potential threat (Bucci & Robinson, 2014; Witter & Amaral, 2021; Luchsinger et al., 2021; Berry et al., 2022; J. A. Brown et al., 2022).

4.1.3 Other considerations and alternative models

Altogether we are proposing a model in which BNST$^{PKC\delta}$ cells may be positioned as threat

detection cells to facilitate risk assessment in the pre-encounter mode of defensive behaviors, and that this perception of threat then leads to increases in anxiety-like behaviors. However many of our findings are also consistent with the possibility that these neurons are involved in encoding an anxiety-like state more broadly. Indeed while there have to-date been few studies examining the function of BNST$^{PKC\delta}$ cells, those that exist have in part attempted to characterize their role in anxiety-like behavior (Y. Wang et al., 2019; X. Wang et al., 2020; Ueda et al., 2021). The EPM in particular is a well-validated tool for measuring changes in anxiety-like behavior, and exposure to stressors such as footshock and restraint are known to increase anxiety-like behaviors (Hogg, 1996; Rodgers & Dalvi, 1997; Carobrez & Bertoglio, 2005; Kraeuter et al., 2019; La-Vu et al., 2020). Anxiety can be defined as a prolonged state of heightened apprehension, and as such, one would hypothesize that a cell population encoding changes in anxiety would show sustained activity in the presence of a stressor. Here our data suggest that BNST$^{PKC\delta}$ cells are only transiently activated by stressors, and indeed with the electrophysiological data, there were subset of PKCδ neurons that would only fire briefly at the onset of activation and then remain inactive for the remainder of the current injection step. Future studies should examine whether there may be any spike adaptation with prolonged activation, as well as conduct a further characterization of the heterogeneity present within the BNST$^{PKC\delta}$ population. Additionally, future work should examine changes in baseline signal that occur throughout the duration of a stressor. The quantification methods used here to examine overall transient frequency and amplitude of transients throughout the duration of a stressor rely on flattening the “baseline” to be level throughout the trace, but there may be important information conveyed in bulk signal baseline fluctuations that are not reflected by brief transients. Future studies should modify the analysis code to better investigate potential sustained activity changes and potential direct contributions of BNST$^{PKC\delta}$ cells to an anxiety-like state. It should also be noted that threat detection and encoding of an anxiety-like state are not mutually exclusive, and aspects of BNST$^{PKC\delta}$ neurons could be involved in the encoding of both.

The absence of a significant increase in cfos protein in BNST$^{PKC\delta}$ cells also warrants further discussion. As mentioned above, this finding supports the hypothesis that this population may be transiently activated by stress in order to facilitate chronic recruitment to stressors.

Nonetheless, it was a surprising result given the stress sensitivity of the BNST as a whole, increased cfos mRNA in BNST$^{PKC\delta}$ neurons after restraint stress, and the increased GCaMP signal seen at the onset of struggle bouts as well as the overall increase in transient frequency and amplitude during RESTRAINT. However, as discussed previously, it is likely that the increases in activity tied to struggle bouts are not frequent or persistent enough to drive changes in cfos expression. Indeed, we have previously shown that time spent participating in whole-body struggle bouts represent a minority of total time during restraint stress (Luchsinger et al., 2021). Further, it should be emphasized that both GCaMP and cfos are proxies of neuronal activity and not direct readouts of action potentials. Increases in intracellular calcium detected by GCaMP is associated with action potential firing, but may also come as a result of release from intracellular stores, often mediated by GPCR signaling. Studies have shown that cfos induction may require presynaptic input rather than just calcium influx, as antidromic stimulation (activation of a neuron's axon resulting in back-propagation to the cell body) is not sufficient for cfos expression (Luckman et al., 1994), and there are several examples of neurons known to be activated within a circuit that do not display increases in cfos (Labiner et al., 1993; Luckman et al., 1994; Figueiredo, Bodie, et al., 2003; K. J. Kovács, 2008). Further, despite it being a common assumption, mRNA expression can be poorly correlated with changes in protein (Koussounadis et al., 2015). In the case of cfos, this may be mediated by miR-7b, a microRNA that inhibits cfos translation, or an AP-1 site on cfos that inhibits its own translation (Konig et al., 1989; H. J. Lee et al., 2006). Indeed miR-7b expression can be regulated by G-protein coupled estrogen receptor 1 (GPER1) and downstream PKC signaling pathways (Xu et al., 2015; J. He et al., 2018), which could underlie our current observation of an increase in cfos mRNA in BNST$^{PKC\delta}$ cells but no corresponding increase in cfos protein. It is also worth considering that the restraint stress used to examine cfos changes took place in dark, sound- and light-attenuating chambers, whereas *in vivo* recordings during RESTRAINT took place in well-lit conditions (to facilitate video recording and DeepLabCut behavioral analysis). The BNST has been shown to mediate the ability of prolonged bright light exposure to potentiate acoustic startle in sustained fear models (Davis et al., 2010), and future studies should examine the contribution of these conditions to the recruitment of PKCδ cells specifically. It would be interesting to investigate potentially differential

GCaMP and/or cfos recruitment under varying light environments.

Finally, it is also interesting to note that, while there was not a significant difference in the number of PKCδ cells expressing cfos protein between no-stress control mice and mice at 90 minute, 3 hours, and 24 hours after stress, there is a population of cfos+ PKCδ cells present in each of these conditions. While BNST-wide we found a low (1-4%) of cells expressing cfos basally, and this increased to about 3-8% of cells after stress, in PKCδ cells specifically we saw an average of 6-10% of neurons co-expressing cfos across groups. This can not be wholly explained away by the fact that there are simply more PKCδ(-) than PKCδ(+) cells, as even when looking at raw numbers, we see that a considerable number of total cfos+ cells at baseline are PKCδ cells, with cfos+ PKCδ cells in fact comprising a significantly larger portion of total cfos+ cells in the no stress, baseline condition than each of the three post-stress timepoints. (Appendix Fig 5.4). It is possible that there exists a basally active subpopulation of $BNST^{PKC\delta}$ cells that may have a distinct function from cfos-resistant cells. Indeed it would be interesting to know whether it is the cells that are basally active or those which are quiescent that are recruited during active stress coping bouts, and also how this maps onto to basal electrophysiological heterogeneity. One interesting hypothesis is that increases in activation of the quiescent PKCδ cells may signal threat, while dampening of the basally active PKCδ population mediates a relative safety signal such as that seen upon entry to the closed arm of the EPM. Future studies should utilize *ex vivo* and *in vivo* calcium imaging technology such as a miniscope to better understand differences in activation dynamics and potential subpopulations present within $BNST^{PKC\delta}$ cells.

4.2 The role of PKCδ expression in the BNST and beyond

One of the key findings that initially prompted this investigation into the function of $BNST^{PKC\delta}$ neurons was the demonstration that there is a significant increase in the number of cells expressing PKCδ mRNA in the BNST of female mice after restraint stress (Fetterly et al., 2019). This new expression of PKCδ occurred at least in part in $BNST^{CRF}$ cells, as there was also a significant increase in the number of neurons co-expressing PKCδ and CRF mRNA, and no change in total CRF cells (Fetterly et al., 2019). We have conducted a number of additional studies to further investigate this finding and whether this mRNA increase is translated to protein

changes, with mixed findings (Appendix 1). Briefly, at 90 minutes after stress, we found no significant changes in the number of PKCδ+ neurons, at 3 hours after stress we found a trend for increased PKCδ+ neurons, and at 24 hours after stress we found a significant decrease in the number of PKCδ neurons in male mice (Fig 5.3). There may be a trend for a decrease in the number of cells expressing *prkcd* (PKCδ mRNA) after 5 days of chronic stress (Fig 5.2), but there is a significant increase in the number of cells expressing PKCδ protein after 5 days and 10 days of chronic stress compared to acute (1 day) of stress (Fig 5.5). This increase in PKCδ cells fits with our finding that $BNST^{PKC\delta}$ cell activation does not desensitize with chronic homotypic stress exposure and, a trend for dose dependence, with there being and even larger increase after 10 days of stress than 5 days) is interesting in the context of PKCδ's role in inflammation stress-induced apoptosis in other disease models (reviewed in Chapter 1.4.1) and the decrease observed in PKCδ expression 24 hours after stress (Fig 5.3A).It is possible that stress could be having a complex interaction with PKCδ in the BNST, simultaneously activating cells which already express PKCδ and also leading to stress-induced neuronal cell death in those cells or in cells in which novel expression is induced, and future studies should further probe the potential timecourse of expression changes in acute and chronic stress.

Although the exact nature of the stress-sensitivity of PKCδ expression in the BNST still requires significant investigation, dynamic regulation does seem to be occurring, and one potential pathway for this regulation could be through the PBN→$BNST^{PKC\delta}$ pathway. We and others have shown that the PBN sends significant input to $BNST^{PKC\delta}$ cells and to the BNST as a whole (Fig 2.3) (Ye & Veinante, 2019), and this input is likely axosomatic, allowing for enhanced influence over the postsynaptic cell. CGRP released from the PBN acts on G_s and G_q- coupled signaling pathways that recruit PKC isoforms including PKCδ, and it is possible that this pathway could play a role in the increased number of cells expressing PKCδ mRNA seen following acute stress and subsequent protein changes.

Efforts to examine stress-sensitive changes in PKCδ expression also underscores a critical question that has yet to be investigated: what is PKCδ doing in the BNST, or in other words *why is it a marker of a specific neuronal subpopulation*? Indeed, each of the other subpopulations within the BNST are marked by expression of various neuropeptides and/or

neuropeptide receptors, which serve a relatively clear function. They are transsynaptic signaling molecules that can be released from one neuron and impact the activity of its postsynaptic targets. PKCδ, however, is a kinase. It is not being released from these neurons (that we know of), so surely it must be playing some key function in the physiology of the neurons within which it is expressed.

We can take some clues from the CeA, where a small number of studies have begun to investigate the functional contribution of PKCδ expression in rats. Venniro et al developed a short hairpin RNA (shRNA) to specifically knock down PKCδ (shPKCδ), and found that cells expressing the shPKCδ showed significantly reduced excitability, firing fewer action potentials in response to varying levels of current injections compared to a control shRNA construct (shCtrl), but no change in resting membrane potential (Venniro et al., 2020). This is interesting when compared with the effects of an shSom virus, which resulted in less of a reduction (though still significant) in excitability, but also significantly hyperpolarized the membrane potential of CeA^{Som} neurons (Venniro et al., 2020), suggesting potentially unique contributions of PKCδ expression to the physiology of neurons compared to neuropeptides. Further, they had previously reported that social choice-induced abstinence blocks incubation of methamphetamine craving and activates $CeA^{PKCδ}$ cells, as measured by increased cfos expression (Venniro et al., 2018). Knocking down PKCδ had no effect on social-choice induced abstinence itself (ie rats still robustly sought social stimuli over drug when given the choice), but it did prevent the protective effect of social choice on incubation of drug craving, and also significantly reduced the cfos induction in $CeA^{PKCδ}$ cells that had been observed after this protocol (Venniro et al., 2020). In another study, Domi et al found that activation of $CeA^{PKCδ}$ neurons was significantly associated with rats displaying compulsive-like alcohol taking, but that knocking down PKCδ in the CeA reduced alcohol seeking in this punishment-resistant group (Domi et al., 2021). Together, this suggests that expression of PKCδ is indeed mediating key aspects of both the physiology and function of the neurons defined by its expression.

In this work, we began to investigate the function of PKCδ expression within the BNST using the same shRNA developed by and Venniro et al and used in both of the aforementioned studies. Unfortunately, a series of complications impeded progress on this front, and thus, future

studies should continue and expand on this work to knock down PKCδ in the BNST of mice and examine its impact. Given our current hypothesis positioning $BNST^{PKC\delta}$ neurons as part of a threat detection circuit, it is intriguing to consider that changes in PKCδ expression could be a mechanism for modulating threat detection sensitivity following exposure to a stressor. Further, given the finding of increased PKCδ mRNA in CRF cells, future studies should investigate the function of PKCδ expression not only within basal $BNST^{PKC\delta}$ neurons, but also in formerly non-PKCδ neurons and how that may impact their function. The finding that knocking down PKCδ in $CeA^{PKC\delta}$ neurons decreases excitability generates an interesting hypothesis that new expression of PKCδ in neurons such as $BNST^{CRF}$ cells could amplify, for example, their anxiogenic and pro-reinstatement output. Additionally, the knockdown approach brings to light an important limitation in our work investigating stress-induced changes in PKCδ expression, in that those studies have focused solely on presence or absence of PKCδ in neuron, rather than relative expression level. It will be important for future studies to take a more nuanced approach in understanding the stress-sensitivity of PKCδ and how varying levels impact function through the use of techniques such as IHC-based fluorescence intensity and/or Western blot analysis.

Finally, it is important to note that the BNST and CeA are not the only brain regions in which PKCδ is expressed. For example, in the lateral septum, immediately neighboring the BNST, there is a large population of neurons expressing PKCδ. Incidental investigation of the function of these neurons (ie off-target viral injections and fiber implants that had been aiming for the BNSTov) suggest that septal PKCδ neurons show high levels of basal activity, and show overlapping but distinct stress-related contexts in which they are recruited (data not shown). It is also interesting to note that in regions like the septum, PKCδ expression is diffuse and not indicated as a marker of a specific subset of neurons. This is in contrast to the CeA and BNST, as well as other regions such as the cerebellum and sensory regions where PKCδ marks a distinct subset of neurons that are otherwise comparable within the region (Garcia et al., 1993; Merchenthaler et al., 1993). Thus, in addition to future studies investigating PKCδ in other regions in general, it is intriguing to consider any potentially unique functional contributions PKCδ expression may play specifically in these regions compared to regions in which PKCδ is not a distinct population marker but rather more ubiquitously expressed. Relatedly, the question of why

it is PKCδ specifically that has emerged as a distinct subpopulation marker, and not other isoforms of PKC that are also expressed in the brain such as PKCε and PKCγ, would be an interesting line of study.

4.3 Conclusions and additional future directions

Here we propose a model in which BNST^{PKCδ} cells are engaged in threat detection, which may facilitate risk assessment in the pre-encounter mode of defensive behaviors. They may act in opposition to CeA^{PKCδ} cells facilitating approach/avoidance decision making and the transition to post-encounter behaviors based on assessment of the situation. It is possible that expression changes in PKCδ expression itself may facilitate this role and engage this population in unique signaling patterns distinct from neuropeptide populations and provide a flexible means of modulating neuronal excitability to allow for a nuanced assessment of one's environment and appropriate action selection in the face of potential threat. Indeed, the ability of an organism to accurately perceive and respond to threat is paramount to its survival and thus evolutionarily adaptive. However, maladaptive threat responses are a central feature of many peripheral and psychiatric disorders including depression, anxiety, PTSD, OCD, and substance use disorders, and it has been shown that stress has complex effects on threat detection and approach/avoidance behaviors in both. Given the demonstration of the stress sensitivity of BNST^{PKCδ} cells, this neuronal population is well-positioned to be a key mediator in this interaction.

This work only begins to scratch the surface of understanding BNST^{PKCδ} neurons, and there exists a wealth of additional possible directions to continue this work, in addition to all of the future directions already described throughout this discussion. Their role in threat detection as well as their larger contribution to anxiety-like behaviors should be further parsed, including through the use of other measures of anxiety like such as NIH, which is one of few anxiety assays modulated by both acute benzodiazepines and chronic antidepressant administration. As mentioned previously, expression of PKCδ globally and in the CeA specifically has been implicated in addictive-like behaviors, and future studies should begin to explore a potential role for both the expression of PKCδ, and cells labelled by its expression, in the BNST in substance use disorders. Of note, despite HPA-axis attenuation and desensitization of regions such as the

BNST, even chronic homotypic stressors increase anxiety- and depressive like behaviors and increase propensity for relapse in both rodent model and human populations (Caspi et al., 2003; Hammen et al., 2004; Dinan, 2005; Blackmore et al., 2007; Patel & Hillard, 2008; Lui et al., 2012; K. T. Ball et al., 2018). The lack of desensitization of BNST$^{PKC\delta}$ cells positions them well to potentially play a key role in the transition from adaptive to maladaptive stress and subsequent disease development, and they may be a potential new therapeutic target warranting further investigation. Indeed there is much work to be done in examining recruitment of BNST$^{PKC\delta}$ cells in chronic stress as a whole, and future studies should fully characterize their recruitment at a variety of timepoints during and after both homotypic and heterotypic chronic stressors. Given the unique possible contribution of GPCR-mediated signaling pathways involving PKCδ expression, these additional studies should be done by examining a variety of different signaling modalities, including but not limited to immediate early gene expression such as cfos and Arc labelling, observation of synaptic plasticity through electrophysiological recordings, *in vivo* measures of calcium-based activation proxies such as GCaMP (Dana et al., 2019; Y. Zhang et al., 2021), measures of more specific GPCR-based signaling such as genetically encoded cAMP/PKA sensors using the FRET/FLIM system (Massengill et al., 2021), more direct measures of firing-based activity through tools such as genetically encoded voltage indicators (GEVIs) (Gong et al., 2015; Bando et al., 2019) and cell-type specific *in vivo* electrophysiology (N. Chou et al., 2022), as well as measures of *in vivo* synaptic input on BNST$^{PKC\delta}$ cells through neurotransmitter sensors such as iGABASnFR (Marvin et al., 2019) and the newest iGluSnFR3 with improved differentiation between synaptic and extrasynaptic glutamate release (Aggarwal et al., 2022).

There is also much work to be done regarding BNST$^{PKC\delta}$ connectivity. The vast majority of anatomically-identified afferents have yet to be functionally validated, and those which we have validated here should be further characterized to investigate their specific contributions and plasticity in a variety of behavioral contexts. It will also be important to begin investigating downstream targets of BNST$^{PKC\delta}$ cells through tools such as synaptophysin-mediated anterograde tracing in conjunction with whole brain imaging. The role of these afferents and efferents could then be further investigated through tools such as the sensors listed above expressed in input regions projecting to BNST$^{PKC\delta}$ cells to record afferent terminal activity or

expressed in BNST^{PKCδ} cells and recording from their terminals in efferent regions. One discussed above, one particular afferent-efferent circuit that warrants further investigation is potential reciprocal connectivity between BNST^{PKCδ} cells and CeA^{PKCδ} cells. This could be done through approaches such as that taken by Pomrenze et al in their investigation of CeA^{CRF}→BNST^{CRF} cells, where they combined Cre-dependent Gq-DREADDs in the CeA with Cre-dependent KORD, an inhibitory kappa-opioid based designer receptor, in the BNST (Pomrenze et al., 2019). It would be very interesting to simultaneously inhibit one PKCδ population while activating the other to dissect specific subpopulation contributions to behavior, either through a similar DREADD based approach, a paired inhibitory and excitatory *in vivo* optogenetic approach, or a hybrid of the two.

It is also likely that there exists considerable intra-BNST connectivity with BNST^{PKCδ} cells, and it is known that many BNST subpopulations can mediate opposing behavioral outputs. For example, BNST^{PKCδ} cells inhibit feeding and increase anxiety-like behaviors, while BNST^{NPY} neurons drive feeding and decrease pain, anxiety, and EtOH binge drinking (Desai et al., 2013; Pleil et al., 2015; Centanni et al., 2022). In the CeA there are complex and important inhibitory microcircuits between CeA^{PKCδ}, CeA^{Som}, and CeA^{CRF} neurons in mediating aspects of fear- and addiction-related behaviors (Moscarello & Penzo, 2022), and future studies should begin to parse any potential parallel microcircuitry between BNST^{PKCδ} neurons and other BNST subpopulations. Additionally, it will be interesting to begin to investigate interactions between BNST^{PKCδ} neurons and astrocytes. Of note, throughout this text, we have used “cells” and “neurons” interchangeably to refer to the BNST^{PKCδ} population, and based on the size and pattern of PKCδ expression, it is likely the majority of PKCδ+ cells in the BNST are indeed neurons. However, it has also been shown that glial cells can express PKCδ (Gott et al., 1994), and future work should examine PKCδ cell identity, using markers such as NeuN to label neurons specifically and quantify the relative proportions.

All of this region- and cell-type-specific work also underscores the need for significant technological advancement in pharmacological and other treatment approaches. Work looking at differential contributions of subregions and subpopulations within those regions make it clear that optimizing treatment efficacy while minimizing off-target side effect will require the ability to safely

and ethically target specific molecules in brain regions. In terms of optimal targets, relative restriction in neuropeptide and PKCδ expression make them potentially better targets than the current pharmacotherapies that target broad NT systems such as GABA. However current clinical trials have had relatively limited success with neuropeptide approaches, which may be due to region-specific functional roles. It is possible that PKCδ could prove an interesting new target because of its restriction to within-neuron actions as opposed to being a synaptic signaling molecule but here still, it's expression in a variety of brain regions with potentially distinct effect could limit it efficacy and/or increase off target effect. Thus future work should also focus on new methods to manipulate cell-type specific activity in discrete regions of interest in humans.

Overall, our current understanding of the function of BNST$^{PKC\delta}$ cells is truly nascent. These cells hold the potential for innumerable interesting and important future studies to further our understanding of threat processing and stress responses. Indeed, despite the prevalence and extraordinary economic, personal, and psychological burden that stress-related psychiatric disorders such as anxiety, depression, and addiction pose, treatment options remain limited and poorly understood, and "cures" a distant dream. By furthering our knowledge of BNST$^{PKC\delta}$ neurons, we may help to fill in one small piece of this complex puzzle of understanding how stress impacts the brain and its interaction with so many debilitating diseases, and hopefully ultimately improve the lives of all those most impacted by its effects.

CHAPTER 5

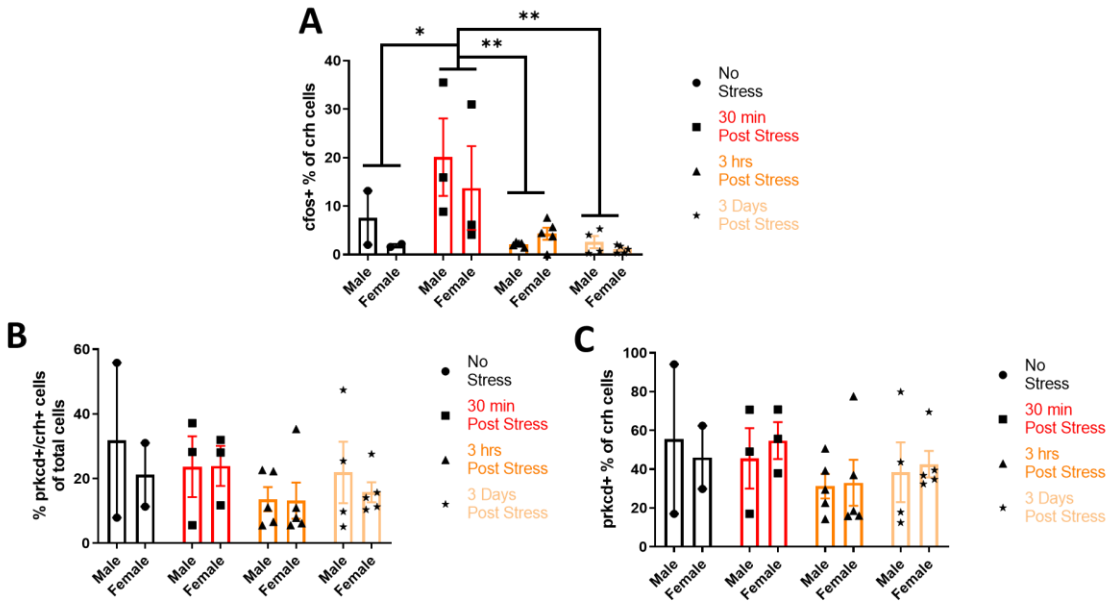
Appendices

5.1. Appendix I: Stress sensitivity of PKCδ and CRF expression in the BNST

We previously found that in there is an increase in the number of BNST neurons expressing PKCδ mRNA (*prkcd*) an increased number of activated *prkcd* neurons (colocalizing with *cfos*) and an increased number of neurons co-expressing *prkcd* and CRF mRNA (*crh*) in female mice 30 minutes after 1 hour of restraint stress. We sought to expand on these findings to examine stress-dependent changes in PKCδ, CRF, and cfos expression in the BNST.

We first examined the timecourse of the changes in the mRNA to see how long the effects might last by repeating the 1 hour restraint stress assay and measuring RNA transcripts at 30 minutes, 3 hours, or 3 days after stress (Fig 5.1). As expected, we found that there was a significant increase in the number of *crh* cells as a percentage of total *crh* cells expressing *cfos* at 30 minutes after stress (Fig 5.1A, No stress vs 30 min p=0.047). *cfos+ crh* cells were significantly increased at 30 minutes compared to 3 hrs (p=0.004) and 3 days (p=0.002) post stress, but there were no significant differences between no stress controls and 3 hrs (p=0.98) or 3 day (p=0.89) after stress. We examined the number of coexpressing *prkcd+/crh+* cells as a percentage of the total cells (Fig 5.1B)and did not see any significant effect of stress (2-way ANOVA, p=0.37), but it is likely underpowered. Interestingly, there does seem to be a trend for a decrease in coexpression at 3 hours compared to 30 minutes after stress. We also examined the number of *prkcd+/crh+* cells as a percentage of total *crh* cells (Fig. 5.1 C) and saw no significant differences (2-way ANOVA stress effect p=0.45) though there again appears to be a trend for decreased coexpression at 3 hours compared to 30 minutes after stress. We did not see any significant sex differences.

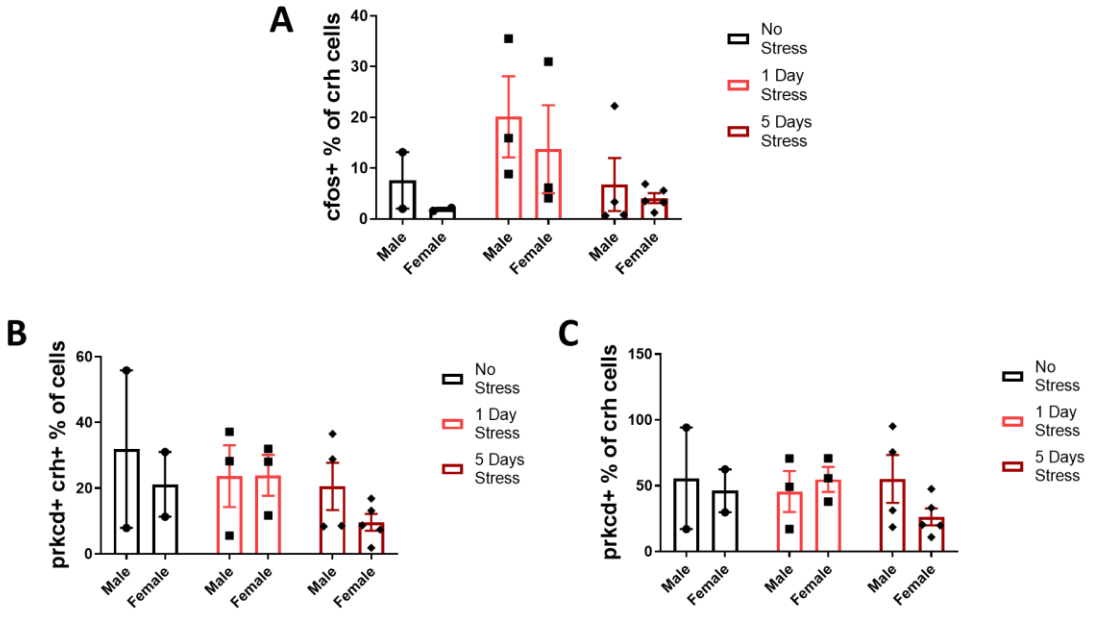
Fig. 5.1 Timecourse Analysis of *prkcd, cfos,* and *crh* cells after acute stress



RNAScope analysis of *prkcd, crh,* and *cfos* mRNA at varying timepoints after 1 hour of restraint stress. **A.** Number of *cfos+ crh* cells as a % of total *crh* cells. 2-way ANOVA. Sex effect p=0.30 F(1,21)=1.14. Timepoint effect p=0.002 F(3,21)=7.05. Tukey's multiple comparisons test. No stress vs 30 min p=0.047. No stress vs 3 hrs p=0.98. No stress vs 3 days p=0.89. 30 min vs 3 hrs p=0.004. 30 min vs 24 hrs p=0.002. 3 hrs vs 3 days p=0.97. **B.** Number of *prkcd+ crh+* coexpressing cells as a % of total cells. 2-way ANOVA. Sex effect p=0.46 F(1,21)=0.56. Timepoint effect p=0.37 F(3,21)=1.11. **C.** Number of *prkcd+ crh+* cells as a % of total *crh* cells. 2-way ANOVA. Sex effect p=0.89 F(1,21)=0.02. Timepoint effect p=0.45 F(3,21)=0.91.

We next examined potential mRNA changes after chronic stress compared to acute stress. We stressed one group of mice for 1 hour on 1 day, and another group for 1 hour for 5 consecutive days and sac'd mice 30 minutes after the last (or only) day of stress. Here we saw a trend for an increase in the number of *cfos+ crh* cells in the acute stress group, compared to both the unstressed controls and the chronic stress group (Fig. 5.2A) (2-way ANOVA, stress effect p=0.087) though it is again likely underpowered. This fits with the idea that BNST^{CRF} cells desensitize to chronic homotypic stress exposure. We did not see any significant effect of sex or stress on the coexpression of *prkcd+/crh+* cells as a percentage of either total BNST cells (Fig 5.2B, 2-way ANOVA sex effect p=0.34, stress effect p=0.37)) or of total *crh* cells (Fig. 5.2C, 2-way ANOVA sex effect p=0.49, stress effect p=0.76). Interestingly, there does seem to be a trend for a decrease in coexpression in females after chronic stress compared to acute stress and no stress controls.

Fig. 5.2 Analysis of *prkcd, cfos,* and *crh* cells after acute stress verses chronic

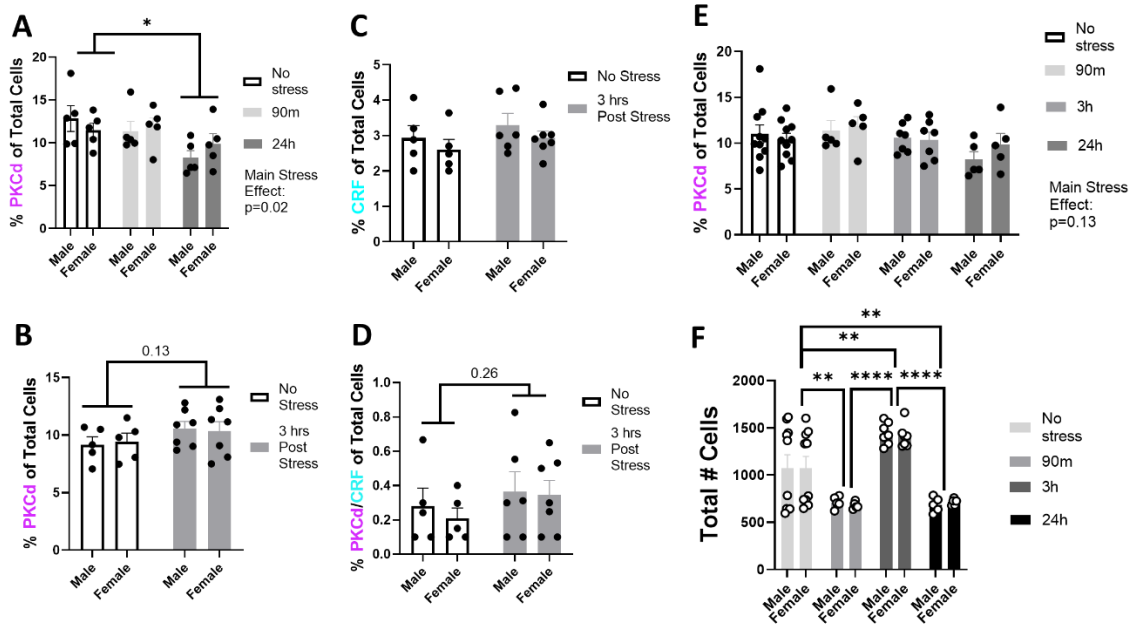


RNAScope analysis of *prkcd, crh,* and *cfos* mRNA at 30 minutes after 1 day or 5 days of 1 hour restraint stress. **A.** Number of *cfos+ crh* cells as a % of total *crh* cells. 2-way ANOVA. Sex effect. P=0.32 F(1,13)=1.08. Stress effect p=0.087 F(2,13)=2.96. **B.** Number of *prkcd+ crh+* coexpressing cells as a % of total cells. 2-way ANOVA. Sex effect p=0.34 F(1,13)=0.98. Stress effect p=0.37 F(2,13)=1.07 **C.** Number of *prkcd+ crh+* cells as a % of total *crh* cells. 2-way ANOVA. Sex effect p=0.49 F(1,13)=0.51. Stress effect p=0.76 F(2,13)=0.28

We next asked whether the changes in *prkcd* mRNA and coexpression with *crh* are translated to functional protein. Unfortunately, due to the density of CRF immunoreactive fibers in the BNST, conducting IHC to stain for CRF cell bodies in infeasible. Thus we sought to examine changes in PKCδ cell numbers alone first. We put mice through 1 hour of restraint stress and then sac'd mice 90 minutes later (when peak cfos protein changes can be observed). We also wanted to investigate the longevity of any potential changes in PKCδ expression, and so sac'd another group of mice 24 hours after restraint. We examined the number of PKCδ+ cells as a percentage of total BNST cells (Fig 5.3A) and saw there was no difference between no stress and 90 minutes after stress ($p=0.95$), but that there was actually a significant decrease in the number of $BNST^{PKC\delta}$ neurons at 24 hours after stress ($p=0.03$).

We thought that perhaps 90 minutes was too early to observe changes in PKCδ protein, and so repeated the experiment looking at 3 hours after stress. We also took advantage of a Crh-VENUS mouse line that expresses fluorescent Venus protein in CRF cells to enable quantification of cell bodies in the BNST and examine changes in coexpression with PKCd cells. Here we found a trend for an increase in the number of PKCδ+ cells as a percentage of total cells at 3 hours after stress compared to no stress controls (Fig 5.3B, 2-way ANOVA stress effect $p=0.13$). We did not see an changes in the number of CRF+ cells as a percentage of total cell (Fig 5.3C) but there was a possible trend for an increase in the number of coexpressing PKCδ+/CRF+ neurons as a percentage of total cells in the BNST (Fig 5.3D, 2-way ANOVA stress effect $p=0.26$). We then combined the results of the two experiments to examine an overall timecourse of changes in PKCδ expression (Fig 5.3E), and here saw a trend for an effect of stress (2-way ANOVA stress effect $p=0.13$). However it should be noted that there was significant experiment/counter variability, with significant differences in the total number of neurons counted between experiments (Fig 5.3F), and this could have contributed to an attenuated effect.

Fig 5.3 Number of PKCd and PKCd/CRF cells after acute stress



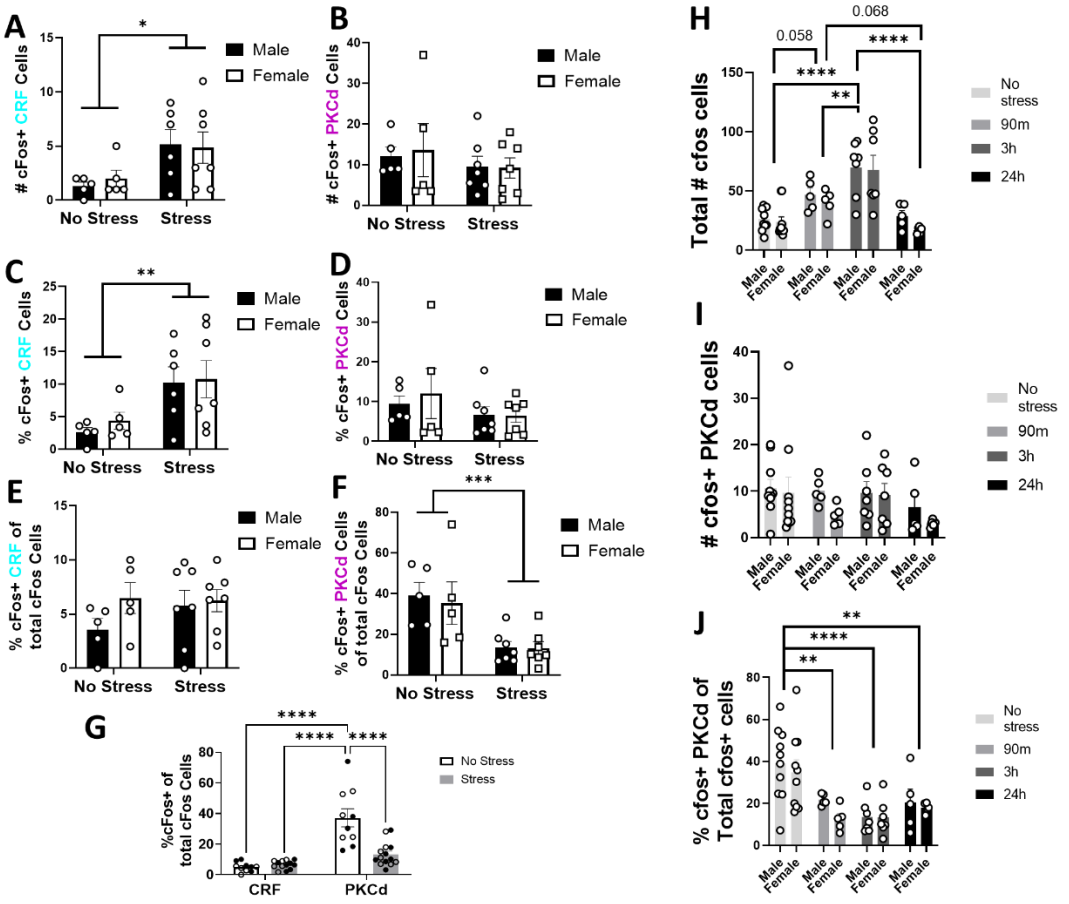
IHC of protein changes in PKCd, CRF, and their coexpression, and total cells at various timepoint after 1 hour of restraint stress. **A.** Number of PKCd cells as a percentage of total cell in the BNST at 90 minutes and 24 hours after stress vs no stress controls. 2-way ANOVA, Sex effect p=0.79, F(1,24)=0.08. Stress effect p=0.026, F(2,24)=4.29. Sidak's multiple comparisons No stress vs 90 min p=0.95, No stress vs 24 hrs p=0.03, 90 min vs 24 hrs p=0.10. **B.** Number of PKCd cells as a percentage of total cells in the BNST 3 hours after stress vs No stress controls. 2-way ANOVA, Sex effect p=0.99, F(1,20) = 0.00004. Stress effect p=0.13, F(1,20)=2.55. **C.** Number of CRF cells as a percentage of total cells in the BNST. 2-way ANOVA, Sex effect p=0.24, F(1,19)=1.48. Stress effect p=0.24, F(1,19)=1.49 **D.** Number PKCd+/CRF+ neurons as percent of total cells in the BNST. 2-way ANOVA, Sex effect p=0.64, F(1,19)=0.23. Stress effect p=0.26, F(1,19)=1.35. **E.** Both experiments combines to assess PKCd expression changes across timepoints. 2-way ANOVA Sex effect p=0.62, F(1,46)=0.25. Stress effect p=0.126, F(3,46)=2.01. **F.** Total number of DAPI cells, averaged across 2-3 slices per mouse and 2 experimenter counts per slice. 2-way ANOVA, Sex effect p=0.91, F(1,46)=0.01. Stress effect p<0.0001, F(3,46)=20.35. Sidak's multiple comparisons No Stress vs 90 min p=0.004, No stress vs 3 hours p=0.003, No stress vs 24 hrs p=0.005, 90 min vs 3 hrs p<0.0001, 90 min vs 24 hrs p>0.9999, 3 hrs vs 24 hrs p<0.0001.

In Chapter 3 of this work, we found no stress-induced increases in cfos protein in PKCδ cells at 90 minutes, 3 hours, or 24 hours after 1 hour of restraint stress. However, we noticed that there seemed to be a considerable amount of PKCδ cells expressing cfos at every time point, including the no stress control condition. So, we sought to examine relative contribution of cfos+ PKCδ cells to overall BNST cfos expression. We first looked at changes in cfos in both CRF and PKCδ neurons at 3 hours after stress Fig 5.4A-G). We found that there is a significant increase in both the raw number (Fig 5.4A, p=0.01) and number of cfos+ CRF cells as a percentage of total CRF cells (Fig 5.4C, p=0.007). We did not see an increase in the raw number of cfos+ PKCδ cells (Fig 5.4B, p=0.23) or number of cfos+ PKCδ cells as a percent of total PKCδ cells (Fig. 5.4D,

p=0.21). We also examined the number of cfos+ CRF cells and PKCδ cells as a percentage of total cfos+ neurons in the BNST. We saw so significant difference in the relative contribution of cfos+ CRF neurons between stressed and unstressed mice (Fig 5.4E, 2-way ANOVA stress effect p=0.24), though there seems to be a possible trend increased contribution in stressed male mice. In contrast, PKCδ cells make a up a significantly larger number percentage of total cfos+ neurons in no stress controls compared to after stress, which aligns with their lack of increased cfos after stress. We did note, however, that it seemed that PKCδ cells overall comprised a larger proportion of total cfos+ cells than CRF cells. We did not observe any significant sex differences, and so combined sexes and directly examined the relative contribution of CRF and PKCδ cells to total cfos cells (Fig 54.G) and found that this was indeed the case, with there being a larger proportion of cfos+ PKCδ cells than cfos+ PKCδ cells (2-way ANOVA cell type effect p<0.0001). This was specifically the case in the unstressed condition (p<0.0001).

We also examined the relative contribution of PKCδ cfos cells to total cfos+ cells in our combined timepoints dataset. When looking at cfos, as with looking at percentages of expression (Fig 3.2B), we found a significant increase in the raw number of cfos+ neurons in the BNST at 3 hours (p=<0.0001), and a trend for an increase at 90 minutes (p=0.058) after stress, and no difference at 24 hours after stress compared to no stress controls (p=0.9997) (Fig 5.4H) Number of total cfos neurons was also significantly higher at 3hrs post stress than 90 minutes (p=0.006) or 24 hours (p<0.0001) post stress, but this is likely due to experimenter counting variability. When looking at raw numbers of cfos+ PKCδ neurons, we saw no significant differences across timepoints (Fig 5.4I) (2-way ANOVA, Sex effect p=0.19, F(1,46)=1.81, Stress effect p=0.21, F(3,46)=1.57). However, when we look at the percentage of total cfos+ neurons that are cfos+/PKCδ+, we see that PKCδ neurons consistently make up a large proportion of total cfos neurons, and this proportion is significantly greater in the no stress control conditions that after stress (Fig 54.J) (Sidak's multiple comparisons, No stress vs 90 min p=0.001, No stress vs 3 hrs p<0.0001, No stress vs 24 hrs p=0.005). The 24 hr comparison is particularly interesting because there is neither a significant increase in global cfos nor decrease in PKCδ+ cfos neurons at this time point, but enough of a trend in each case to result in this significant difference. Together this suggests there may be a basally active population of $BNST^{PKC\delta}$ neurons that is actually downregulated after stress.

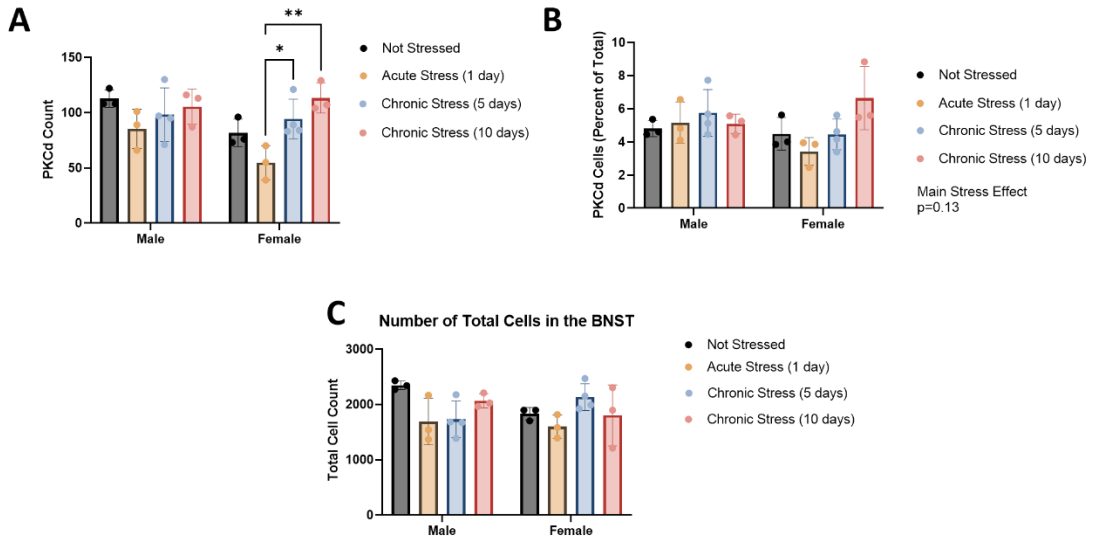
Fig 5.4 Cfos activation in BNST^{PKCd} and BNST^{CRF} cells after acute stress



IHC of protein changes at various timepoint after stress to examine relative contributions of cfos+ CRF neurons and cfos+ PKCd neurons to total cfos in the BNST. **A.** Total number of cfos+ CRF neurons. 2-way ANOVA, Sex effect p=0.87, F(1,19)=0.03. Stress effect p=0.01, F(1,19)=7.57. **B.** Total number of cfos+ PKCd cells. 2-way ANOVA, Sex effect p=0.87, F(1,20)=0.03, Stress effect p=0.234 F(1,20)=0.94. **C.** Number of cfos+CRF cells as a % of total CRF cells. 2-way ANOVA, Sex effect p=0.61, F(1,19)=0.26. Stress effect p=0.007, F(1,19)=9.19. **D.** Number of cfos+PKCd cells as a % of total PKCd cells. 2-way ANOVA, Sex effect p=0.71, F(1,20)=0.14. Stress effect p=0.21 F(1,20)=0.21 **E.** Number of cfos+ CRF neurons as a % of total cfos+ neurons. 2-way ANOVA Sex effect p=0.24, F(1,19)=1.02. Stress effect p=0.24, F(1,19)=1.50. **F.** Number of cfos+ PKCd cells as a % of total cfos+ cells. 2-way ANOVA p=0.73 F(1,20)=0.12. Stress effect p=0.0005 F(1,20)=17.32. **G.** Sexes combined, Filled circles=female (F), Open circles=male (M). Examining number of cfos+ CRF cells vs cfos+ PKCd cell as % of total cfos cells. 2-way ANOVA, Stress effect p=0.0003, F(1,43)=15.33. Cell type effect p<0.0001 F(1,43)=46.66. Interaction p<0.0001 F(1,43)=19.55. Tukey's multiple comparisons test. CRF no stress vs CRF stress p=0.98. PKCd no stress vs PKCd stress p<0.0001. CRF no stress vs PKCd no stress p<0.0001. CRF stress vs PKCd stress p=0.27. CRF stress vs PKCd no stress p<0.0001. CRF no stress vs PKCd stress p=0.18 **H-J.** Combined two data sets, one with No Stress controls, 90 min post-stress, and 24-hours post stress, and one with No Stress controls and 3 hours post-stress. **H.** Total number of cfos+ cells. 2-way ANOVA, Sex effect p=0.2456, F(1,46)=1.383. Stress effect p=<0.0001, F(3,46)=21.00. Post-hoc Sidaks multiple comparisons. No stress vs 90 min p=0.0575. No stress vs 3 hrs p<0.0001. No stress vs 24 hs p=0.9997. 90 min vs 3 hrs p=0.006. 90 min vs 24 hrs p=0.07. 3 hrs vs 24 hrs p<0.0001. **I.** Total number of cfos+PKCd cells. 2-way ANOVA Sex effect p=0.19 F(1,46)=1.81. Stress effect p=0.21, F(3,46)=1.57. **J.** Number of cfos+ PKCd cells as a % of total cfos cells. 2-way ANOVA. Sex effect p=0.27, F(1,46)=1.25. Stress effect p<0.0001, F(3,46)=11.42. Sidak's multiple comparison's No stress vs 90 min p=0.001, No stress vs 3 hrs p<0.0001, No stress vs 24 hrs p=0.005. 90 min vs 3 hrs p=0.98. 90 min vs 24 hrs p=0.9995. 3 hrs vs 24 hrs p=0.85.

Finally, we sought to examine changes in the expression of PKCδ after chronic stress compared to acute stress. We sac'd mice at 90 minutes after 1 day, 5 days, or 10 days of 1 hour restraint stress. We found that there was a significant effect of stress exposure on the raw number of PKCδ cells (Fig 5.5A, 2-way ANOVA, sex effect p=0.0.45, stress effect p=0.006). In female mice specifically, there was a significant increase in the number PKCδ cells after both 5 days (p=0.03) and 10 days (p=0.003) compared to 1 day of stress, with a trend for a decreased in the number of PKCδ cells after acute stress compared to no stress controls (p=0.24). Male mice also tended to generally followed this general pattern of decrease after acute stress and dose-dependent stair-stepping increase after 5 and 10 days of stress.

Fig 5.5 Number of PKCd cells after chronic stress



IHC of PKCd protein changes at 90 minutes after 1 day, 5 days, or 10 days of 1 hour restraint stress. **A.** Number of PKCd cells after acute and chronic stress. 2-way ANOVA, Sex effect p=0.045 F(1,18)=4.62. Stress effect p=0.006 F(3,18)=5.75. Tukey's multiple comparisons test, Males: No stress vs 1 day p=0.23. No stress vs 5 days p=0.68. no stress vs 10 days p=0.95. 1 day vs 5 days p=0.75. 1 day vs 10 days p=0.49. 5 days vs 10 days p=0.95. Females: no stress vs 1 day p=0.24. no stress vs 5 days p=0.77. no stress vs 10 days p=0.14. 1 day vs 5 days p=0.03. 1 day vs 10 days p=0.003. 5 days vs 10 days p=0.47. **B.** Number of PKCd cells as a percent of total cells in the BNST. 2-way ANOVA Sex effect p=0.33 F(1,18)=0.99. Stress effect p=0.13 F(3,18)=2.12. Interaction p=0.09 F(3,18)=2.55. **C.** Total number of cells (DAPI) in the BNST. 2-way ANOVA, Sex effect p=0.34 F(1,18)=0.97. Stress effect p=0.11 F(3,18)=2.30. Interaction p=0.06 F(3,18)=2.97.

Overall, it seems that there are interesting changes and trends for stress-dependent regulation of PKCδ expression level in the BNST, and future studies should further examine the stress sensitivity of this peptide and neurons withing which it is expresses.

5.2. Appendix II: Numbered references for Figure 1.1

1. Rood, B. D. *et al.* Site of origin of and sex differences in the vasopressin innervation of the mouse (Mus musculus) brain. *J. Comp. Neurol.* **521**, 2321–2358 (2013).
2. Rood, B. D. & De Vries, G. J. Vasopressin innervation of the mouse (Mus musculus) brain and spinal cord. *J. Comp. Neurol.* **519**, 2434–2474 (2011).
3. Otero-Garcia, M. *et al.* Extending the socio-sexual brain: Arginine-vasopressin immunoreactive circuits in the telencephalon of mice. *Brain Struct. Funct.* **219**, 1055–1081 (2014).
4. van Leeuwen, F. & Caffé, R. Vasopressin-immunoreactive cell bodies in the bed nucleus of the stria terminalis of the rat. *Cell Tissue Res.* **228**, 525–534 (1983).
5. Fink, G., Sumner, B. E. H., Rosie, R., Grace, O. & Quinn, J. P. Estrogen control of central neurotransmission: Effect on mood, mental state, and memory. *Cell. Mol. Neurobiol.* **16**, 325–344 (1996).
6. Buijs, R. M. & Van Leeuwen, F. W. Sex Differences in Vasopressin and Other Neurotransmitter Systems in the Brain. *Prog. Brain Res.* **61**, 185–203 (1984).
7. Rigney, N., Whylings, J., Mieda, M., De Vries, G. J. & Petrulis, A. Sexually dimorphic vasopressin cells modulate social investigation and communication in sex-specific ways. *eNeuro* **6**, (2019).
8. Meddle, S. L., Bishop, V. R., Gkoumassi, E., Van Leeuwen, F. W. & Douglas, A. J. Dynamic changes in oxytocin receptor expression and activation at parturition in the rat brain. *Endocrinology* **148**, 5095–5104 (2007).
9. Naik, R. R. & de Jong, T. R. Transient and persistent behavioral and molecular changes in primiparous female Wistar rats. *Eur. J. Neurosci.* **45**, 797–804 (2017).
10. Yohn, C. N., Leithead, A. B. & Becker, E. A. Increased vasopressin expression in the BNST accompanies paternally induced territoriality in male and female California mouse offspring. *Horm. Behav.* **93**, 9–17 (2017).
11. Bosch, O. J., Pförtsch, J., Beiderbeck, D. I., Landgraf, R. & Neumann, I. D. Maternal behaviour is associated with vasopressin release in the medial preoptic area and bed nucleus of the stria terminalis in the rat. *J. Neuroendocrinol.* **22**, 420–429 (2010).
12. Duque-Wilckens, N. *et al.* Inhibition of vasopressin V1a receptors in the medioventral bed nucleus of the stria terminalis has sex- and context-specific anxiogenic effects. *Neuropharmacology* **110**, 59–68 (2016).
13. Veenema, A. H., Beiderbeck, D. I., Lukas, M. & Neumann, I. D. Distinct correlations of vasopressin release within the lateral septum and the bed nucleus of the stria terminalis with the display of intermale aggression. *Horm. Behav.* **58**, 273–281 (2010).
14. Steinman, M. Q. *et al.* Hypothalamic vasopressin systems are more sensitive to the long term effects of social defeat in males versus females. *Psychoneuroendocrinology* **51**, 122–134 (2015).
15. Bamshad, M., Cooper, T. T., Karom, M. & Albers, H. E. Glutamate and vasopressin interact to control scent marking in Syrian hamsters(Mesocricetus auratus). *Brain Res.* **731**, 213–216 (1996).
16. Whylings, J., Rigney, N., Peters, N. V., de Vries, G. J. & Petrulis, A. Sexually dimorphic role of BNST vasopressin cells in sickness and social behavior in male and female mice. *Brain. Behav. Immun.* **83**, 68–77 (2020).
17. Giardino, W. J. *et al.* Parallel circuits from the bed nuclei of stria terminalis to the lateral hypothalamus drive opposing emotional states. *Nat. Neurosci.* **21**, 1084–1095 (2018).
18. Silberman, Y., Matthews, R. T. & Winder, D. G. A Corticotropin Releasing Factor Pathway for Ethanol Regulation of the Ventral Tegmental Area in the Bed Nucleus of the Stria Terminalis. *J. Neurosci.* **33**, 950–960 (2013).

19. Silberman, Y. & Winder, D. G. Emerging role for corticotropin releasing factor signaling in the bed nucleus of the stria terminalis at the intersection of stress and reward. *Front. psychiatry* **4**, 42 (2013).
20. Bruzsik, B. *et al.* Somatostatin neurons of the bed nucleus of stria terminalis enhance associative fear memory consolidation in mice. *J. Neurosci.* JN-RM-1944-20 (2021) doi:10.1523/jneurosci.1944-20.2020.
21. Wang, L. *et al.* Comparison of CRF-immunoreactive neurons distribution in mouse and rat brains and selective induction of Fos in rat hypothalamic CRF neurons by abdominal surgery. *Brain Res.* **1415**, 34–46 (2011).
22. Cummings, S., Elde, R., Ells, J. & Lindall, A. Corticotropin-releasing factor immunoreactivity is widely distributed within the central nervous system of the rat: an immunohistochemical study. *J. Neurosci.* **3**, 1355–68 (1983).
23. Ju, G., Swanson, L. W. & Simerly, R. B. Studies on the cellular architecture of the bed nuclei of the stria terminalis in the rat: II. chemoarchitecture. *J. Comp. Neurol.* **280**, 603–621 (1989).
24. Uchida, K. *et al.* Female-biased sexual dimorphism of corticotropin-releasing factor neurons in the bed nucleus of the stria terminalis. *Biol. Sex Differ.* **10**, 6 (2019).
25. Day, H. E. W., Curran, E. J., Watson, S. J. & Akil, H. Distinct neurochemical populations in the rat central nucleus of the amygdala and bed nucleus of the stria terminalis: Evidence for their selective activation by interleukin-1? *J. Comp. Neurol.* **413**, 113–128 (1999).
26. Shimada, S. *et al.* Coexistence of peptides (corticotropin releasing factor/neurotensin and substance P/somatostatin) in the bed nucleus of the stria terminalis and central amygdaloid nucleus of the rat. *Neuroscience* **30**, 377–383 (1989).
27. Daniel, S. E. & Rainnie, D. G. Stress Modulation of Opposing Circuits in the Bed Nucleus of the Stria Terminalis. *Neuropsychopharmacology* **41**, 1–23 (2015).
28. Makino, S., Gold, P. W. & Schulkin, J. Effects of corticosterone on CRH mRNA and content in the bed nucleus of the stria terminalis; comparison with the effects in the central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus. *Brain Res.* **657**, 141–149 (1994).
29. Watts, A. G., Kelly, A. B. & Sanchez-Watts, G. Neuropeptides and thirst: The temporal response of corticotropin-releasing hormone and neurotensin/neuromedin N gene expression in rat limbic forebrain neurons to drinking hypertonic saline. *Behav. Neurosci.* **109**, 1146–1157 (1995).
30. Shalev, U., Morales, M., Hope, B., Yap, J. & Shaham, Y. Time-dependent changes in extinction behavior and stress-induced reinstatement of drug seeking following withdrawal from heroin in rats. *Psychopharmacology (Berl).* **156**, 98–107 (2001).
31. Funk, D., Li, Z. & Lê, A. D. Effects of environmental and pharmacological stressors on c-fos and corticotropin-releasing factor mRNA in rat brain: Relationship to the reinstatement of alcohol seeking. *Neuroscience* **138**, 235–243 (2006).
32. Kim, S.-J. *et al.* Effects of repeated tianeptine treatment on CRF mRNA expression in non-stressed and chronic mild stress-exposed rats. *Neuropharmacology* **50**, 824–833 (2006).
33. Fetterly, T. L. *et al.* α2A-Adrenergic Receptor Activation Decreases Parabrachial Nucleus Excitatory Drive onto BNST CRF Neurons and Reduces Their Activity In Vivo. *J. Neurosci.* **39**, 472–484 (2019).
34. Sahuque, L. L. *et al.* Anxiogenic and aversive effects of corticotropin-releasing factor (CRF) in the bed nucleus of the stria terminalis in the rat: role of CRF receptor subtypes. *Psychopharmacology (Berl).* **186**, 122–132 (2006).
35. Lee, Y. & Davis, M. Role of the hippocampus, the bed nucleus of the stria terminalis, and the

amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. *J. Neurosci.* **17**, 6434–6446 (1997).
36. Klampfl, S. M., Brunton, P. J., Bayerl, D. S. & Bosch, O. J. Hypoactivation of CRF receptors, predominantly type 2, in the medial-posterior BNST is vital for adequate maternal behavior in lactating rats. *J. Neurosci.* **34**, 9665–9676 (2014).
37. Klampfl, S. M. *et al.* Brain CRF-binding protein modulates aspects of maternal behavior under stressful conditions and supports a hypo-anxious state in lactating rats. *Horm. Behav.* **84**, 136–144 (2016).
38. Klampfl, S. M., Brunton, P. J., Bayerl, D. S. & Bosch, O. J. CRF-R1 activation in the anterior-dorsal BNST induces maternal neglect in lactating rats via an HPA axis-independent central mechanism. *Psychoneuroendocrinology* **64**, 89–98 (2016).
39. Lee, Y., Fitz, S., Johnson, P. L. & Shekhar, A. Repeated stimulation of CRF receptors in the BNST of rats selectively induces social but not panic-like anxiety. *Neuropsychopharmacology* **33**, 2586–2594 (2008).
40. Jasnow, A. M., Davis, M. & Huhman, K. L. Involvement of central amygdalar and bed nucleus of the stria terminalis corticotropin-releasing factor in behavioral responses to social defeat. *Behav. Neurosci.* **118**, 1052–1061 (2004).
41. Cooper, M. A. & Huhman, K. L. Blocking corticotropin-releasing factor-2 receptors, but not corticotropin-releasing factor-1 receptors or glucocorticoid feedback, disrupts the development of conditioned defeat. *Physiol. Behav.* **101**, 527–532 (2010).
42. Crowley, N. A. *et al.* Dynorphin Controls the Gain of an Amygdalar Anxiety Circuit. *Cell Rep.* **14**, 2774–2783 (2016).
43. Poulin, J.-F. F., Arbour, D., Laforest, S. & Drolet, G. Neuroanatomical characterization of endogenous opioids in the bed nucleus of the stria terminalis. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* **33**, 1356–1365 (2009).
44. Neal, C. R., Swann, J. M. & Newman, S. W. The colocalization of substance P and prodynorphin immunoreactivity in neurons of the medial preoptic area, bed nucleus of the stria terminalis and medial nucleus of the amygdala of the Syrian hamster. *Brain Res.* **496**, 1–13 (1989).
45. Normandeau, C. P., Torruella Suárez, M. L., Sarret, P., McElligott, Z. A. & Dumont, E. C. Neurotensin and dynorphin Bi-Directionally modulate CeA inhibition of oval BNST neurons in male mice. *Neuropharmacology* **143**, 113–121 (2018).
46. Land, B. B. *et al.* The dysphoric component of stress is encoded by activation of the dynorphin κ-opioid system. *J. Neurosci.* **28**, 407–414 (2008).
47. Chung, S. *et al.* Desipramine and citalopram attenuate pretest swim-induced increases in prodynorphin immunoreactivity in the dorsal bed nucleus of the stria terminalis and the lateral division of the central nucleus of the amygdala in the forced swimming test. *Neuropeptides* **48**, 273–280 (2014).
48. Robles, C. F. *et al.* Effects of kappa opioid receptors on conditioned place aversion and social interaction in males and females. *Behav. Brain Res.* **262**, 84–93 (2014).
49. Bruchas, M. R., Land, B. B., Lemos, J. C. & Chavkin, C. CRF1-R activation of the dynorphin/kappa opioid system in the mouse basolateral amygdala mediates anxiety-like behavior. *PLoS One* **4**, (2009).
50. Li, C. *et al.* Presynaptic inhibition of gamma-aminobutyric acid release in the bed nucleus of the stria terminalis by kappa opioid receptor signaling. *Biol. Psychiatry* **71**, 725–732 (2012).
51. Veinante, P., Stoeckel, M.-E., Lasbennes, F. & Freund-Mercier, M.-J. c-Fos and peptide immunoreactivities in the central extended amygdala of morphine-dependent rats after naloxone-precipitated withdrawal. *Eur. J. Neurosci.* **18**, 1295–1305 (2003).

52. Walker, J. R., Ahmed, S. H., Gracy, K. N. & Koob, G. F. Microinjections of an opiate receptor antagonist into the bed nucleus of the stria terminalis suppress heroin self-administration in dependent rats. *Brain Res.* **854**, 85–92 (2000).
53. Poulin, J. F., Chevalier, B., Laforest, S. & Drolet, G. Enkephalinergic afferents of the centromedial amygdala in the rat. *J. Comp. Neurol.* **496**, 859–876 (2006).
54. Gray, T. S. & Magnuson, D. J. Neuropeptide neuronal efferents from the bed nucleus of the stria terminalis and central amygdaloid nucleus to the dorsal vagal complex in the rat. *J. Comp. Neurol.* **262**, 365–374 (1987).
55. Miller, M. A., Kolb, P. E. & Raskind, M. A. Extra-hypothalamic vasopressin neurons coexpress galanin messenger RNA as shown by double in situ hybridization histochemistry. *J. Comp. Neurol.* **329**, 378–384 (1993).
56. Rajendren, G., Levenkova, N. & Gibson, M. J. Galanin immunoreactivity in mouse basal forebrain: sex differences and discrete projections of galanin-containing cells beyond the blood-brain barrier. *Neuroendocrinology* **71**, 27–33 (2000).
57. Cecchi, M., Khoshbouei, H., Javors, M. & Morilak, D. A. Modulatory effects of norepinephrine in the lateral bed nucleus of the stria terminalis on behavioral and neuroendocrine responses to acute stress. *Neuroscience* **112**, 13–21 (2002).
58. Miller, M. A., Kolb, P. E. & Raskind, M. A. Testosterone regulates galanin gene expression in the bed nucleus of the stria terminalis. *Brain Res.* **611**, 338–41 (1993).
59. Neal, C. R. *et al.* Localization of orphanin FQ (nociceptin) peptide and messenger RNA in the central nervous system of the rat. *J. Comp. Neurol.* **406**, 503–547 (1999).
60. Rodriguez-Romaguera, J. *et al.* Prepronociceptin-Expressing Neurons in the Extended Amygdala Encode and Promote Rapid Arousal Responses to Motivationally Salient Stimuli. *Cell Rep.* **33**, 108362 (2020).
61. Dawe, K. L., Wakerley, J. B. & Fulford, A. J. Nociceptin/orphanin FQ and the regulation of neuronal excitability in the rat bed nucleus of the stria terminalis: Interaction with glucocorticoids. *Stress* **13**, 516–527 (2010).
62. Ciccocioppo, R. *et al.* The Bed Nucleus Is a Neuroanatomical Substrate for the Anorectic Effect of Corticotropin-Releasing Factor and for Its Reversal by Nociceptin/Orphanin FQ. *J. Neurosci.* **23**, 9445 (2003).
63. Rodi, D. *et al.* Functional antagonism between nociceptin/orphanin FQ (N/OFQ) and corticotropin-releasing factor (CRF) in the rat brain: Evidence for involvement of the bed nucleus of the stria terminalis. *Psychopharmacology (Berl).* **196**, 523–531 (2008).
64. Pleil, K. E. *et al.* Chronic stress alters neuropeptide Y signaling in the bed nucleus of the stria terminalis in DBA/2J but not C57BL/6J mice. *Neuropharmacology* **62**, 1777–1786 (2012).
65. Chronwall, B. M. *et al.* The anatomy of neuropeptide-y-containing neurons in rat brain. *Neuroscience* **15**, 1159–1181 (1985).
66. Sharko, A. C., Kaigler, K. F., Fadel, J. R. & Wilson, M. A. Ethanol-induced anxiolysis and neuronal activation in the amygdala and bed nucleus of the stria terminalis. *Alcohol* **50**, 19–25 (2016).
67. Ide, S. *et al.* Opposing roles of corticotropin-releasing factor and neuropeptide Y within the dorsolateral bed nucleus of the stria terminalis in the negative affective component of pain in rats. *J Neurosci* **33**, 5881–94 (2013).
68. Tasan, R. O. *et al.* The central and basolateral amygdala are critical sites of neuropeptide Y/Y2 receptor-mediated regulation of anxiety and depression. *J. Neurosci.* **30**, 6282–6290 (2010).
69. Desai, S. J., Borkar, C. D., Nakhate, K. T., Subhedar, N. K. & Kokare, D. M. Neuropeptide Y attenuates anxiety- and depression-like effects of cholecystokinin-4 in mice. *Neuroscience*

277, 818–830 (2014).
70. Kash, T. L. & Winder, D. G. Neuropeptide Y and corticotropin-releasing factor bi-directionally modulate inhibitory synaptic transmission in the bed nucleus of the stria terminalis. *Neuropharmacology* **51**, 1013–1022 (2006).
71. Hawley, D. F. *et al.* Neurobiological constituents of active, passive, and variable coping strategies in rats: Integration of regional brain neuropeptide y levels and cardiovascular responses. *Stress* **13**, 172–183 (2010).
72. D'Este, L., Casini, A., Pontieri, F. E. & Renda, T. G. Changes in neuropeptide FF and NPY immunohistochemical patterns in rat brain under heroin treatment. *Brain Res.* **1083**, 151–158 (2006).
73. Desai, S. J., Upadhya, M. A., Subhedar, N. K. & Kokare, D. M. NPY mediates reward activity of morphine, via NPY Y1 receptors, in the nucleus accumbens shell. *Behav. Brain Res.* **247**, 79–91 (2013).
74. Pleil, K. E. *et al.* NPY signaling inhibits extended amygdala CRF neurons to suppress binge alcohol drinking. *Nat. Neurosci.* **18**, 545–552 (2015).
75. Kotagale, N. R. *et al.* Agmatine attenuates nicotine induced conditioned place preference in mice through modulation of neuropeptide Y system. *Behav. Brain Res.* **262**, 118–124 (2014).
76. Rangani, R. J., Upadhya, M. A., Nakhate, K. T., Kokare, D. M. & Subhedar, N. K. Nicotine evoked improvement in learning and memory is mediated through NPY Y1 receptors in rat model of Alzheimer's disease. *Peptides* **33**, 317–328 (2012).
77. Beal, M. F. *et al.* Somatostatin and neuropeptide Y concentrations in pathologically graded cases of huntington's disease. *Ann. Neurol.* **23**, 562–569 (1988).
78. Gray, T. S. & Magnuson, D. J. Peptide immunoreactive neurons in the amygdala and the bed nucleus of the stria terminalis project to the midbrain central gray in the rat. *Peptides* **13**, 451–460 (1992).
79. Ju, G. & Han, Z. Coexistence of corticotropin releasing factor and neurotensin within oval nucleus neurons in the bed nuclei of the stria terminalis in the rat. *Neurosci. Lett.* **99**, 246–250 (1989).
80. Schroeder, L. E. *et al.* Mapping the populations of neurotensin neurons in the male mouse brain. *Neuropeptides* **76**, 101930 (2019).
81. Normandeau, C. P. *et al.* A Key Role for Neurotensin in Chronic-Stress-Induced Anxiety-Like Behavior in Rats. *Neuropsychopharmacology* **43**, 285–293 (2018).
82. Krawczyk, M. *et al.* D1 dopamine receptor-mediated LTP at GABA synapses encodes motivation to self-administer cocaine in rats. *J. Neurosci.* **33**, 11960–11971 (2013).
83. Ubaldi, M. *et al.* Activation of Hypocretin-1/Orexin-A Neurons Projecting to the Bed Nucleus of the Stria Terminalis and Paraventricular Nucleus Is Critical for Reinstatement of Alcohol Seeking by Neuropeptide S. *Biol. Psychiatry* **79**, 452–462 (2016).
84. Peyron, C. *et al.* Neurons Containing Hypocretin (Orexin) Project to Multiple Neuronal Systems. *J. Neurosci.* **18**, 9996–10015 (1998).
85. Ch'ng, S. S. & Lawrence, A. J. Distribution of the orexin-1 receptor (OX1R) in the mouse forebrain and rostral brainstem: A characterisation of OX1R-eGFP mice. *J. Chem. Neuroanat.* **66**–**67**, 1–9 (2015).
86. Ciriello, J., Rosas-Arellano, M. P., Solano-Flores, L. P. & De Oliveira, C. V. R. Identification of neurons containing orexin-B (hypocretin-2) immunoreactivity in limbic structures. *Brain Res.* **967**, 123–131 (2003).
87. James, M. H., Mahler, S. V., Moorman, D. E. & Aston-Jones, G. A Decade of Orexin/Hypocretin and Addiction: Where Are We Now? *Curr. Top. Behav. Neurosci.* **33**, 247 (2017).

88. Conrad, K. L. *et al.* Yohimbine Depresses Excitatory Transmission in BNST and Impairs Extinction of Cocaine Place Preference Through Orexin-Dependent, Norepinephrine-Independent Processes. *Neuropsychopharmacology* **37**, 2253–2266 (2012).
89. Lungwitz, E. A. *et al.* Orexin-A induces anxiety-like behavior through interactions with glutamatergic receptors in the bed nucleus of the stria terminalis of rats. *Physiol. Behav.* **107**, 726–732 (2012).
90. Kodani, S., Soya, S. & Sakurai, T. Excitation of GABAergic Neurons in the Bed Nucleus of the Stria Terminalis Triggers Immediate Transition from Non-Rapid Eye Movement Sleep to Wakefulness in Mice. *J. Neurosci.* **37**, 7164 (2017).
91. Duque-Wilckens, N. *et al.* Extrahypothalamic oxytocin neurons drive stress-induced social vigilance and avoidance. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 26406–26413 (2020).
92. Steinman, M. Q. *et al.* Sex-Specific Effects of Stress on Oxytocin Neurons Correspond With Responses to Intranasal Oxytocin. *Biol. Psychiatry* **80**, 406–414 (2016).
93. Dumais, K. M., Bredewold, R., Mayer, T. E. & Veenema, A. H. Sex differences in oxytocin receptor binding in forebrain regions: Correlations with social interest in brain region- and sex- specific ways. *Horm. Behav.* **64**, 693–701 (2013).
94. Dabrowska, J. *et al.* Neuroanatomical evidence for reciprocal regulation of the corticotrophin-releasing factor and oxytocin systems in the hypothalamus and the bed nucleus of the stria terminalis of the rat: Implications for balancing stress and affect. *Psychoneuroendocrinology* **36**, 1312–1326 (2011).
95. Moffitt, J. R. *et al.* Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region. *Science (80-.).* **362**, (2018).
96. Calcagnoli, F. *et al.* Local oxytocin expression and oxytocin receptor binding in the male rat brain is associated with aggressiveness. *Behav. Brain Res.* **261**, 315–322 (2014).
97. Consiglio, A. R., Borsoi, A., Pereira, G. A. M. & Lucion, A. B. Effects of oxytocin microinjected into the central amygdaloid nucleus and bed nucleus of stria terminalis on maternal aggressive behavior in rats. *Physiol. Behav.* **85**, 354–362 (2005).
98. Jarrett, T. M., McMurray, M. S., Walker, C. H. & Johns, J. M. Cocaine treatment alters oxytocin receptor binding but not mRNA production in postpartum rat dams. *Neuropeptides* **40**, 161–167 (2006).
99. Dumais, K. M., Alonso, A. G., Immormino, M. A., Bredewold, R. & Veenema, A. H. Involvement of the oxytocin system in the bed nucleus of the stria terminalis in the sex-specific regulation of social recognition. *Psychoneuroendocrinology* **64**, 79–88 (2016).
100. Moaddab, M. & Dabrowska, J. Oxytocin receptor neurotransmission in the dorsolateral bed nucleus of the stria terminalis facilitates the acquisition of cued fear in the fear-potentiated startle paradigm in rats. *Neuropharmacology* **121**, 130–139 (2017).
101. Martinon, D. *et al.* Oxytocin receptors in the dorsolateral bed nucleus of the stria terminalis (BNST) bias fear learning toward temporally predictable cued fear. *Transl. Psychiatry* **9**, (2019).
102. De Bundel, D. *et al.* Dopamine D2 receptors gate generalization of conditioned threat responses through mTORC1 signaling in the extended amygdala. *Mol. Psychiatry* **21**, 1545–1553 (2016).
103. Ye, J. & Veinante, P. Cell-type specific parallel circuits in the bed nucleus of the stria terminalis and the central nucleus of the amygdala of the mouse. *Brain Struct. Funct.* **224**, 1067–1095 (2019).
104. Wang, Y. *et al.* A bed nucleus of stria terminalis microcircuit regulating inflammation-associated modulation of feeding. *Nat. Commun.* **10**, 2769 (2019).
105. Wang, X. *et al.* Anxiety-related cell-type-specific neural circuits in the anterior-dorsal bed

nucleus of the stria terminalis. *Sci. Bull.* **65**, 1203–1216 (2020).
106. Ueda, S. *et al.* Distinctive Regulation of Emotional Behaviors and Fear-Related Gene Expression Responses in Two Extended Amygdala Subnuclei With Similar Molecular Profiles. *Front. Mol. Neurosci.* **14**, 186 (2021).
107. Xiao, Q. *et al.* A new GABAergic somatostatin projection from the BNST onto accumbal parvalbumin neurons controls anxiety. *Mol. Psychiatry* 1–23 (2020) doi:10.1038/s41380-020-0816-3.
108. Ahrens, S. *et al.* A Central Extended Amygdala Circuit That Modulates Anxiety. *J. Neurosci.* **38**, 5567–5583 (2018).
109. Verney, C., Gaspar, P., Febvret, A. & Berger, B. Transient tyrosine hydroxylase-like immunoreactive neurons contain somatostatin and substance P in the developing amygdala and bed nucleus of the stria terminalis of the rat. *Dev. Brain Res.* **42**, 45–58 (1988).
110. Damalama, M. & Swann, J. Substance P and neurokinin A are colocalized in the central chemosensory pathway of the male golden hamster. *Neuropeptides* **24**, 327–334 (1993).
111. Mayer, M. L. & MacLeod, N. K. The excitatory action of substance P and stimulation of the stria terminalis bed nucleus on preoptic neurones. *Brain Res.* **166**, 206–210 (1979).
112. Swann, J. M. & Macchione, N. Photoperiodic regulation of substance P immunoreactivity in the mating behavior pathway of the male golden hamster. *Brain Res.* **590**, 29–38 (1992).
113. Rasri, K., Mason, P., Govitrapong, P., Pevet, P. & Klosen, P. Testosterone-driven seasonal regulation of vasopressin and galanin in the bed nucleus of the stria terminalis of the Djungarian hamster (Phodopus sungorus). *Neuroscience* **157**, 174–187 (2008).
114. Flint Beal, M. *et al.* A detailed examination of substance P in pathologically graded cases of Huntington's disease. *J. Neurol. Sci.* **84**, 51–61 (1988).
115. Ebner, K. & Singewald, N. The role of substance P in stress and anxiety responses. *Amino Acids* **31**, 251–272 (2006).
116. Iftikhar, K., Siddiq, A., Baig, S. G. & Zehra, S. Substance P: A neuropeptide involved in the psychopathology of anxiety disorders. *Neuropeptides* vol. 79 101993 (2020).
117. Piggins, H. D., Stamp, J. A., Burns, J., Rusak, B. & Semba, K. Distribution of pituitary adenylate cyclase activating polypeptide (PACAP) immunoreactivity in the hypothalamus and extended amygdala of the rat. *J. Comp. Neurol.* **376**, 278–294 (1996).
118. Hashimoto, H. *et al.* Distribution of the mRNA for a pituitary adenylate cyclase-activating polypeptide receptor in the rat brain: An in situ hybridization study. *J. Comp. Neurol.* **371**, 567–577 (1996).
119. Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K. & Nagata, S. Functional expression and tissue distribution of a novel receptor for vasoactive intestinal polypeptide. *Neuron* **8**, 811–819 (1992).
120. Sheward, W. J., Lutz, E. M. & Harmar, A. J. The distribution of vasoactive intestinal peptide2 receptor messenger RNA in the rat brain and pituitary gland as assessed by in situ hybridization. *Neuroscience* **67**, 409–418 (1995).
121. Kozicz, T., Vigh, S. & Arimura, A. Immunohistochemical evidence for PACAP and VIP interaction with Met-enkephalin and CRF containing neurons in the bed nucleus of the stria terminalis. in *Annals of the New York Academy of Sciences* vol. 865 523–528 (New York Academy of Sciences, 1998).
122. Kozicz, T., Vigh, S. & Arimura, A. Axon terminals containing PACAP- and VIP-immunoreactivity form synapses with CRF-immunoreactive neurons in the dorsolateral division of the bed nucleus of the stria terminalis in the rat. *Brain Res.* **767**, 109–119 (1997).
123. Agarwal, A., Halvorson, L. M. & Legradi, G. Pituitary adenylate cyclase-activating polypeptide (PACAP) mimics neuroendocrine and behavioral manifestations of stress: Evidence for PKA-

mediated expression of the corticotropin-releasing hormone (CRH) gene. *Brain Res. Mol. Brain Res.* **138**, 45 (2005).
124. Stroth, N. & Eiden, L. E. Stress hormone synthesis in mouse hypothalamus and adrenal gland triggered by restraint is dependent on PACAP signaling. *Neuroscience* **165**, 1025 (2010).
125. Stroth, N., Liu, Y., Aguilera, G. & Eiden, L. E. Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Controls Stimulus-Transcription Coupling in the Hypothalamic-Pituitary-Adrenal Axis to Mediate Sustained Hormone Secretion During Stress. *J. Neuroendocrinol.* **23**, 944 (2011).
126. Hammack, S. E. *et al.* Chronic stress increases pituitary adenylate cyclase-activating peptide (PACAP) and brain-derived neurotrophic factor (BDNF) mRNA expression in the bed nucleus of the stria terminalis (BNST): Roles for PACAP in anxiety-like behavior. *Psychoneuroendocrinology* **34**, 833–843 (2009).
127. Lezak, K. R. *et al.* Regulation of Bed Nucleus of the Stria Terminalis PACAP Expression by Stress and Corticosterone. *J. Mol. Neurosci.* **54**, 477–484 (2014).
128. Roman, C. W. *et al.* PAC1 receptor antagonism in the bed nucleus of the stria terminalis (BNST) attenuates the endocrine and behavioral consequences of chronic stress. *Psychoneuroendocrinology* **47**, 151–165 (2014).
129. King, S. B. *et al.* The Effects of Prior Stress on Anxiety-Like Responding to Intra-BNST Pituitary Adenylate Cyclase Activating Polypeptide in Male and Female Rats. *Neuropsychopharmacology* **42**, 1679–1687 (2017).
130. Hashimoto, H. *et al.* Altered psychomotor behaviors in mice lacking pituitary adenylate cyclase-activating polypeptide (PACAP). *Proc. Natl. Acad. Sci. U. S. A.* **98**, 13355–60 (2001).
131. Otto, C. *et al.* Altered emotional behavior in PACAP-type-I-receptor-deficient mice. *Mol. Brain Res.* **92**, 78–84 (2001).
132. Kocho-Schellenberg, M. *et al.* PACAP in the BNST Produces Anorexia and Weight Loss in Male and Female Rats. *Neuropsychopharmacology* **39**, 1614–1623 (2014).

References

Abbafati, C., Abbas, K. M., Abbasi-Kangevari, M., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abegaz, K. H., Abolhassani, H., Aboyans, V., Abreu, L. G., Abrigo, M. R. M., Abualhasan, A., Abu-Raddad, L. J., Abushouk, A. I., Adabi, M., Adekanmbi, V., Adeoye, A. M., Adetokunboh, O. O., … Amini, S. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet (London, England)*, *396*(10258), 1204. https://doi.org/10.1016/S0140-6736(20)30925-9

Aggarwal, A., Liu, R., Chen, Y., Ralowicz, A. J., Bergerson, S. J., Tomaska, F., Hanson, T. L., Hasseman, J. P., Reep, D., Tsegaye, G., Yao, P., Ji, X., Kloos, M., Walpita, D., Patel, R., Mohr, M. A., Tilberg, P. W., Mohar, B., GENIE Project Team, T., … Podgorski Visualization, K. (2022). Glutamate indicators with improved activation kinetics and localization for imaging synaptic transmission. *BioRxiv*, 2022.02.13.480251. https://doi.org/10.1101/2022.02.13.480251

Ahrens, S., Wu, M. V., Furlan, A., Hwang, G. R., Paik, R., Li, H., Penzo, M. A., Tollkuhn, J., & Li, B. (2018). A Central Extended Amygdala Circuit That Modulates Anxiety. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *38*(24), 5567–5583. https://doi.org/10.1523/JNEUROSCI.0705-18.2018

Alcántara, C., Gallo, L. C., Wen, J., Dudley, K. A., Wallace, D. M., Mossavar-Rahmani, Y., Sotres-Alvarez, D., Zee, P. C., Ramos, A. R., Petrov, M. E., Casement, M. D., Hall, M. H., Redline, S., & Patel, S. R. (2019). Employment status and the association of sociocultural stress with sleep in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). *Sleep*, *42*(4). https://doi.org/10.1093/SLEEP/ZSZ002

Alheid, G. F. (2003). Extended amygdala and basal forebrain. *Annals of the New York Academy of Sciences*, *985*, 185–205. https://doi.org/10.1111/J.1749-6632.2003.TB07082.X

Alheid, G. F. F., Beltramino, C. A. A., de Olmos, J. S. S., Forbes, M. S. S., Swanson, D. J. J., & Heimer, L. (1998). The neuronal organization of the supracapsular part of the stria terminalis in the rat: The dorsal component of the extended amygdala. *Neuroscience*, *84*(4), 967–996. https://doi.org/10.1016/S0306-4522(97)00560-5

Allen-Petersen, B. L., Miller, M. R., Neville, M. C., Anderson, S. M., Nakayama, K. I., & Reyland, M. E. (2010). Loss of protein kinase C delta alters mammary gland development and apoptosis. *Cell Death & Disease*, *1*(1), e17. https://doi.org/10.1038/CDDIS.2009.20

Allen, H. N., Chaudhry, S., Hong, V. M., Lewter, L. A., Sinha, G. P., Carrasquillo, Y., Taylor, B. K., & Kolber, B. J. (2022). A parabrachial-to-amygdala circuit that determines hemispheric lateralization of somatosensory processing. *Biological Psychiatry*, *0*(0). https://doi.org/10.1016/j.biopsych.2022.09.010

Alvarez, R. P., Kirlic, N., Misaki, M., Bodurka, J., Rhudy, J. L., Paulus, M. P., & Drevets, W. C. (2015). Increased anterior insula activity in anxious individuals is linked to diminished perceived control. *Translational Psychiatry*, *5*(6). https://doi.org/10.1038/TP.2015.84

Amaro, H., Sanchez, M., Bautista, T., & Cox, R. (2021). Social vulnerabilities for substance use: Stressors, socially toxic environments, and discrimination and racism. *Neuropharmacology*, *188*, 108518. https://doi.org/10.1016/J.NEUROPHARM.2021.108518

American Psychiatric Association. (2013). Diagnostic and Statistical Manual of Mental Disorders. *Diagnostic and Statistical Manual of Mental Disorders*. https://doi.org/10.1176/APPI.BOOKS.9780890425596

American Psychological Association. (2023). *APA Dictionary of Psychology - Stress*. https://dictionary.apa.org/stress?_ga=2.266806862.1766047099.1676574733-2006045312.1676574730

Andersson, B., & Larsson, B. (1961). Influence of local temperature changes in the preoptic area and rostral hypothalamus on the regulation of food and water intake. *Acta Physiologica Scandinavica*,

52(1), 75–89. https://doi.org/10.1111/J.1748-1716.1961.TB02203.X

APA Div. 12 (Society of Clinical Psychology). (2017, July). *What is Cognitive Behavioral Therapy?* https://www.apa.org/ptsd-guideline/patients-and-families/cognitive-behavioral

Aschbacher, K., Kornfeld, S., Picard, M., Puterman, E., Havel, P. J., Stanhope, K., Lustig, R. H., & Epel, E. (2014). Chronic Stress Increases Vulnerability to Diet-Related Abdominal Fat, Oxidative Stress, and Metabolic Risk. *Psychoneuroendocrinology*, *46*, 14. https://doi.org/10.1016/J.PSYNEUEN.2014.04.003

Ashcraft, M. H., & Kirk, E. P. (2001). The Relationships Among Working Memory, Math Anxiety, and Performance. *Journal of Experimental Psychology: General*, *130*(2), 224–237. https://doi.org/10.1037/0096-3445.130.2.224

Asok, A., Draper, A., Hoffman, A. F., Schulkin, J., Lupica, C. R., & Rosen, J. B. (2018). Optogenetic silencing of a corticotropin-releasing factor pathway from the central amygdala to the bed nucleus of the stria terminalis disrupts sustained fear. *Molecular Psychiatry*, *23*(4), 914–922. https://doi.org/10.1038/MP.2017.79

Avery, S. N., Clauss, J. A., & Blackford, J. U. (2016). The Human BNST: Functional Role in Anxiety and Addiction. *Neuropsychopharmacology*, *41*(1), 126–141. https://doi.org/10.1038/npp.2015.185

Babb, J. A., Masini, C. V., Day, H. E. W., & Campeau, S. (2014). Habituation of hypothalamic–pituitary–adrenocortical axis hormones to repeated homotypic stress and subsequent heterotypic stressor exposure in male and female rats. *Stress (Amsterdam, Netherlands)*, *17*(3), 224. https://doi.org/10.3109/10253890.2014.905534

Babb, J. A., Masini, C. V, Day, H. E. W., & Campeau, S. (2013). Sex differences in activated corticotropin-releasing factor neurons within stress-related neurocircuitry and hypothalamic-pituitary-adrenocortical axis hormones following restraint in rats. *Neuroscience*, *234*, 40–52. https://doi.org/10.1016/j.neuroscience.2012.12.051

Bailey, J. E., Papadopoulos, A., Diaper, A., Phillips, S., Schmidt, M. E., Van Der Ark, P., Dourish, C. T., Dawson, G. R., & Nutt, D. J. (2011). Preliminary evidence of anxiolytic effects of the CRF(1) receptor antagonist R317573 in the 7.5% CO(2) proof-of-concept experimental model of human anxiety. *Journal of Psychopharmacology (Oxford, England)*, *25*(9), 1199–1206. https://doi.org/10.1177/0269881111400650

Bajo, M., Cruz, M. T., Siggins, G. R., Messing, R., & Roberto, M. (2008). Protein kinase C epsilon mediation of CRF- and ethanol-induced GABA release in central amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(24), 8410. https://doi.org/10.1073/PNAS.0802302105

Ball, K. T., Stone, E., Best, O., Collins, T., Edson, H., Hagan, E., Nardini, S., Neuciler, P., Smolinsky, M., Tosh, L., & Woodlen, K. (2018). Chronic restraint stress during withdrawal increases vulnerability to drug priming-induced cocaine seeking via a dopamine D1-like receptor-mediated mechanism. *Drug and Alcohol Dependence*, *187*, 327. https://doi.org/10.1016/J.DRUGALCDEP.2018.03.024

Ball, T. M., & Gunaydin, L. A. (2022). Measuring maladaptive avoidance: from animal models to clinical anxiety. *Neuropsychopharmacology*, *47*(5), 978. https://doi.org/10.1038/S41386-021-01263-4

Ballaz, S. J., Bourin, M., Akil, H., & Watson, S. J. (2020). BLOCKADE OF THE CHOLECYSTOKININ CCK-2 RECEPTOR PREVENTS THE NORMALIZATION OF ANXIETY LEVELS IN THE RAT. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *96*, 109761. https://doi.org/10.1016/J.PNPBP.2019.109761

Balon, R., & Starcevic, V. (2020). Role of benzodiazepines in anxiety disorders. *Advances in Experimental Medicine and Biology*, *1191*, 367–388. https://doi.org/10.1007/978-981-32-9705-0_20

Bandelow, B., & Michaelis, S. (2022). Epidemiology of anxiety disorders in the 21st century. *Https://Doi.Org/10.31887/DCNS.2015.17.3/Bbandelow*, *17*(3), 327–335. https://doi.org/10.31887/DCNS.2015.17.3/BBANDELOW

Bandelow, B., Reitt, M., Röver, C., Michaelis, S., Görlich, Y., & Wedekind, D. (2015). Efficacy of treatments for anxiety disorders: A meta-analysis. *International Clinical Psychopharmacology*, *30*(4), 183–192. https://doi.org/10.1097/YIC.0000000000000078

Bando, Y., Sakamoto, M., Kim, S., Ayzenshtat, I., & Yuste, R. (2019). Comparative Evaluation of Genetically Encoded Voltage Indicators. *Cell Reports*, *26*(3), 802-813.e4. https://doi.org/10.1016/j.celrep.2018.12.088

Bandoli, G., Jelliffe-Pawlowski, L. L., Feuer, S. K., Liang, L., Oltman, S. P., Paynter, R., Ross, K. M., Schetter, C. D., Ryckman, K. K., & Chambers, C. D. (2018). Second trimester serum cortisol and preterm birth: an analysis by timing and subtype. *Journal of Perinatology : Official Journal of the California Perinatal Association*, *38*(8), 973. https://doi.org/10.1038/S41372-018-0128-5

Banihashemi, L., & Rinaman, L. (2006). Noradrenergic inputs to the bed nucleus of the stria terminalis and paraventricular nucleus of the hypothalamus underlie hypothalamic-pituitary-adrenal axis but not hypophagic or conditioned avoidance responses to systemic yohimbine. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(44), 11442–11453. https://doi.org/10.1523/JNEUROSCI.3561-06.2006

Banihashemi, L., Sheu, L. K., Midei, A. J., & Gianaros, P. J. (2015). Childhood physical abuse predicts stressor-evoked activity within central visceral control regions. *Social Cognitive and Affective Neuroscience*, *10*(4), 474. https://doi.org/10.1093/SCAN/NSU073

Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J., & Van Ijzendoorn, M. H. (2007). Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychological Bulletin*, *133*(1), 1–24. https://doi.org/10.1037/0033-2909.133.1.1

Barbier, M., González, J. A., Houdayer, C., Burdakov, D., Risold, P. Y., & Croizier, S. (2021). Projections from the dorsomedial division of the bed nucleus of the stria terminalis to hypothalamic nuclei in the mouse. *The Journal of Comparative Neurology*, *529*(5), 929. https://doi.org/10.1002/CNE.24988

Barnett, S. A. (1963). The rat: A study in behavior. In *The Rat: A Study in Behavior* (1st ed.). Taylor and Francis. https://doi.org/10.4324/9781315134468/RAT-BARNETT

Basu, A., & Pal, D. (2010). Two Faces of Protein Kinase Cδ: The Contrasting Roles of PKCδ in Cell Survival and Cell Death. *The Scientific World Journal*, *10*, 2272. https://doi.org/10.1100/TSW.2010.214

Bäumler, G. (1994). On the validity of the Yerkes-Dodson law. *Studia Psychologica*, *36*(3), 205. http://proxy.library.vanderbilt.edu/login?url=https://www.proquest.com/scholarly-journals/on-validity-yerkes-dodson-law/docview/1306136110/se-2?accountid=14816

Bäumler, G., & Lienert, G. A. (1993). Re-evaluation of the Yerkes-Dodson law by nonparametric tests of trend. *Studia Psychologica*, *35*, 431–436.

Bayless, D. W., Yang, T., Mason, M. M., Susanto, A. A. T., Lobdell, A., & Shah, N. M. (2019). Limbic Neurons Shape Sex Recognition and Social Behavior in Sexually Naive Males. *Cell*, *176*(5), 1190-1205.e20. https://doi.org/10.1016/j.cell.2018.12.041

Bays, H. E., Kulkarni, A., German, C., Satish, P., Iluyomade, A., Dudum, R., Thakkar, A., Rifai, M. Al, Mehta, A., Thobani, A., Al-Saiegh, Y., Nelson, A. J., Sheth, S., & Toth, P. P. (2022). Ten things to know about ten cardiovascular disease risk factors – 2022. *American Journal of Preventive Cardiology*, *10*, 100342. https://doi.org/10.1016/J.AJPC.2022.100342

Bechtholt, A. J., Valentino, R. J., & Lucki, I. (2007). Overlapping and Distinct Brain Regions Associated with the Anxiolytic Effects of Chlordiazepoxide and Chronic Fluoxetine. *Neuropsychopharmacology 2008 33:9*, *33*(9), 2117–2130. https://doi.org/10.1038/sj.npp.1301616

Bell, K. A., Kobayashi, I., Akeeb, A., Lavela, J., & Mellman, T. A. (2019). Emotional response to perceived racism and nocturnal heart rate variability in young adult African Americans. *Journal of Psychosomatic Research*, *121*, 88–92. https://doi.org/10.1016/J.JPSYCHORES.2019.03.180

Benjamin, E. J., Blaha, M. J., Chiuve, S. E., Cushman, M., Das, S. R., Deo, R., De Ferranti, S. D., Floyd, J., Fornage, M., Gillespie, C., Isasi, C. R., Jim'nez, M. C., Jordan, L. C., Judd, S. E.,

Lackland, D., Lichtman, J. H., Lisabeth, L., Liu, S., Longenecker, C. T., … Muntner, P. (2017). Heart Disease and Stroke Statistics—2017Update: A Report From the American Heart Association. *Circulation*, *135*(10), e146. https://doi.org/10.1161/CIR.0000000000000485

Benner, A. D., Wang, Y., Shen, Y., Boyle, A. E., Polk, R., & Cheng, Y. P. (2018). Racial/ethnic discrimination and well-being during adolescence: A meta-analytic review. *The American Psychologist*, *73*(7), 855. https://doi.org/10.1037/AMP0000204

Bernard, D. L., Smith, Q., & Lanier, P. (2022). Racial discrimination and other adverse childhood experiences as risk factors for internalizing mental health concerns among Black youth. *Journal of Traumatic Stress*, *35*(2), 473–483. https://doi.org/10.1002/JTS.22760

Berry, S. C., Lawrence, A. D., Lancaster, T. M., Casella, C., Aggleton, J. P., & Postans, M. (2022). Subiculum–BNST structural connectivity in humans and macaques. *Neuroimage*, *253*. https://doi.org/10.1016/J.NEUROIMAGE.2022.119096

Betley, J. N., Cao, Z. F. H., Ritola, K. D., & Sternson, S. M. (2013). Parallel, redundant circuit organization for homeostatic control of feeding behavior. *Cell*, *155*(6). https://doi.org/10.1016/J.CELL.2013.11.002

Beyeler, A., & Dabrowska, J. (2020). Neuronal diversity of the amygdala and the bed nucleus of the stria terminalis. In *Handbook of Behavioral Neuroscience* (Vol. 26, pp. 63–100). Elsevier B.V. https://doi.org/10.1016/B978-0-12-815134-1.00003-9

Bhatnagar, S., & Dallman, M. F. (1999). The paraventricular nucleus of the thalamus alters rhythms in core temperature and energy balance in a state-dependent manner. *Brain Research*, *851*(1–2), 66–75. https://doi.org/10.1016/S0006-8993(99)02108-3

Bienkowski, M. S., & Rinaman, L. (2013). Common and distinct neural inputs to the medial central nucleus of the amygdala and anterior ventrolateral bed nucleus of stria terminalis in rats. *Brain Structure and Function*, *218*(1), 187–208. https://doi.org/10.1007/s00429-012-0393-6

Blackmore, E. R., Stansfeld, S. A., Weller, I., Munce, S., Zagorski, B. M., & Stewart, D. E. (2007). Major Depressive Episodes and Work Stress: Results From a National Population Survey. *American Journal of Public Health*, *97*(11), 2088. https://doi.org/10.2105/AJPH.2006.104406

Block, C. H., & Hoffman, G. E. (1987). Neuropeptide and monoamine components of the parabrachial pontine complex. *Peptides*, *8*(2), 267–283. https://doi.org/10.1016/0196-9781(87)90102-1

Bodnoff, S. R., Suranyi-Cadotte, B., Aitken, D. H., Quirion, R., & Meaney, M. J. (1988). The effects of chronic antidepressant treatment in an animal model of anxiety. *Psychopharmacology*, *95*(3), 298–302. https://doi.org/10.1007/BF00181937

Bosch, O. J., Pförtsch, J., Beiderbeck, D. I., Landgraf, R., & Neumann, I. D. (2010). Maternal behaviour is associated with vasopressin release in the medial preoptic area and bed nucleus of the stria terminalis in the rat. *Journal of Neuroendocrinology*, *22*(5), 420–429. https://doi.org/10.1111/j.1365-2826.2010.01984.x

Botta, P., Demmou, L., Kasugai, Y., Markovic, M., Xu, C., Fadok, J. P., Lu, T., Poe, M. M., Xu, L., Cook, J. M., Rudolph, U., Sah, P., Ferraguti, F., & Lüthi, A. (2015). Regulating anxiety with extrasynaptic inhibition. *Nature Neuroscience*, *18*(10), 1493–1500. https://doi.org/10.1038/nn.4102

Bourin, M. (2015). Animal models for screening anxiolytic-like drugs: a perspective. *Dialogues in Clinical Neuroscience*, *17*(3), 295. https://doi.org/10.31887/DCNS.2015.17.3/MBOURIN

Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology*, *463*(1–3), 55–65. https://doi.org/10.1016/S0014-2999(03)01274-3

Bourne, L. E., & Yaroush, R. A. (2003). *Stress and Cognition 1 STRESS AND COGNITION: A COGNITIVE PSYCHOLOGICAL PERSPECTIVE National Aeronautics and Space Administration Grant Number NAG2-1561 Stress and Cognition 2 Outline Stress and Cognition: A Cognitive Psychological Perspective Introduction Cognition in Emergency and other Abnormal Situations Acute and Chronic Stress Teamwork under Emergencies*. https://human-factors.arc.nasa.gov/flightcognition/download/misc/Stress and Cognition.pdf

Bowers, B. J., Owen, E. H., Collins, A. C., Abeliovich, A., Tonegawa, S., & Wehner, J. M. (1999).

Decreased Ethanol Sensitivity and Tolerance Development in γ-Protein Kinase C Null Mutant Mice Is Dependent on Genetic Background. *Alcoholism: Clinical and Experimental Research*, *23*(3), 387–397. https://doi.org/10.1111/J.1530-0277.1999.TB04127.X

Bowers, B. J., & Wehner, J. M. (2001). Ethanol Consumption and Behavioral Impulsivity Are Increased in Protein Kinase Cγ Null Mutant Mice. *The Journal of Neuroscience*, *21*(21), RC180. https://doi.org/10.1523/JNEUROSCI.21-21-J0004.2001

Boyle, M. P., Brewer, J. A., Funatsu, M., Wozniak, D. F., Tsien, J. Z., Izumi, Y., & Muglia, L. J. (2005). Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(2), 473–478. https://doi.org/10.1073/PNAS.0406458102

Brewin, C. R. (1996). Theoretical foundations of cognitive-behavior therapy for anxiety and depression. *Annual Review of Psychology*, *47*, 33–57. https://doi.org/10.1146/ANNUREV.PSYCH.47.1.33

Bria, A., Bernaschi, M., Guarrasi, M., & Iannello, G. (2019). Exploiting Multi-Level Parallelism for Stitching Very Large Microscopy Images. *Frontiers in Neuroinformatics*, *13*. https://doi.org/10.3389/FNINF.2019.00041

Briand, L. A., Vassoler, F. M., Pierce, R. C., Valentino, R. J., & Blendy, J. A. (2010). Ventral tegmental afferents in stress-induced reinstatement: The role of cAMP response element-binding protein. *Journal of Neuroscience*, *30*(48), 16149–16159. https://doi.org/10.1523/JNEUROSCI.2827-10.2010

Britton, D. R., & Britton, K. T. (1981). A sensitive open field measure of anxiolytic drug activity. *Pharmacology, Biochemistry, and Behavior*, *15*(4), 577–582. https://doi.org/10.1016/0091-3057(81)90212-4

Broadbent, D. E. (1963). Differences and Interactions between Stresses. *Quarterly Journal of Experimental Psychology*, *15*(3), 205–211. https://doi.org/10.1080/17470216308416325

Brondolo, E., Love, E. E., Pencille, M., Schoenthaler, A., & Ogedegbe, G. (2011). Racism and hypertension: a review of the empirical evidence and implications for clinical practice. *American Journal of Hypertension*, *24*(5), 518–529. https://doi.org/10.1038/AJH.2011.9

Brown, G. R., & Nemes, C. (2008). The exploratory behaviour of rats in the hole-board apparatus: is head-dipping a valid measure of neophilia? *Behavioural Processes*, *78*(3), 442–448. https://doi.org/10.1016/J.BEPROC.2008.02.019

Brown, J. A., Petersen, N., Centanni, S. W., Jin, A. Y., Yoon, H. J., Cajigas, S. A., Bedenbaugh, M. N., Luchsinger, J. R., Patel, S., Calipari, E. S., Simerly, R. B., & Winder, D. G. (2022). An ensemble recruited by α2a-adrenergic receptors is engaged in a stressor-specific manner in mice. *Neuropsychopharmacology 2022*, 1–11. https://doi.org/10.1038/s41386-022-01442-x

Brown, T. M., & Fee, E. (2002). Walter Bradford Cannon: Pioneer Physiologist of Human Emotions. *American Journal of Public Health*, *92*(10), 1594. /pmc/articles/PMC1447286/

Brown, W. P. (1965). The Yerkes-Dodson Law Repealed. *Psychological Reports*, *17*(2), 663–666. https://doi.org/10.2466/PR0.1965.17.2.663

Bruce, S. E., Yonkers, K. A., Otto, M. W., Eisen, J. L., Weisberg, R. B., Pagano, M., Shea, M. T., & Keller, M. B. (2005). Influence of psychiatric comorbidity on recovery and recurrence in generalized anxiety disorder, social phobia, and panic disorder: A 12-year prospective study. *American Journal of Psychiatry*, *162*(6), 1179–1187. https://doi.org/10.1176/APPI.AJP.162.6.1179

Bruijnzeel, A. W., Stam, R., Compaan, J. C., Croiset, G., Akkermans, L. M. A., Olivier, B., & Wiegant, V. M. (1999). Long-term sensitization of Fos-responsivity in the rat central nervous system after a single stressful experience. *Brain Research*, *819*(1–2), 15–22. https://doi.org/10.1016/S0006-8993(98)01350-X

Bruzsik, B., Biro, L., Zelena, D., Sipos, E., Szebik, H., Sarosdi, K. R., Horvath, O., Farkas, I., Csillag, V., Finszter, C. K., Mikics, E., & Toth, M. (2021). Somatostatin neurons of the bed nucleus of stria terminalis enhance associative fear memory consolidation in mice. *The Journal of Neuroscience*, JN-RM-1944-20. https://doi.org/10.1523/jneurosci.1944-20.2020

Bucci, D. J., & Robinson, S. (2014). Toward a Conceptualization of Retrohippocampal Contributions to Learning and Memory. *Neurobiology of Learning and Memory*, *0*, 197. https://doi.org/10.1016/J.NLM.2014.05.007

Burger, J. M., & Arkin, R. M. (1980). Prediction, control, and learned helplessness. *Journal of Personality and Social Psychology*, *38*(3), 482–491. https://doi.org/10.1037/0022-3514.38.3.482

Buynitsky, T., & Mostofsky, D. I. (2009). Restraint stress in biobehavioral research: Recent developments. *Neuroscience & Biobehavioral Reviews*, *33*(7), 1089–1098. http://www.sciencedirect.com/science/article/pii/S0149763409000748?via%3Dihub

Bystritsky, A. (2006). Treatment-resistant anxiety disorders. *Molecular Psychiatry*, *11*(9), 805–814. https://doi.org/10.1038/SJ.MP.4001852

Cai, H., Haubensak, W., Anthony, T. E., & Anderson, D. J. (2014). Central amygdala PKC-δ+ neurons mediate the influence of multiple anorexigenic signals. *Nature Neuroscience*, *17*(9), 1240–1248. https://doi.org/10.1038/nn.3767

Campi, K. L., Jameson, C. E., & Trainor, B. C. (2013). Sexual dimorphism in the brain of the monogamous california mouse (peromyscus californicus). *Brain, Behavior and Evolution*, *81*(4), 236–249. https://doi.org/10.1159/000353260

Campos, C. A., Bowen, A. J., Roman, C. W., & Palmiter, R. D. (2018). Encoding of danger by parabrachial CGRP neurons Coding of painful stimuli by CGRP PBN neurons. *Nature Publishing Group*. https://doi.org/10.1038/nature25511

Cannon, W. B. (1915). *Bodily changes in pain, hunger, fear, and rage: an account of recent researches into the function of emotional excitement* (1st ed.). D. Appleton and Company. https://archive.org/details/cu31924022542470/mode/1up

Cannon, W. B. (1926). Physiological regulation of normal states: some tentative postulates concerning biological homeostatics. In: Pettit A, editor. A Charles Richet: ses amis, ses collègues, ses élèves (in French). *Paris: Les Éditions Médicales*, 91.

Canteras, N. S., & Swanson, L. W. (1992). Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: a PHAL anterograde tract-tracing study in the rat. *The Journal of Comparative Neurology*, *324*(2), 180–194. https://doi.org/10.1002/CNE.903240204

Cantley, J., Boslem, E., Laybutt, D. R., Cordery, D. V., Pearson, G., Carpenter, L., Leitges, M., & Biden, T. J. (2011). Deletion of protein kinase Cδ in mice modulates stability of inflammatory genes and protects against cytokine-stimulated beta cell death in vitro and in vivo. *Diabetologia*, *54*(2), 380–389. https://doi.org/10.1007/S00125-010-1962-Y

Carboni, E., Silvagni, A., Rolando, M. T., & Di Chiara, G. (2000). Stimulation of in vivo dopamine transmission in the bed nucleus of stria terminalis by reinforcing drugs. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(20), RC102–RC102. https://doi.org/10.1523/jneurosci.20-20-j0002.2000

Carobrez, A. P., & Bertoglio, L. J. (2005). Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. *Neuroscience & Biobehavioral Reviews*, *29*(8), 1193–1205. https://doi.org/10.1016/J.NEUBIOREV.2005.04.017

Carrasquillo, Y., & Gereau, R. W. (2008). Hemispheric lateralization of a molecular signal for pain modulation in the amygdala. *Molecular Pain*, *4*, 24. https://doi.org/10.1186/1744-8069-4-24

Carson, E. A., Golinelli, D., & Statisticians, B. (2014). Prisoners in 2012. *Bureau of Justice Statistics*. https://www.ebony.com/wp-content/uploads/2014/06/p12tar9112.pdf

Carter, M. E., Soden, M. E., Zweifel, L. S., & Palmiter, R. D. (2013). Genetic identification of a neural circuit that suppresses appetite. *Nature*, *503*(7474), 111. https://doi.org/10.1038/NATURE12596

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H. L., McClay, J., Mill, J., Martin, J., Braithwaite, A., & Poulton, R. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science (New York, N.Y.)*, *301*(5631), 386–389. https://doi.org/10.1126/SCIENCE.1083968

Catania, C., Sotiropoulos, I., Silva, R., Onofri, C., Breen, K. C., Sousa, N., & Almeida, O. F. X. (2009). The amyloidogenic potential and behavioral correlates of stress. *Molecular Psychiatry*, *14*(1), 95–105. https://doi.org/10.1038/SJ.MP.4002101

Cecchi, M., Khoshbouei, H., Javors, M., & Morilak, D. A. (2002). Modulatory effects of norepinephrine in the lateral bed nucleus of the stria terminalis on behavioral and neuroendocrine responses to acute stress. *Neuroscience*, *112*(1), 13–21. https://doi.org/10.1016/S0306-4522(02)00062-3

Centanni, S. W., Bedse, G., Patel, S., & Winder, D. G. (2019). Driving the Downward Spiral: Alcohol-Induced Dysregulation of Extended Amygdala Circuits and Negative Affect. *Alcoholism: Clinical and Experimental Research*, *43*(10), 2000–2013. https://doi.org/10.1111/acer.14178

Centanni, S. W., Brown, J. A., Williford, K. M., Flook, E. A., Luchsinger, J. R., & Winder, D. G. (2022). Bed Nucleus of the Stria Terminalis (BNST) circuits. In N. W. Gilpin (Ed.), *Neurocircuitry of Addiction* (pp. 321–374). Academic Press. https://shop.elsevier.com/books/neurocircuitry-of-addiction/gilpin/978-0-12-823453-2

Centanni, S. W., Morris, B. D., Luchsinger, J. R., Bedse, G., Fetterly, T. L., Patel, S., & Winder, D. G. (2019). Endocannabinoid control of the insular-bed nucleus of the stria terminalis circuit regulates negative affective behavior associated with alcohol abstinence. *Neuropsychopharmacology*, *44*(3), 526–537. https://doi.org/10.1038/s41386-018-0257-8

Ch'ng, S., Fu, J., Brown, R. M., Mcdougall, S. J., Lawrence, A. J., Ch, S., Fu, J., Brown, R. M., Mcdougall, S. J., Lawrence, A. J., Ch'ng, S., Fu, J., Brown, R. M., Mcdougall, S. J., & Lawrence, A. J. (2018). The intersection of stress and reward: BNST modulation of aversive and appetitive states. *Progress in Neuropsychopharmacology & Biological Psychiatry*, *87*(August 2017), 108–125. https://doi.org/10.1016/j.pnpbp.2018.01.005

Chastrette, N., Pfaff, D. W., & Gibbs, R. B. (1991). Effects of daytime and nighttime stress on Fos-like immunoreactivity in the paraventricular nucleus of the hypothalamus, the habenula, and the posterior paraventricular nucleus of the thalamus. *Brain Research*, *563*(1–2), 339–344. https://doi.org/10.1016/0006-8993(91)91559-J

Cheadle, J. E., Goosby, B. J., Jochman, J. C., Tomaso, C. C., Yancey, C. B. K., & Nelson, T. D. (2020). Race and ethnic variation in college students' allostatic regulation of racism-related stress. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(49), 31053–31062. https://doi.org/10.1073/PNAS.1922025117/-/DCSUPPLEMENTAL

Chen, J. Y., Campos, C. A., Jarvie, B. C., & Palmiter, R. D. (2018). Parabrachial CGRP neurons establish and sustain aversive taste memories. *Neuron*, *100*(4), 891. https://doi.org/10.1016/J.NEURON.2018.09.032

Chesnut, M., Harati, S., Paredes, P., Khan, Y., Foudeh, A., Kim, J., Bao, Z., & Williams, L. M. (2021). Stress Markers for Mental States and Biotypes of Depression and Anxiety: A Scoping Review and Preliminary Illustrative Analysis. *Chronic Stress*, *5*. https://doi.org/10.1177/24705470211000338

Chichger, H., Grinnell, K. L., Casserly, B., Chung, C. S., Braza, J., Lomas-Neira, J., Ayala, A., Rounds, S., Klinger, J. R., & Harrington, E. O. (2012). Genetic disruption of protein kinase Cδ reduces endotoxin-induced lung injury. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, *303*(10), L880. https://doi.org/10.1152/AJPLUNG.00169.2012

Choi, D. C., Furay, A. R., Evanson, N. K., Ostrander, M. M., Ulrich-Lai, Y. M., & Herman, J. P. (2007). Bed Nucleus of the Stria Terminalis Subregions Differentially Regulate Hypothalamic-Pituitary-Adrenal Axis Activity: Implications for the Integration of Limbic Inputs. *Journal of Neuroscience*, *27*(8), 2025–2034. https://doi.org/10.1523/JNEUROSCI.4301-06.2007

Choi, D. C., Furay, A. R., Evanson, N. K., Ulrich-Lai, Y. M., Nguyen, M. M. N., Ostrander, M. M., & Herman, J. P. (2008). The role of the posterior medial bed nucleus of the stria terminalis in modulating hypothalamic-pituitary-adrenocortical axis responsiveness to acute and chronic stress. *Psychoneuroendocrinology*, *33*(5), 659–669. https://doi.org/10.1016/j.psyneuen.2008.02.006

Choi, D., Wei, W., Deitchman, J. K., Kharazia, V. N., Lesscher, H. M. B., Mcmahon, T., Wang, D., Qi, Z., Sieghart, W., Zhang, C., Shokat, K. M., Mody, I., & Messing, R. O. (2008). Protein Kinase C delta Regulates Ethanol Intoxication and Enhancement of GABA-Stimulated Tonic Current. *J*

Neurosci, *28*(46), 11890–11899. https://doi.org/10.1523/JNEUROSCI.3156-08.2008

Chou, N., Shin, H., Kim, K., Chae, U., Jang, M., Jeong, U. J., Hwang, K. S., Yi, B., Lee, S. E., Woo, J., Cho, Y., Lee, C., Baker, B. J., Oh, S. J., Nam, M. H., Choi, N., & Cho, I. J. (2022). A Multimodal Multi-Shank Fluorescence Neural Probe for Cell-Type-Specific Electrophysiology in Multiple Regions across a Neural Circuit. *Advanced Science*, *9*(2), 2103564. https://doi.org/10.1002/ADVS.202103564

Chou, W. H., & Messing, R. O. (2008). Hypertensive encephalopathy and the blood-brain barrier: is δPKC a gatekeeper? *The Journal of Clinical Investigation*, *118*(1), 17. https://doi.org/10.1172/JCI34516

Christianson, S. Å. (1992). Emotional stress and eyewitness memory: a critical review. *Psychological Bulletin*, *112*(2), 284–309. https://doi.org/10.1037/0033-2909.112.2.284

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nature Reviews Endocrinology 2009 5:7*, *5*(7), 374–381. https://doi.org/10.1038/NRENDO.2009.106

Ciccocioppo, R., Fedeli, A., Economidou, D., Policani, F., Weiss, F., & Massi, M. (2003). The Bed Nucleus Is a Neuroanatomical Substrate for the Anorectic Effect of Corticotropin-Releasing Factor and for Its Reversal by Nociceptin/Orphanin FQ. *The Journal of Neuroscience*, *23*(28), 9445. https://doi.org/10.1523/jneurosci.23-28-09445.2003

Ciocchi, S., Herry, C., Grenier, F., Wolff, S. B. E., Letzkus, J. J., Vlachos, I., Ehrlich, I., Sprengel, R., Deisseroth, K., Stadler, M. B., Müller, C., & Lüthi, A. (2010a). Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature 2010 468:7321*, *468*(7321), 277–282. https://doi.org/10.1038/nature09559

Ciocchi, S., Herry, C., Grenier, F., Wolff, S. B. E., Letzkus, J. J., Vlachos, I., Ehrlich, I., Sprengel, R., Deisseroth, K., Stadler, M. B., Müller, C., & Lüthi, A. (2010b). Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature*, *468*(7321), 277–282. https://doi.org/10.1038/nature09559

Cisler, J. M., & Koster, E. H. W. (2010). Mechanisms of attentional biases towards threat in anxiety disorders: An integrative review. *Clinical Psychology Review*, *30*(2), 203–216. https://doi.org/10.1016/J.CPR.2009.11.003

Clark, R., Anderson, N. B., Clark, V. R., & Williams, D. R. (1999). Racism as a stressor for African Americans. A biopsychosocial model. *The American Psychologist*, *54*(10), 805–816. https://doi.org/10.1037//0003-066X.54.10.805

Clark, T. T., Salas-Wright, C. P., Vaughn, M. G., & Whitfield, K. E. (2015). Everyday Discrimination and Mood and Substance Use Disorders: A Latent Profile Analysis with African Americans and Caribbean Blacks. *Addictive Behaviors*, *40*, 119. https://doi.org/10.1016/J.ADDBEH.2014.08.006

Cohen, S. (1980). Aftereffects of stress on human performance and social behavior: A review of research and theory. *Psychological Bulletin*, *88*(1), 82–108. https://doi.org/10.1037/0033-2909.88.1.82

Cohen, S., Janicki-Deverts, D., Doyle, W. J., Miller, G. E., Frank, E., Rabin, B. S., & Turner, R. B. (2012). Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(16), 5995. https://doi.org/10.1073/PNAS.1118355109

Cohen, S., & Weinstein, N. (1981). Nonauditory Effects of Noise on Behavior and Health. *Journal of Social Issues*, *37*(1), 36–70. https://doi.org/10.1111/J.1540-4560.1981.TB01057.X

Cole, L. W. (1911). The relation of strength of stimulus to rate of learning in the chick. *Journal of Animal Behavior*, *1*(2), 111–124. https://doi.org/10.1037/H0074224

Commons, K. G., Connolley, K. R., & Valentino, R. J. (2003). A neurochemically distinct dorsal raphe-limbic circuit with a potential role in affective disorders. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *28*(2), 206–215. https://doi.org/10.1038/SJ.NPP.1300045

Comoli, E., Ribeiro-Barbosa, É. R., Negrão, N., Goto, M., & Canteras, N. S. (2005). Functional mapping

of the prosencephalic systems involved in organizing predatory behavior in rats. *Neuroscience*, *130*(4), 1055–1067. https://doi.org/10.1016/J.NEUROSCIENCE.2004.10.020

Companion, M. A., & Thiele, T. E. (2018). Assessment of ventral tegmental area-projecting GABAergic neurons from the bed nucleus of the stria terminalis in modulating binge-like ethanol intake. *European Journal of Neuroscience*, *48*(11), 3335–3343. https://doi.org/10.1111/ejn.14222

Conrad, K. L., Louderback, K. M., Gessner, C. P., & Winder, D. G. (2011). Stress-induced alterations in anxiety-like behavior and adaptations in plasticity in the bed nucleus of the stria terminalis. *Physiology and Behavior*, *104*(2), 248–256. https://doi.org/10.1016/j.physbeh.2011.03.001

Cook, C. J. (2004). Stress induces CRF release in the paraventricular nucleus, and both CRF and GABA release in the amygdala. *Physiology and Behavior*, *82*(4), 751–762. https://doi.org/10.1016/j.physbeh.2004.06.013

Cooper, A., Sneider, J., Cohen-Gilbert, J., Blackford, J., Potter, A., & Silveri, M. (2021). Brain Mechanisms of Perceived Discrimination in Black Youth: Results From the ABCD Study. *ACNP 60th Annual Meeting: Poster Abstracts P1 - P275*. https://doi.org/10.1038/s41386-021-01236-7

Cooper, C. L., & Dewe, P. (2007). *Stress : a Brief History.* John Wiley & Sons. https://www.google.com/books/edition/Stress/nQZEssR1RBUC?hl=en&gbpv=0

Cryan, J. F., & Sweeney, F. F. (2011). The age of anxiety: role of animal models of anxiolytic action in drug discovery. *British Journal of Pharmacology*, *164*(4), 1129. https://doi.org/10.1111/J.1476-5381.2011.01362.X

Cui, Y., Lv, G., Jin, S., Peng, J., Yuan, J., He, X., Gong, H., Xu, F., Xu, T., & Li, H. (2017). A Central Amygdala-Substantia Innominata Neural Circuitry Encodes Aversive Reinforcement Signals. *Cell Reports*, *21*(7), 1770–1782. https://doi.org/10.1016/J.CELREP.2017.10.062

Cullinan, W. E., Herman, J. P., & Watson, S. J. (1993). Ventral subicular interaction with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis. *The Journal of Comparative Neurology*, *332*(1), 1–20. https://doi.org/10.1002/CNE.903320102

Dabrowska, J., Hazra, R., Ahern, T. H., Guo, J.-D., McDonald, A. J., Mascagni, F., Muller, J. F., Young, L. J., & Rainnie, D. G. (2011). Neuroanatomical evidence for reciprocal regulation of the corticotrophin-releasing factor and oxytocin systems in the hypothalamus and the bed nucleus of the stria terminalis of the rat: Implications for balancing stress and affect. *Psychoneuroendocrinology*, *36*(9), 1312–1326. https://doi.org/10.1016/J.PSYNEUEN.2011.03.003

Dana, H., Sun, Y., Mohar, B., Hulse, B. K., Kerlin, A. M., Hasseman, J. P., Tsegaye, G., Tsang, A., Wong, A., Patel, R., Macklin, J. J., Chen, Y., Konnerth, A., Jayaraman, V., Looger, L. L., Schreiter, E. R., Svoboda, K., & Kim, D. S. (2019). High-performance calcium sensors for imaging activity in neuronal populations and microcompartments. *Nature Methods 2019 16:7*, *16*(7), 649–657. https://doi.org/10.1038/s41592-019-0435-6

Daniel, S. E., Guo, J., & Rainnie, D. G. (2017). A comparative analysis of the physiological properties of neurons in the anterolateral bed nucleus of the stria terminalis in the *Mus musculus* , *Rattus norvegicus* , and *Macaca mulatta*. *Journal of Comparative Neurology*, *525*(9), 2235–2248. https://doi.org/10.1002/cne.24202

Daniel, S. E., & Rainnie, D. G. (2015). Stress Modulation of Opposing Circuits in the Bed Nucleus of the Stria Terminalis. *Neuropsychopharmacology*, *41*(1), 1–23. https://doi.org/10.1038/npp.2015.178

Davies, K. J. A. (2016). Adaptive Homeostasis. *Molecular Aspects of Medicine*, *49*, 1. https://doi.org/10.1016/J.MAM.2016.04.007

Davis, M., Walker, D. L., & Lee, Y. (1997). Roles of the amygdala and bed nucleus of the stria terminalis in fear and anxiety measured with the acoustic startle reflex. Possible relevance to PTSD. *Annals of the New York Academy of Sciences*, *821*, 305–331. https://doi.org/10.1111/J.1749-6632.1997.TB48289.X

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs sustained fear in rats and humans: Role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology*, *35*(1), 105–135.

https://doi.org/10.1038/npp.2009.109

de Guglielmo, G., Kallupi, M., Pomrenze, M. B., Crawford, E., Simpson, S., Schweitzer, P., Koob, G. F., Messing, R. O., & George, O. (2019). Inactivation of a CRF-dependent amygdalofugal pathway reverses addiction-like behaviors in alcohol-dependent rats. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-09183-0

De Kloet, E. R. (2013). Functional profile of the binary brain corticosteroid receptor system: Mediating, multitasking, coordinating, integrating. *European Journal of Pharmacology*, *719*(1–3), 53–62. https://doi.org/10.1016/J.EJPHAR.2013.04.053

de Voogd, L. D., Hagenberg, E., Zhou, Y. J., de Lange, F. P., & Roelofs, K. (2022). Acute threat enhances perceptual sensitivity without affecting the decision criterion. *Scientific Reports*, *12*(1). https://doi.org/10.1038/S41598-022-11664-0

Dean, K. M., Roudot, P., Welf, E. S., Danuser, G., & Fiolka, R. (2015). Deconvolution-free Subcellular Imaging with Axially Swept Light Sheet Microscopy. *Biophysical Journal*, *108*(12), 2807–2815. https://doi.org/10.1016/j.bpj.2015.05.013

Delfs, J. M., Zhu, Y., Druhan, J. P., & Aston-Jones, G. (2000). Noradrenaline in the ventral forebrain is critical for opiate withdrawal-induced aversion. *Nature*, *403*(6768), 430–434. https://doi.org/10.1038/35000212

Desai, S. J., Upadhya, M. A., Subhedar, N. K., & Kokare, D. M. (2013). NPY mediates reward activity of morphine, via NPY Y1 receptors, in the nucleus accumbens shell. *Behavioural Brain Research*, *247*, 79–91. https://doi.org/10.1016/j.bbr.2013.03.018

Dhabhar, F. S. (2009). Enhancing versus Suppressive Effects of Stress on Immune Function: Implications for Immunoprotection and Immunopathology. *Neuroimmunomodulation*, *16*(5), 300. https://doi.org/10.1159/000216188

Dilly, G. A., Kittleman, C. W., Kerr, T. M., Messing, R. O., & Mayfield, R. D. (2022). Cell-type specific changes in PKC-delta neurons of the central amygdala during alcohol withdrawal. *Translational Psychiatry*, *12*(1). https://doi.org/10.1038/S41398-022-02063-0

Dinan, T. G. (2005). Stress: the shared common component in major mental illnesses. *European Psychiatry : The Journal of the Association of European Psychiatrists*, *20 Suppl 3*(SUPPL. 3). https://doi.org/10.1016/S0924-9338(05)80184-1

Dobolyi, A., Irwin, S., Makara, G., Usdin, T. B., & Palkovits, M. (2005). Calcitonin gene-related peptide-containing pathways in the rat forebrain. *The Journal of Comparative Neurology*, *489*(1), 92–119. https://doi.org/10.1002/CNE.20618

Dodson, J. D. (1915). The relation of strength of stimulus to rapidity of habit-formation in the kitten. *Journal of Animal Behavior*, *5*(4), 330–336. https://doi.org/10.1037/H0073415

Domi, E., Xu, L., Toivainen, S., Nordeman, A., Gobbo, F., Venniro, M., Shaham, Y., Messing, R. O., Visser, E., van den Oever, M. C., Holm, L., Barbier, E., Augier, E., & Heilig, M. (2021). A neural substrate of compulsive alcohol use. *Science Advances*, *7*(34). https://doi.org/10.1126/SCIADV.ABG9045

Dong, H.-W., & Swanson, L. W. (2004). Projections from bed nuclei of the stria terminalis, posterior division: Implications for cerebral hemisphere regulation of defensive and reproductive behaviors. *The Journal of Comparative Neurology*, *471*(4), 396–433. https://doi.org/10.1002/cne.20002

Dong, H.-W., & Swanson, L. W. (2006). Projections from bed nuclei of the stria terminalis, magnocellular nucleus: Implications for cerebral hemisphere regulation of micturition, defecation, and penile erection. *The Journal of Comparative Neurology*, *494*(1), 108–141. https://doi.org/10.1002/cne.20789

Dong, H., Petrovich, G. D., Watts, A. G., & Swanson, L. W. (2001). *Basic Organization of Projections From the Oval and Fusiform Nuclei of the Bed Nuclei of the Stria Terminalis in Adult Rat Brain*. *455*(April), 430–455.

Dong, H., & Swanson, L. W. (2004). *Organization of Axonal Projections from the Anterolateral Area of the Bed Nuclei of the Stria Terminalis*. *298*(June 2003), 277–298.

https://doi.org/10.1002/cne.10949

Dong, H. W., Petrovich, G. D., & Swanson, L. W. (2001). Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Research Reviews*, *38*(1–2), 192–246. https://doi.org/10.1016/S0165-0173(01)00079-0

Dong, H. W., & Swanson, L. W. (2006a). Projections from bed nuclei of the stria terminalis, anteromedial area: Cerebral hemisphere integration of neuroendocrine, autonomic, and behavioral aspects of energy balance. *Journal of Comparative Neurology*, *494*(1), 142–178. https://doi.org/10.1002/cne.20788

Dong, H. W., & Swanson, L. W. (2006b). Projections from bed nuclei of the stria terminalis, dorsomedial nucleus: Implications for cerebral hemisphere integration of neuroendocrine, autonomic, and drinking responses. *Journal of Comparative Neurology*, *494*(1), 75–107. https://doi.org/10.1002/cne.20790

Dulawa, S. C., & Hen, R. (2005). Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. *Neuroscience and Biobehavioral Reviews*, *29*(4–5), 771–783. https://doi.org/10.1016/J.NEUBIOREV.2005.03.017

Dumais, K. M., Alonso, A. G., Immormino, M. A., Bredewold, R., & Veenema, A. H. (2016). Involvement of the oxytocin system in the bed nucleus of the stria terminalis in the sex-specific regulation of social recognition. *Psychoneuroendocrinology*, *64*, 79–88. https://doi.org/10.1016/j.psyneuen.2015.11.007

Dunn, J. D., & Williams, T. J. (1995). Cardiovascular responses to electrical stimulation of the bed nucleus of the stria terminalis. *The Journal of Comparative Neurology*, *352*(2), 227–234. https://doi.org/10.1002/cne.903520206

Duque-Wilckens, N., Steinman, M. Q., Busnelli, M., Chini, B., Yokoyama, S., Pham, M., Laredo, S. A., Hao, R., Perkeybile, A. M., Minie, V. A., Tan, P. B., Bales, K. L., & Trainor, B. C. (2018). Oxytocin Receptors in the Anteromedial Bed Nucleus of the Stria Terminalis Promote Stress-Induced Social Avoidance in Female California Mice. *Biological Psychiatry*, *83*(3), 203–213. https://doi.org/10.1016/j.biopsych.2017.08.024

Duque-Wilckens, N., Torres, L. Y., Yokoyama, S., Minie, V. A., Tran, A. M., Petkova, S. P., Hao, R., Ramos-Maciel, S., Rios, R. A., Jackson, K., Flores-Ramirez, F. J., Garcia-Carachure, I., Pesavento, P. A., Iniguez, S. D., Grinevich, V., & Trainor, B. C. (2020). Extrahypothalamic oxytocin neurons drive stress-induced social vigilance and avoidance. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(42), 26406–26413. https://doi.org/10.1073/pnas.2011890117

Duvarci, S., Bauer, E. P., & Paré, D. (2009). The Bed Nucleus of the Stria Terminalis Mediates Inter-individual Variations in Anxiety and Fear. *The Journal of Neuroscience*, *29*(33), 10357. https://doi.org/10.1523/JNEUROSCI.2119-09.2009

Duvarci, S., Popa, D., & Paré, D. (2011). Central Amygdala Activity during Fear Conditioning. *Journal of Neuroscience*, *31*(1), 289–294. https://doi.org/10.1523/JNEUROSCI.4985-10.2011

Easterbrook, J. A. (1959). The effect of emotion on cue utilization and the organization of behavior. *Psychological Review*, *66*(3), 183–201. https://doi.org/10.1037/H0047707

Ebner, K., Muigg, P., Singewald, G., & Singewald, N. (2008). Substance P in Stress and Anxiety. *Annals of the New York Academy of Sciences*, *1144*(1), 61–73. https://doi.org/10.1196/annals.1418.018

Ebner, K., & Singewald, N. (2006). The role of substance P in stress and anxiety responses. *Amino Acids*, *31*(3), 251–272. https://doi.org/10.1007/S00726-006-0335-9

Edwards, J. R., & Cooper, C. L. (1990). The person-environment fit approach to stress: Recurring problems and some suggested solutions. *Journal of Organizational Behavior*, *11*(4), 293–307. https://doi.org/10.1002/JOB.4030110405

Egan, L. J., & Dennis-Tiwary, T. A. (2018). Dynamic Measures of Anxiety-Related Threat Bias: Links to Stress Reactivity. *Motivation and Emotion*, *42*(4), 546. https://doi.org/10.1007/S11031-018-9674-6

Egli, R. E., & Winder, D. G. (2003). Dorsal and Ventral Distribution of Excitable and Synaptic Properties of Neurons of the Bed Nucleus of the Stria Terminalis. *Journal of Neurophysiology*, *90*(1), 405–414. https://doi.org/10.1152/jn.00228.2003

Elias, A., & Paradies, Y. (2021). The Costs of Institutional Racism and its Ethical Implications for Healthcare. *Journal of Bioethical Inquiry*, *18*(1), 45. https://doi.org/10.1007/S11673-020-10073-0

Emmons, R., Sadok, T., Rovero, N. G., Belnap, M. A., Henderson, H. J. M., Quan, A. J., Del Toro, N. J., & Halladay, L. R. (2021). Chemogenetic manipulation of the bed nucleus of the stria terminalis counteracts social behavioral deficits induced by early life stress in C57BL/6J mice. *Journal of Neuroscience Research*, *99*(1), 90–109. https://doi.org/10.1002/jnr.24644

Ericsson, A., Kovács, K. J., & Sawchenko, P. E. (1994). A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. *Journal of Neuroscience*, *14*(2), 897–913. https://doi.org/10.1523/JNEUROSCI.14-02-00897.1994

Everson-Rose, S. A., & Lewis, T. T. (2005). Psychosocial factors and cardiovascular diseases. *Annual Review of Public Health*, *26*, 469–500. https://doi.org/10.1146/ANNUREV.PUBLHEALTH.26.021304.144542

Eysenck Royal Holloway, M. W., Press HOVE, X., York Hove, N., & York, N. (2014). Anxiety and Cognition : A Unified Theory. *Anxiety and Cognition*. https://doi.org/10.4324/9781315804606

Fadok, J. P., Krabbe, S., Markovic, M., Courtin, J., Xu, C., Massi, L., Botta, P., Bylund, K., Müller, C., Kovacevic, A., Tovote, P., & Lüthi, A. (2017). A competitive inhibitory circuit for selection of active and passive fear responses. *Nature*, *542*(7639), 96–100. https://doi.org/10.1038/nature21047

Fali, T., Vallet, H., & Sauce, D. (2018). Impact of stress on aged immune system compartments: Overview from fundamental to clinical data. *Experimental Gerontology*, *105*, 19–26. https://doi.org/10.1016/J.EXGER.2018.02.007

Falk, J. L., & Bindra, D. (1954). Judgment of time as a function of serial position and stress. *Journal of Experimental Psychology*, *47*(4), 279–282. https://doi.org/10.1037/H0061946

Fanselow, M. S., Lester, L. S., & Helmstetter, F. J. (1988). Changes in feeding and foraging patterns as an antipredator defensive strategy: a laboratory simulation using aversive stimulation in a closed economy. *Journal of the Experimental Analysis of Behavior*, *50*(3), 361. https://doi.org/10.1901/JEAB.1988.50-361

Fendt, M., Siegl, S., & Steiniger-Brach, B. (2005). Noradrenaline transmission within the ventral bed nucleus of the stria terminalis is critical for fear behavior induced by trimethylthiazoline, a component of fox odor. *Journal of Neuroscience*, *25*(25), 5998–6004. https://doi.org/10.1523/JNEUROSCI.1028-05.2005

Fenno, L., Yizhar, O., & Deisseroth, K. (2011). The development and application of optogenetics. *Annual Review of Neuroscience*, *34*(1), 389–412. https://doi.org/10.1146/annurev-neuro-061010-113817

Fetterly, T. L., Basu, A., Nabit, B. P., Awad, E., Williford, K. M., Centanni, S. W., Matthews, R. T., Silberman, Y., & Winder, D. G. (2019). α2A-Adrenergic Receptor Activation Decreases Parabrachial Nucleus Excitatory Drive onto BNST CRF Neurons and Reduces Their Activity In Vivo. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *39*(3), 472–484. https://doi.org/10.1523/JNEUROSCI.1035-18.2018

Figueiredo, H. F., Bodie, B. L., Tauchi, M., Dolgas, C. M., & Herman, J. P. (2003). Stress integration after acute and chronic predator stress: differential activation of central stress circuitry and sensitization of the hypothalamo-pituitary-adrenocortical axis. *Endocrinology*, *144*(12), 5249–5258. https://doi.org/10.1210/EN.2003-0713

Figueiredo, H. F., Bruestle, A., Bodie, B., Dolgas, C. M., & Herman, J. P. (2003). The medial prefrontal cortex differentially regulates stress-induced c-fos expression in the forebrain depending on type of stressor. *The European Journal of Neuroscience*, *18*(8), 2357–2364. https://doi.org/10.1046/J.1460-9568.2003.02932.X

File, S. E. (2001). Factors controlling measures of anxiety and responses to novelty in the mouse.

Behavioural Brain Research, *125*(1–2), 151–157. https://doi.org/10.1016/S0166-4328(01)00292-3

Flanigan, M. E., & Kash, T. L. (2022). Coordination of social behaviors by the bed nucleus of the stria terminalis. *The European Journal of Neuroscience*, *55*(9–10), 2404. https://doi.org/10.1111/EJN.14991

Flavin, S. A., & Winder, D. G. (2013). Noradrenergic Control of the Bed Nucleus of the Stria Terminalis in Stress and Reward. *Neuropharmacology*, *70*, 324. https://doi.org/10.1016/J.NEUROPHARM.2013.02.013

Flavin, S. a, Matthews, R. T., Wang, Q., Muly, E. C., & Winder, D. G. (2014). α2A-Adrenergic Receptors Filter Parabrachial Inputs to the Bed Nucleus of the Stria Terminalis. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *34*(28), 9319–9331. https://doi.org/10.1523/JNEUROSCI.0822-14.2014

Forman, E. M., Chapman, J. E., Herbert, J. D., Goetter, E. M., Yuen, E. K., & Moitra, E. (2012). Using session-by-session measurement to compare mechanisms of action for acceptance and commitment therapy and cognitive therapy. *Behavior Therapy*, *43*(2), 341–354. https://doi.org/10.1016/J.BETH.2011.07.004

Forray, M. I., & Gysling, K. (2004). Role of noradrenergic projections to the bed nucleus of the stria terminalis in the regulation of the hypothalamic-pituitary-adrenal axis. *Brain Research Reviews*, *47*(1–3), 145–160. https://doi.org/10.1016/j.brainresrev.2004.07.011

Forray, M. I., Gysling, K., Andrés, M. E., Bustos, G., & Araneda, S. (2000). Medullary noradrenergic neurons projecting to the bed nucleus of the stria terminalis express mRNA for the NMDA-NR1 receptor. *Brain Research Bulletin*, *52*(3), 163–169. https://doi.org/10.1016/S0361-9230(00)00229-X

Fox, A. S., Oler, J. A., Shackman, A. J., Shelton, S. E., Raveendran, M., McKay, D. R., Converse, A. K., Alexander, A., Davidson, R. J., Blangero, J., Rogers, J., & Kalin, N. H. (2015). Intergenerational neural mediators of early-life anxious temperament. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(29), 9118–9122. https://doi.org/10.1073/PNAS.1508593112/-/DCSUPPLEMENTAL

Francesconi, W., Szücs, A., Berton, F., Koob, G. F., Vendruscolo, L. F., & Sanna, P. P. (2017). Opiate dependence induces cell type-specific plasticity of intrinsic membrane properties in the rat juxtacapsular bed nucleus of stria terminalis (jcBNST). *Psychopharmacology*, *234*(23–24), 3485–3498. https://doi.org/10.1007/s00213-017-4732-4

Fredriksson, I., Applebey, S. V., Minier-Toribio, A., Shekara, A., Bossert, J. M., & Shaham, Y. (2020). Effect of the dopamine stabilizer (-)-OSU6162 on potentiated incubation of opioid craving after electric barrier-induced voluntary abstinence. *Neuropsychopharmacology*, *45*(5), 770. https://doi.org/10.1038/S41386-020-0602-6

Freedman, L. J., & Cassell, M. D. (1994). Distribution of dopaminergic fibers in the central division of the extended amygdala of the rat. *Brain Research*, *633*(1–2), 243–252. https://doi.org/10.1016/0006-8993(94)91545-8

Frick, A., Ahs, F., Linnman, C., Jonasson, M., Appel, L., Lubberink, M., Långström, B., Fredrikson, M., & Furmark, T. (2015). Increased neurokinin-1 receptor availability in the amygdala in social anxiety disorder: a positron emission tomography study with [11C]GR205171. *Translational Psychiatry*, *5*(7), e597. https://doi.org/10.1038/TP.2015.92

Frisch, J. U., Häusser, J. A., & Mojzisch, A. (2015). The Trier Social Stress Test as a paradigm to study how people respond to threat in social interactions. *Frontiers in Psychology*, *6*(FEB). https://doi.org/10.3389/FPSYG.2015.00014

Frone, M. R. (2016). Work Stress and Alcohol Use: Developing and Testing a Biphasic Self-Medication Model. *Work and Stress*, *30*(4), 374–394. https://doi.org/10.1080/02678373.2016.1252971

Funk, D., Li, Z., & Lê, A. D. (2006). Effects of environmental and pharmacological stressors on c-fos and corticotropin-releasing factor mRNA in rat brain: Relationship to the reinstatement of alcohol seeking. *Neuroscience*, *138*(1), 235–243. https://doi.org/10.1016/J.NEUROSCIENCE.2005.10.062

Furay, A. R., Bruestle, A. E., & Herman, J. P. (2008). The Role of the Forebrain Glucocorticoid Receptor in Acute and Chronic Stress. *Endocrinology*, *149*(11), 5482. https://doi.org/10.1210/EN.2008-0642

Garakani, A., Murrough, J. W., Freire, R. C., Thom, R. P., Larkin, K., Buono, F. D., & Iosifescu, D. V. (2020). Pharmacotherapy of Anxiety Disorders: Current and Emerging Treatment Options. *Frontiers in Psychiatry*, *11*. https://doi.org/10.3389/FPSYT.2020.595584

Garcia, M. M., Cusick, C. G., & Harlan, R. E. (1993). Protein kinase C-delta in rat brain: association with sensory neuronal hierarchies. *The Journal of Comparative Neurology*, *331*(3), 375–388. https://doi.org/10.1002/CNE.903310307

Gauss, R., & Seifert, R. (2000). Pacemaker oscillations in heart and brain: a key role for hyperpolarization-activated cation channels. *Chronobiology International*, *17*(4), 453–469. http://www.ncbi.nlm.nih.gov/pubmed/10908123

Georges, F., & Aston-Jones, G. (2001). Potent regulation of midbrain dopamine neurons by the bed nucleus of the stria terminalis. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *21*(16). https://doi.org/10.1523/JNEUROSCI.21-16-J0003.2001

Georges, F., & Aston-Jones, G. (2002). Activation of ventral tegmental area cells by the bed nucleus of the stria terminalis: a novel excitatory amino acid input to midbrain dopamine neurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(12), 5173–5187. https://doi.org/10.1523/JNEUROSCI.22-12-05173.2002

Geronimus, A. T. (1996). Black/white differences in the relationship of maternal age to birthweight: A population-based test of the weathering hypothesis. *Social Science and Medicine*, *42*(4), 589–597. https://doi.org/10.1016/0277-9536(95)00159-X

Geronimus, A. T., Hicken, M., Keene, D., & Bound, J. (2006). "Weathering" and Age Patterns of Allostatic Load Scores Among Blacks and Whites in the United States. *American Journal of Public Health*, *96*(5), 826. https://doi.org/10.2105/AJPH.2004.060749

Giardino, W. J., Eban-Rothschild, A., Christoffel, D. J., Li, S. Bin, Malenka, R. C., & de Lecea, L. (2018). Parallel circuits from the bed nuclei of stria terminalis to the lateral hypothalamus drive opposing emotional states. *Nature Neuroscience*, *21*(8), 1084–1095. https://doi.org/10.1038/s41593-018-0198-x

Gilsbach, R., Albarrán-Juárez, J., & Hein, L. (2011). Pre- versus Postsynaptic Signaling by α 2-Adrenoceptors. In *Current Topics in Membranes* (Vol. 67). Curr Top Membr. https://doi.org/10.1016/B978-0-12-384921-2.00007-0

Glavin, G. B., Paré, W. P., Sandbak, T., Bakke, H.-K. K., & Murison, R. (1994). Restraint stress in biomedical research: An update. *Neuroscience & Biobehavioral Reviews*, *18*(2). https://doi.org/10.1016/0149-7634(94)90027-2

Glue, P., Medlicott, N. J., Harland, S., Neehoff, S., Anderson-Fahey, B., Le Nedelec, M., Gray, A., & McNaughton, N. (2017). Ketamine's dose-related effects on anxiety symptoms in patients with treatment refractory anxiety disorders. *Journal of Psychopharmacology (Oxford, England)*, *31*(10), 1302–1305. https://doi.org/10.1177/0269881117705089

Glynn, R. M., Rosenkranz, J. A., Wolf, M. E., Caccamise, A., Shroff, F., Smith, A. B., & Loweth, J. A. (2018). Repeated restraint stress exposure during early withdrawal accelerates incubation of cue-induced cocaine craving. *Addiction Biology*, *23*(1), 80. https://doi.org/10.1111/ADB.12475

Godoy, L. D., Rossignoli, M. T., Delfino-Pereira, P., Garcia-Cairasco, N., & Umeoka, E. H. de L. (2018). A comprehensive overview on stress neurobiology: Basic concepts and clinical implications. *Frontiers in Behavioral Neuroscience*, *12*, 127. https://doi.org/10.3389/FNBEH.2018.00127/BIBTEX

Goeders, N. E. (2002). The HPA axis and cocaine reinforcement. *Psychoneuroendocrinology*, *27*(1–2), 13–33. https://doi.org/10.1016/S0306-4530(01)00034-8

Gold, P. W., Goodwin, F. K., & Chrousos, G. P. (1988). Clinical and biochemical manifestations of depression. Relation to the neurobiology of stress (2). *The New England Journal of Medicine*, *319*(7), 413–420. https://doi.org/10.1056/NEJM198808183190706

Gong, Y., Huang, C., Li, J. Z., Grewe, B. F., Zhang, Y., Eismann, S., & Schnitzer, M. J. (2015). High-speed recording of neural spikes in awake mice and flies with a fluorescent voltage sensor. *Science*, *350*(6266), 1361–1366. https://doi.org/10.1126/science.aab0810

Goode, T. D., & Maren, S. (2017). Role of the bed nucleus of the stria terminalis in aversive learning and memory. *Learning & Memory*, *24*(9), 480. https://doi.org/10.1101/LM.044206.116

Goodson, J. L., & Kabelik, D. (2009). Dynamic limbic networks and social diversity in vertebrates: From neural context to neuromodulatory patterning. In *Frontiers in Neuroendocrinology* (Vol. 30, Issue 4, pp. 429–441). Academic Press. https://doi.org/10.1016/j.yfrne.2009.05.007

Gordon, A. S., Yao, L., Wu, Z. L., Coe, I. R., & Diamond, I. (1997). Ethanol alters the subcellular localization of delta- and epsilon protein kinase C in NG108-15 cells. *Molecular Pharmacology*, *52*(4), 554–559. https://doi.org/10.1124/MOL.52.4.554

Gorka, A. X., LaBar, K. S., & Hariri, A. R. (2016). Variability in emotional responsiveness and coping style during active avoidance as a window onto psychological vulnerability to stress. *Physiology & Behavior*, *158*, 90. https://doi.org/10.1016/J.PHYSBEH.2016.02.036

Gott, A. L., Mallon, B. S., Paton, A., Groome, N., & Rumsby, M. G. (1994). Rat brain glial cells in primary culture and subculture contain the delta, epsilon and zeta subspecies of protein kinase C as well as the conventional subspecies. *Neuroscience Letters*, *171*(1–2), 117–120. https://doi.org/10.1016/0304-3940(94)90619-X

Gozzi, A., Jain, A., Giovanelli, A., Bertollini, C., Crestan, V., Schwarz, A. J., Tsetsenis, T., Ragozzino, D., Gross, C. T., & Bifone, A. (2010). A neural switch for active and passive fear. *Neuron*, *67*(4), 656–666. https://doi.org/10.1016/j.neuron.2010.07.008

Graving, J. M., Chae, D., Naik, H., Li, L., Koger, B., Costelloe, B. R., & Couzin, I. D. (2019). DeepPoseKit, a software toolkit for fast and robust animal pose estimation using deep learning. *ELife*, *8*. https://doi.org/10.7554/ELIFE.47994

Greene, M. W., Burrington, C. M., Lynch, D. T., Davenport, S. K., Johnson, A. K., Horsman, M. J., Chowdhry, S., Zhang, J., Sparks, J. D., & Tirrell, P. C. (2014). Lipid Metabolism, Oxidative Stress and Cell Death Are Regulated by PKC Delta in a Dietary Model of Nonalcoholic Steatohepatitis. *PLoS ONE*, *9*(1). https://doi.org/10.1371/JOURNAL.PONE.0085848

Griessner, J., Pasieka, M., Böhm, V., Grössl, F., Kaczanowska, J., Pliota, P., Kargl, D., Werner, B., Kaouane, N., Strobelt, S., Kreitz, S., Hess, A., & Haubensak, W. (2021). Central amygdala circuit dynamics underlying the benzodiazepine anxiolytic effect. *Molecular Psychiatry*, *26*(2), 534–544. https://doi.org/10.1038/S41380-018-0310-3

Grillon, C., Robinson, O. J., Cornwell, B., & Ernst, M. (2019). Modeling anxiety in healthy humans: a key intermediate bridge between basic and clinical sciences. *Neuropsychopharmacology*, *44*(12), 1999. https://doi.org/10.1038/S41386-019-0445-1

Grissom, N., & Bhatnagar, S. (2009). Habituation to repeated stress: get used to it. *Neurobiology of Learning and Memory*, *92*(2), 215. https://doi.org/10.1016/J.NLM.2008.07.001

Grueter, B. A., Gosnell, H. B., Olsen, C. M., Schramm-Sapyta, N. L., Nekrasova, T., Landreth, G. E., & Winder, D. G. (2006). Extracellular-signal regulated kinase 1-dependent metabotropic glutamate receptor 5-induced long-term depression in the bed nucleus of the stria terminalis is disrupted by cocaine administration. *Journal of Neuroscience*, *26*(12), 3210–3219. https://doi.org/10.1523/JNEUROSCI.0170-06.2006

Gungor, N. Z., Yamamoto, R., Paré, D., Pare, D., & Paré, D. (2015). Optogenetic study of the projections from the bed nucleus of the stria terminalis to the central amygdala. *Journal of Neurophysiology*, *114*(5), 2903–2911. https://doi.org/10.1152/jn.00677.2015

Guo, J. D., & Rainnie, D. G. (2010). Presynaptic 5-HT(1B) receptor-mediated serotonergic inhibition of glutamate transmission in the bed nucleus of the stria terminalis. *Neuroscience*, *165*(4), 1390–1401. https://doi.org/10.1016/J.NEUROSCIENCE.2009.11.071

Guo, S., Li, F., Wills, M., Yip, J., Wehbe, A., Peng, C., Geng, X., & Ding, Y. (2022). Chlorpromazine and Promethazine (C+P) Reduce Brain Injury after Ischemic Stroke through the PKC- δ/NOX/MnSOD

Pathway. *Mediators of Inflammation*, *2022*. https://doi.org/10.1155/2022/6886752

Hamed, S., Bradby, H., Ahlberg, B. M., & Thapar-Björkert, S. (2022). Racism in healthcare: a scoping review. *BMC Public Health*, *22*(1). https://doi.org/10.1186/S12889-022-13122-Y

Hammack, S. E., Mania, I., & Rainnie, D. G. (2007). Differential Expression of Intrinsic Membrane Currents in Defined Cell Types of the Anterolateral Bed Nucleus of the Stria Terminalis. *Journal of Neurophysiology*, *98*(2), 638–656. https://doi.org/10.1152/jn.00382.2007.

Hammack, S. E., Roman, C. W., Lezak, K. R., Kocho-Shellenberg, M., Grimmig, B., Falls, W. A., Braas, K., & May, V. (2010). Roles for Pituitary Adenylate Cyclase-Activating Peptide (PACAP) Expression and Signaling in the Bed Nucleus of the Stria Terminalis (BNST) in Mediating the Behavioral Consequences of Chronic Stress. *Journal of Molecular Neuroscience*, *42*(3), 327–340. https://doi.org/10.1007/s12031-010-9364-7

Hammen, C., Brennan, P. A., & Shih, J. H. (2004). Family discord and stress predictors of depression and other disorders in adolescent children of depressed and nondepressed women. *Journal of the American Academy of Child and Adolescent Psychiatry*, *43*(8), 994–1002. https://doi.org/10.1097/01.CHI.0000127588.57468.F6

Hancock, P. A., & Ganey, H. C. N. (2003). From the Inverted-U to the Extended-U: The Evolution of a Law of Psychology. *Journal of Human Performance in Extreme Environments*, *7*(1). https://doi.org/10.7771/2327-2937.1023

Hancock, P. A., & Warm, J. S. (2003). A Dynamic Model of Stress and Sustained Attention. *Journal of Human Performance in Extreme Environments*, *7*(1). https://doi.org/10.7771/2327-2937.1024

Hancock, P., & Szalma, J. (Eds.). (2008). *Performance Under Stress*. Ashgate Publishing Company. https://sciences.ucf.edu/psychology/perl/wp-content/uploads/sites/29/2013/01/Hancock-Szalma-2008-Stress-and-Performance.pdf

Hardaway, J. A., Crowley, N. A., Bulik, C. M., & Kash, T. L. (2015). Integrated circuits and molecular components for stress and feeding: implications for eating disorders. *Genes, Brain, and Behavior*, *14*(1), 85–97. https://doi.org/10.1111/GBB.12185

Harischandra, D. S., Kondru, N., Martin, D. P., Kanthasamy, A., Jin, H., Anantharam, V., & Kanthasamy, A. G. (2014). Role of proteolytic activation of protein kinase Cδ in the pathogenesis of prion disease. *Prion*, *8*(1), 143–153. https://doi.org/10.4161/PRI.28369

Harris, N. A., Austin, X., Isaac, T., Günther, A., Merkel, K., Melchior, X. J., Xu, M., Eguakun, E., Perez, R., Nabit, B. P., Flavin, S., Gilsbach, R., Shonesy, B., Lutz Hein, X., Abel, T., Baumann, X. A., Matthews, X. R., Centanni, S. W., & Winder, D. G. (2018). Dorsal BNST 2A-Adrenergic Receptors Produce HCN-Dependent Excitatory Actions That Initiate Anxiogenic Behaviors. *The Journal of Neuroscience*, *38*(42), 8922–8942. https://doi.org/10.1523/JNEUROSCI.0963-18.2018

Harris, N. A., Isaac, A. T., Günther, A., Merkel, K., Melchior, J., Xu, M., Eguakun, E., Perez, R., Nabit, B. P., Flavin, S., Gilsbach, R., Shonesy, B., Hein, L., Abel, T., Baumann, A., Matthews, R., Centanni, S. W., Winder, D. G., Austin, X., … Winder, D. G. (2018). Dorsal BNST alpha2A-Adrenergic Receptors Produce HCN-Dependent Excitatory Actions That Initiate Anxiogenic Behaviors. *The Journal of Neuroscience*, *38*(42), 8922–8942. https://doi.org/10.1523/JNEUROSCI.0963-18.2018

Harris, N. A., & Winder, D. G. (2018). Synaptic Plasticity in the Bed Nucleus of the Stria Terminalis: Underlying Mechanisms and Potential Ramifications for Reinstatement of Drug- and Alcohol-Seeking Behaviors. *ACS Chemical Neuroscience*, *9*(9), 2173–2187. https://doi.org/10.1021/acschemneuro.8b00169

Harris, R. A., McQuilkin, S. J., Paylor, R., Abeliovich, A., Tonegawa, S., & Wehner, J. M. (1995). Mutant mice lacking the gamma isoform of protein kinase C show decreased behavioral actions of ethanol and altered function of gamma-aminobutyrate type A receptors. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(9), 3658. https://doi.org/10.1073/PNAS.92.9.3658

Hashimoto, H., Ishihara, T., Shigemoto, R., Mori, K., & Nagata, S. (1993). Molecular cloning and tissue distribution of a receptor for pituitary adenylate cyclase-activating polypeptide. *Neuron*, *11*(2),

333–342. https://doi.org/10.1016/0896-6273(93)90188-W

Haubensak, W., Kunwar, P. S., Cai, H., Ciocchi, S., Wall, N. R., Ponnusamy, R., Biag, J., Dong, H.-W. W., Deisseroth, K., Callaway, E. M., Fanselow, M. S., Lüthi, A., & Anderson, D. J. (2010). Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature*, *468*(7321), 270–276. https://doi.org/10.1038/nature09553

He, H., Xiang, Y., Gao, F., Bai, L., Gao, F., Fan, Y., Lyu, J., & Ma, X. (2019). Comparative efficacy and acceptability of first-line drugs for the acute treatment of generalized anxiety disorder in adults: A network meta-analysis. *Journal of Psychiatric Research*, *118*, 21–30. https://doi.org/10.1016/J.JPSYCHIRES.2019.08.009

He, J., Zhang, J., Wang, Y., Liu, W., Gou, K., Liu, Z., & Cui, S. (2018). MiR-7 Mediates the Zearalenone Signaling Pathway Regulating FSH Synthesis and Secretion by Targeting FOS in Female Pigs. *Endocrinology*, *159*(8), 2993–3006. https://doi.org/10.1210/EN.2018-00097

He, Y., & Wang, Z. J. (2015). Nociceptor Beta II, Delta, and Epsilon Isoforms of PKC Differentially Mediate Paclitaxel-Induced Spontaneous and Evoked Pain. *The Journal of Neuroscience*, *35*(11), 4614. https://doi.org/10.1523/JNEUROSCI.1580-14.2015

He, Y., Wilkie, D. J., Nazari, J., Wang, R., Messing, R. O., DeSimone, J., Molokie, R. E., & Wang, Z. J. (2016). PKCδ-targeted intervention relieves chronic pain in a murine sickle cell disease model. *The Journal of Clinical Investigation*, *126*(8), 3053. https://doi.org/10.1172/JCI86165

Hebb, D. O. (1955). Drives and the C. N. S. (conceptual nervous system). *Psychological Review*, *62*(4), 243–254. https://doi.org/10.1037/H0041823

Heck, A. L., & Handa, R. J. (2019). Sex differences in the hypothalamic–pituitary–adrenal axis' response to stress: an important role for gonadal hormones. *Neuropsychopharmacology*, *44*(1), 45. https://doi.org/10.1038/S41386-018-0167-9

Hedde, P. N., & Gratton, E. (2018). Selective plane illumination microscopy with a light sheet of uniform thickness formed by an electrically tunable lens. *Microscopy Research and Technique*, *81*(9), 924–928. https://doi.org/10.1002/JEMT.22707

Hedegaard, H., Minino, A. M., & Warner, M. (2018). *Drug Overdose Deaths in the United States, 1999-2017*. https://www.cdc.gov/nchs/products/databriefs/db329.htm

Henry, A. M., & Hohmann, J. G. (2012). High-resolution gene expression atlases for adult and developing mouse brain and spinal cord. *Mammalian Genome : Official Journal of the International Mammalian Genome Society*, *23*(9–10), 539–549. https://doi.org/10.1007/S00335-012-9406-2

Herbert, J. D., & Forman, E. M. (2013). Caution: the differences between CT and ACT may be larger (and smaller) than they appear. *Behavior Therapy*, *44*(2), 218–223. https://doi.org/10.1016/J.BETH.2009.09.005

Herman, J. P., Adams, D., & Prewitt, C. (1995). Regulatory changes in neuroendocrine stress-integrative circuitry produced by a variable stress paradigm. *Neuroendocrinology*, *61*(2), 180–190. https://doi.org/10.1159/000126839

Herman, J. P., Cullinan, W. E., Morano, M. I., Akil, H., & Watson, S. J. (1995). Contribution of the ventral subiculum to inhibitory regulation of the hypothalamo-pituitary-adrenocortical axis. *Journal of Neuroendocrinology*, *7*(6), 475–482. https://doi.org/10.1111/J.1365-2826.1995.TB00784.X

Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., & Cullinan, W. E. (2003). Central mechanisms of stress integration: Hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Frontiers in Neuroendocrinology*, *24*(3), 151–180. https://doi.org/10.1016/j.yfrne.2003.07.001

Herman, J. P., & Larson, B. R. (2001). Differential regulation of forebrain glutamic acid decarboxylase mRNA expression by aging and stress. *Brain Research*, *912*(1), 60–66. https://doi.org/10.1016/S0006-8993(01)02641-5

Herman, J. P., Ostrander, M. M., Mueller, N. K., & Figueiredo, H. (2005). Limbic system mechanisms of stress regulation: Hypothalamo-pituitary- adrenocortical axis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *29*(8), 1201–1213.

https://doi.org/10.1016/j.pnpbp.2005.08.006

Hermosura, A. H., Haynes, S. N., & Kaholokula, J. K. (2018). A Preliminary Study of the Relationship between Perceived Racism and Cardiovascular Reactivity and Recovery in Native Hawaiians. *Journal of Racial and Ethnic Health Disparities*, *5*(5), 1142. https://doi.org/10.1007/S40615-018-0463-4

Hessel, M., Pape, H. C., & Seidenbecher, T. (2020). Stimulation of 5-HT receptors in anterodorsal BNST guides fear to predictable and unpredictable threat. *European Neuropsychopharmacology*, *39*, 56–69. https://doi.org/10.1016/j.euroneuro.2020.08.006

Hieu, T. H., Dibas, M., Surya Dila, K. A., Sherif, N. A., Hashmi, M. U., Mahmoud, M., Trang, N. T. T., Abdullah, L., Nghia, T. L. B., Mai Nhu, Y., Hirayama, K., & Huy, N. T. (2019). Therapeutic efficacy and safety of chamomile for state anxiety, generalized anxiety disorder, insomnia, and sleep quality: A systematic review and meta-analysis of randomized trials and quasi-randomized trials. *Phytotherapy Research : PTR*, *33*(6), 1604–1615. https://doi.org/10.1002/PTR.6349

Hill, M. N., Hellemans, K. G. C., Verma, P., Gorzalka, B. B., & Weinberg, J. (2012). Neurobiology of chronic mild stress: Parallels to major depression. *Neuroscience and Biobehavioral Reviews*, *36*(9), 2085. https://doi.org/10.1016/J.NEUBIOREV.2012.07.001

Hobson, J. M., Moody, M. D., Sorge, R. E., & Goodin, B. R. (2022). The neurobiology of social stress resulting from Racism: Implications for pain disparities among racialized minorities. *Neurobiology of Pain (Cambridge, Mass.)*, *12*. https://doi.org/10.1016/J.YNPAI.2022.100101

Hodge, C. W., Mehmert, K. K., Kelley, S. P., McMahon, T., Haywood, A., Olive, M. F., Wang, D., Sanchez-Perez, A. M., & Messing, R. O. (1999). Supersensitivity to allosteric GABA(A) receptor modulators and alcohol in mice lacking PKCepsilon. *Nature Neuroscience*, *2*(11), 997–1002. https://doi.org/10.1038/14795

Hodge, C. W., Raber, J., McMahon, T., Walter, H., Sanchez-Perez, A. M., Olive, M. F., Mehmert, K., Morrow, A. L., & Messing, R. O. (2002). Decreased anxiety-like behavior, reduced stress hormones, and neurosteroid supersensitivity in mice lacking protein kinase Cε. *The Journal of Clinical Investigation*, *110*(7), 1003. https://doi.org/10.1172/JCI15903

Hofmann, S. G., Asmundson, G. J. G., & Beck, A. T. (2013a). The science of cognitive therapy. *Behavior Therapy*, *44*(2), 199–212. https://doi.org/10.1016/J.BETH.2009.01.007

Hofmann, S. G., Asmundson, G. J. G., & Beck, A. T. (2013b). The Science of Cognitive Therapy. *Behavior Therapy*, *44*(2), 199–212. https://doi.org/10.1016/J.BETH.2009.01.007

Hofmann, S. G., & Hay, A. C. (2018). Rethinking Avoidance: Toward a Balanced Approach to Avoidance in Treating Anxiety Disorders. *Journal of Anxiety Disorders*, *55*, 14. https://doi.org/10.1016/J.JANXDIS.2018.03.004

Hogg, S. (1996). A review of the validity and variability of the Elevated Plus-Maze as an animal model of anxiety. *Pharmacology Biochemistry and Behavior*, *54*(1), 21–30. https://doi.org/10.1016/0091-3057(95)02126-4

Holleran, K. M., Wilson, H. H., Fetterly, T. L., Bluett, R. J., Centanni, S. W., Gilfarb, R. A., Rocco, L. E. R., Patel, S., & Winder, D. G. (2016). Ketamine and MAG Lipase Inhibitor-Dependent Reversal of Evolving Depressive-Like Behavior During Forced Abstinence From Alcohol Drinking. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *41*(8), 2062–2071. https://doi.org/10.1038/npp.2016.3

Holsboer, F. (2000). The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *23*(5), 477–501. https://doi.org/10.1016/S0893-133X(00)00159-7

Huang, C. W., Lui, C. C., Chang, W. N., Lu, C. H., Wang, Y. L., & Chang, C. C. (2009). Elevated basal cortisol level predicts lower hippocampal volume and cognitive decline in Alzheimer's disease. *Journal of Clinical Neuroscience*, *16*(10), 1283–1286. https://doi.org/10.1016/j.jocn.2008.12.026

Hurley, K. M., Herbert, H., Moga, M. M., & Saper, C. B. (1991). Efferent projections of the infralimbic cortex of the rat. *The Journal of Comparative Neurology*, *308*(2), 249–276.

https://doi.org/10.1002/CNE.903080210

Hutmacher, F. (2021). Putting Stress in Historical Context: Why It Is Important That Being Stressed Out Was Not a Way to Be a Person 2,000 Years Ago. *Frontiers in Psychology*, *12*, 1333. https://doi.org/10.3389/FPSYG.2021.539799/BIBTEX

Hwa, L. S., Neira, S., Flanigan, M. E., Stanhope, C. M., Pina, M. M., Pati, D., Hon, O. J., Yu, W., Kokush, E., Calloway, R., Boyt, K., & Kash, T. L. (2020). Alcohol drinking alters stress response to predator odor via bnst kappa opioid receptor signaling in male mice. *ELife*, *9*, 1–53. https://doi.org/10.7554/eLife.59709

Ide, S., Hara, T., Ohno, A., Tamano, R., Koseki, K., Naka, T., Maruyama, C., Kaneda, K., Yoshioka, M., & Minami, M. (2013). Opposing roles of corticotropin-releasing factor and neuropeptide Y within the dorsolateral bed nucleus of the stria terminalis in the negative affective component of pain in rats. *J Neurosci*, *33*(14), 5881–5894. https://doi.org/10.1523/JNEUROSCI.4278-12.2013

Inoue, N. (2014). Stress and atherosclerotic cardiovascular disease. *Journal of Atherosclerosis and Thrombosis*, *21*(5), 391–401. https://doi.org/10.5551/JAT.21709

Jackson, M. (2013). *The Age of Stress: Science and the Search for Stability*. OUP Oxford. https://www.google.com/books/edition/The_Age_of_Stress/xwfDB5Q1TU4C?hl=en&gbpv=0

Jacobson, L. (2014). Hypothalamic-pituitary-adrenocortical axis: neuropsychiatric aspects. *Comprehensive Physiology*, *4*(2), 715–738. https://doi.org/10.1002/CPHY.C130036

Janak, P. H., & Tye, K. M. (2015). From circuits to behaviour in the amygdala. *Nature 2015 517:7534*, *517*(7534), 284–292. https://doi.org/10.1038/NATURE14188

Jaramillo, A. A., Brown, J. A., & Winder, D. G. (2021). Danger and distress: Parabrachial-extended amygdala circuits. *Neuropharmacology*, *198*, 108757. https://doi.org/10.1016/J.NEUROPHARM.2021.108757

Jaramillo, A. A., Williford, K. M., Marshall, C., Winder, D. G., & Centanni, S. W. (2020). BNST transient activity associates with approach behavior in a stressful environment and is modulated by the parabrachial nucleus. *Neurobiology of Stress*, *13*. https://doi.org/10.1016/j.ynstr.2020.100247

Jasnow, A. M., Davis, M., & Huhman, K. L. (2004). Involvement of central amygdalar and bed nucleus of the stria terminalis corticotropin-releasing factor in behavioral responses to social defeat. *Behavioral Neuroscience*, *118*(5), 1052–1061. https://doi.org/10.1037/0735-7044.118.5.1052

Jennings, J. H., Rizzi, G., Stamatakis, A. M., Ung, R. L., & Stuber, G. D. (2013). The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding. *Science*, *341*(6153), 1517–1521. https://doi.org/10.1126/science.1241812

Jennings, J. H., Sparta, D. R., Stamatakis, A. M., Ung, R. L., Pleil, K. E., Kash, T. L., & Stuber, G. D. (2013). Distinct extended amygdala circuits for divergent motivational states. *Nature*, *496*(7444), 224–228. https://doi.org/10.1038/nature12041

Jeong, Y. H., Park, C. H., Yoo, J., Shin, K. Y., Ahn, S., Kim, H., Lee, S. H., Emson, P. C., & Suh, Y. (2006). Chronic stress accelerates learning and memory impairments and increases amyloid deposition in APPV717I-CT100 transgenic mice, an Alzheimer's disease model. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *20*(6), 729–731. https://doi.org/10.1096/FJ.05-4265FJE

Jia, Y., Li, S., Guo, X., Lei, B., Hu, J., Xu, X. H., & Zhang, W. (2022). Selfee, self-supervised features extraction of animal behaviors. *ELife*, *11*. https://doi.org/10.7554/ELIFE.76218

Jin, H., Kanthasamy, A., Harischandra, D. S., Kondru, N., Ghosh, A., Panicker, N., Anantharam, V., Rana, A., & Kanthasamy, A. G. (2014). Histone hyperacetylation up-regulates protein kinase Cδ in dopaminergic neurons to induce cell death: relevance to epigenetic mechanisms of neurodegeneration in Parkinson disease. *The Journal of Biological Chemistry*, *289*(50), 34743–34767. https://doi.org/10.1074/jbc.M114.576702

Johnson, V., & Pandina, R. J. (1993). A longitudinal examination of the relationships among stress, coping strategies, and problems associated with alcohol use. *Alcoholism, Clinical and Experimental Research*, *17*(3), 696–702. https://doi.org/10.1111/J.1530-0277.1993.TB00822.X

Jolkkonen, E., Miettinen, R., & Pitkänen, A. (2001). Projections from the amygdalo-piriform transition area to the amygdaloid complex: A PHA-l study in rat. *Journal of Comparative Neurology*, *432*(4), 440–465. https://doi.org/10.1002/CNE.1113

Jolly, S., Vittinghoff, E., Chattopadhyay, A., & Bibbins-Domingo, K. (2010). Higher cardiovascular disease prevalence and mortality among younger blacks compared to whites. *The American Journal of Medicine*, *123*(9), 811–818. https://doi.org/10.1016/J.AMJMED.2010.04.020

Ju, G., & Swanson, L. W. (1989). Studies on the cellular architecture of the bed nuclei of the stria terminalis in the rat: I. cytoarchitecture. *The Journal of Comparative Neurology*, *280*(4), 587–602. https://doi.org/10.1002/cne.902800409

Ju, G., Swanson, L. W., & Simerly, R. B. (1989). Studies on the cellular architecture of the bed nuclei of the stria terminalis in the rat: II. chemoarchitecture. *The Journal of Comparative Neurology*, *280*(4), 603–621. https://doi.org/10.1002/cne.902800410

Julian, L. J. (2011). Measures of Anxiety. *Arthritis Care & Research*, *63*(0 11). https://doi.org/10.1002/ACR.20561

Kaasinen, S. K., Goldsteins, G., Alhonen, L., Jänne, J., & Koistinaho, J. (2002). Induction and activation of protein kinase Cδ in hippocampus and cortex after kainic acid treatment. *Experimental Neurology*, *176*(1), 203–212. https://doi.org/10.1006/exnr.2002.7919

Kahneman, D. (1973). *Attention and Effort*. Prentice-Hall inc.

Kalivas, P. W., & Duffy, P. (1995). Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. *Brain Research*, *675*(1–2), 325–328. https://doi.org/10.1016/0006-8993(95)00013-G

Kane, G. A., Lopes, G., Saunders, J. L., Mathis, A., & Mathis, M. W. (2020). Real-time, low-latency closed-loop feedback using markerless posture tracking. *ELife*, *9*, 1–29. https://doi.org/10.7554/ELIFE.61909

Kash, T. L., Pleil, K. E., Marcinkiewcz, C. A., Lowery-Gionta, E. G., Crowley, N., Mazzone, C., Sugam, J., Hardaway, J. A., & McElligott, Z. A. (2015). Neuropeptide Regulation of Signaling and Behavior in the BNST. *Molecules and Cells*, *38*(1), 1–13. https://doi.org/10.14348/molcells.2015.2261

Kelly, R. R., McDonald, L. T., Jensen, N. R., Sidles, S. J., & LaRue, A. C. (2019). Impacts of Psychological Stress on Osteoporosis: Clinical Implications and Treatment Interactions. *Frontiers in Psychiatry*, *10*. https://doi.org/10.3389/FPSYT.2019.00200

Kemppainen, S., Jalkkonen, E., & Pitkänen, A. (2002). Projections from the posterior cortical nucleus of the amygdala to the hippocampal formation and parahippocampal region in rat. *Hippocampus*, *12*(6), 735–755. https://doi.org/10.1002/HIPO.10020

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, *62*(6), 593. https://doi.org/10.1001/archpsyc.62.6.593

Kessler, R. C., Chiu, W. T., Demler, O., Walters, E. E., Walters, E. E., Wai, T. C., Demler, O., & Walters, E. E. (2005). Prevalence, Severity, and Comorbidity of 12-Month DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, *62*(6). https://doi.org/10.1001/archpsyc.62.6.617

Kessler, R. C., Petukhova, M., Sampson, N. A., Zaslavsky, A. M., & Wittchen, H. U. (2012). Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. *International Journal of Methods in Psychiatric Research*, *21*(3), 169–184. https://doi.org/10.1002/MPR.1359

Kilts, C. D., & Anderson, C. M. (1986). The simultaneous quantification of dopamine, norepinephrine and epinephrine in micropunched rat brain nuclei by on-line trace enrichment HPLC with electrochemical detection: Distribution of catecholamines in the limbic system. *Neurochemistry International*, *9*(3), 437–445. https://doi.org/10.1016/0197-0186(86)90086-0

Kim, B., Yoon, S., Nakajima, R., Lee, H. J., Lim, H. J., Lee, Y. K., Choi, J. S., Yoon, B. J., Augustine, G.

J., & Baik, J. H. (2018). Dopamine D2 receptor-mediated circuit from the central amygdala to the bed nucleus of the stria terminalis regulates impulsive behavior. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(45), E10730–E10739. https://doi.org/10.1073/PNAS.1811664115/-/DCSUPPLEMENTAL

Kim, J. H., Kromm, G. H., Barnhill, O. K., Sperber, J., Heuer, L. B., Loomis, S., Newman, M. C., Han, K., Gulamali, F. F., Legan, T. B., Jensen, K. E., Funderburk, S. C., Krashes, M. J., & Carter, M. E. (2022). A discrete parasubthalamic nucleus subpopulation plays a critical role in appetite suppression. *ELife*, *11*. https://doi.org/10.7554/ELIFE.75470

Kim, J., Zhang, X., Muralidhar, S., LeBlanc, S. A., & Tonegawa, S. (2017). Basolateral to Central Amygdala Neural Circuits for Appetitive Behaviors. *Neuron*, *93*(6), 1464-1479.e5. https://doi.org/10.1016/j.neuron.2017.02.034

Kim, S.-Y., Adhikari, A., Lee, S. Y., Marshel, J. H., Kim, C. K., Mallory, C. S., Lo, M., Pak, S., Mattis, J., Lim, B. K., Malenka, R. C., Warden, M. R., Neve, R., Tye, K. M., & Deisseroth, K. (2013). Diverging neural pathways assemble a behavioural state from separable features in anxiety. *Nature*, *496*(7444), 219–223. https://doi.org/10.1038/nature12018

Kim, S., Im, E. O., Liu, J., & Ulrich, C. (2020). Maternal Age Patterns of Preterm Birth: Exploring the Moderating Roles of Chronic Stress and Race/Ethnicity. *Annals of Behavioral Medicine: A Publication of the Society of Behavioral Medicine*, *54*(9), 653. https://doi.org/10.1093/ABM/KAAA008

Kim, Y., Venkataraju, K. U., Pradhan, K., Mende, C., Taranda, J., Turaga, S. C., Arganda-Carreras, I., Ng, L., Hawrylycz, M. J., Rockland, K. S., Seung, H. S., & Osten, P. (2015). Mapping social behavior-induced brain activation at cellular resolution in the mouse. *Cell Reports*, *10*(2), 292–305. https://doi.org/10.1016/j.celrep.2014.12.014

Kinlein, S. A., & Karatsoreos, I. N. (2020). The hypothalamic-pituitary-adrenal axis as a substrate for stress resilience: interactions with the circadian clock. *Frontiers in Neuroendocrinology*, *56*, 100819. https://doi.org/10.1016/J.YFRNE.2019.100819

Kinlein, S. A., Wallace, N. K., Savenkova, M. I., & Karatsoreos, I. N. (2022). Chronic hypothalamic-pituitary-adrenal axis disruption alters glutamate homeostasis and neural responses to stress in male C57Bl6/N mice. *Neurobiology of Stress*, *19*. https://doi.org/10.1016/J.YNSTR.2022.100466

Kirlic, N., Young, J., & Aupperle, R. L. (2017). Animal to Human Translational Paradigms Relevant for Approach Avoidance Conflict Decision Making. *Behaviour Research and Therapy*, *96*, 14. https://doi.org/10.1016/J.BRAT.2017.04.010

Kirouac, G. J. (2021). The Paraventricular Nucleus of the Thalamus as an Integrating and Relay Node in the Brain Anxiety Network. *Frontiers in Behavioral Neuroscience*, *15*, 21. https://doi.org/10.3389/FNBEH.2021.627633/BIBTEX

Klampfl, S. M., & Bosch, O. J. (2019). When mothers neglect their offspring: an activated CRF system in the BNST is detrimental for maternal behavior. *Archives of Women's Mental Health*, *22*(3), 409–415. https://doi.org/10.1007/s00737-018-0897-z

Klampfl, S. M., Schramm, M. M., Stinnett, G. S., Bayerl, D. S., Seasholtz, A. F., & Bosch, O. J. (2016). Brain CRF-binding protein modulates aspects of maternal behavior under stressful conditions and supports a hypo-anxious state in lactating rats. *Hormones and Behavior*, *84*, 136–144. https://doi.org/10.1016/j.yhbeh.2016.06.009

Konig, H., Ponta, H., Rahmsdorf, U., Buscher, M., Schonthal, A., Rahmsdorf, H. J., & Herrlich, P. (1989). Autoregulation of fos: the dyad symmetry element as the major target of repression. *EMBO Journal*, *8*(9), 2559–2566. https://doi.org/10.1002/J.1460-2075.1989.TB08394.X

Koob, G. F. (2008). A Role for Brain Stress Systems in Addiction. *Neuron*, *59*(1), 11. https://doi.org/10.1016/J.NEURON.2008.06.012

Koob, G. F. (2013). Addiction is a Reward Deficit and Stress Surfeit Disorder. *Frontiers in Psychiatry*, *4*(AUG). https://doi.org/10.3389/FPSYT.2013.00072

Koob, G. F., & Heinrichs, S. C. (1999). A role for corticotropin releasing factor and urocortin in

behavioral responses to stressors. *Brain Research*, *848*(1–2), 141–152. https://doi.org/10.1016/S0006-8993(99)01991-5

Koob, G. F., & Le Moal, M. (2008). Addiction and the brain antireward system. *Annual Review of Psychology*, *59*, 29–53. https://doi.org/10.1146/annurev.psych.59.103006.093548

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, *35*(1), 217–238. https://doi.org/10.1038/npp.2009.110

Koponen, S., Goldsteins, G., Keinänen, R., & Koistinaho, J. (2000). Induction of protein kinase Cdelta subspecies in neurons and microglia after transient global brain ischemia. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *20*(1), 93–102. https://doi.org/10.1097/00004647-200001000-00013

Koussounadis, A., Langdon, S. P., Um, I. H., Harrison, D. J., & Smith, V. A. (2015). Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Scientific Reports 2015 5:1*, *5*(1), 1–9. https://doi.org/10.1038/srep10775

Kovács, K. J. (2008). Measurement of Immediate-Early Gene Activation- c-fos and Beyond. *Journal of Neuroendocrinology*, *20*(6), 665–672. https://doi.org/10.1111/j.1365-2826.2008.01734.x

Kovács, L. Á., Füredi, N., Ujvári, B., Golgol, A., & Gaszner, B. (2022). Age-Dependent FOSB/ΔFOSB Response to Acute and Chronic Stress in the Extended Amygdala, Hypothalamic Paraventricular, Habenular, Centrally-Projecting Edinger-Westphal, and Dorsal Raphe Nuclei in Male Rats. *Frontiers in Aging Neuroscience*, *14*, 403. https://doi.org/10.3389/FNAGI.2022.862098/BIBTEX

Kovner, R., Oler, J. A., & Kalin, N. H. (2019). Cortico-Limbic Interactions Mediate Adaptive and Maladaptive Responses Relevant to Psychopathology. *The American Journal of Psychiatry*, *176*(12), 987–999. https://doi.org/10.1176/APPI.AJP.2019.19101064

Kozicz, T., Vigh, S., & Arimura, A. (1997). Axon terminals containing PACAP- and VIP-immunoreactivity form synapses with CRF-immunoreactive neurons in the dorsolateral division of the bed nucleus of the stria terminalis in the rat. *Brain Research*, *767*(1), 109–119. https://doi.org/10.1016/S0006-8993(97)00737-3

Kraeuter, A. K., Guest, P. C., & Sarnyai, Z. (2019). The Elevated Plus Maze Test for Measuring Anxiety-Like Behavior in Rodents. *Methods in Molecular Biology*, *1916*, 69–74. https://doi.org/10.1007/978-1-4939-8994-2_4/COVER

Krajniak, M. I., Anderson, K., & Eisen, A. R. (2022). Separation Anxiety. *Encyclopedia of Mental Health: Second Edition*, 128–132. https://doi.org/10.1016/B978-0-12-397045-9.00251-2

Kräplin, A., Joshanloo, M., Wolff, M., Krönke, K. M., Goschke, T., Bühringer, G., & Smolka, M. N. (2022). The relationship between executive functioning and addictive behavior: new insights from a longitudinal community study. *Psychopharmacology*, *239*(11), 3507. https://doi.org/10.1007/S00213-022-06224-3

Krawczyk, M., Georges, F., Sharma, R., Mason, X., Berthet, A., Bézard, E., & Dumont, É. C. (2011). Double-dissociation of the catecholaminergic modulation of synaptic transmission in the oval bed nucleus of the stria terminalis. *Journal of Neurophysiology*, *105*(1), 145–153. https://doi.org/10.1152/jn.00710.2010

Kreitzer, A. C. (2009). Physiology and Pharmacology of Striatal Neurons. *Annual Review of Neuroscience*, *32*(1), 127–147. https://doi.org/10.1146/annurev.neuro.051508.135422

Krettek, J. E., & Price, J. L. (1978). Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. *The Journal of Comparative Neurology*, *178*(2), 225–253. https://doi.org/10.1002/cne.901780204

Krishnan, V., & Nestler, E. J. (2008). The molecular neurobiology of depression. *Nature*, *455*(7215), 894. https://doi.org/10.1038/NATURE07455

Kudlow, P. A., McIntyre, R. S., & Lam, R. W. (2014). Early switching strategies in antidepressant non-responders: current evidence and future research directions. *CNS Drugs*, *28*(7), 601–609. https://doi.org/10.1007/S40263-014-0171-5

Kudo, T., Uchigashima, M., Miyazaki, T., Konno, K., Yamasaki, M., Yanagawa, Y., Minami, M., & Watanabe, M. (2012). Three types of neurochemical projection from the bed nucleus of the stria terminalis to the ventral tegmental area in adult mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *32*(50), 18035–18046. https://doi.org/10.1523/JNEUROSCI.4057-12.2012

Kugelmann, R. (1992). *Stress : the nature and history of engineered grief*. Praeger.

Kulesskaya, N., & Voikar, V. (2014). Assessment of mouse anxiety-like behavior in the light-dark box and open-field arena: role of equipment and procedure. *Physiology & Behavior*, *133*, 30–38. https://doi.org/10.1016/J.PHYSBEH.2014.05.006

La-Vu, M., Tobias, B. C., Schuette, P. J., & Adhikari, A. (2020). To Approach or Avoid: An Introductory Overview of the Study of Anxiety Using Rodent Assays. *Frontiers in Behavioral Neuroscience*, *14*, 145. https://doi.org/10.3389/FNBEH.2020.00145/BIBTEX

Labiner, D. M., Butler, L. S., Cao, Z., Hosford, D. A., Shin, C., & McNamara, J. O. (1993). Induction of c-fos mRNA by kindled seizures: complex relationship with neuronal burst firing. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *13*(2), 744–751. https://doi.org/10.1523/JNEUROSCI.13-02-00744.1993

Laflamme, N., Nappi, R. E., Drolet, G., Labrie, C., & Rivest, S. (1998). Expression and neuropeptidergic characterization of estrogen receptors (ERα and ERβ) throughout the rat brain: Anatomical evidence of distinct roles of each subtype. *Journal of Neurobiology*, *36*(3), 357–378. https://doi.org/10.1002/(SICI)1097-4695(19980905)36:3<357::AID-NEU5>3.0.CO;2-V

Lange, M. D., Daldrup, T., Remmers, F., Szkudlarek, H. J., Lesting, J., Guggenhuber, S., Ruehle, S., Jüngling, K., Seidenbecher, T., Lutz, B., & Pape, H. C. (2017). Cannabinoid CB1 receptors in distinct circuits of the extended amygdala determine fear responsiveness to unpredictable threat. *Molecular Psychiatry*, *22*(10), 1422–1430. https://doi.org/10.1038/mp.2016.156

Lebow, M. A., & Chen, A. (2016). Overshadowed by the amygdala: the bed nucleus of the stria terminalis emerges as key to psychiatric disorders. *Molecular Psychiatry*, *21*(4), 450–463. https://doi.org/10.1038/mp.2016.1

Lebow, M., Neufeld-Cohen, A., Kuperman, Y., Tsoory, M., Gil, S., & Chen, A. (2012). Susceptibility to PTSD-like behavior is mediated by corticotropin-releasing factor receptor type 2 levels in the bed nucleus of the stria terminalis. *Journal of Neuroscience*, *32*(20), 6906–6916. https://doi.org/10.1523/JNEUROSCI.4012-11.2012

LeDoux, J. (2003). The emotional brain, fear, and the amygdala. *Cellular and Molecular Neurobiology*, *23*(4–5), 727–738. https://doi.org/10.1023/A:1025048802629

LeDoux, J. E. (2003). Emotion Circuits in the Brain. *Https://Doi.Org/10.1146/Annurev.Neuro.23.1.155*, *23*, 155–184. https://doi.org/10.1146/ANNUREV.NEURO.23.1.155

Lee, D. B., Peckins, M. K., Heinze, J. E., Miller, A. L., Assari, S., & Zimmerman, M. A. (2018). Psychological pathways from racial discrimination to cortisol in African American males and females. *Journal of Behavioral Medicine*, *41*(2), 208–220. https://doi.org/10.1007/S10865-017-9887-2/TABLES/3

Lee, H. J., Palkovits, M., & Young, W. S. (2006). miR-7b, a microRNA up-regulated in the hypothalamus after chronic hyperosmolar stimulation, inhibits Fos translation. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(42), 15669–15674. https://doi.org/10.1073/PNAS.0605781103/ASSET/29DECDFA-E5FD-4C01-8E3E-69FD96DCE392/ASSETS/GRAPHIC/ZPQ0400637160005.JPEG

Lee, S., Kim, S. J., Kwon, O. Bin, Lee, J. H., & Kim, J. H. (2013). Inhibitory networks of the amygdala for emotional memory. *Frontiers in Neural Circuits*, *7*(JUL), 129. https://doi.org/10.3389/FNCIR.2013.00129/BIBTEX

Lee, Y., & Davis, M. (1997). Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. *Journal of Neuroscience*, *17*(16), 6434–6446. https://doi.org/10.1523/jneurosci.17-16-06434.1997

Leri, F., Flores, J., Rodaros, D., & Stewart, J. (2002). Blockade of stress-induced but not cocaine-induced reinstatement by infusion of noradrenergic antagonists into the bed nucleus of the stria terminalis or the central nucleus of the amygdala. *Journal of Neuroscience*, *22*(13), 5713–5718. https://doi.org/10.1523/jneurosci.22-13-05713.2002

Lesur, A., Gaspar, P., Alvarez, C., & Berger, B. (1989). Chemoanatomic compartments in the human bed nucleus of the stria terminalis. *Neuroscience*, *32*(1), 181–194. https://doi.org/10.1016/0306-4522(89)90117-6

Lewis, T. T., Lampert, R., Charles, D., & Katz, S. (2019). Expectations of Racism and Carotid Intima Media Thickness in African-American Women. *Psychosomatic Medicine*, *81*(8), 759. https://doi.org/10.1097/PSY.0000000000000684

Li, C. shan R., & Sinha, R. (2008). Inhibitory Control and Emotional Stress Regulation: Neuroimaging Evidence for Frontal-Limbic Dysfunction in Psycho-stimulant Addiction. *Neuroscience and Biobehavioral Reviews*, *32*(3), 581. https://doi.org/10.1016/J.NEUBIOREV.2007.10.003

Lin, X., Itoga, C. A., Taha, S., Li, M. H., Chen, R., Sami, K., Berton, F., Francesconi, W., & Xu, X. (2018). c-Fos mapping of brain regions activated by multi-modal and electric foot shock stress. *Neurobiology of Stress*, *8*, 92. https://doi.org/10.1016/J.YNSTR.2018.02.001

Lister, R. G. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology*, *92*(2), 180–185. https://doi.org/10.1007/BF00177912

Logan, J. G., & Barksdale, D. J. (2008). Allostasis and allostatic load: Expanding the discourse on stress and cardiovascular disease. *Journal of Clinical Nursing*, *17*(7B), 201–208. https://doi.org/10.1111/j.1365-2702.2008.02347.x

Lowry, C. A., Hale, M. W., Evans, A. K., Heerkens, J., Staub, D. R., Gasser, P. J., & Shekhar, A. (2008). Serotonergic systems, anxiety, and affective disorder: focus on the dorsomedial part of the dorsal raphe nucleus. *Annals of the New York Academy of Sciences*, *1148*, 86–94. https://doi.org/10.1196/ANNALS.1410.004

Lu, D., Palmer, J. R., Rosenberg, L., Shields, A. E., Orr, E. H., DeVivo, I., & Cozier, Y. C. (2019). Perceived racism in relation to telomere length among African-American women in the Black Women's Health Study. *Annals of Epidemiology*, *36*, 33. https://doi.org/10.1016/J.ANNEPIDEM.2019.06.003

Lu, J., Cheng, Y., Xie, X., Woodson, K., Bonifacio, J., Disney, E., Barbee, B., Wang, X., Zaidi, M., & Wang, J. (2021). Whole-brain mapping of direct inputs to dopamine D1 and D2 receptor-expressing medium spiny neurons in the posterior dorsomedial striatum. *ENeuro*, *8*(1), 1–15. https://doi.org/10.1523/ENEURO.0348-20.2020

Lu, L., & Shaham, Y. (2005). The role of stress in opiate and psychostimulant addiction: evidence from animal models. In J. M. H.M, N. Reul, T. Kalin, & T. Steckler (Eds.), *Handbook of Stress and the Brain Part 2: Stress: Integrative and Clinical Aspects* (Vol. 15, pp. 315–332). Elsevier.

Lucassen, P. J., Pruessner, J., Sousa, N., Almeida, O. F. X., Van Dam, A. M., Rajkowska, G., Swaab, D. F., & Czéh, B. (2014). Neuropathology of stress. *Acta Neuropathologica*, *127*(1), 109–135. https://doi.org/10.1007/S00401-013-1223-5/FIGURES/9

Luchsinger, J. R., Fetterly, T. L., Williford, K. M., Salimando, G. J., Doyle, M. A., Maldonado, J., Simerly, R. B., Winder, D. G., & Centanni, S. W. (2021). Delineation of an insula-BNST circuit engaged by struggling behavior that regulates avoidance in mice. *Nature Communications*, *12*(1). https://doi.org/10.1038/S41467-021-23674-Z

Luckman, S. M., Dyball, R. E. J., & Leng, G. (1994). Induction of c-fos expression in hypothalamic magnocellular neurons requires synaptic activation and not simply increased spike activity. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *14*(8), 4825–4830. https://doi.org/10.1523/JNEUROSCI.14-08-04825.1994

Lui, P., Padow, V. A., Franco, D., Hall, B. S., Park, B., Klein, Z. A., & Romeo, R. D. (2012). Divergent Stress-Induced Neuroendocrine and Behavioral Responses Prior to Puberty. *Physiology & Behavior*, *107*(1), 104. https://doi.org/10.1016/J.PHYSBEH.2012.06.011

Lupien, S. J., De Leon, M., De Santi, S., Convit, A., Tarshish, C., Nair, N. P. V., Thakur, M., McEwen, B. S., Hauger, R. L., & Meaney, M. J. (1998). Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nature Neuroscience*, *1*(1), 69–73. https://doi.org/10.1038/271

Luskin, A. T., Bhatti, D. L., Mulvey, B., Pedersen, C. E., Girven, K. S., Oden-Brunson, H., Kimbell, K., Blackburn, T., Sawyer, A., Gereau, R. W., Dougherty, J. D., & Bruchas, M. R. (2021). Extended amygdala-parabrachial circuits alter threat assessment and regulate feeding. *Science Advances*, *7*(9). https://doi.org/10.1126/SCIADV.ABD3666

Macatee, R. J., Albanese, B. J., Schmidt, N. B., & Cougle, J. R. (2017). Attention Bias towards Negative Emotional Information and its Relationship with Daily Worry in the Context of Acute Stress: An Eye-Tracking Study. *Behaviour Research and Therapy*, *90*, 96. https://doi.org/10.1016/J.BRAT.2016.12.013

Magarin˜os, A. M., & McEwen, B. S. (1995). Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience*, *69*(1), 89–98. https://doi.org/10.1016/0306-4522(95)00259-L

Maita, I., Roepke, T. A., & Samuels, B. A. (2022). Chronic stress-induced synaptic changes to corticotropin-releasing factor-signaling in the bed nucleus of the stria terminalis. *Frontiers in Behavioral Neuroscience*, *16*. https://doi.org/10.3389/FNBEH.2022.903782

Makino, S., Gold, P. W., & Schulkin, J. (1994a). Corticosterone effects on corticotropin-releasing hormone mRNA in the central nucleus of the amygdala and the parvocellular region of the paraventricular nucleus of the hypothalamus. *Brain Research*, *640*(1–2), 105–112. https://doi.org/10.1016/0006-8993(94)91862-7

Makino, S., Gold, P. W., & Schulkin, J. (1994b). Effects of corticosterone on CRH mRNA and content in the bed nucleus of the stria terminalis; comparison with the effects in the central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus. *Brain Research*, *657*(1–2), 141–149. https://doi.org/10.1016/0006-8993(94)90961-X

Makino, S., Smith, M. A., & Gold, P. W. (1995). Increased expression of corticotropin-releasing hormone and vasopressin messenger ribonucleic acid (mRNA) in the hypothalamic paraventricular nucleus during repeated stress: association with reduction in glucocorticoid receptor mRNA levels. *Endocrinology*, *136*(8), 3299–3309. https://doi.org/10.1210/ENDO.136.8.7628364

Mantsch, J. R., Baker, D. A., Funk, D., Lê, A. D., & Shaham, Y. (2016). Stress-induced reinstatement of drug seeking: 20 years of progress. *Neuropsychopharmacology*, *41*(1), 335–356. https://doi.org/10.1038/npp.2015.142

Mantsch, J. R., Saphier, D., & Goeders, N. E. (1998). Corticosterone Facilitates the Acquisition of Cocaine Self-Administration in Rats: Opposite Effects of the Type II Glucocorticoid Receptor Agonist Dexamethasone. *Journal of Pharmacology and Experimental Therapeutics*, *287*(1).

Marcinkiewcz, C. A., Bierlein-De La Rosa, G., Dorrier, C. E., McKnight, M., Diberto, J. F., Pati, D., Gianessi, C. A., Hon, O. J., Tipton, G., McElligott, Z. A., Delpire, E., & Kash, T. L. (2019). Sex-dependent modulation of anxiety and fear by 5-HT1A receptors in the bed nucleus of the stria terminalis. *ACS Chemical Neuroscience*, *10*(7), 3154–3166. https://doi.org/10.1021/acschemneuro.8b00594

Marcinkiewcz, C. A., Dorrier, C. E., Lopez, A. J., & Kash, T. L. (2015). Ethanol induced adaptations in 5-HT2c receptor signaling in the bed nucleus of the stria terminalis: Implications for anxiety during ethanol withdrawal. *Neuropharmacology*, *89*, 157–167. https://doi.org/10.1016/j.neuropharm.2014.09.003

Maren, S., & Quirk, G. J. (2004). Neuronal signalling of fear memory. *Nature Reviews. Neuroscience*, *5*(11), 844–852. https://doi.org/10.1038/NRN1535

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., & Wu, C. (2004). Interneurons of the neocortical inhibitory system. *Nature Reviews Neuroscience*, *5*(10), 793–807. https://doi.org/10.1038/nrn1519

Martí, O., & Armario, A. (1998). Anterior pituitary response to stress: time-related changes and adaptation. *International Journal of Developmental Neuroscience : The Official Journal of the*

International Society for Developmental Neuroscience, *16*(3–4), 241–260. https://doi.org/10.1016/S0736-5748(98)00030-6

Martianova, E., Aronson, S., & Proulx, C. D. (2019). Multi-Fiber Photometry to Record Neural Activity in Freely-Moving Animals. *Journal of Visualized Experiments : JoVE*, *2019*(152). https://doi.org/10.3791/60278

Martin, J. A., & Osterman, M. J. K. (2018). Describing the Increase in Preterm Births in the United States, 2014-2016 Key findings Data from the National Vital Statistics System. *NCHS Data Brief*. https://www.cdc.gov/nchs/data/databriefs/db312_table.pdf#3.

Martínez, A. D., Mercado, E., Barbieri, M., Kim, S. Y., & Granger, D. A. (2021). The Importance of Biobehavioral Research to Examine the Physiological Effects of Racial and Ethnic Discrimination in the Latinx Population. *Frontiers in Public Health*, *9*. https://doi.org/10.3389/FPUBH.2021.762735

Martinon, D., & Dabrowska, J. (2018). Corticotropin-Releasing Factor Receptors Modulate Oxytocin Release in the Dorsolateral Bed Nucleus of the Stria Terminalis (BNST) in Male Rats. *Frontiers in Neuroscience*, *12*(MAR), 183. https://doi.org/10.3389/fnins.2018.00183

Marvin, J. S., Shimoda, Y., Magloire, V., Leite, M., Kawashima, T., Jensen, T. P., Kolb, I., Knott, E. L., Novak, O., Podgorski, K., Leidenheimer, N. J., Rusakov, D. A., Ahrens, M. B., Kullmann, D. M., & Looger, L. L. (2019). A genetically encoded fluorescent sensor for in vivo imaging of GABA. *Nature Methods*, *16*(8), 763–770. https://doi.org/10.1038/S41592-019-0471-2

Marx, W., Lane, M., Rocks, T., Ruusunen, A., Loughman, A., Lopresti, A., Marshall, S., Berk, M., Jacka, F., & Dean, O. M. (2019). Effect of saffron supplementation on symptoms of depression and anxiety: a systematic review and meta-analysis. *Nutrition Reviews*, *77*(8), 557–571. https://doi.org/10.1093/NUTRIT/NUZ023

Massengill, C. I., Day-Cooney, J., Mao, T., & Zhong, H. (2021). Genetically encoded sensors towards imaging cAMP and PKA activity in vivo. *Journal of Neuroscience Methods*, *362*, 109298. https://doi.org/10.1016/J.JNEUMETH.2021.109298

Mathé, A. A., Michaneck, M., Berg, E., Charney, D. S., & Murrough, J. W. (2020). A Randomized Controlled Trial of Intranasal Neuropeptide Y in Patients With Major Depressive Disorder. *International Journal of Neuropsychopharmacology*, *23*(12), 783. https://doi.org/10.1093/IJNP/PYAA054

Mathews, A., & MacLeod, C. (1994). Cognitive approaches to emotion and emotional disorders. *Annual Review of Psychology*, *45*, 25–50. https://doi.org/10.1146/ANNUREV.PS.45.020194.000325

Matsumoto, K., Hamatani, S., & Shimizu, E. (2021). Effectiveness of Videoconference-Delivered Cognitive Behavioral Therapy for Adults With Psychiatric Disorders: Systematic and Meta-Analytic Review. *Journal of Medical Internet Research*, *23*(12). https://doi.org/10.2196/31293

Mazzone, C. M., Pati, D., Michaelides, M., DiBerto, J., Fox, J. H., Tipton, G., Anderson, C., Duffy, K., McKlveen, J. M., Hardaway, J. A., Magness, S. T., Falls, W. A., Hammack, S. E., McElligott, Z. A., Hurd, Y. L., & Kash, T. L. (2018). Acute engagement of Gq-mediated signaling in the bed nucleus of the stria terminalis induces anxiety-like behavior. *Molecular Psychiatry*, *23*(1), 143–153. https://doi.org/10.1038/mp.2016.218

McCullough, K. M., Daskalakis, N. P., Gafford, G., Morrison, F. G., & Ressler, K. J. (2018). Cell-type-specific interrogation of CeA Drd2 neurons to identify targets for pharmacological modulation of fear extinction. *Translational Psychiatry*, *8*(1). https://doi.org/10.1038/S41398-018-0190-Y

Mccullough, K. M., Morrison, F. G., Hartmann, J., Carlezon, W. A., & Ressler, K. J. (2018). Quantified Coexpression Analysis of Central Amygdala Subpopulations. *ENeuro*. https://doi.org/10.1523/ENEURO.0010-18.2018

McCullough, K. M., Morrison, F. G., Hartmann, J., Carlezon, W. A., & Ressler, K. J. (2018). Quantified Coexpression Analysis of Central Amygdala Subpopulations. *ENeuro*, *5*(1). https://doi.org/10.1523/ENEURO.0010-18.2018

Mcdonald, A. J. (1998). Cortical pathways to the mammalian amygdala. *Progress in Neurobiology*, *55*(3), 257–332. https://doi.org/10.1016/S0301-0082(98)00003-3

Mcelligott, Z. A., Klug, J. R., Nobis, W. P., Patel, S., Grueter, B. A., & Kash, T. L. (2010). *Distinct forms of G q -receptor-dependent plasticity of excitatory transmission in the BNST are differentially affected by stress*. *107*(5). https://doi.org/10.1073/pnas.0905568107

McElligott, Z. A., & Winder, D. G. (2008). α1-Adrenergic receptor-induced heterosynaptic long-term depression in the bed nucleus of the stria terminalis is disrupted in mouse models of affective disorders. *Neuropsychopharmacology*, *33*(10), 2313–2323. https://doi.org/10.1038/sj.npp.1301635

McEwen, B. S. (2017). Neurobiological and Systemic Effects of Chronic Stress. *Chronic Stress*, *1*. https://doi.org/10.1177/2470547017692328

McEwen, B. S., & Stellar, E. (1993). Stress and the Individual: Mechanisms Leading to Disease. *Archives of Internal Medicine*, *153*(18), 2093–2101. https://doi.org/10.1001/ARCHINTE.1993.00410180039004

McFadden, K., Griffin, T. A., Levy, V., Wolfe, J. H., & Valentino, R. J. (2012). Overexpression of corticotropin-releasing factor in Barrington's nucleus neurons by adeno-associated viral transduction: effects on bladder function and behavior. *European Journal of Neuroscience*, *36*(10), 3356–3364. https://doi.org/10.1111/j.1460-9568.2012.08250.x

McGaugh, J. L. (2000). Neuroscience - Memory - a century of consolidation. *Science*, *287*(5451), 248–251. https://doi.org/10.1126/science.287.5451.248

McHenry, J. A., Rubinow, D. R., & Stuber, G. D. (2015). Maternally responsive neurons in the bed nucleus of the stria terminalis and medial preoptic area: Putative circuits for regulating anxiety and reward. *Frontiers in Neuroendocrinology*, *38*, 65–72. https://doi.org/10.1016/J.YFRNE.2015.04.001

McKinley, M. J., Pennington, G. L., & Ryan, P. J. (2021). The median preoptic nucleus: A major regulator of fluid, temperature, sleep, and cardiovascular homeostasis. *Handbook of Clinical Neurology*, *179*, 435–454. https://doi.org/10.1016/B978-0-12-819975-6.00028-5

McMenamin, B. W., Langeslag, S. J. E., Sirbu, M., Padmala, S., & Pessoa, L. (2014). Network Organization Unfolds over Time during Periods of Anxious Anticipation. *The Journal of Neuroscience*, *34*(34), 11261. https://doi.org/10.1523/JNEUROSCI.1579-14.2014

Melchior, J. R., Perez, R. E., Salimando, G. J., Luchsinger, J. R., Basu, A., & Winder, D. G. (2021). Cocaine Augments Dopamine-Mediated Inhibition of Neuronal Activity in the Dorsal Bed Nucleus of the Stria Terminalis. *Journal of Neuroscience*, *41*(27), 5876–5893. https://doi.org/10.1523/JNEUROSCI.0284-21.2021

Meller, W. H., Kathol, R. G., Samuelson, S. D., Gehris, T. L., Carroll, B. T., Pitts, A. F., & Clayton, P. J. (1995). CRH challenge test in anxious depression. *Biological Psychiatry*, *37*(6), 376–382. https://doi.org/10.1016/0006-3223(94)00132-M

Meloni, E. G., Gerety, L. P., Knoll, A. T., Cohen, B. M., & Carlezon, W. A. (2006). Behavioral and anatomical interactions between dopamine and corticotropin-releasing factor in the rat. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(14), 3855–3863. https://doi.org/10.1523/JNEUROSCI.4957-05.2006

Mennis, J., Stahler, G. J., & Mason, M. J. (2016). Risky Substance Use Environments and Addiction: A New Frontier for Environmental Justice Research. *International Journal of Environmental Research and Public Health*, *13*(6). https://doi.org/10.3390/IJERPH13060607

Merali, Z., Levac, C., & Anisman, H. (2003). Validation of a simple, ethologically relevant paradigm for assessing anxiety in mice. *Biological Psychiatry*, *54*(5), 552–565. https://doi.org/10.1016/S0006-3223(02)01827-9

Merchenthaler, I., Liposits, Z., Reid, J. J., & Wetsel, W. C. (1993). Light and electron microscopic immunocytochemical localization of PKCδ immunoreactivity in the rat central nervous system. *Journal of Comparative Neurology*, *336*(3), 378–399. https://doi.org/10.1002/CNE.903360306

Merriam-Webster.com Dictionary. (2023). *Stress*. https://www.merriam-webster.com/dictionary/stress

Messing, R. O., Petersen, P. J., & Henrich, C. J. (1991). Chronic ethanol exposure increases levels of protein kinase C delta and epsilon and protein kinase C-mediated phosphorylation in cultured

neural cells. *Journal of Biological Chemistry*, *266*(34), 23428–23432. https://doi.org/10.1016/S0021-9258(18)54514-2

Micioni Di Bonaventura, M. V., Ciccocioppo, R., Romano, A., Bossert, J. M., Rice, K. C., Ubaldi, M., St. Laurent, R., Gaetani, S., Massi, M., Shaham, Y., & Cifani, C. (2014). Role of Bed Nucleus of the Stria Terminalis Corticotrophin-Releasing Factor Receptors in Frustration Stress-Induced Binge-Like Palatable Food Consumption in Female Rats with a History of Food Restriction. *The Journal of Neuroscience*, *34*(34), 11316. https://doi.org/10.1523/JNEUROSCI.1854-14.2014

Miettinen, S., Roivainen, R., Keinänen, R., Hökfelt, T., & Koistinaho, J. (1996). Specific induction of protein kinase C delta subspecies after transient middle cerebral artery occlusion in the rat brain: inhibition by MK-801. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *16*(19), 6236–6245. https://doi.org/10.1523/JNEUROSCI.16-19-06236.1996

Mitra, R., Jadhav, S., McEwen, B. S., Vyas, A., & Chattarji, S. (2005). Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(26), 9371–9376. https://doi.org/10.1073/PNAS.0504011102

Moffitt, J. R., Bambah-Mukku, D., Eichhorn, S. W., Vaughn, E., Shekhar, K., Perez, J. D., Rubinstein, N. D., Hao, J., Regev, A., Dulac, C., & Zhuang, X. (2018). Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region. *Science*, *362*(6416). https://doi.org/10.1126/science.aau5324

Moga, M. M., Saper, C. B., & Gray, T. S. (1989). Bed nucleus of the stria terminalis: cytoarchitecture, immunohistochemistry, and projection to the parabrachial nucleus in the rat. *J Comp Neurol*, *283*(3), 315–332. https://doi.org/10.1002/cne.902830302 [doi]

Mondrinos, M. J., Kennedy, P. A., Lyons, M., Deutschman, C. S., & Kilpatrick, L. E. (2013). Protein Kinase C and Acute Respiratory Distress Syndrome. *Shock (Augusta, Ga.)*, *39*(6), 467. https://doi.org/10.1097/SHK.0B013E318294F85A

Monteggia, L. M., Eisch, A. J., Tang, M. D., Kaczmarek, L. K., & Nestler, E. J. (2000). Cloning and localization of the hyperpolarization-activated cyclic nucleotide-gated channel family in rat brain. *Molecular Brain Research*, *81*(1–2), 129–139. https://doi.org/10.1016/S0169-328X(00)00155-8

Moscarello, J. M., & Penzo, M. A. (2022). The central nucleus of the amygdala and the construction of defensive modes across the threat-imminence continuum. *Nature Neuroscience*, *25*(8), 999–1008. https://doi.org/10.1038/S41593-022-01130-5

Mula, M., Pini, S., & Cassano, G. B. (2007). The role of anticonvulsant drugs in anxiety disorders: A critical review of the evidence. *Journal of Clinical Psychopharmacology*, *27*(3), 263–272. https://doi.org/10.1097/JCP.0B013E318059361A

Murrough, J. W., Yaqubi, S., Sayed, S., & Charney, D. S. (2015). Emerging Drugs for the Treatment of Anxiety. *Expert Opinion on Emerging Drugs*, *20*(3), 393. https://doi.org/10.1517/14728214.2015.1049996

Myers, B., Mark Dolgas, C., Kasckow, J., Cullinan, W. E., & Herman, J. P. (2014). Central stress-integrative circuits: forebrain glutamatergic and GABAergic projections to the dorsomedial hypothalamus, medial preoptic area, and bed nucleus of the stria terminalis. *Brain Structure & Function*, *219*(4), 1287–1303. https://doi.org/10.1007/S00429-013-0566-Y

Nakamura, K. (2011). Central circuitries for body temperature regulation and fever. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *301*(5). https://doi.org/10.1152/AJPREGU.00109.2011

National Research Council. (2014). The Growth of Incarceration in the United States: Exploring Causes and ConseTravis, Jeremy Western, Bruce Redburn, F.quences. In J. Travis, B. Western, & F. Redburn (Eds.), *Publications and Research*. National Academies Press. https://academicworks.cuny.edu/jj_pubs/27

Nelson, R. J., & Trainor, B. C. (2007). Neural mechanisms of aggression. In *Nature Reviews Neuroscience* (Vol. 8, Issue 7, pp. 536–546). Nature Publishing Group. https://doi.org/10.1038/nrn2174

New, D. C., & Wong, Y. H. (2007). Molecular mechanisms mediating the G protein-coupled receptor regulation of cell cycle progression. *Journal of Molecular Signaling*, *2*(0). https://doi.org/10.1186/1750-2187-2-2

Newcomb, M. D., & Harlow, L. L. (1986). Life events and substance use among adolescents: mediating effects of perceived loss of control and meaninglessness in life. *Journal of Personality and Social Psychology*, *51*(3), 564–577. https://doi.org/10.1037//0022-3514.51.3.564

Newton, A. C. (2001). Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. *Chemical Reviews*, *101*(8), 2353–2364. https://doi.org/10.1021/CR0002801

NIMH. (2017). *Any Anxiety Disorder*. National Institute of Mental Health Statistics. https://www.nimh.nih.gov/health/statistics/any-anxiety-disorder.shtml

Nixon, P. G. (1982). The human function curve - a paradigm for our times. *Activitas Nervosa Superior*, *Suppl 3*(Pt 1), 130–133.

Nixon, P. G. F. (1976). The human function curve; with special reference to cardiovascular disorders: part II. *Practitioner*, *217*(1302), 935–944.

Nollet, M., Hicks, H., McCarthy, A. P., Wu, H., Möller-Levet, C. S., Laing, E. E., Malki, K., Lawless, N., Wafford, K. A., Dijk, D. J., & Winsky-Sommerer, R. (2019). REM sleep's unique associations with corticosterone regulation, apoptotic pathways, and behavior in chronic stress in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(7), 2733–2742. https://doi.org/10.1073/PNAS.1816456116/-/DCSUPPLEMENTAL

Norman, D. A., & Bobrow, D. G. (1975). On Data-limited and Resource-limited Processes. *Cognitive Psychology*.

NSDUH. (2021). *2021 National Survey of Drug Use and Health (NSDUH) Releases*. https://www.samhsa.gov/data/release/2021-national-survey-drug-use-and-health-nsduh-releases

O'Driscoll, K. R., Teng, K. K., Fabbro, D., Greene, L. A., & Weinstein, I. B. (1995). Selective translocation of protein kinase C-delta in PC12 cells during nerve growth factor-induced neuritogenesis. *Molecular Biology of the Cell*, *6*(4), 449. https://doi.org/10.1091/MBC.6.4.449

O'Leary, T. P., Kendrick, R. M., Bristow, B. N., Sullivan, K. E., Wang, L., Clements, J., Lemire, A. L., & Cembrowski, M. S. (2022). Neuronal cell types, projections, and spatial organization of the central amygdala. *IScience*, *25*(12). https://doi.org/10.1016/J.ISCI.2022.105497

Olive, M. F., Mehmert, K. K., Messing, R. O., & Hodge, C. W. (2000). Reduced operant ethanol self-administration and in vivo mesolimbic dopamine responses to ethanol in PKCepsilon-deficient mice. *The European Journal of Neuroscience*, *12*(11), 4131–4140. https://doi.org/10.1046/J.1460-9568.2000.00297.X

Ong, A. D., Williams, D. R., Nwizu, U., & Gruenewald, T. L. (2017). Everyday Unfair Treatment and Multisystem Biological Dysregulation in African American Adults. *Cultural Diversity & Ethnic Minority Psychology*, *23*(1), 27. https://doi.org/10.1037/CDP0000087

Ontario, H. Q. (2017). Psychotherapy for Major Depressive Disorder and Generalized Anxiety Disorder: A Health Technology Assessment. *Ontario Health Technology Assessment Series*, *17*(15), 1. /pmc/articles/PMC5709536/

Pacak, K., Tjurmina, O., Palkovits, M., Goldstein, D. S., Koch, C. A., Hoff, T., & Chrousos, G. P. (2002). Chronic hypercortisolemia inhibits dopamine synthesis and turnover in the nucleus accumbens: an in vivo microdialysis study. *Neuroendocrinology*, *76*(3), 148–157. https://doi.org/10.1159/000064522

Packard, A. E. B., Egan, A. E., & Ulrich-Lai, Y. M. (2016). HPA axis- Interaction with Behavioral Systems. *Comprehensive Physiology*, *6*(4), 1897. https://doi.org/10.1002/CPHY.C150042

Padilla-Coreano, N., Batra, K., Patarino, M., Chen, Z., Rock, R. R., Zhang, R., Hausmann, S. B., Weddington, J. C., Patel, R., Zhang, Y. E., Fang, H. S., Mishra, S., LeDuke, D. O., Revanna, J., Li, H., Borio, M., Pamintuan, R., Bal, A., Keyes, L. R., … Tye, K. M. (2022). Cortical ensembles orchestrate social competition via hypothalamic outputs. *Nature*, *603*(7902), 667.

https://doi.org/10.1038/S41586-022-04507-5

Palij, P., & Stamford, J. A. (1992). Real-time monitoring of endogenous noradrenaline release in rat brain slices using fast cyclic voltammetry: 1. Characterisation of evoked noradrenaline efflux and uptake from nerve terminals in the bed nucleus of stria terminalis, pars ventralis. *Brain Research*, *587*(1), 137–146. https://doi.org/10.1016/0006-8993(92)91438-K

Palmiter, R. D. (2018). The Parabrachial Nucleus: CGRP Neurons Function as a General Alarm. *Trends in Neurosciences*, *41*(5), 280–293. https://doi.org/10.1016/j.tins.2018.03.007

Pape, H.-C., & Pare, D. (2010). Plastic Synaptic Networks of the Amygdala for the Acquisition, Expression, and Extinction of Conditioned Fear. *Physiological Reviews*, *90*(2), 419–463. https://doi.org/10.1152/physrev.00037.2009

Paradies, Y., Ben, J., Denson, N., Elias, A., Priest, N., Pieterse, A., Gupta, A., Kelaher, M., & Gee, G. (2015). Racism as a Determinant of Health: A Systematic Review and Meta-Analysis. *PLoS ONE*, *10*(9). https://doi.org/10.1371/JOURNAL.PONE.0138511

Paré, W. P., & Glavin, G. B. (1986). Restraint stress in biomedical research: A review. *Neuroscience & Biobehavioral Reviews*, *10*(3), 339–370. https://doi.org/10.1016/0149-7634(86)90017-5

Paretkar, T., & Dimitrov, E. (2018). The central amygdala corticotropin-releasing hormone (CRH) neurons modulation of anxiety-like behavior and hippocampus-dependent memory in mice. *Neuroscience*, *390*, 187. https://doi.org/10.1016/J.NEUROSCIENCE.2018.08.019

Park, J., Bucher, E. S., Fontillas, K., Owesson-White, C., Ariansen, J. L., Carelli, R. M., & Wightman, R. M. (2013). Opposing catecholamine changes in the bed nucleus of the stria terminalis during intracranial self-stimulation and its extinction. *Biological Psychiatry*, *74*(1), 69–76. https://doi.org/10.1016/j.biopsych.2012.11.008

Park, J., Kile, B. M., & Wightman, M. R. (2009). In vivo voltammetric monitoring of norepinephrine release in the rat ventral bed nucleus of the stria terminalis and anteroventral thalamic nucleus. *The European Journal of Neuroscience*, *30*(11), 2121–2133. https://doi.org/10.1111/J.1460-9568.2009.07005.X

Park, J., Wheeler, R. A., Fontillas, K., Keithley, R. B., Carelli, R. M., & Wightman, R. M. (2012). Catecholamines in the bed nucleus of the stria terminalis reciprocally respond to reward and aversion. *Biological Psychiatry*, *71*(4), 327–334. https://doi.org/10.1016/j.biopsych.2011.10.017

Park, Y. G., Sohn, C. H., Chen, R., McCue, M., Yun, D. H., Drummond, G. T., Ku, T., Evans, N. B., Oak, H. C., Trieu, W., Choi, H., Jin, X., Lilascharoen, V., Wang, J., Truttmann, M. C., Qi, H. W., Ploegh, H. L., Golub, T. R., Chen, S. C., … Chung, K. (2019). Protection of tissue physicochemical properties using polyfunctional crosslinkers. *Nature Biotechnology*, *37*(1), 73. https://doi.org/10.1038/nbt.4281

Partridge, J. G., Forcelli, P. A., Luo, R., Cashdan, J. M., Schulkin, J., Valentino, R. J., & Vicini, S. (2016). Stress increases GABAergic neurotransmission in CRF neurons of the central amygdala and bed nucleus stria terminalis. *Neuropharmacology*, *107*, 239–250. https://doi.org/10.1016/j.neuropharm.2016.03.029

Paskitti, M. E., McCreary, B. J., & Herman, J. P. (2000). Stress regulation of adrenocorticosteroid receptor gene transcription and mRNA expression in rat hippocampus: Time-course analysis. *Molecular Brain Research*, *80*(2), 142–152. https://doi.org/10.1016/S0169-328X(00)00121-2

Patel, S., & Hillard, C. J. (2008). Adaptations in Endocannabinoid Signaling in Response to Repeated Homotypic Stress: A Novel Mechanism for Stress Habituation. *The European Journal of Neuroscience*, *27*(11), 2821. https://doi.org/10.1111/J.1460-9568.2008.06266.X

Patel, S., Roelke, C. T., Rademacher, D. J., & Hillard, C. J. (2005). Inhibition of restraint stress-induced neural and behavioural activation by endogenous cannabinoid signalling. *European Journal of Neuroscience*, *21*(4), 1057–1069. https://doi.org/10.1111/j.1460-9568.2005.03916.x

Pavesi, E., Canteras, N. S., & Carobrez, A. P. (2011). Acquisition of Pavlovian Fear Conditioning Using β-Adrenoceptor Activation of the Dorsal Premammillary Nucleus as an Unconditioned Stimulus to Mimic Live Predator-Threat Exposure. *Neuropsychopharmacology 2011 36:5*, *36*(5), 926–939.

https://doi.org/10.1038/npp.2010.231

Penninx, B. W., Pine, D. S., Holmes, E. A., & Reif, A. (2021). Anxiety disorders. *The Lancet*, *397*(10277), 914–927. https://doi.org/10.1016/S0140-6736(21)00359-7

Penzo, M. A., Robert, V., Tucciarone, J., De Bundel, D., Wang, M., Van Aelst, L., Darvas, M., Parada, L. F., Palmiter, R. D., He, M., Huang, Z. J., & Li, B. (2015). The paraventricular thalamus controls a central amygdala fear circuit. *Nature*, *519*(7544), 455–459. https://doi.org/10.1038/nature13978

Pereira, G. M. V., Pimentel, V. M., Surita, F. G., Silva, A. D., & Brito, L. G. O. (2022). Perceived racism or racial discrimination and the risk of adverse obstetric outcomes: a systematic review. *São Paulo Medical Journal*, *140*(5), 705. https://doi.org/10.1590/1516-3180.2021.0505.R1.07042022

Perez, R. E., Basu, A., Nabit, B. P., Harris, N. A., Folkes, O. M., Patel, S., Gilsbach, R., Hein, L., & Winder, D. G. (2020). α2A-adrenergic heteroreceptors are required for stress-induced reinstatement of cocaine conditioned place preference. *Neuropsychopharmacology*, *45*(9), 1473–1481. https://doi.org/10.1038/s41386-020-0641-z

Perkins, H. W. (1999). Stress-motivated drinking in collegiate and postcollegiate young adulthood: life course and gender patterns. *Journal of Studies on Alcohol*, *60*(2), 219–227. https://doi.org/10.15288/JSA.1999.60.219

Peterson, L. M., Stock, M. L., Monroe, J., Molloy-Paolillo, B. K., & Lambert, S. F. (2020). Racial exclusion causes acute cortisol release among emerging-adult African Americans: The role of reduced perceived control. *Https://Doi-Org.Proxy.Library.Vanderbilt.Edu/10.1080/00224545.2020.1729685*, *160*(5), 658–674. https://doi.org/10.1080/00224545.2020.1729685

Petrulis, A., & Johnston, R. E. (1999). Lesions centered on the medial amygdala impair scent-marking and sex- odor recognition but spare discrimination of individual odors in female golden hamsters. *Behavioral Neuroscience*, *113*(2), 345–357. https://doi.org/10.1037/0735-7044.113.2.345

Phelix, C. F., Liposits, Z., & Paull, W. K. (1992). Monoamine innervation of bed nucleus of stria terminalis: An electron microscopic investigation. *Brain Research Bulletin*, *28*(6), 949–965. https://doi.org/10.1016/0361-9230(92)90218-M

Piazza, P. V., & Le Moal, M. (1996). Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annual Review of Pharmacology and Toxicology*, *36*, 359–378. https://doi.org/10.1146/ANNUREV.PA.36.040196.002043

Pina, M. M., & Cunningham, C. L. (2017). Ethanol-seeking behavior is expressed directly through an extended amygdala to midbrain neural circuit. *Neurobiology of Learning and Memory*, *137*, 83–91. https://doi.org/10.1016/j.nlm.2016.11.013

Pina, M. M., Pati, D., Hwa, L. S., Wu, S. Y., Mahoney, A. A., Omenyi, C. G., Navarro, M., & Kash, T. L. (2020). The kappa opioid receptor modulates GABA neuron excitability and synaptic transmission in midbrainprojections from the insular cortex. *Neuropharmacology*, *165*. https://doi.org/10.1016/j.neuropharm.2019.107831

Pina, M. M., Young, E. A., Ryabinin, A. E., & Cunningham, C. L. (2015). The bed nucleus of the stria terminalis regulates ethanol-seeking behavior in mice. *Neuropharmacology*, *99*, 627–638. https://doi.org/10.1016/j.neuropharm.2015.08.033

Pleil, K. E., Rinker, J. A., Lowery-Gionta, E. G., Mazzone, C. M., McCall, N. M., Kendra, A. M., Olson, D. P., Lowell, B. B., Grant, K. A., Thiele, T. E., & Kash, T. L. (2015). NPY signaling inhibits extended amygdala CRF neurons to suppress binge alcohol drinking. *Nature Neuroscience*, *18*(4), 545–552. https://doi.org/10.1038/nn.3972

Pollak, D. D., Rey, C. E., & Monje, F. J. (2010). Rodent models in depression research: classical strategies and new directions. *Annals of Medicine*, *42*(4), 252–264. https://doi.org/10.3109/07853891003769957

Pomrenze, M. B., Tovar-Diaz, J., Blasio, A., Maiya, R., Giovanetti, S. M., Lei, K., Morikawa, H., Woodward Hopf, F., & Messing, R. O. (2019). A corticotropin releasing factor network in the

extended amygdala for anxiety. *Journal of Neuroscience*, *39*(6), 1030–1043. https://doi.org/10.1523/JNEUROSCI.2143-18.2018

Priscilla Wong, by, is, W., & Wong, P. (2010). Selective Mutism: A Review of Etiology, Comorbidities, and Treatment. *Psychiatry (Edgmont)*, *7*(3), 23. /pmc/articles/PMC2861522/

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, *463*(1–3), 3–33. https://doi.org/10.1016/S0014-2999(03)01272-X

Qiu, H., Lu, H., Pei, J., Zhang, Y., Ma, Y., Xing, C., Wang, X., & Zhu, X. (2023). Effects of chronic stress on smartphone addiction: A moderated mediation model. *Frontiers in Public Health*, *11*. https://doi.org/10.3389/FPUBH.2023.1048210

Radley, J. J., Arias, C. M., & Sawchenko, P. E. (2006). Regional Differentiation of the Medial Prefrontal Cortex in Regulating Adaptive Responses to Acute Emotional Stress. *Journal of Neuroscience*, *26*(50), 12967–12976. https://doi.org/10.1523/JNEUROSCI.4297-06.2006

Radley, J. J., Gosselink, K. L., & Sawchenko, P. E. (2009). A discrete GABAergic relay mediates medial prefrontal cortical inhibition of the neuroendocrine stress response. *Journal of Neuroscience*, *29*(22), 7330–7340. https://doi.org/10.1523/JNEUROSCI.5924-08.2009

Radley, J. J., & Johnson, S. B. (2018). Anteroventral bed nuclei of the stria terminalis neurocircuitry: Towards an integration of HPA axis modulation with coping behaviors - Curt Richter Award Paper 2017. In *Psychoneuroendocrinology* (Vol. 89, pp. 239–249). https://doi.org/10.1016/j.psyneuen.2017.12.005

Radley, J. J., Rocher, A. B., Rodriguez, A., Ehlenberger, D. B., Dammann, M., Mcewen, B. S., Morrison, J. H., Wearne, S. L., & Hof, P. R. (2008). REPEATED STRESS ALTERS DENDRITIC SPINE MORPHOLOGY IN THE RAT MEDIAL PREFRONTAL CORTEX. *The Journal of Comparative Neurology*, *507*(1), 1141. https://doi.org/10.1002/CNE.21588

Radley, J., Morilak, D., Viau, V., & Campeau, S. (2015). Chronic stress and brain plasticity: Mechanisms underlying adaptive and maladaptive changes and implications for stress-related CNS disorders. *Neuroscience and Biobehavioral Reviews*, *58*, 79–91. https://doi.org/10.1016/j.neubiorev.2015.06.018

Rangani, R. J., Upadhya, M. A., Nakhate, K. T., Kokare, D. M., & Subhedar, N. K. (2012). Nicotine evoked improvement in learning and memory is mediated through NPY Y1 receptors in rat model of Alzheimer's disease. *Peptides*, *33*(2), 317–328. https://doi.org/10.1016/j.peptides.2012.01.004

Ren, J., Wang, Q., Morgan, S., Si, Y., Ravichander, A., Dou, C., Kent, K. C., & Liu, B. (2014). Protein Kinase C-δ (PKCδ) Regulates Proinflammatory Chemokine Expression through Cytosolic Interaction with the NF-κB Subunit p65 in Vascular Smooth Muscle Cells. *The Journal of Biological Chemistry*, *289*(13), 9013. https://doi.org/10.1074/JBC.M113.515957

Repina, N. A., Rosenbloom, A., Mukherjee, A., Schaffer, D. V., & Kane, R. S. (2017). At light speed: Advances in optogenetic systems for regulating cell signaling and behavior. *Annual Review of Chemical and Biomolecular Engineering*, *8*, 13–39. https://doi.org/10.1146/annurev-chembioeng-060816-101254

Ressler, K. J., Berretta, S., Bolshakov, V. Y., Rosso, I. M., Meloni, E. G., Rauch, S. L., & Carlezon, W. A. (2022). Post-traumatic stress disorder: clinical and translational neuroscience from cells to circuits. *Nature Reviews. Neurology*, *18*(5), 273. https://doi.org/10.1038/S41582-022-00635-8

Reyland, M. E. (2009). Protein kinase C isoforms: Multi-functional regulators of cell life and death. *Frontiers in Bioscience (Landmark Edition)*, *14*(6), 2386. https://doi.org/10.2741/3385

Reyland, M. E., & Jones, D. N. M. (2016). Multifunctional roles of PKCδ: Opportunities for targeted therapy in human disease. *Pharmacology & Therapeutics*, *165*, 1. https://doi.org/10.1016/J.PHARMTHERA.2016.05.001

Ricardo, J. A., & Tongju Koh, E. (1978). Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Research*, *153*(1), 1–26. https://doi.org/10.1016/0006-8993(78)91125-3

Rich-Edwards, J. W., Buka, S. L., Brennan, R. T., & Earls, F. (2003). Diverging associations of maternal age with low birthweight for black and white mothers. *International Journal of Epidemiology*, *32*(1), 83–90. https://doi.org/10.1093/IJE/DYG008

Rinker, J. A., Marshall, S. A., Mazzone, C. M., Lowery-Gionta, E. G., Gulati, V., Pleil, K. E., Kash, T. L., Navarro, M., & Thiele, T. E. (2017). Extended Amygdala to Ventral Tegmental Area Corticotropin-Releasing Factor Circuit Controls Binge Ethanol Intake. *Biological Psychiatry*, *81*(11), 930–940. https://doi.org/10.1016/j.biopsych.2016.02.029

Risch, N., Herrell, R., Lehner, T., Liang, K. Y., Eaves, L., Hoh, J., Griem, A., Kovacs, M., Ott, J., & Merikangas, K. R. (2009). Interaction Between the Serotonin Transporter Gene (5-HTTLPR), Stressful Life Events, and Risk of Depression: A Meta-analysis. *JAMA*, *301*(23), 2462–2471. https://doi.org/10.1001/JAMA.2009.878

Roberts, G. W., Woodhams, P. L., Polak, J. M., & Crow, T. J. (1982). Distribution of neuropeptides in the limbic system of the rat: The amygdaloid complex. *Neuroscience*, *7*(1), 99–131. https://doi.org/10.1016/0306-4522(82)90156-7

Roder, S., & Ciriello, J. (1993). Contribution of bed nucleus of the stria terminalis to the cardiovascular responses elicited by stimulation of the amygdala. *Journal of the Autonomic Nervous System*, *45*(1), 61–75. https://doi.org/10.1016/0165-1838(93)90362-X

Rodgers, R. J., & Dalvi, A. (1997). Anxiety, defence and the elevated plus-maze. *Neuroscience & Biobehavioral Reviews*, *21*(6), 801–810. https://doi.org/10.1016/S0149-7634(96)00058-9

Rodriguez-Romaguera, J., Ung, R. L., Nomura, H., Otis, J. M., Basiri, M. L., Namboodiri, V. M. K., Zhu, X., Elliott Robinson, J., McHenry, J. A., Kosyk, O., Jhou, T. C., Kash, T. L., Bruchas, M. R., & Stuber, G. D. (2020). Prepronociceptin expressing neurons in the extended amygdala encode and promote rapid arousal responses to motivationally salient stimuli. In *bioRxiv* (p. 2020.01.21.914341). bioRxiv. https://doi.org/10.1101/2020.01.21.914341

Rodriguez-Romaguera, J., Ung, R. L., Nomura, H., Otis, J. M., Basiri, M. L., Namboodiri, V. M. K., Zhu, X., Robinson, J. E., van den Munkhof, H. E., McHenry, J. A., Eckman, L. E. H., Kosyk, O., Jhou, T. C., Kash, T. L., Bruchas, M. R., & Stuber, G. D. (2020). Prepronociceptin-Expressing Neurons in the Extended Amygdala Encode and Promote Rapid Arousal Responses to Motivationally Salient Stimuli. *Cell Reports*, *33*(6), 108362. https://doi.org/10.1016/J.CELREP.2020.108362

Rodríguez-Sierra, O. E., Turesson, H. K., & Pare, D. (2013). Contrasting distribution of physiological cell types in different regions of the bed nucleus of the stria terminalis. *Journal of Neurophysiology*, *110*(9), 2037–2049. https://doi.org/10.1152/jn.00408.2013

Rodriguez, B. F., Weisberg, R. B., Pagano, M. E., Bruce, S. E., Spencer, M. A., Culpepper, L., & Keller, M. B. (2006). Characteristics and predictors of full and partial recovery from generalized anxiety disorder in primary care patients. *Journal of Nervous and Mental Disease*, *194*(2), 91–97. https://doi.org/10.1097/01.NMD.0000198140.02154.32

Rohrmann, S., Hennig, J., & Netter, P. (1999). Changing psychobiological stress reactions by manipulating cognitive processes. *International Journal of Psychophysiology*, *33*(2), 149–161. https://doi.org/10.1016/S0167-8760(99)00036-7

Roland, B. L., & Sawchenko, P. E. (1993). Local origins of some GABAergic projections to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *The Journal of Comparative Neurology*, *332*(1), 123–143. https://doi.org/10.1002/CNE.903320109

Romero, L. M., & Butler, L. K. (2007). Endocrinology of Stress. *International Journal of Comparative Psychology*, *20*, 89–95.

Ross, R. A., Foster, S. L., & Ionescu, D. F. (2017). The Role of Chronic Stress in Anxious Depression. *Chronic Stress*, *1*. https://doi.org/10.1177/2470547016689472

Roth, B. L. (2016). DREADDs for Neuroscientists. *Neuron*, *89*(4), 683–694. https://doi.org/10.1016/j.neuron.2016.01.040

Rothbaum, B. O., Meadows, E. A., Resick, P., & Foy, D. W. (2000). Cognitive-behavioral therapy. In *Effective treatments for PTSD: Practice guidelines from the International Society for Traumatic*

Stress Studies. (pp. 320–325). The Guilford Press.

Russell, F. A., King, R., Smillie, S. J., Kodji, X., & Brain, S. D. (2014). Calcitonin Gene-Related Peptide: Physiology and Pathophysiology. *Physiological Reviews*, *94*(4), 1099. https://doi.org/10.1152/PHYSREV.00034.2013

Saal, D., Dong, Y., Bonci, A., & Malenka, R. C. (2003). Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron*, *37*(4), 577–582. https://doi.org/10.1016/S0896-6273(03)00021-7

SAH, P., FABER, E. S. L., LOPEZ DE ARMENTIA, M., & POWER, J. (2003). The Amygdaloid Complex: Anatomy and Physiology. *Physiological Reviews*, *83*(3), 803–834. https://doi.org/10.1152/physrev.00002.2003

Salimando, G. J., Hyun, M., Boyt, K. M., & Winder, D. G. (2020). BNST GluN2D-Containing NMDA Receptors Influence Anxiety- and Depressive-like Behaviors and ModulateCell-Specific Excitatory/Inhibitory Synaptic Balance. *The Journal of Neuroscience*, *40*(20), 3949. https://doi.org/10.1523/JNEUROSCI.0270-20.2020

Salzer, E., Santos-Valente, E., Keller, B., Warnatz, K., & Boztug, K. (2016). Protein Kinase C δ: a Gatekeeper of Immune Homeostasis. *Journal of Clinical Immunology*, *36*(7), 631. https://doi.org/10.1007/S10875-016-0323-0

Samidurai, M., Palanisamy, B. N., Bargues-Carot, A., Hepker, M., Kondru, N., Manne, S., Zenitsky, G., Jin, H., Anantharam, V., Kanthasamy, A. G., & Kanthasamy, A. (2021). PKC Delta Activation Promotes Endoplasmic Reticulum Stress (ERS) and NLR Family Pyrin Domain-Containing 3 (NLRP3) Inflammasome Activation Subsequent to Asynuclein-Induced Microglial Activation: Involvement of Thioredoxin-Interacting Protein (TXNIP)/Thioredoxin (Trx) Redoxisome Pathway. *Frontiers in Aging Neuroscience*, *13*. https://doi.org/10.3389/FNAGI.2021.661505

Sanford, C. A., Soden, M. E., Baird, M. A., Miller, S. M., Schulkin, J., Palmiter, R. D., Clark, M., & Zweifel, L. S. (2017). A Central Amygdala CRF Circuit Facilitates Learning about Weak Threats. *Neuron*, *93*(1), 164–178. https://doi.org/10.1016/j.neuron.2016.11.034

Santomauro, D. F., Mantilla Herrera, A. M., Shadid, J., Zheng, P., Ashbaugh, C., Pigott, D. M., Abbafati, C., Adolph, C., Amlag, J. O., Aravkin, A. Y., Bang-Jensen, B. L., Bertolacci, G. J., Bloom, S. S., Castellano, R., Castro, E., Chakrabarti, S., Chattopadhyay, J., Cogen, R. M., Collins, J. K., … Ferrari, A. J. (2021). Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. *Lancet (London, England)*, *398*(10312), 1700. https://doi.org/10.1016/S0140-6736(21)02143-7

Savage, K., Firth, J., Stough, C., & Sarris, J. (2018). GABA-modulating phytomedicines for anxiety: A systematic review of preclinical and clinical evidence. *Phytotherapy Research : PTR*, *32*(1), 3–18. https://doi.org/10.1002/PTR.5940

Sayed, S., Van Dam, N. T., Horn, S. R., Kautz, M. M., Parides, M., Costi, S., Collins, K. A., Iacoviello, B., Iosifescu, D. V., Mathé, A. A., Southwick, S. M., Feder, A., Charney, D. S., & Murrough, J. W. (2018). A Randomized Dose-Ranging Study of Neuropeptide Y in Patients with Posttraumatic Stress Disorder. *International Journal of Neuropsychopharmacology*, *21*(1), 3. https://doi.org/10.1093/IJNP/PYX109

Schlund, M. W., Carter, H., Cudd, G., Murphy, K., Ahmed, N., Dymond, S., & Tone, E. B. (2021). Human social defeat and approach-avoidance: Escalating social-evaluative threat and threat of aggression increases social avoidance. *Journal of the Experimental Analysis of Behavior*, *115*(1), 157. https://doi.org/10.1002/JEAB.654

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice. *Journal of Visualized Experiments*, *96*, 1–6. https://doi.org/10.3791/52434

Selye, H. (1936). A Syndrome produced by Diverse Nocuous Agents. *Nature 1936 138:3479*, *138*(3479), 32–32. https://doi.org/10.1038/138032a0

Selye, H. (1946). THE GENERAL ADAPTATION SYNDROME AND THE DISEASES OF ADAPTATION. *The Journal of Clinical Endocrinology & Metabolism*, *6*(2), 117–230.

https://doi.org/10.1210/JCEM-6-2-117

Selye, H. (1976). Forty years of stress research: principal remaining problems and misconceptions. *Canadian Medical Association Journal*, *115*(1), 53. /pmc/articles/PMC1878603/?report=abstract

Seung, H. S., & Sümbül, U. (2014). Neuronal cell types and connectivity: lessons from the retina. *Neuron*, *83*(6), 1262–1272. https://doi.org/10.1016/j.neuron.2014.08.054

Shackman, A. J., & Fox, A. S. (2016). Contributions of the Central Extended Amygdala to Fear and Anxiety. *The Journal of Neuroscience*, *36*(31), 8050. https://doi.org/10.1523/JNEUROSCI.0982-16.2016

Shaham, Y., & Stewart, J. (1995). Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. *Psychopharmacology*, *119*(3), 334–341. http://www.ncbi.nlm.nih.gov/pubmed/7675970

Shalev, U., Morales, M., Hope, B., Yap, J., & Shaham, Y. (2001). Time-dependent changes in extinction behavior and stress-induced reinstatement of drug seeking following withdrawal from heroin in rats. *Psychopharmacology*, *156*(1), 98–107. https://doi.org/10.1007/s002130100748

Shiels, M. S., Freedman, N. D., Thomas, D., & De Gonzalez, A. B. (2018). Trends in U.S. Drug Overdose Deaths in non-Hispanic blacks, Hispanics, and non-Hispanic whites: 2000–2015. *Annals of Internal Medicine*, *168*(6), 453. https://doi.org/10.7326/M17-1812

Shimada, S., Inagaki, S., Kubota, Y., Kito, S., Funaki, H., & Takagi, H. (1989). Light and electron microscopic studies of calcitonin gene-related peptide-like immunoreactive terminals in the central nucleus of the amygdala and the bed nucleus of the stria terminalis of the rat. *Experimental Brain Research*, *77*(1), 217–220. https://doi.org/10.1007/BF00250584

Silberman, Y., Matthews, R. T., & Winder, D. G. (2013). A Corticotropin Releasing Factor Pathway for Ethanol Regulation of the Ventral Tegmental Area in the Bed Nucleus of the Stria Terminalis. *Journal of Neuroscience*, *33*(3), 950–960. https://doi.org/10.1523/JNEUROSCI.2949-12.2013

Šimić, G., Tkalčić, M., Vukić, V., Mulc, D., Španić, E., Šagud, M., Olucha-Bordonau, F. E., Vukšić, M., & Hof, P. R. (2021). Understanding Emotions: Origins and Roles of the Amygdala. *Biomolecules*, *11*(6). https://doi.org/10.3390/BIOM11060823

Sinha, R. (2008). Chronic Stress, Drug Use, and Vulnerability to Addiction. *Ann N Y Acad Sci*, *1141*, 105–130. https://doi.org/10.1196/annals.1441.030

Slutske, W. S., Deutsch, A. R., Statham, D. J., & Martin, N. G. (2015). Local Area Disadvantage and Gambling Involvement and Disorder: Evidence for Gene-Environment Correlation and Interaction. *Journal of Abnormal Psychology*, *124*(3), 606. https://doi.org/10.1037/ABN0000071

Snyder, A. E., Salimando, G. J., Winder, D. G., & Silberman, Y. (2019). Chronic intermittent ethanol and acute stress similarly modulate BNST CRF neuron activity via noradrenergic signaling. *Alcoholism, Clinical and Experimental Research*, *43*(8), 1695. https://doi.org/10.1111/ACER.14118

Somerville, L. H., Wagner, D. D., Wig, G. S., Moran, J. M., Whalen, P. J., & Kelley, W. M. (2013). Interactions Between Transient and Sustained Neural Signals Support the Generation and Regulation of Anxious Emotion. *Cerebral Cortex (New York, NY)*, *23*(1), 49. https://doi.org/10.1093/CERCOR/BHR373

Speidel, J. T., Affandi, T., Jones, D. N. M., Ferrara, S. E., & Reyland, M. E. (2020). Functional proteomic analysis reveals roles for PKCδ in regulation of cell survival and cell death: Implications for cancer pathogenesis and therapy. *Advances in Biological Regulation*, *78*, 100757. https://doi.org/10.1016/J.JBIOR.2020.100757

Spierling, S. R., & Zorrilla, E. P. (2017). Don't stress about CRF: Assessing the translational failures of CRF1 antagonists. *Psychopharmacology*, *234*(9–10), 1467. https://doi.org/10.1007/S00213-017-4556-2

Spiga, F., Walker, J. J., Terry, J. R., & Lightman, S. L. (2014). HPA axis-rhythms. *Comprehensive Physiology*, *4*(3), 1273–1298. https://doi.org/10.1002/CPHY.C140003

Spinhoven, P., van Hemert, A. M., & Penninx, B. W. J. H. (2017). Experiential Avoidance and Bordering

Psychological Constructs as Predictors of the Onset, Relapse and Maintenance of Anxiety Disorders: One or Many? *Cognitive Therapy and Research*, *41*(6), 867. https://doi.org/10.1007/S10608-017-9856-7

Staal, M. A. (2004). Stress, Cognition, and Human Performance: A Literature Review and Conceptual Framework [National Aeronautics and Space Administration]. In *NASA Scientific and Technical Information*. https://human-factors.arc.nasa.gov/flightcognition/Publications/IH_054_Staal.pdf

Stamatakis, A. M., Sparta, D. R., Jennings, J. H., Mcelligott, Z. A., Decot, H., & Stuber, G. D. (2014). Amygdala and Bed Nucleus of the Stria Terminalis Circuitry: Implications for addiction-related behaviors. *Neuropharmacology*, *76*(0 0), 320–328. https://doi.org/10.1016/J.NEUROPHARM.2013.05.046

Steenen, S. A., Van Wijk, A. J., Van Der Heijden, G. J. M. G., Van Westrhenen, R., De Lange, J., & De Jongh, A. (2016). Propranolol for the treatment of anxiety disorders: Systematic review and meta-analysis. *Journal of Psychopharmacology*, *30*(2), 128–139. https://doi.org/10.1177/0269881115612236

Steimer, T. (2002). The biology of fear- and anxiety-related behaviors. *Dialogues in Clinical Neuroscience*, *4*(3), 231. https://doi.org/10.31887/DCNS.2002.4.3/TSTEIMER

Steimer, T. (2011). Animal models of anxiety disorders in rats and mice: some conceptual issues. *Dialogues in Clinical Neuroscience*, *13*(4), 495. https://doi.org/10.31887/DCNS.2011.13.4/TSTEIMER

Sterling, P., & Eyer, J. (1988). Allostasis: A new paradigm to explain arousal pathology. In *Handbook of life stress, cognition and health.* (pp. 629–649). John Wiley & Sons.

Sterrenburg, L., Gaszner, B., Boerrigter, J., Santbergen, L., Bramini, M., Elliott, E., Chen, A., Peeters, B. W. M. M., Roubos, E. W., & Kozicz, T. (2011). Chronic stress induces sex-specific alterations in methylation and expression of corticotropin-releasing factor gene in the rat. *PloS One*, *6*(11). https://doi.org/10.1371/JOURNAL.PONE.0028128

Stokes, A. F., & Kite, K. (2000). On Grasping a Nettle and Becoming Emotional. *Stress, Workload, and Fatigue*, 107–132. https://doi.org/10.1201/B12791-1.4

Strawn, J. R., Compton, S. N., Robertson, B., Albano, A. M., Hamdani, M., & Rynn, M. A. (2017). Extended Release Guanfacine in Pediatric Anxiety Disorders: A Pilot, Randomized, Placebo-Controlled Trial. *Journal of Child and Adolescent Psychopharmacology*, *27*(1), 29–37. https://doi.org/10.1089/CAP.2016.0132

Ströhle, A., & Holsboer, F. (2003). Stress responsive neurohormones in depression and anxiety. *Pharmacopsychiatry*, *36 Suppl 3*(SUPPL. 3). https://doi.org/10.1055/S-2003-45132

Sullivan, G. M., Apergis, J., Bush, D. E. A., Johnson, L. R., Hou, M., & Ledoux, J. E. (2004). Lesions in the bed nucleus of the stria terminalis disrupt corticosterone and freezing responses elicited by a contextual but not by a specific cue-conditioned fear stimulus. *Neuroscience*, *128*(1), 7–14. https://doi.org/10.1016/J.NEUROSCIENCE.2004.06.015

Szücs, A., Berton, F., Nowotny, T., Sanna, P., & Francesconi, W. (2010). Consistency and Diversity of Spike Dynamics in the Neurons of Bed Nucleus of Stria Terminalis of the Rat: A Dynamic Clamp Study. *PLoS ONE*, *5*(8), e11920. https://doi.org/10.1371/journal.pone.0011920

Takagishi, M., & Chiba, T. (1991). Efferent projections of the infralimbic (area 25) region of the medial prefrontal cortex in the rat: an anterograde tracer PHA-L study. *Brain Research*, *566*(1–2), 26–39. https://doi.org/10.1016/0006-8993(91)91677-S

Takahashi, H., Takada, Y., Nagai, N., Urano, T., & Takada, A. (1998). Effects of nicotine and footshock stress on dopamine release in the striatum and nucleus accumbens. *Brain Research Bulletin*, *45*(2), 157–162. https://doi.org/10.1016/S0361-9230(97)00332-8

Tamashiro, K. L., Sakai, R. R., Shively, C. A., Karatsoreos, I. N., & Reagan, L. P. (2011). Chronic stress, metabolism, and metabolic syndrome. *Stress (Amsterdam, Netherlands)*, *14*(5), 468–474. https://doi.org/10.3109/10253890.2011.606341

Tan, S. Y., & Yip, A. (2018). Hans Selye (1907–1982): Founder of the stress theory. *Singapore Medical*

Journal, *59*(4), 170. https://doi.org/10.11622/SMEDJ.2018043

Tang, Y., Soroush, F., Sun, S., Liverani, E., Langston, J. C., Yang, Q., Kilpatrick, L. E., & Kiani, M. F. (2018). Protein kinase C-delta inhibition protects blood-brain barrier from sepsis-induced vascular damage. *Journal of Neuroinflammation*, *15*(1), 1–12. https://doi.org/10.1186/S12974-018-1342-Y/FIGURES/6

Tank, A. W., & Wong, D. L. (2015). Peripheral and Central Effects of Circulating Catecholamines. *Comprehensive Physiology*, *5*(1), 1–15. https://doi.org/10.1002/CPHY.C140007

Tasan, R. O., Verma, D., Wood, J., Lach, G., Hörmer, B., de Lima, T. C. M., Herzog, H., & Sperk, G. (2016). The role of Neuropeptide Y in fear conditioning and extinction. *Neuropeptides*, *55*, 111–126. https://doi.org/10.1016/J.NPEP.2015.09.007

Taylor, J. H., Landeros-Weisenberger, A., Coughlin, C., Mulqueen, J., Johnson, J. A., Gabriel, D., Reed, M. O., Jakubovski, E., & Bloch, M. H. (2018). Ketamine for Social Anxiety Disorder: A Randomized, Placebo-Controlled Crossover Trial. *Neuropsychopharmacology*, *43*(2), 325. https://doi.org/10.1038/NPP.2017.194

Terlizzi, E., & Villarroel, M. (2020). *Symptoms of generalized anxiety disorder among adults: United States, 2019. NCHS Data Brief, no 378.* https://www.cdc.gov/nchs/products/databriefs/db378.htm#Suggested_citation

Thierry, A. M., Tassin, J. P., Blanc, G., & Glowinski, J. (1976). Selective activation of mesocortical DA system by stress. *Nature*, *263*(5574), 242–244. https://doi.org/10.1038/263242A0

Thompson, E. (2015). Hamilton Rating Scale for Anxiety (HAM-A). *Occupational Medicine*, *65*(7), 601–601. https://doi.org/10.1093/OCCMED/KQV054

Timmers, I., Quaedflieg, C. W. E. M., Hsu, C., Heathcote, L. C., Rovnaghi, C. R., & Simons, L. E. (2019). The interaction between stress and chronic pain through the lens of threat learning. *Neuroscience and Biobehavioral Reviews*, *107*, 641. https://doi.org/10.1016/J.NEUBIOREV.2019.10.007

Ting, J. T., Daigle, T. L., Chen, Q., & Feng, G. (2014). Acute brain slice methods for adult and aging animals: application of targeted patch clamp analysis and optogenetics. *Methods in Molecular Biology (Clifton, N.J.)*, *1183*, 221–242. https://doi.org/10.1007/978-1-4939-1096-0_14

Torrisi, S., O'Connell, K., Davis, A., Reynolds, R., Balderston, N., Fudge, J. L., Grillon, C., & Ernst, M. (2015). Resting state connectivity of the bed nucleus of the stria terminalis at ultra-high field. *Human Brain Mapping*, *36*(10), 4076–4088. https://doi.org/10.1002/HBM.22899

Turner, R. J., & Lloyd, D. A. (2003). Cumulative adversity and drug dependence in young adults: racial/ethnic contrasts. *Addiction (Abingdon, England)*, *98*(3), 305–315. https://doi.org/10.1046/J.1360-0443.2003.00312.X

Ueda, S., Hosokawa, M., Arikawa, K., Takahashi, K., Fujiwara, M., Kakita, M., Fukada, T., Koyama, H., Horigane, S. I., Itoi, K., Kakeyama, M., Matsunaga, H., Takeyama, H., Bito, H., & Takemoto-Kimura, S. (2021). Distinctive Regulation of Emotional Behaviors and Fear-Related Gene Expression Responses in Two Extended Amygdala Subnuclei With Similar Molecular Profiles. *Frontiers in Molecular Neuroscience*, *14*, 186. https://doi.org/10.3389/FNMOL.2021.741895/BIBTEX

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural Regulation of Endocrine and Autonomic Stress Responses. *Nature Reviews. Neuroscience*, *10*(6), 397. https://doi.org/10.1038/NRN2647

Ulrich-Lai, Y. M., Xie, W., Meij, J. T. A., Dolgas, C. M., Yu, L., & Herman, J. P. (2006). Limbic and HPA axis function in an animal model of chronic neuropathic pain. *Physiology & Behavior*, *88*(1–2), 67–76. https://doi.org/10.1016/J.PHYSBEH.2006.03.012

Vail, G., & Roepke, T. A. (2019). Membrane-Initiated Estrogen Signaling via Gq-coupled GPCR in the Central Nervous System. *Steroids*, *142*, 77. https://doi.org/10.1016/J.STEROIDS.2018.01.010

Vale, W., Rivier, C., Yang, L., Minick, S., & Guillemin, R. (1978). Effects of Purified Hypothalamic Corticotropin-Releasing Factor and Other Substances on the Secretion of Adrenocorticotropin and β-Endorphin-Like Immunoactivities in Vitro. *Endocrinology*, *103*(5), 1910–1915.

https://doi.org/10.1210/ENDO-103-5-1910

Vale, W., Spiess, J., Rivier, C., & Rivier, J. (1981). Characterization of a 41-Residue Ovine Hypothalamic Peptide That Stimulates Secretion of Corticotropin and β-Endorphin. *Science*, *213*(4514), 1394–1397. https://doi.org/10.1126/SCIENCE.6267699

van Aerde, K. I., & Feldmeyer, D. (2015). Morphological and Physiological Characterization of Pyramidal Neuron Subtypes in Rat Medial Prefrontal Cortex. *Cerebral Cortex*, *25*(3), 788–805. https://doi.org/10.1093/cercor/bht278

Van Bockstaele, B., Verschuere, B., Tibboel, H., De Houwer, J., Crombez, G., & Koster, E. H. W. (2014). A review of current evidence for the causal impact of attentional bias on fear and anxiety. *Psychological Bulletin*, *140*(3), 682–721. https://doi.org/10.1037/A0034834

van den Berg, H. (2022). Evaluating the validity of animal models of mental disorder: from modeling syndromes to modeling endophenotypes. *History and Philosophy of the Life Sciences*, *44*(4), 1–26. https://doi.org/10.1007/S40656-022-00537-4/TABLES/2

Vekaria, K. M., O'Connell, K., Rhoads, S. A., Brethel-Haurwitz, K. M., Cardinale, E. M., Robertson, E. L., Walitt, B., VanMeter, J. W., & Marsh, A. A. (2020). Activation in bed nucleus of the stria terminalis (BNST) corresponds to everyday helping. *Cortex*, *127*, 67–77. https://doi.org/10.1016/j.cortex.2020.02.001

Venniro, M., Reverte, I., Ramsey, L. A., Papastrat, K. M., D'Ottavio, G., Milella, M. S., Li, X., Grimm, J. W., & Caprioli, D. (2021). Factors modulating the incubation of drug and non-drug craving andtheir clinical implications. *Neuroscience and Biobehavioral Reviews*, *131*, 847. https://doi.org/10.1016/J.NEUBIOREV.2021.09.050

Venniro, M., Russell, T. I., Ramsey, L. A., Richie, C. T., Lesscher, H. M. B., Giovanetti, S. M., Messing, R. O., & Shaham, Y. (2020). Abstinence-dependent dissociable central amygdala microcircuits control drug craving. *PNAS*, *117*(14), 8126–8134. https://doi.org/10.1073/PNAS.2001615117/SUPPL_FILE/PNAS.2001615117.SAPP.PDF

Venniro, M., Zhang, M., Caprioli, D., Hoots, J. K., Golden, S. A., Heins, C., Morales, M., Epstein, D. H., & Shaham, Y. (2018). Volitional social interaction prevents drug addiction in rat models. *Nature Neuroscience*, *21*(11), 1520–1529. https://doi.org/10.1038/S41593-018-0246-6

Ventura-Silva, A. P., Pêgo, J. M., Sousa, J. C., Marques, A. R., Rodrigues, A. J., Marques, F., Cerqueira, J. J., Almeida, O. F. X., & Sousa, N. (2012). Stress shifts the response of the bed nucleus of the stria terminalis to an anxiogenic mode. *European Journal of Neuroscience*, *36*(10), 3396–3406. https://doi.org/10.1111/j.1460-9568.2012.08262.x

Verissimo, A. D. O., Grella, C. E., Amaro, H., & Gee, G. C. (2014). Discrimination and Substance Use Disorders Among Latinos: The Role of Gender, Nativity, and Ethnicity. *American Journal of Public Health*, *104*(8), 1421. https://doi.org/10.2105/AJPH.2014.302011

Vertes, R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse (New York, N.Y.)*, *51*(1), 32–58. https://doi.org/10.1002/SYN.10279

Vilsaint, C. L., NeMoyer, A., Fillbrunn, M., Sadikova, E., Kessler, R. C., Sampson, N. A., Alvarez, K., Green, J. G., McLaughlin, K. A., Chen, R., Williams, D. R., Jackson, J. S., & Alegría, M. (2019). Racial/Ethnic Differences in 12-Month Prevalence and Persistence of Mood, Anxiety, and Substance Use Disorders: Variation by Nativity and Socioeconomic Status. *Comprehensive Psychiatry*, *89*, 52. https://doi.org/10.1016/J.COMPPSYCH.2018.12.008

Vitlic, A., Lord, J. M., & Phillips, A. C. (2014). Stress, ageing and their influence on functional, cellular and molecular aspects of the immune system. *Age (Dordrecht, Netherlands)*, *36*(3), 1169–1185. https://doi.org/10.1007/S11357-014-9631-6

Vranjkovic, O., Pina, M., Kash, T. L., & Winder, D. G. (2017). The bed nucleus of the stria terminalis in drug-associated behavior and affect: A circuit-based perspective. *Neuropharmacology*, *122*, 100–106. https://doi.org/10.1016/j.neuropharm.2017.03.028

Vyas, A., Bernal, S., & Chattarji, S. (2003). Effects of chronic stress on dendritic arborization in the central and extended amygdala. *Brain Research*, *965*(1–2), 290–294.

https://doi.org/10.1016/S0006-8993(02)04162-8

Vyas, A., Mitra, R., Shankaranarayana Rao, B. S., & Chattarji, S. (2002). Chronic Stress Induces Contrasting Patterns of Dendritic Remodeling in Hippocampal and Amygdaloid Neurons. *The Journal of Neuroscience*, *22*(15), 6810. https://doi.org/10.1523/JNEUROSCI.22-15-06810.2002

Wadhwa, P. D., Culhane, J. F., Rauh, V., Barve, S. S., Hogan, V., Sandman, C. A., Hobel, C. J., Chicz-DeMet, A., Dunkel-Schetter, C., Garite, T. J., & Glynn, L. (2001). Stress, infection and preterm birth: a biobehavioural perspective. *Paediatric and Perinatal Epidemiology*, *15 Suppl 2*(SUPPL. 2), 17–29. https://doi.org/10.1046/J.1365-3016.2001.00005.X

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols*, *2*(2), 322. https://doi.org/10.1038/NPROT.2007.44

Walker, D. L., Miles, L. A., & Davis, M. (2009). Selective participation of the bed nucleus of the stria terminalis and CRF in sustained anxiety-like versus phasic fear-like responses. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *33*(8), 1291–1308. https://doi.org/10.1016/j.pnpbp.2009.06.022

Walker, D. L., Toufexis, D. J., & Davis, M. (2003). Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *European Journal of Pharmacology*, *463*(1–3), 199–216. https://doi.org/10.1016/S0014-2999(03)01282-2

Walter, A., Mai, J. K. K., Lanta, L., & Görcs, T. (1991). Differential distribution of immunohistochemical markers in the bed nucleus of the stria terminalis in the human brain. *Journal of Chemical Neuroanatomy*, *4*(4), 281–298. https://doi.org/10.1016/0891-0618(91)90019-9

Wang, H. H., Lee, D. K., Liu, M., Portincasa, P., & Wang, D. Q. H. (2020). Novel Insights into the Pathogenesis and Management of the Metabolic Syndrome. *Pediatric Gastroenterology, Hepatology & Nutrition*, *23*(3), 189. https://doi.org/10.5223/PGHN.2020.23.3.189

Wang, X., Cen, X., & Lu, L. (2001). Noradrenaline in the bed nucleus of the stria terminalis is critical for stress-induced reactivation of morphine-conditioned place preference in rats. *European Journal of Pharmacology*, *432*(2–3), 153–161. https://doi.org/10.1016/S0014-2999(01)01487-X

Wang, X., Zhang, Y., Wang, X., Dai, J., Hua, R., Zeng, S., & Li, H. (2020). Anxiety-related cell-type-specific neural circuits in the anterior-dorsal bed nucleus of the stria terminalis. *Science Bulletin*, *65*(14), 1203–1216. https://doi.org/10.1016/J.SCIB.2020.03.028

Wang, Y., Kim, J., Schmit, M. B., Cho, T. S., Fang, C., & Cai, H. (2019). A bed nucleus of stria terminalis microcircuit regulating inflammation-associated modulation of feeding. *Nature Communications*, *10*(1), 2769. https://doi.org/10.1038/s41467-019-10715-x

Wang, Y., Krabbe, S., Eddison, M., Henry, F. E., Fleishman, G., Lemire, A. L., Wang, L., Korff, W., Tillberg, P. W., Lüthi, A., & Sternson, S. M. (2023). Multimodal mapping of cell types and projections in the central nucleus of the amygdala. *ELife*, *12*. https://doi.org/10.7554/ELIFE.84262

Webb, E. K., Bird, C. M., Deroon-Cassini, T. A., Weis, C. N., Huggins, A. A., Fitzgerald, J. M., Miskovich, T., Bennett, K., Krukowski, J., Torres, L., & Larson, C. L. (2022). Racial Discrimination and Resting-State Functional Connectivity of Salience Network Nodes in Trauma-Exposed Black Adults in the United States. *JAMA Network Open*, *5*(1), E2144759. https://doi.org/10.1001/JAMANETWORKOPEN.2021.44759

Wei, N. L., Quan, Z. F., Zhao, T., Yu, X. D., Xie, Q., Zeng, J., Ma, F. K., Wang, F., Tang, Q. S., Wu, H., & Zhu, J. H. (2019). Chronic stress increases susceptibility to food addiction by increasing the levels of DR2 and MOR in the nucleus accumbens. *Neuropsychiatric Disease and Treatment*, *15*, 1211. https://doi.org/10.2147/NDT.S204818

Welch, J. D., Kozareva, V., Ferreira, A., Vanderburg, C., Martin, C., & Macosko, E. Z. (2019). Single-Cell Multi-omic Integration Compares and Contrasts Features of Brain Cell Identity. *Cell*, *177*(7), 1873-1887.e17. https://doi.org/10.1016/j.cell.2019.05.006

Wetsel, W. C., Khan, W. A., Merchenthaler, I., Rivera, H., Halpern, A. E., Phung, H. M., Negro-Vilar, A., & Hannun, Y. A. (1992). Tissue and cellular distribution of the extended family of protein kinase C isoenzymes. *The Journal of Cell Biology*, *117*(1), 121. https://doi.org/10.1083/JCB.117.1.121

Whiston, A., Bockting, C. L. H., & Semkovska, M. (2019). Towards personalising treatment: a systematic review and meta-analysis of face-to-face efficacy moderators of cognitive-behavioral therapy and interpersonal psychotherapy for major depressive disorder. *Psychological Medicine*, *49*(16), 2657–2668. https://doi.org/10.1017/S0033291719002812

Whittle, N., Fadok, J., MacPherson, K. P., Nguyen, R., Botta, P., Wolff, S. B. E., Müller, C., Herry, C., Tovote, P., Holmes, A., Singewald, N., Lüthi, A., & Ciocchi, S. (2021). Central amygdala micro-circuits mediate fear extinction. *Nature Communications*, *12*(1). https://doi.org/10.1038/S41467-021-24068-X

Wichmann, S., Kirschbaum, C., Böhme, C., & Petrowski, K. (2017). Cortisol stress response in post-traumatic stress disorder, panic disorder, and major depressive disorder patients. *Psychoneuroendocrinology*, *83*, 135–141. https://doi.org/10.1016/J.PSYNEUEN.2017.06.005

Wieser, M. J., & Keil, A. (2020). Attentional threat biases and their role in anxiety: A neurophysiological perspective. *International Journal of Psychophysiology*, *153*, 148–158. https://doi.org/10.1016/J.IJPSYCHO.2020.05.004

Wilensky, A. E., Schafe, G. E., Kristensen, M. P., & LeDoux, J. E. (2006). Rethinking the Fear Circuit: The Central Nucleus of the Amygdala Is Required for the Acquisition, Consolidation, and Expression of Pavlovian Fear Conditioning. *Journal of Neuroscience*, *26*(48), 12387–12396. https://doi.org/10.1523/JNEUROSCI.4316-06.2006

Willner, P. (1984). The validity of animal models of depression. *Psychopharmacology*, *83*(1), 1–16. https://doi.org/10.1007/BF00427414/METRICS

Willner, P. (1986). Validation criteria for animal models of human mental disorders: learned helplessness as a paradigm case. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *10*(6), 677–690. https://doi.org/10.1016/0278-5846(86)90051-5

Wills, T. A., Vaccaro, D., & McNamara, G. (1992). The role of life events, family support, and competence in adolescent substance use: a test of vulnerability and protective factors. *American Journal of Community Psychology*, *20*(3), 349–374. https://doi.org/10.1007/BF00937914

Wilson, D. A., Kadohisa, M., & Fletcher, M. L. (2006). Cortical contributions to olfaction: Plasticity and perception. *Seminars in Cell & Developmental Biology*, *17*(4), 462–470. https://doi.org/10.1016/J.SEMCDB.2006.04.008

Wilson, T. D., Valdivia, S., Khan, A., Ahn, H. S., Adke, A. P., Gonzalez, S. M., Sugimura, Y. K., & Carrasquillo, Y. (2019). Dual and Opposing Functions of the Central Amygdala in the Modulation of Pain. *Cell Reports*, *29*(2), 332-346.e5. https://doi.org/10.1016/J.CELREP.2019.09.011

Witter, M. P., & Amaral, D. G. (2021). The entorhinal cortex of the monkey: VI. Organization of projections from the hippocampus, subiculum, presubiculum, and parasubiculum. *The Journal of Comparative Neurology*, *529*(4), 828. https://doi.org/10.1002/CNE.24983

Wolff, M., Enge, S., Kräplin, A., Krönke, K. M., Bühringer, G., Smolka, M. N., & Goschke, T. (2021). Chronic stress, executive functioning, and real-life self-control: An experience sampling study. *Journal of Personality*, *89*(3), 402–421. https://doi.org/10.1111/JOPY.12587

Woody, M. L., Bell, E. C., Cruz, N. A., Wears, A., Anderson, R. E., & Price, R. B. (2022). Racial Stress and Trauma and the Development of AdolescentDepression: A Review of the Role of Vigilance Evoked by Racism-RelatedThreat. *Chronic Stress*, *6*. https://doi.org/10.1177/24705470221118574

Xiao, Q., Zhou, X., Wei, P., Xie, L., Han, Y., Wang, J., Cai, A., Xu, F., Tu, J., & Wang, L. (2020). A new GABAergic somatostatin projection from the BNST onto accumbal parvalbumin neurons controls anxiety. *Molecular Psychiatry*, 1–23. https://doi.org/10.1038/s41380-020-0816-3

Xu, H., Guo, S., Li, W., & Yu, P. (2015). The circular RNA Cdr1as, via miR-7 and its targets, regulates insulin transcription and secretion in islet cells. *Scientific Reports 2015 5:1*, *5*(1), 1–12. https://doi.org/10.1038/srep12453

Yang, F., Liu, Y., Chen, S., Dai, Z., Yang, D., Gao, D., Shao, J., Wang, Y., Wang, T., Zhang, Z., Zhang, L., Lu, W. W., Li, Y., & Wang, L. (2020). A GABAergic neural circuit in the ventromedial hypothalamus mediates chronic stress-induced bone loss. *The Journal of Clinical Investigation*,

130(12), 6539–6554. https://doi.org/10.1172/JCI136105

Yang, L., Zhao, Y., Wang, Y., Liu, L., Zhang, X., Li, B., & Cui, R. (2015). The Effects of Psychological Stress on Depression. *Current Neuropharmacology*, *13*(4), 494. https://doi.org/10.2174/1570159X1304150831150507

Yang, Q., Langston, J. C., Tang, Y., Kiani, M. F., & Kilpatrick, L. E. (2019). The Role of Tyrosine Phosphorylation of Protein Kinase C Delta in Infection and Inflammation. *International Journal of Molecular Sciences*, *20*(6). https://doi.org/10.3390/IJMS20061498

Yarkoni, T., Poldrack, R. A., Nichols, T. E., Van Essen, D. C., & Wager, T. D. (2011). Large-scale automated synthesis of human functional neuroimaging data. *Nature Methods*, *8*(8), 665. https://doi.org/10.1038/NMETH.1635

Ye, J., & Veinante, P. (2019). Cell-type specific parallel circuits in the bed nucleus of the stria terminalis and the central nucleus of the amygdala of the mouse. *Brain Structure & Function*, *224*(3), 1067–1095. https://doi.org/10.1007/S00429-018-01825-1

Yehuda, R. (2009). Status of glucocorticoid alterations in post-traumatic stress disorder. *Annals of the New York Academy of Sciences*, *1179*, 56–69. https://doi.org/10.1111/J.1749-6632.2009.04979.X

Yehuda, R., Giller, E. L., Southwick, S. M., Lowy, M. T., & Mason, J. W. (1991). Hypothalamic-pituitary-adrenal dysfunction in posttraumatic stress disorder. *Biological Psychiatry*, *30*(10), 1031–1048. https://doi.org/10.1016/0006-3223(91)90123-4

Yohn, C. N., Leithead, A. B., & Becker, E. A. (2017). Increased vasopressin expression in the BNST accompanies paternally induced territoriality in male and female California mouse offspring. *Hormones and Behavior*, *93*, 9–17. https://doi.org/10.1016/j.yhbeh.2017.03.008

Yoon, Y. R., & Baik, J. H. (2015). Melanocortin 4 receptor and dopamine D2 receptor expression in brain areas involved in food intake. *Endocrinology and Metabolism*, *30*(4), 576–583. https://doi.org/10.3803/EnM.2015.30.4.576

Yu, S., François, M., Huesing, C., & Münzberg, H. (2018). The Hypothalamic Preoptic Area and Body Weight Control. *Neuroendocrinology*, *106*(2), 187. https://doi.org/10.1159/000479875

Zhang, H., Li, X., Pang, J., Zhao, X., Cao, S., Wang, X., Wang, X., & Li, H. (2020). Predicting SSRI-Resistance: Clinical Features and tagSNPs Prediction Models Based on Support Vector Machine. *Frontiers in Psychiatry*, *11*. https://doi.org/10.3389/FPSYT.2020.00493/FULL

Zhang, L., Pan, J., Chen, W., Jiang, J., & Huang, J. (2020). Chronic stress-induced immune dysregulation in cancer: implications for initiation, progression, metastasis, and treatment. *American Journal of Cancer Research*, *10*(5), 1294. /pmc/articles/PMC7269780/

Zhang, Y., Rózsa, M., Liang, Y., Bushey, D., Wei, Z., Zheng, J., Reep, D., Broussard, G. J., Tsang, A., Tsegaye, G., Narayan, S., Obara, C. J., Lim, J.-X., Patel, R., Zhang, R., Ahrens, M. B., Turner, G. C., Wang, S. S.-H., Korff, W. L., … Looger, L. L. (2021). Fast and sensitive GCaMP calcium indicators for imaging neural populations. *BioRxiv*, 2021.11.08.467793. https://doi.org/10.1101/2021.11.08.467793

Zhang, Z. M., Chen, S., & Liang, Y. Z. (2010). Baseline correction using adaptive iteratively reweighted penalized least squares. *The Analyst*, *135*(5), 1138–1146. https://doi.org/10.1039/B922045C

Zhao, D., Wang, D., Wang, W., Dai, J., Cui, M., Wu, M., Liu, C., Liu, J., Meng, F., Wang, K., Hu, F., Liu, D., Qiu, C., Li, W., & Li, C. (2022). The altered sensitivity of acute stress induced anxiety-related behaviors by modulating insular cortex-paraventricular thalamus-bed nucleus of the stria terminalis neural circuit. *Neurobiology of Disease*, *174*, 105890. https://doi.org/10.1016/J.NBD.2022.105890

Zhao, N., & Zhou, G. (2021). COVID-19 Stress and Addictive Social Media Use (SMU): Mediating Role of Active Use and Social Media Flow. *Frontiers in Psychiatry*, *12*. https://doi.org/10.3389/FPSYT.2021.635546

Zimmerman, J. M., Rabinak, C. A., McLachlan, I. G., & Maren, S. (2007). The central nucleus of the amygdala is essential for acquiring and expressing conditional fear after overtraining. *Learning & Memory*, *14*(9), 634. https://doi.org/10.1101/LM.607207

Zwanzger, P., Domschke, K., & Bradwejn, J. (2012). Neuronal network of panic disorder: the role of the neuropeptide cholecystokinin. *Depression and Anxiety*, *29*(9), 762–774. https://doi.org/10.1002/DA.21919