DOPAMINE AND THE POSITIVELY REINFORCING PROPERTIES OF AGGRESSION

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To my wonderful mother and father; for with their numerous struggles and unending support, made my education possible.

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CHAPTER I

INTRODUCTION

Overview

Aggression is an important component of adaptive behavioral repertoires aimed at ensuring survival. Like other behaviors that fall into this pallet of behaviors, such as feeding, drinking and sex, it has been hypothesized that acts of aggression are positively reinforcing. Though the neural mechanisms underlying several different forms of aggression have been well documented, the link between these mechanisms and mechanisms of positive reinforcement has yet to be approached.

One neural characteristic continually associated with positively reinforcing stimuli is the release of dopamine (DA) into the Nucleus Accumbens (NAC) from afferent projections from the Ventral Tegmental Area (VTA). This fact has led to several innovative methods used to treat addictions to positively reinforcing stimuli. Due to the pressing need to control species atypical aggression around the world, it would be quite valuable to determine if the same mechanisms underlie to positively reinforcing properties of aggression; in hopes of adopting similar innovative treatment options for individuals who struggle with controlling aggression.

In efforts to understand the mechanisms involved in the positively reinforcing properties of aggression, the overall aim of this work is to A) Further

understand the positively rewarding characteristics of aggression and B) to examine DA, DA metabolites and DA receptors (DA1-like and DA2-like) in the NAC and the Pre Frontal Cortex (PFC) and their role in positively reinforcing aggression.

Why Study Aggression?

Although aggression is evolutionally adaptive in many instances, atypical levels of aggression have become a heavy burden on societies around the world, especially in the United States. In 2003, 5.3% of children age 12-18 report being a victim of violent crime. Also in 2003, there were a total of 5.4 million violent crimes committed in the U.S. (U.S. Bureau of Justice Statistics. 2006). Around the world, 1 in 3 women have reported being beaten, raped or otherwise physically abused at least once in their lifetime (Family Violence Prevention Fund. 2006).

In addition to the increasing burden violence places on the entire population at large, aggression occurs at an unusually high rate among developmentally delayed and mentally ill populations. Aggression is a major symptom of a range of neurodevelopmental and psychologically challenging disabilities including, but not limited to, Schizophrenia, autism spectrum disorder (ASD), and Alzheimer's disease. It is likely that an improved understanding of the underlying causes of aggression, not only as a symptom but as a maladaptive condition itself, will shed light on the mechanisms of action of the neurodevelopmental disorder it accompanies as well. The cost of violence in the

normally developing population —including medical costs, quality of life, and loss of productivity—reached more than $158 billion in 2000 (SfN.org; Brain Research Success Stories). This estimate is not even including the financing of additional personnel (e.g., additional police force, additional hospital or private care staff) needed to manage aggression in a variety of environments.

Due to the ever-present need for understanding the entire picture involved with maladaptive aggression, efforts from several avenues of science, including molecular, behavioral and clinical research, have been launched. Though some advances have been made, the above described societal burdens still persist due to the yet unanswered questions about the link between aggression and positive reinforcement.

What is Aggression?

Aggression is a complex social behavior that evolved in the context of defending or obtaining resources. Although there are broad similarities across species, some features of aggression are species-specific. When studying aggression in a species-specific manner, species-specific behavior topographies are of interest. However, when investigating aggression across species, studies show that many of the same neurochemical and anatomical systems are activated during aggressive behavior in humans and non-human animals, even though the specific behavioral outputs can differ greatly.

Traditionally, aggression has been defined as overt behavior that has the intention of inflicting physical damage on another individual (Moyer, 1971).

Although aggression can yield competitive advantages in an evolutional sense, it is time-consuming and can be dangerous. Pathological aggression is considered so when it is exaggerated, persistent or expressed out of context. From an ethological perspective, aggression is used for obtaining or defending food or mates from competitors; from a psychiatric perspective, it is thought to be motivated by hypothetical constructs such as anger, irritation, frustration, fear and, in some cases, as is discussed in the following work, pleasure. Two subtypes of aggression have been identified in humans: the instrumental subtype and the affective subtype (Vitiello & Stoff, 1997). Affective aggression is considered to be more impulsive (it is usually associated with anger), whereas instrumental aggression is considered to be more purposeful and goal-oriented. Affective aggression is considered reactive and can result in sudden, inappropriate aggressive responses. This type of aggression is thought to account for the majority of aggressive crime and aggression associated with psychiatric/developmental disorders. However, higher profile incidents, such as serial killings, genocides and assassinations, are thought to be caused by a more instrumental mechanism of aggression. Instrumental aggression is thought to be regulated by higher cortical systems and less dependent on the hypothalamic and limbic systems that are known to mediate affective aggression.

What is Positive Reinforcement?

Behavioral theory embraces the notion that responses are selected by the individual based on the consequences of behavior (Skinner, 1953). According to

a behavior-analytic approach, the environment evokes behavior. Once the behavior is emitted it can be reinforced or punished by some change in the environment, thus increasing or decreasing (respectively) the probability of the response occurring in the future under similar conditions. Over time, an organism learns to discriminate the availability of the stimulus event and responds according to the contingencies of reinforcement. Behavior under control of consequences in the environment is called an operant. Operants under stimulus control are differentially reinforced in the presence of contextual stimuli while other responses are extinguished. Thus, responding occurs in the presence of discriminative stimuli that set the occasion for reinforcement if responding occurs

Basic behavioral processes involved in shaping operant behaviors include positive and negative reinforcement (Catania, 1998). Positive reinforcement is a process by which the rate of responding increases contingent upon the presentation of a pleasant stimulus. For most people, receiving food, money, or preferred activities are considered positively reinforcing events. When these stimulus events are available, people will engage in behaviors that access these stimuli. Conversely, negative reinforcement is a process by which the rate of responding increases contingent upon the subsequent removal of a noxious stimulus. People who find loud noise or the presentation of a task aversive may engage in certain behaviors, such as leaving the room, to avoid the stimulus. Whether a stimulus is considered pleasant or noxious is idiosyncratic to the organism.

Neurobiology of Aggression

Work in the mid-1930's initially implicated the limbic-hypothalamic-periaquiductal grey's (PAG) role in the performance of aggressive behavior (Grinker & Serota, 1938; Wortis & Maurer, 1941). Through these efforts and modern extensions of them it is thought that there are two distinct circuits that underlie affective and instrumental aggression (Monroe, 1978; Monroe, 1985; Reeves & Plum, 1969; Siegel et al., 1999).

The neurons essential for the performance of affective aggression behaviors reside in both the medial hypothalamus (MH) and the PAG. The MH sends efferents which synapse on PAG neurons. PAG neurons then send efferents to the brain stem and spinal cord. Though the MH and the PAG are essential for the expression of affective aggression, many other structures such as the amygdale, bed nucleus of the stria terminalis, frontal cortex and the lateral hypothalamus (LH) are thought to play important modulatory roles (Potegal et al., 1996; Raine et al., 1994; Raine et al., 1998; Raine et al. 1999; Reeves & Plum, 1969; Siegel et al., 1999).

Neurons essential for the expression of instrumental aggression are located in the LH. These neurons project directly to the trigeminal motor nucleus, locus cerruleus, pons, VTA, and the ventral portion of the PAG (Siegel et al., 1999).

Three important caveats are to be considered when utilizing the above two models of aggression. First, although the above mentioned studies conducted on humans offer converging evidence in support of these models, most of the

controlled experimentation used to elucidate these pathways were conducted on animals (predominantly cats). Second, much less work has been done on the model of instrumental aggression (as compared to the work completed on affective aggression). Third, the modulatory impact of other brain areas is not yet adequately understood.

Neurobiology of Positive Reinforcement

The scientific literature investigating the biology of Positive Reinforcement indicates that there are many limbic structures that play important modulatory roles in positive reinforcement processing such as the bed nucleus of the stria terminalis and the amygdala; however, it is widely thought that the ventral tegmental area's dopaminergic projections to the nucleus accumbens (NAc) and the pre-frontal cortex (PFC) (Mesocorticolimbic pathway; see Fig. 1) are integral for the processing of positive reinforcement (Bozarth, 1987; Bozarth, 1991; Fibiger & Phillips,1979; Olds & Milner,1954; Staley & Mash, 1996; Wise, 2002). The release of dopamine (DA) in the NAc has been highly associated with positive reinforcement.

Brain-Environment Interactions

The ventral striatum is activated during early learning experiences with environmental contingencies (Berridge & Robinson, 1998; Ikemoto & Panksepp, 1999). When a stimulus predicts reinforcement, DA is released from the substantia nigra and ventral tegemental area onto DA receptors in the ventral

and dorsal striatum. DA transmission in the ventral striatum subsequently enhances the effects of a stimulus to predict reinforcement, which determines the reinforcer value of the stimulus.

Most of the evidence for DA involvement in mediating the acquisition of instrumental behavior comes from DA antagonism of D1 and D2 receptors in the striatum. For example, pre-trial blockade of D1 receptors with the DA antagonist, SCH23390, impairs the acquisition of a lever-press response in rats (Hernandez, Andrzejewski, Sadeghian, Panksepp, & Kelley, 2005). Fowler and Liou (1994) conducted a study on the effects of DA antagonists on lever pressing in rats to obtain water reinforcement. It was concluded that D1 antagonists prevent learning, while D2 antagonism with drugs, like raclopride, enhances learning. However, raclopride has also been shown to reduce operant behavior on intermittent Schedules of reinforcement, suggesting D2 antagonists most likely influence movement more than motivation (Nakajima & Baker, 1989).

When D1 and D2 antagonists are given simultaneously, D2 receptor antagonists abolish the effects of D1 antagonists. For example, SCH23390 reduces running speed of pups attempting to obtain nipple reinforcement from a dam (McDougall, Crawford, & Nonneman, 1992). The D2 receptor antagonist, sulpiride, reinstated running speed. It was concluded that D1 and D2 receptors have a synergistic effect on reinforcement, although D2 antagonism alone influences primarily movement. Therefore, D1 receptors are more importantly involved in the positively reinforcing properties of stimuli.

The findings related to the effects of D1 and D2 antagonists confirm those previously mentioned about the effects of DA receptors on stereotypy as an operant behavior (Capper-Loup et al., 2002; Keefe & Gerfen, 1995; Kuczenski & Segal, 1999; Waszczak et al., 2002). That is, activating both D1 and D2 receptors produces movement in the context of reinforcing stimuli. For movement to occur, DA receptors must be activated. If DA receptors are activated, it is in the context of an experience that is either (a) novel to the organism, or (b) an environment that no longer produces reinforcement. Thus, DA projections from ventral and dorsal regions of the striatum play a role in mediating the positively reinforcing effects of various environmental stimuli. (Bardo, 1998; Beninger & Miller, 1998; Blackburn, Phillips, Jakubovic, & Fibiger, 1989; Robbins & Everitt, 1996; Schultz, Apicella, & Ljungberg, 1993).

This DA release in the NAc that is coupled with the hedonic experience of positive reinforcement occurs in one of three regions of the NAc; the core, the shell and the rostral pole. These subdivisions of the NAc are based upon staining appearance and anatomical connections. The shell is thought to be much more anatomically and chemically diverse than the core, whereas the rostral pole is much less studied than both the shell and core (Zahm, 2000). Research with a variety of species and reinforcers suggests that the medial shell is strongly associated with the positively reinforcing effects of a stimulus, whereas the core contributes to behavioral activation. This hypothesis is strongly supported by evidence ranging from c-fos mRNA expression studies to studies

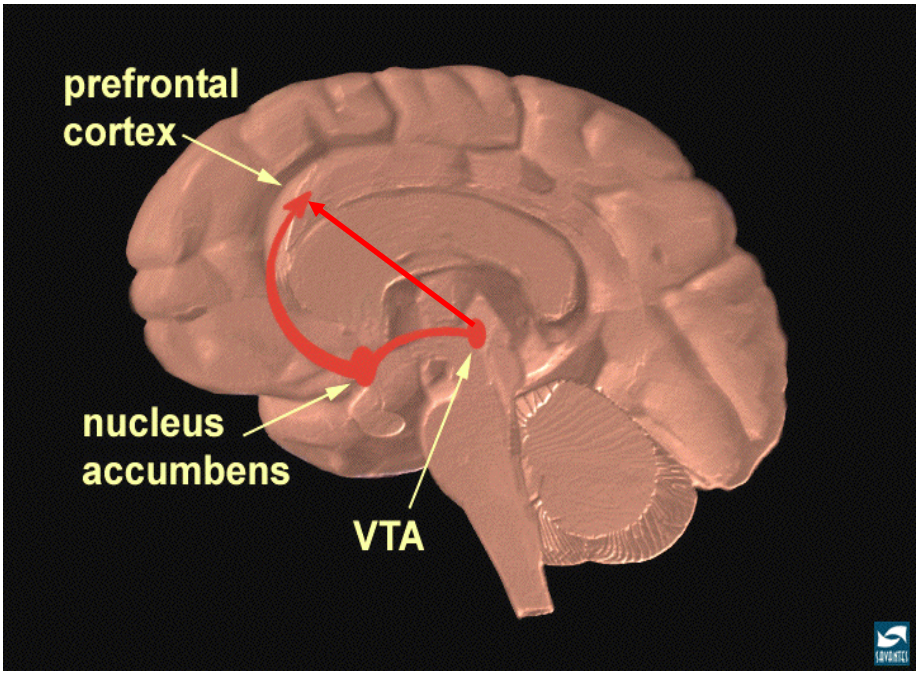

Figure 1. Mesocorticolimbic DA system. Adapted from the National Institute on Drug Abuse website.

utilizing 6-Hydroxydopmaine lesions in conjunction with a variety of positive reinforcement-response analysis paradigms (Sham et al., 2007; Floresco et al., 2006; Selling's & Clark, 2006; Hara & Pickle, 2005; Kimono et al., 2005; Selling's & Clark, 2003).

Thought the NAc is most often studied in conjunction to positive reinforcement, the PFC has also demonstrated an important role in positive reinforcement. The PFC is thought to be involved in the cognitive processes of executive functioning. Executive functions include the ability to differentiate among conflicting thoughts, determine value among stimuli, determine 'same-ness' and 'different-ness', initiation of goal directed behavior, prediction of outcomes, expectations based on actions, and social "control". Positive reinforcement plays an integrate role in each of these executive functions in that the value of a stimulus effects an organisms approach or avoidance behavior towards that stimulus. Thus, it is necessary that the area integral for processing positive reinforcement stimuli (NAc) be intimately related to the area that initiates complex decisions about that stimulus. The intimate relation that these two brain areas share is that the activation of medial PFC glutamatergic neurons causes DA release in the NAc (Wise, 2002). In addition, suppression of glutamatergic activation, for example through serotonergic activation, suppresses the release of DA in the NAc. By these mechanisms, the PFC strongly modulates NAc activation and positive reinforcement.

How to Study Aggression as a Reinforcer

Behavioral Analysis Meets Neuroscience

As described above, theoretical and empirical evidence is mounting that suggests a relation between brain function and environmental contingencies (Kennedy, Caruso & Thompson, 2001; Cohen & Leckman, 1994; Nelson & Bloom, 1997). Until recently, however, behavior analysis and neurobiology have been studied in parallel. Whereas the former is concerned with the observation and measurement of behavior, the latter is concerned with the study of cellular, neurophysiological, and biochemical processes in the nervous system. In a majority of modern neuroscience, a long term goal of basic research is to some day affect therapy of nervous system disorders. Through the years of treating a vast array of disorders ranging from very physical disorders like Parkinsons disease to psychological disorders such as Schizophrenia, clinical evidence suggests that the most effective treatments plans include a combination of both external treatment (such as physical therapy, counseling etc.) and internal treatments (such as surgery, drug therapy etc.). Thus, current research investigating complex neurological disorders has finally begun to be guided by this clinical evidence. Basic science researchers are now approaching neuroscience questions from both points of view; with outside behavioral observation and manipulation in conjunction with internal neurochemical observation and manipulation. There are a variety of ways that the study of behavior and neuroscience are being integrated, from clinical questionnaires in combination to drug therapies to new emerging imaging techniques. For the

purposes of the studies described here, basic animal models utilizing behavioral pharmacological method will be the focus.

Using Animal Models

A preponderance of research on the neurobiology of aggression comes from murine models. Rodents and humans have similar brain structures involving movement, memory, and emotions (Bear, Connors, & Paradaiso, 2001). Rodent models are an efficient means for examining neurobiological influences on aggression because (a) behavioral processes of rodents are representative of more complex species and are thought to be highly conserved, (b) experimenters have greater control over the experiences of rodents, and (c) neurotransmitter systems found in rodents parallel those found in humans. Therefore, many researchers find rodent models to be an appropriate model for human stereotypy.

Is Aggression Positively Reinforcing?

Behavioral Evidence Supporting Aggression as a Positive Reinforcer

Beginning in the 1950's it started to become clear, behaviorally at least, that aggression might have some self-reinforcing properties. In 1951, Scott and Fredrickson noted that animals that were victorious in a battle were more likely to engage in aggression than those animals that were not victorious (Scott & Fredrickson, 1951). While this study did show that victory could be positively reinforcing and it allowed for the idea that aggression might be positively reinforcing, it did not definitively demonstrate the positively reinforcing aspects of

aggression itself. Later experiments conducted by et al. (1969) used a T-maze paradigm to demonstrate that aggression was positively reinforcing (Tellegen et al., 1969). In these experiments, mice were given a choice to run to a side of the T-maze where they had previously been conditioned to associate aggressive encounters or to a side of the T-maze where they had never experienced aggression. A vast majority of these trials resulted in the mice choosing the arm of the maze where they had previously experienced the aggression. These results were later replicated by multiple groups in the mid-1990 by using similar paradigms (Meisel & Joppa, 1994; Martinez, 1995). More compelling are the experiments where fighting fish (also demonstrated in fighting cocks) will complete elaborate mazes, going over, under and through barriers to even get a glimpse of a potential opponent engaging in aggressive posturing (Thompson, 1964; Thompson & Bloom, 1964; Thompson, 1969).

Advancing this line of research has been more elaborate work conducted on rodents. In these experiments, male resident rodents have been trained to perform an operant response in order to obtain the opportunity to aggress against a conspecific intruder (Fish et al., 2002; Miczek, 2002; Tellegen & Horn, 1972). This response has been maintained on a variety of reinforcement Schedules including fixed ratio, fixed interval, variable ratio and progressive ratio (PR) and has been shown to be sensitive to extinction paradigms. While results from PR Schedules reveal that aggression may not be as positively reinforcing as food, it does prove to be a potent positive reinforcer with mice maintaining PR

Schedules as high as PR40 (Kennedy, May & Couppis, unpublished observations).

Anatomical Evidence In Support of Aggression as a Positive Reinforcer

In addition to the behavioral data massing to support an interaction between mechanisms of positive reinforcement and those of aggression, there is substantial anatomical data that also suggests a plausible link. Due to the fact that the NAc is the structure most often implicated in positive reinforcement processing, it is important to look at its anatomy and projections. The NAc is divided into three regions based upon primary efferents and afferents: the core, shell and rostral pole. Through extensive work on the anatomy of each region, the shell has been most vigorously implicated in positive reinforcement processing due to its elaborate interconnections with structures associated with emotive behavior. The shell's primary efferents are to the ventromedial ventral pallidum, ventral tegmental area, prefrontal cortex, PAG and a dense innervation of the LH (Brog et al., 1993, Zahm, 2000). The efferents to the PAG and the LH are of specific interest as they are both thought to play central roles in aggression (Bandler R, 1988; Gregg & Seigel 2001; Seigel, 1999, Zahm, 2000). The shell receives dense innervation by limbic structures including various nuclei of the amygdala, the extended amygdala, prefrontal cortex and the LH (French & Totterdell, 1983; Zahm, 2000). These afferents are of interest as well due to studies implicating each in modulating aggressive output (Adolfs, 1994; Pietrini et al., 2000; Potegal et al., 1996; Raine et al., 1994; Raine et al., 1998; Volkow &

Tancredi, 1987). In addition, it has been observed that neurons in the shell region will respond to excitatory input from more than one source, making it an excellent candidate for serving an integrative function (Zahm, 2000). Less controlled evidence for the NAc's involvement in aggression and emotion processing related to aggression comes from studies documenting impaired anger recognition in humans with ventral striatum damage (Calder et al., 2004).

Neurochemical Evidence In Support of Aggression as a Positive Reinforcer

Evidence more strongly supporting the idea that aggression is positively reinforcing comes from neurochemical and pharmacological studies. Efforts as early as the 1960's began to incriminate dopamine in the investigation of aggression. These early observations, using post mortem analyses, reported increases in cortical and NAc DA following aggression in mice (Miczek et al., 2002). Though it was a start, these early endeavors lacked the ability to link changes in DA levels to specific time points within an aggressive episode. With the development of in vivo microdialysis, it became possible to measure fluctuations in DA before, during and after aggressive behaviors. In rats, Van Erp et al. (2000) demonstrated that DA levels significantly increased in the NAc during and after an aggressive bout peaking 20-30 minutes after the encounter. In the PFC, DA was also noted to increase up to 120% above baseline following an aggressive encounter (Van Erp & Miczek, 2000). It is not to be ignored that both significant increases and decreases in DA levels have been observed in a variety of socially stressful situations (Kalivas & Duffy, 1995). However, work

conducted by Ferrari et al. (2003) show NAc DA increases associated with expected aggression even when the socially stressing event is omitted. In these experiments rats were trained to have an aggressive encounter at a specific time of day for 10 days. On the 11th day, the encounter was omitted. Samples showed the expected 40% and 150 % (above baseline) DA increases in NAc during and after the aggressive encounter, respectively. Surprisingly, samples of the 11th day session (where Scheduled aggression was omitted) showed an astonishing 60%-70% rise in NAc DA even in the absence of the socially stressful bout (Ferrari et al., 2003). Though these rises in NAc and PFC DA are seen primarily during and after an aggressive episode, it is also plausible that DA could play an important role prior to aggression. In both cat and rat, electrical stimulation of neurons in the VTA lowered latency to attack (Shaikh et al., 1991). Though this does not offer direct evidence in support of a link between positive reinforcement and aggression, taken together with the NAc and PFC microdialysis studies, one could speculate that the lowered attack latency in these studies might be mediated through mesocorticolimbic circuitry.

Genetic Evidence In Support of Aggression as a Positive Reinforcer

Another line of research that is becoming increasingly important to the study of positive reinforcement and aggression is that which links genetic polymorphisms to aggression. Genes of interest are those that encode for proteins that are thought to be important for proper DA neurotransmission such as Monoamine Oxidase (MAO), Catecho-O-Methyl Transferase (COMT), DA

receptors and the DA transporter (DAT). Though these studies can only supply permissive evidence, at best, due to the lack of controlled neurochemical data available in humans, they are highly valuable.

Studies of a psychiatric subpopulation in the Netherlands powerfully linked a point mutation in the gene encoding MAO-A to aggression (Brunner et al.., 1993). Since this finding, several initiatives to study the behavioral relevance of polymorphisms in the MAO-A gene has arisen. In Rhesus monkeys, gene variation yielding lower MAO-A activity, coupled with abnormal rearing, has been shown to result in high levels of aggression (Newman et al., 2003). Work looking at platelet levels of MAO-A have consistently found correlations between low MAO-A activity and aggression (Skondras et al., 2004). Criticisms of work on MAO-A in relation to a DA hypothesis of aggression are that MAO-A also affects serotonin levels. This fact causes difficulty when interpreting findings relating MAO-A and aggression, especially since samples of cerebrospinal fluid from aggressive subjects consistently show abnormalities in serotonin metabolites but not so consistently show abnormalities in DA metabolites.

These facts make the progress relating COMT, DA receptors and DAT activity to positive reinforcement and aggression ever more imperative. Studies generating COMT knockout mice have shown elevated levels of aggression (Volavka et al., 2004). Several studies looking at COMT levels in human subjects also show a correlation between low COMT activity and aggression Volavka et al., 2004).

Similarly, DAT1 knockout mice show problematic social interaction; one issue being increased aggressive behavior towards cage mates (Rodriguez et al., 2004). In humans, DAT1 polymorphism has also been linked to aggression by studies in pathologically aggressive adolescents. In this same population, a D2 receptor polymorphism was also observed (Chen et al., 2005). D2 receptor is not the only receptor linked through correlation to aggression. Polymorphisms in the D3 receptor gene has been found among populations of adult violent criminal offenders (Retz et al., 2003) and D4 receptor polymorphisms have been found in dog breeds scoring high on aggressive behavior scales (Ito et al., 2004). Integrating the above genetic data, it is likely that aggression in humans is related to increased DA tone and transmission through D2 receptor subtype. It is also conceivable that these abnormalities in DA neurotransmission underlie the behavioral link noted between aggression and positive reinforcement.

Pharmacological Evidence In Support of Aggression as a Positive Reinforcer

Early efforts to pharmacologically manipulate aggression through dopaminergic mechanisms were based on observations of clinical practices that used antipsychotics to tame aggression and mania. Antipsychotic drugs primarily act on DA receptors. However, it is also known that several commonly prescribed antipsychotic drugs also have high affinities for receptor types other than DA. Due to the inability to discern whether the anti-aggressive effects of antipsychotics were due to action on DA receptors alone, or if other medications that patients were taking could confound observations, pharmacological

manipulation of aggression through dopaminergic mechanisms moved into the controlled environment of the laboratory.

In laboratory studies, compounds with higher affinity and specificity for different DA receptor subtypes have been used to explore DA's role in aggression. Studies administering apomorphine (a semi-selective D2 receptor agonist) and amphetamine (DAT blocker) report increased aggressive behavior (Miczek et al., 2000; Seigel et al., 1999). One study, using a DA-agonist reportedly even more potent than apomorphine, N-n-Propyl-Norapomorphine, showed a faciliatory effect on aggression under predatory, foot-shock and isolation induced aggression conditions (Baggio & Ferrari, 1980). In addition to drugs that increase DA neurotransmission, studies administering DA antagonists, aimed at decreasing DA neurotransmission, have reported decreases in aggression (Miczek et al., 2000; Seigel et al., 1999). Haloperidol and raclopride, both D2 receptor antagonists, have been associated with lowered aggression in both rodents and humans, though their use and data interpretation are problematic due to undesired motor side effects (Miczek et al., 2000). It has also been observed that administration of haloperidol prior to administration of apomorphine blocked apomorphine's faciliatory effect on aggression without any motor side effects (Seigel et al., 1999).

Studies demonstrating the role of D1 receptors in the modulation of aggression have been less successful. SKF-38393, a selective DA1 receptor agonist, was not shown to effect affective aggression in cats (Seigel et al., 1999) and actually shown to reduce aggression when administered at very high doses

in mice (Miczek et al., 2000). SCH 23390, a D1 receptor antagonist, has been reported to reduce aggressive behavior in rodents (Rodriguez-Arias et al., 1998), an effect not able to be replicated in cat (Seigel et al., 1999).

Preliminary Studies Exploring Aggression as a Positive Reinforcer

In order to understand biologically how aggression might function as a positive reinforcer, it is first necessary to have a complete behavioral portrait of aggression as a reinforcer. Previously in our laboratory at Vanderbilt University, we have replicated and extended the behavioral findings of Miczek et al. (2000) in regards to aggression functioning as a positive reinforcer. Using the resident-intruder model of aggression in conjunction with an operant nose-poke response paradigm (Miczek et al., 2000), we have shaped male mice to respond on various Schedules of reinforcement to gain access to aggression with male conspecifics. We have established access to aggression on fixed ratio (FR), fixed interval (FI) and progressive ratio Schedules (PR).

In the experiments exploring FR responding for access to aggression, we demonstrated contingent nose pokes to access a conspecific for aggression could be established on a FR 8 reinforcement Schedule. That is, resident mice emitted a required number of nose pokes in order to gain access to an intruder mouse. The response rate decreased to near zero levels of responding when the aggression contingency was removed. Baseline responding recovered when the reinforcement contingency was re-established (see Fig. 2). Since responding was maintained when the aggression-event contingency was present, and was

not maintained when the aggression-event contingency was removed, the aggression positively reinforced nose pokes. The Inter-response time (IRT) distribution obtained for resident mice demonstrated that nose pokes occurred in rapid bursts (see Fig. 3). Longer IRTs would suggest nose pokes could be maintained by some other stimulus event, and that contact with the aggression contingency was coincidental. Since short IRT were seen in our experiments, it is likely that we established stimulus control using access to aggression as a reinforcer.

The FR experiments demonstrated that rapid Schedule completion on an FR reinforcement Schedule could be reinforced with an aggressive event with a conspecific mouse. The next experiment completed by our laboratory demonstrated that nose pokes were maintained on a FI reinforcement Schedule when aggression was the reinforcer. This was demonstrated by the sustained response rate over time, a decrease in responding when the aggression contingency was removed, and recovery of the response rate when the aggression contingency was re-instated (see Fig. 4). Furthermore, the Index of curvature (IC; is calculated for FI responding to quantify the acceleration of response rate toward the end of an interval; Bannai et al. 2007) obtained for each mouse suggested responding was minimal during the early minutes of an interval and increased as time progressed toward the expiration of the interval (see Fig. 5). Finally, each mouse nearly maximized all reinforcement opportunities, suggesting aggression as a reinforcer can sustain responding.

Though the above findings in our laboratory added support to the idea that aggression can be positively reinforcing, the extent to which aggression as a reinforcer sustains responding is not known. The FR and FI experiments imposed time constraints, limiting the number of reinforcers earned in a given session. Thus, even though responding was sustained over the course of the session there is no evidence of the value of aggression as a reinforcer beyond the parameters of the experimental time. The third experiment that was completed by our lab was an attempt to establish the reinforcer value of aggression by allowing each mouse to respond freely without time constraints. By doing this, we could establish a point at which aggression no longer sustains nose pokes.

Experiment 3 showed nose pokes during the PR 2 (see Fig. 6) reinforcement Schedule were maintained by access to aggression as reinforcement. Mice successfully completed between 5 and 10 PR 2 reinforcement Schedules before aggression no longer maintained nose pokes. When the aggression contingency was removed, nose pokes quickly ceased. Post-reinforcement pauses for two mice consistently increased as the PR Schedule value increased. The pause durations were recovered when baseline was re-instated (see Fig. 7). Experiment 3 established the extent aggression was a reinforcer for mice on a PR reinforcement Schedule. This was demonstrated by a stable rate of Schedule completion for each mouse. Furthermore, PRPs were demonstrated to increase as a function of the PR 2 Schedule value for two mice.

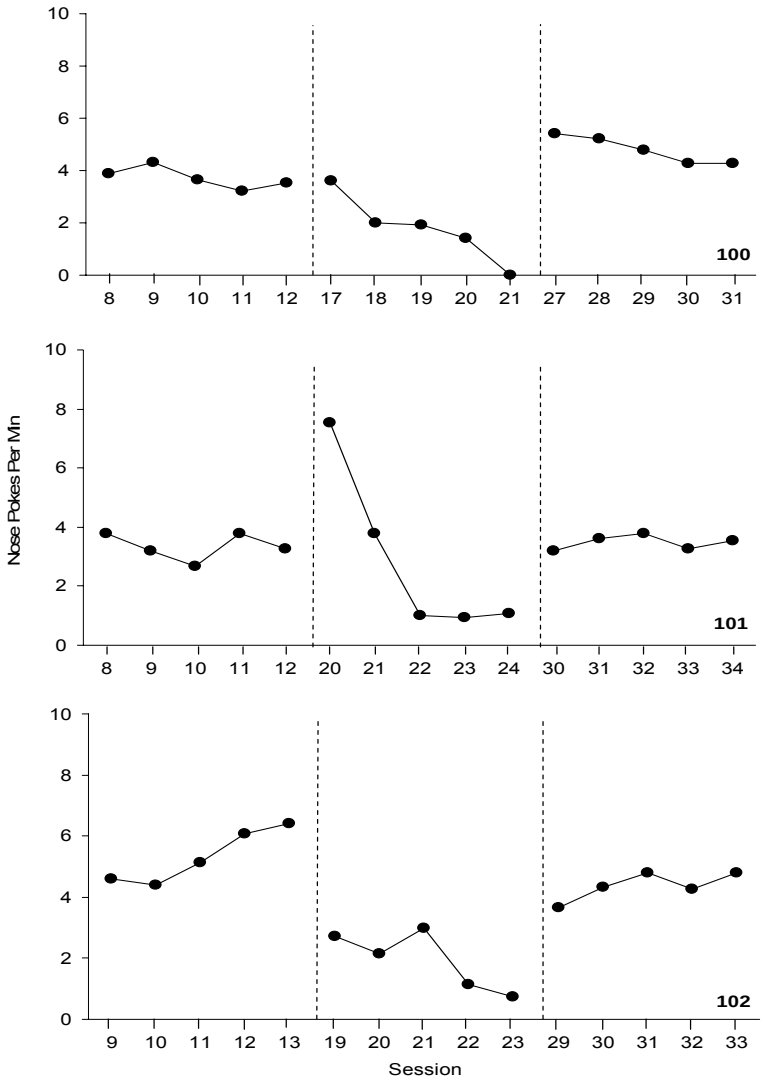

Figure 2. The frequency of nose pokes per min over the last 5 sessions on the FR reinforcement Schedule. Baseline and extinction conditions are separated by dashed phase lines. The vertical axis represents the number of nose pokes per min. The horizontal axis represents the session number. The top panel represents nose pokes for Mouse 100, the middle panel represents nose pokes for Mouse 101, and the bottom panel represents nose pokes for Mouse 102.

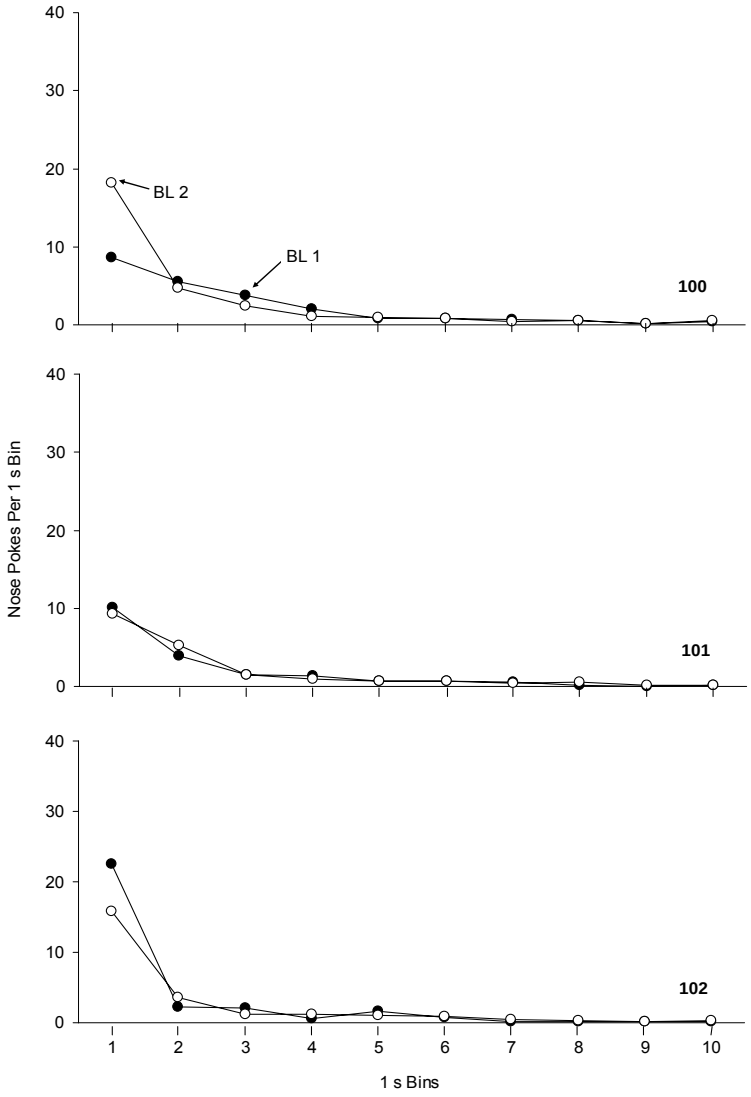

Figure 3. The average IRT per session over the last 5 sessions in 1.0 s time bins on the FR reinforcement Schedule. Black circles represent the first baseline condition and open circles represent the second baseline condition. The vertical axis represents the frequency of nose pokes in 1.0 s time bins. The horizontal axis represents 1.0 s time bins. The top panel represents IRTs for Mouse 100, the middle panel represents IRTs for Mouse 101, and the bottom panel represents IRTs for Mouse 102.

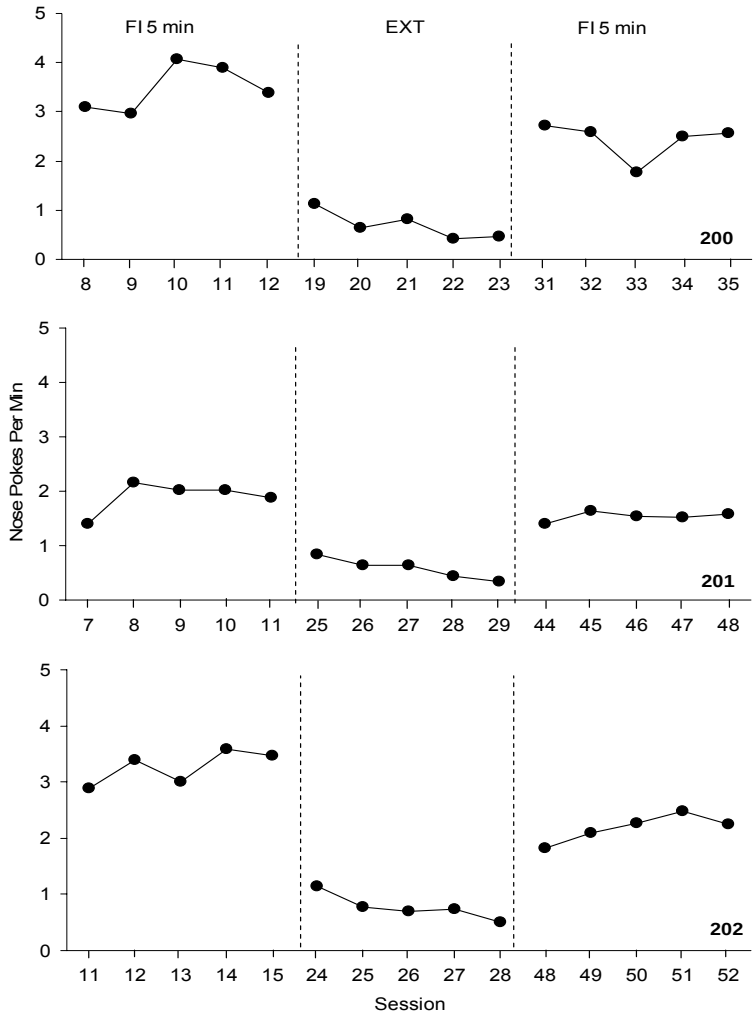

Figure 4. The frequency of nose pokes per min over the last 5 sessions on the FI reinforcement Schedule. Baseline and extinction conditions are separated by dashed phase lines. The vertical axis represents the number of nose pokes per min. The horizontal axis represents the session number. The top panel represents nose pokes for Mouse 200, the middle panel represents nose pokes for Mouse 201, and the bottom panel represents nose pokes for Mouse 202.

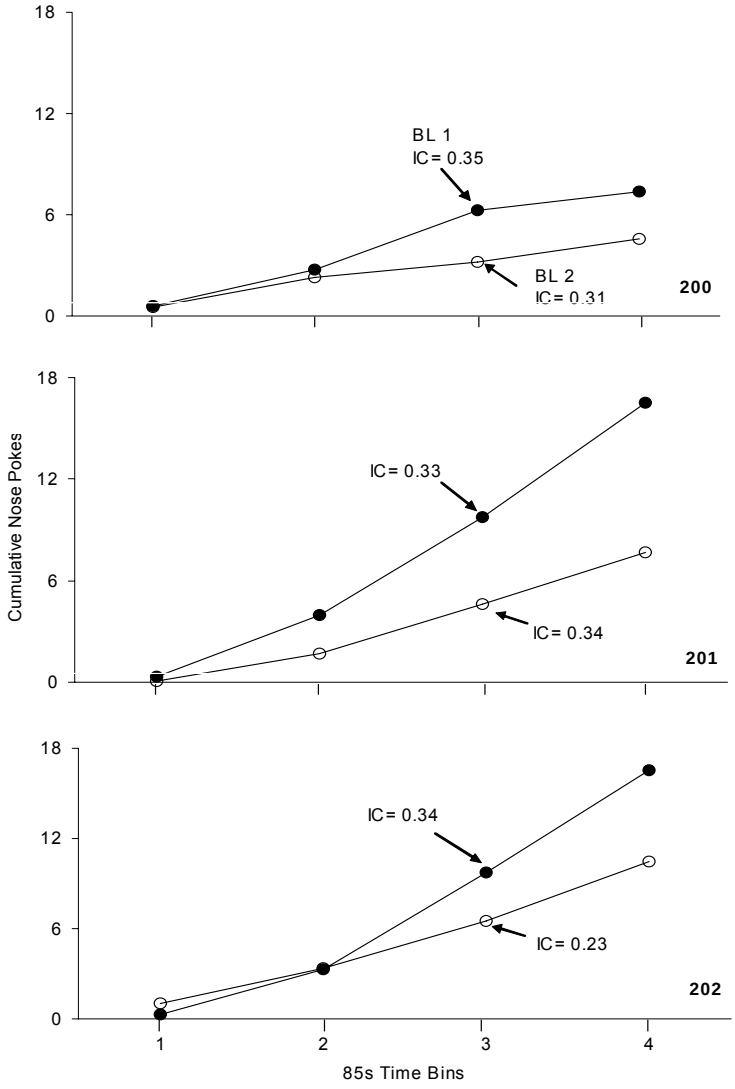

Figure 5. The cumulative rate of nose pokes per quarter and corresponding IC across the FI 5 min reinforcement Schedule over the last 5 sessions. Black circles represent the first baseline condition and open circles represent the second baseline condition. The vertical represents the cumulative number of nose pokes per quarter. The horizontal axis represents each quarter in a 5 min interval. The top panel represents the cumulative graph and IC for Mouse 200, the middle panel represents the cumulative graph and IC for Mouse 201 and the bottom panel represents the cumulative graph and IC for Mouse 202.

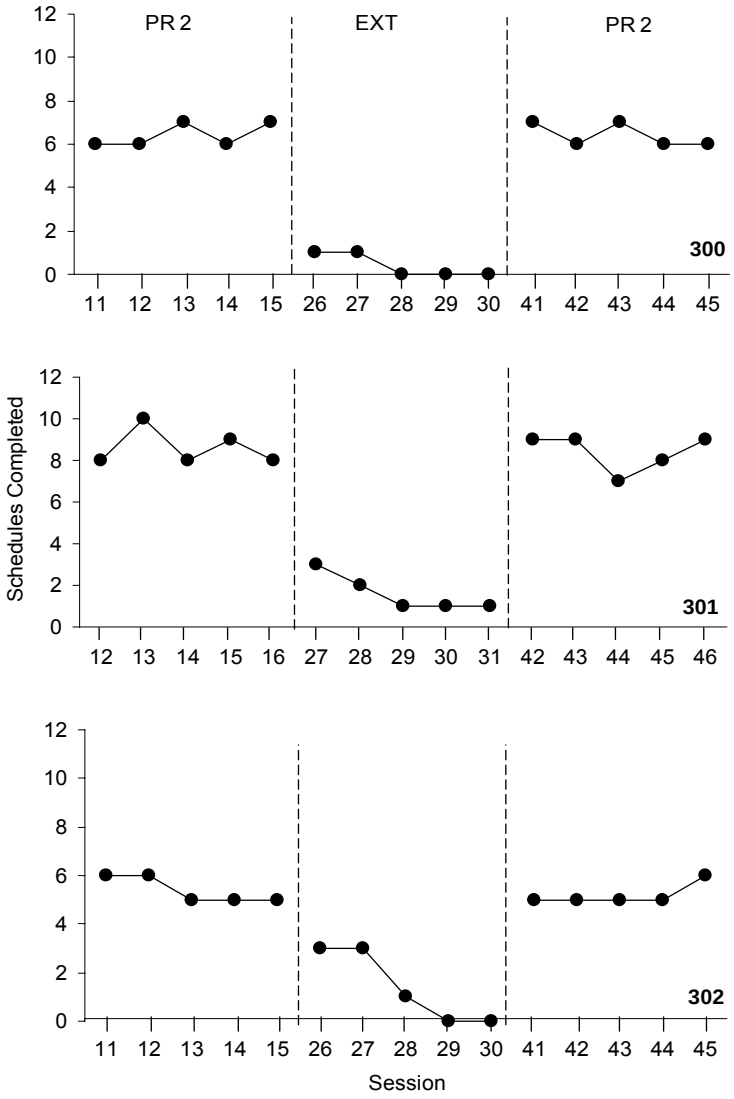

Figure 6. The number of completed Schedules over the last 5 sessions on the PR 2 reinforcement Schedule. Baseline and extinction conditions are separated by dashed phase lines. The vertical axis represents the number of Schedules completed. The horizontal axis represents the session number. The top panel represents completed Schedules for Mouse 300, the middle panel represents completed Schedules for Mouse 301, and the bottom panel represents completed Schedules for Mouse 302.

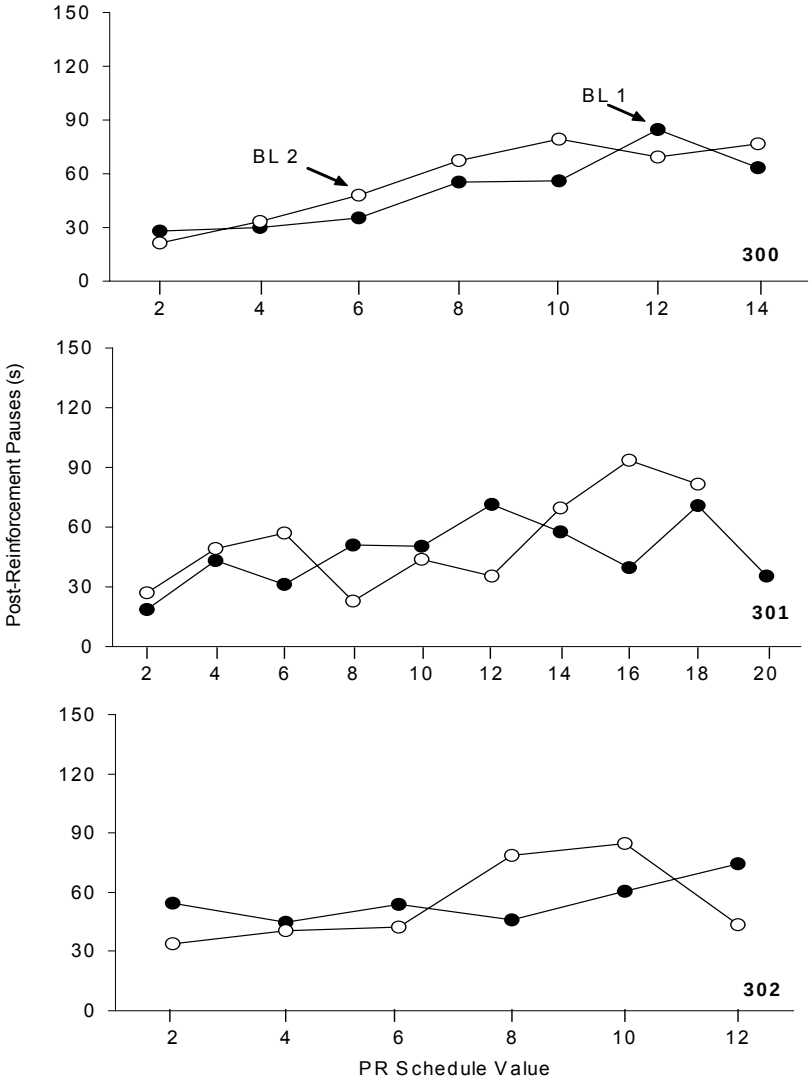

Figure 7. The average PRP durations per session in seconds over the last 5 sessions in on the PR reinforcement Schedule. Black circles represent the first baseline condition and open circles represent the second baseline condition. The vertical axis represents the PRPs per PR 2 Schedule value. The horizontal axis represents PR 2 Schedule values. The top panel represents PRPs for Mouse 300, the middle panel represents PRPs for Mouse 301, and the bottom panel represents PRPs for Mouse 302.

Dopamine Receptors and Drugs

DA receptors

In common with virtually all other neuroreceptors, DA receptors are now known to exist in multiple subtypes. Like other G-protein coupled receptors, the 5 DA receptors have 7 putative membrane spanning helices which forms a narrow dihedral hydrophobic cleft surrounded by 3 extracellular and 3 intracellular loops (see Fig. 8). The receptor polypeptides are thought to be further anchored to the membranes in which they exist through palmitoylation of a conserved Cys residue found in their C-tails (Civelli, 2000).

The first indication that the DA receptors could be differentiated into two subfamilies came in their cloned primary sequences. In their putative transmembrane domains, the D1 and D5 receptors are 79% identical but are only approximately 43% identical to D2, D3 and D4 receptors (Civelli, 2000). In addition, the D2, D3 and D4 receptors share up to 75% homology with each other. Though the DA receptors primary sequences were the first indication that these 5 receptors (D1-D5) could be divided into two subfamilies, the definitive evidence dividing these receptors was made on pharmacological grounds; D1 and D2 receptors are coupled to different postsynaptic transduction mechanisms. The D1 receptors are positively coupled to Gi/o whereas D2 are negatively coupled to Gs. As a result of this differential coupling to G-proteins, activation of each of these receptors results in differential cellular activity. For example, in the dorsal striatum, DA has excitatory effects at D1 receptors while D2 receptors are inhibitory.

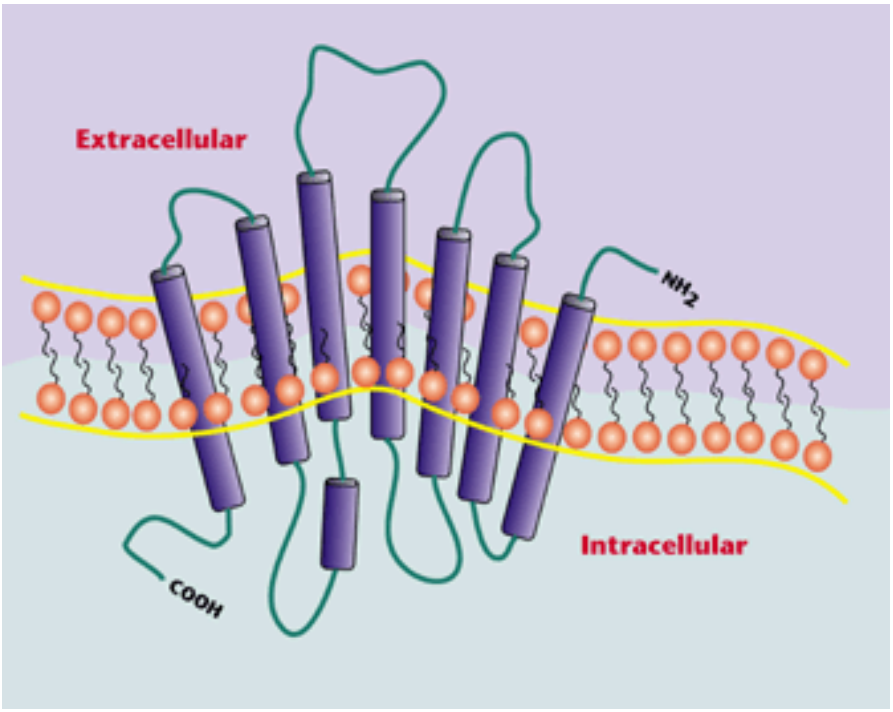

Figure 8. Dopamine Receptor

D1 and D2 receptors are present in all brain regions that receive a dopaminergic projection, both subtypes are expressed at a high level in the dorsal and ventral striatum, olfactory tubercle, and lower levels are present in the septum, hypothalamus and cortex; though D1 receptors predominate in PFC. D2 receptors, but not D1 receptors are found in the substantia nigra and VTA. Conversely, D1 is found in the amygdala, where little if any D2 receptors reside. DA autoreceptors are of the D2 subtype, with a possible D3 contribution; there are no D1 autoreceptors. D3 and D4 receptors are localized almost exclusively within 'limbic' areas, particularly the nucleus accumbens shell, and so are of particular interest in relation to affective disorders (Civelli, 2000). The localization of the D5 receptors is highly specific; in the hippocampus, hypothalamus and the parafascicular nucleus of the thalamus (Civelli, 2000).

D1 Antagonist: SCH-23390

SCH-23390 is a potent D1-like receptor antagonist of the benzazepine family of compounds. SCH-23390 is also known as R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride with a molecular formula of $C_{17}H_{18}ClNO \cdot HCl$ and molecular weight of 324.24 (see Fig. 9). The active enantiomer of SCH-23390 is the (R) in contrast to the in Active (S) enantiomer. This compound inhibits the stimulation of adenylyl cyclase caused by dopamine binding to D1-like receptors.

Most studies *in vivo* utilize radioactively labeled SCH-23390 in order to assess pharmacokinetic activity. K_d values for these compounds have ranged from 0.14 nM in rat striatum (Andersen et al., 1985) to 1.83 nM human putamen

(Raisman et al.., 1985). In experiments conducted by Cumming et al. (1999), it was observed that after 2 min of intravenous infusion of [11C]SCH-23390, untransformed [11C]SCH 23390 comprised 50% of plasma radioactivity at 7 min and only 10% of plasma activity at 30 min. Studies conducted by Sossi et al.. demonstrate that in the occipital cortex, [11C]SCH-23390 reaches equilibrium in the first 15 min of administration. These data taken together indicate that SCH-23390 is a fast acting and rapidly metabolized drug (Cumming et al., 1999; Sossi et al., 2000).

Recently it has been noted that SCH-23390 has some affinity for serotonin (5-HT) 2A receptor. Though there has been a variety of opinions about what SCH-23390's affinity for 5-HT 2A receptors is *in vivo*, it appears to be somewhere in the range of 6-10 fold lower than for D1-like receptors (Bourne, 2001). Though some researchers claim that as much as one-fourth of SCH-23390 *in vivo* cortical activation is through 5-HT 2A receptors (Ekelund et al., 2007), this is not widely agreed upon and SCH-23390 still remains the D1 antagonist of choice in a staggering amount of literature assessing the function of D1-like receptors.

D2 Receptor Antagonist: Sulpride

Sulpiride is a potent D2-like receptor antagonist of the benzamide family of compounds. Sulpiride is also known as (*S*)-5-Aminosulfonyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide, has a molecular formula of $C_{15}H_{23}N_3O_4S$ and a molecular weight of 341.43 (see Fig. 10).

Like SCH-23390, there are two optical enantiomers of sulpiride; (+)-sulpiride or D-sulpiride and (-)-sulpiride or L-sulpiride. The L-sulpiride is the pharmacologically active isomer. Bioavailability of Sulpiride is 30% due to poor and slow gastrointestinal absorption (Kostowski, 1993). Sulpiride's peak plasma concentration is reached after 4.5 hrs with a half-life approximately 5-8 hrs. Using 14C-sulpiride, Dross and Hopf found that in the rat brain, 90% of the radioactivity consisted of unchanged sulpiride, not metabolized sulpiride. In rat waste, about one-third of total 14C-sulpiride was metabolized while two-thirds were excreted unchanged (Dross & Hopf, 1979). These findings indicate that sulpiride is not readily metabolized and is stable in the rodent. K_d for sulpiride ranges between 2-10 nM for D2 and D3 receptors, while it has been reported to be up to 1000 nM for D4 receptors (Strange, 2001).

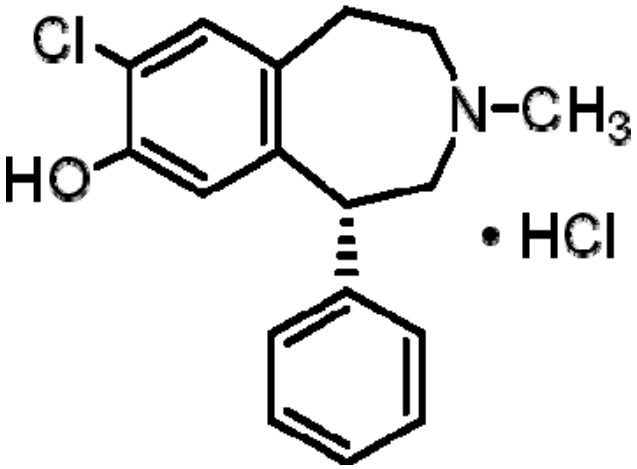

Figure 9. Molecular structure of SCH-23390

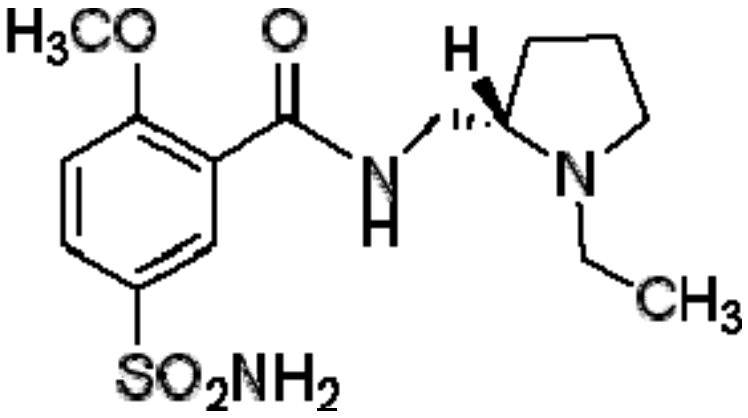

Figure 10. Molecular structure of sulpiride

CHAPTER II

SPECIFIC AIM I: *IS AGGRESSION POSITIVELY REINFORCEING? IF SO, WHAT PART OF THE AGGRESSIVE ENCOUNTER SERVES AS THE POSITIVELY REINFORCING EVENT?*

Rational

While it has been demonstrated that an aggressive encounter in a resident-intruder paradigm can be positively reinforcing, it is still unclear the actual stimulus in the event that functions as the positive reinforcer. An aggressive encounter is a complex one, rich in sight, sound, physical contact, physical activity and olfactory stimulation. Any of these stimuli potentially serve as a positive reinforcer. When studying aggression as a positive reinforcer, it is essential to first determine if aggression itself is positively reinforcing; as opposed to the many other stimuli that the resident mouse comes into contact with during an aggressive encounter.

Materials and Methods

Subjects

Male Swiss Webster albino mice (n = 5) were maintained on a 12:12 h light/dark cycle (lights on at 6:00 A.M.) with experimental sessions occurring during the light-on cycle. At 28 d postpartum, “resident males” were individually housed with a same-strain female. The sire and dam were housed together for

the duration of the experiment. Following a similar timeline, “intruder males” were group-housed (5 males per cage) throughout the experiment. Cages were clear polycarbonate plastic (29 cm x 17 cm x 53 cm) with standard stainless-steel wire lids and CareFresh paper bedding. All mice had *ad libitum* access to rodent chow (Purina, St. Louis, MO) and water. The protocol was reviewed and approved by the Vanderbilt Institutional Animal Care and Use Committee and followed National Institutes of Health guidelines.

Apparatus

The nose-poke apparatus contains two nose-poke sensors (only the right sensor is operative during the experiment), a houselight (illumination at onset of session), and two jeweled stimulus lights (not operative during experiment) (see Fig. 11). Also placed in the home cage prior to the start of the experiment, was a wire mesh screen. The wire mesh screen was placed parallel to and 5 in away from the nose poke apparatus (see Fig. 12). The wire mesh screen served to separate a section of the home cage where the resident mouse could see and smell, but not access physically. The nose-poke instrument panel is controlled by software developed by the Vanderbilt Kennedy Center Computer Services Department, and run on a MSDOS-based personal computer through a Med Associates interface.

Aggression screening

Aggression was assessed by introducing an intruder mouse into the home cage of the male resident mouse with female removed (Miczek & O'Donnell, 1978). Aggression screening involved three separate 10 min resident-intruder encounters each separated by three days. If a resident emitted aggression in 2 or more test sessions, it was included in a subsequent behavioral analysis. For the aggression screening test, aggression was defined as biting or boxing only.

Behavioral contingency

During all behavioral contingency tests, the dam/pups were removed from the resident cage and the operant conditioning panel and mesh screen were inserted. All behavioral contingency sessions were run once daily. All sessions began with house light illumination and lasted for 15 min. Mice meeting criteria in the aggression screening were taught to nose poke via shaping successive approximations with the introduction of an intruder mouse into the resident cage for 6 sec as a consequent stimulus. Resident mice were trained to nose poke on a variable-ratio (VR) 5 reinforcement Schedule to earn access to the intruder mouse. Each time the VR-5 contingency requirement was met, the house light turned off for 0.5 sec and the stimulus mouse was introduced for 6 sec. Mice were required to exhibit aggression toward the intruder on 90% of the opportunities where they earned access to the intruder. If the resident mice did not exhibit aggression on 90% of the opportunities to aggress, they were discontinued. Aggression during the behavioral contingency task was defined as tail rattle, sideways threat, boxing or biting.

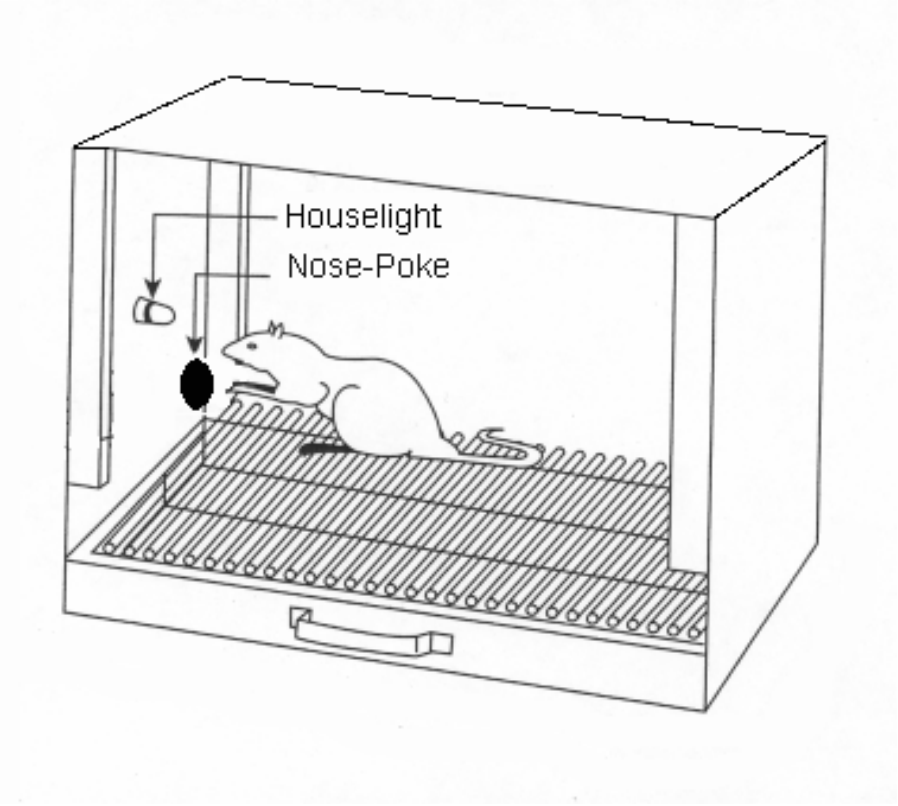

Figure 11. Operant conditioning panel.

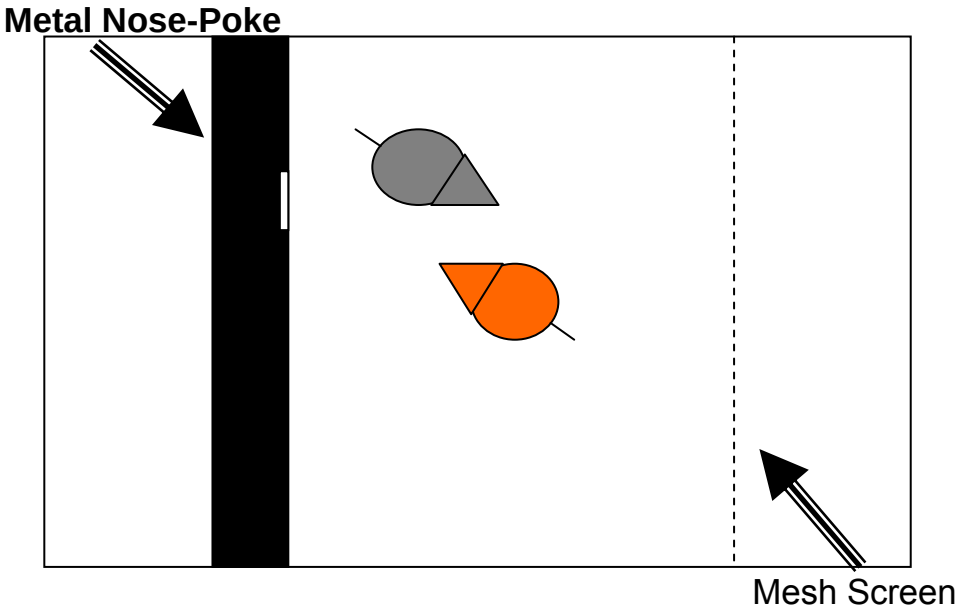

Figure 12. Operant conditioning chamber with mesh screen.

The intruder mouse in the baseline condition was place on the side of the mesh screen that was physically accessible to the resident mouse. The intruder mouse was places approximately 3 in, frontally oriented to the resident mouse upon the resident mouse's completion of the VR-5 contingency. After a steady baseline rate of nose-poke was observed (baseline criteria was that the last three response rates were within the range of the first 5 response rates), subjects were then exposed to an extinction condition. In the extinction condition, after the resident mouse completed the VR-5 contingency, the intruder mouse was placed on the opposite side of the mesh screen; where the resident mouse could see and smell the intruder mouse but not physically attack it. Upon extinguishing the nose-poke rate to the pre-training nose-poke rate, the subjects were then re-exposed to the baseline condition until nose-poke rate was recovered to at least pre-extinction response rate.

Statistical analysis

Within-subjects, repeated measures Analysis of Variance (ANOVA) with Tukey-Kramer post-hoc analyses were used to analyze differences in behavioral response in each baseline condition and extinction. Values included in the statistical analyses were those after steady state in each condition had been reached.

Results

Under the above conditions, nose-poke rate was successfully extinguished by removing physical access to the intruder mouse. Each resident

mouse could still see and smell the intruder mouse from the opposite side of the wire mesh screen after the completion of the VR-5 behavioral contingency. However, without being able to physically attack the intruder mouse, each resident mouse ceased responding over time. There was a significant difference between each of the baseline conditions and the extinction condition ($F_{(2,21)}$ = 61.55; $p < .001$). In the first baseline condition, the mean response rate was 3.80 pokes/min with an *SEM* = .14. In the extinction condition, the mean response rate was .22 pokes/min with an *SEM* = .07. In the second baseline condition, the mean response rate was 4.12 pokes/min with an *SEM* = .19. The mean days for responding to extinguish was 13.80 days with an SEM = 1.77. The mean days for the recovery of baseline nose-poke rate was 3.8 days with an SEM = .37 (see Figs. 13 and 14).

Discussion

In the above analysis it was established that upon the removal of physical access to an intruder mouse, a resident mouse will no longer nose-poke for the opportunity to aggress. By placing a wire mesh screen between the resident and intruder mice, it was clearly demonstrated that physical aggression, not visual stimulation, novelty, social interaction or olfactory stimulation, was the positively reinforcing event in an aggressive encounter. This finding is important for several reasons. First, before this analysis, it had not been established under classic behavioral definitions that aggression itself is positively reinforcing. Much of the literature exploring aggression as a reinforcer use the behavioral

phenomena that animals will perform tasks for the opportunity to aggress. However, there has been no published literature attempting to demonstrate exclusive stimulus control over aggression as a reinforcer. Second, though there was strong evidence in support of aggression being positively reinforcing, there was no insight into what part of the aggressive encounter acted as the positive reinforcer. Due to the fact that an aggressive encounter is a complex one, rich with a variety of stimuli (i.e. olfactory stimulation, visual stimulation, social interaction, tactile stimulation, novelty, heightened physical activity), it was possible the actual act of aggression might not be the positively reinforcing stimulus in fight. In previous literature this issue had not been directly addressed. In fact, work conducted with *Beta splendins* indicate that these fish will complete elaborate mazes just to get a visual image of an opponent (Tellegen, 1969). In addition, humans enjoy watching violent sport and cinema. However, upon experiencing repeated aggressive encounters, as was the case with subjects in this study, it appears clear that aggressive contact is necessary component of an aggressive encounter to establish response contingency.

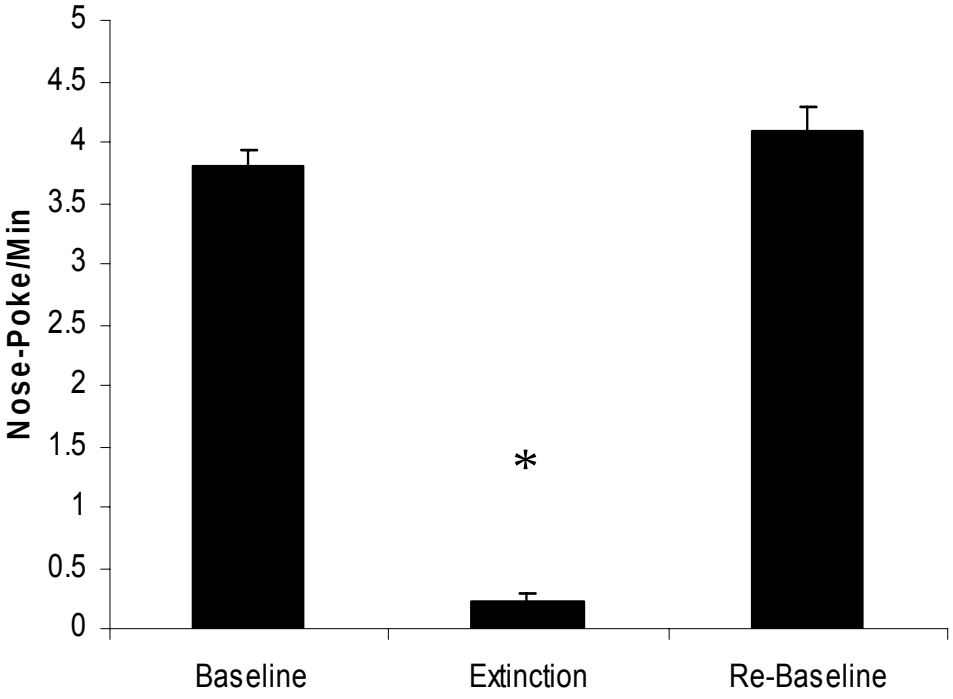

Figure 13. Mean response rate in each behavioral condition in Aim 1. Each bar represents the mean value in each condition after subjects reached steady responding (see above description) in each condition. Error bars represent *SEM*. Asterisks represent significant difference from baseline.

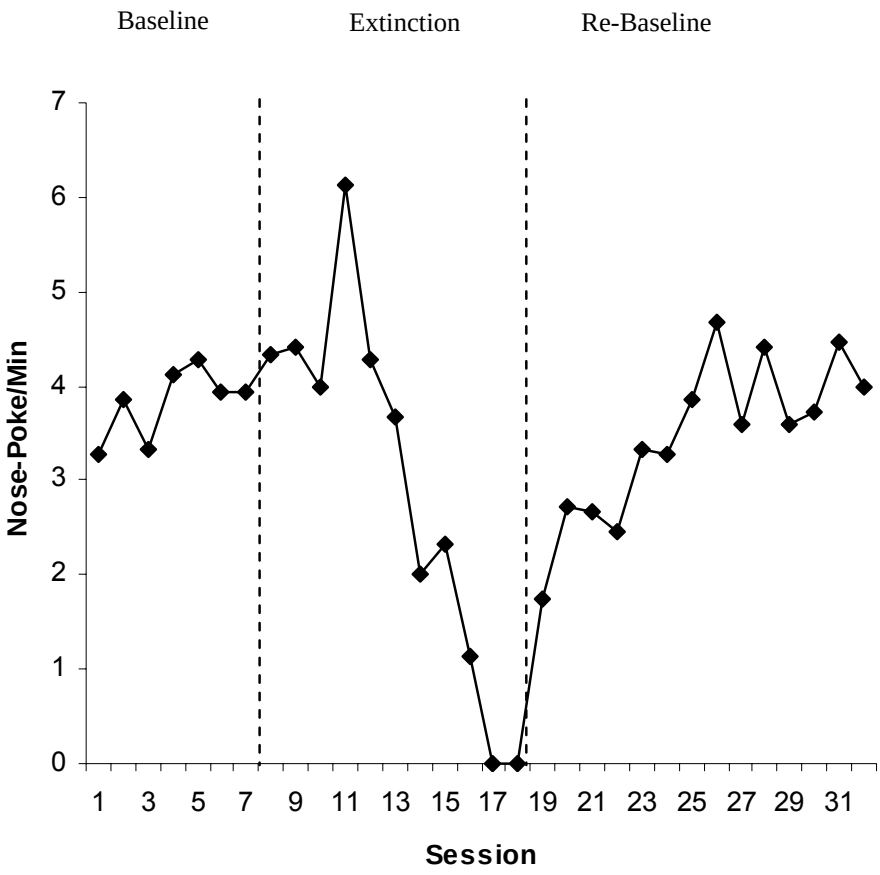

Figure 14. Single-subject response rate across sessions of Aim 1. Baseline, Extinction and Re-Baseline are divided by vertical dashed lines. The values at session 1 began after a steady baseline (see above definition) had been established.

CHAPTER III

SPECIFIC AIM 2: *DO DA 1/5 AND/OR DA 2/3 RECEPTORS IN THE NAC MEDIATE ACCESS TO AGGRESSION AS POSITIVE REINFORCEMENT?*

Rational

The collective problem with present pharmacology work investigating the role of the mesocorticolimbic system in aggression is that they have, thus far, been ineffective at targeting the mesocorticolimbic DA circuit exclusively. Due to the challenges of locally administering drugs in rodents and humans, the pharmacological evidence for the role of positive reinforcement mechanisms in aggression is confounded and must be interpreted in conjunction with behavioral and neurochemical data. What is needed for understanding DA's role in aggression as a positive reinforcer are experiments that carefully separate DA's role in the motivating aspects of aggression from DA's role in generalized movement. Thus, the following experiment is proposed.

Materials and Methods

Subjects

Male Swiss Webster albino mice were maintained on a 12:12 h light/dark cycle (lights on at 6:00 A.M.) with experimental sessions occurring during the light-on cycle. At 28 d postpartum, "resident males" were individually housed with a same-strain female. The sire and dam were housed together for the

duration of the experiment. Following a similar timeline, “intruder males” were group-housed (5 males per cage) throughout the experiment. Cages were clear polycarbonate plastic (29 x 17 x 53 cm) with standard stainless-steel wire lids and CareFresh paper bedding. All mice had *ad libitum* access to rodent chow (Purina, St. Louis, MO) and water. The protocol was reviewed and approved by the Vanderbilt Institutional Animal Care and Use Committee and followed National Institutes of Health guidelines.

Surgical procedures

At 60 to 75 d postpartum, resident males were unilaterally implanted with guide cannula (CMA7, CMA Microdialysis, Solna, Sweden) positioned directly above the nucleus accumbens (AP, 1.6 mm; ML, 7.5 mm; DV, 4.5 mm)(Paxinos and Franklin, 2001). Before surgery, subjects were anesthetized with 125 mg/kg ketamine and 10 mg/kg xylazine. Cannulae were adhered to the skull using Geristore dental adhesive (Denmat Corporation, Santa Maria, CA). The skin was replaced over the base of the guide cannula and sutured closed. After surgery, 7 d of isolated recovery occurred. Mice were then paired with the original female mate and left to acclimate for 7 to 14 d. After the acclamation period, mice were screened for aggression.

Aggression screening

Aggression was assessed by introducing an intruder mouse into the home cage of the male resident mouse with female removed (Miczek and O’Donnell

1978). Aggression screening involved three separate 10 min resident-intruder encounters each separated by three days. If a resident emitted aggression in 2 or more test sessions, it was included in a subsequent pharmacological analysis.

Aggression as positive reinforcement apparatus

The operant conditioning panel (29 cm x 17 cm x 0.6 cm) was comprised of two nose-poke sensors (only the right sensor was operative during the experiment) and a house light. The instrument panel, which was inserted into the resident vivarium cage, was controlled by software developed by the Vanderbilt Kennedy Center and run on a MSDOS-based personal computer through a Med Associates interface (see Fig. 11).

Behavioral contingency

During all behavioral contingency tests, the dam/pups were removed from the resident cage and the operant conditioning panel was inserted. All behavioral contingency sessions were run once daily. Mice meeting criteria in the aggression screening were taught to nose poke via shaping successive approximations with the introduction of an intruder mouse into the resident cage for 6 sec as a consequent stimulus. Resident mice were trained to nose poke on a variable-ratio (VR) 5 reinforcement Schedule to earn access to the intruder mouse. All sessions began with house light illumination and lasted for 15 min. Each time the VR-5 contingency requirement was met, the house light turned off for 0.5 sec and the stimulus mouse was introduced for 6 sec. Mice were required

to exhibit aggression toward the intruder on 90% of the opportunities where they earned access to the intruder. If the resident mice did not exhibit aggression on 90% of the opportunities to aggress, they were discontinued. Aggression during the behavioral contingency task was defined as tail rattle, sideways threat, boxing or biting. Along with the automatically recorded nose pokes, sessions were videotaped and scored for locomotion/aggression as described below.

Dopamine antagonist tests

After subjects demonstrated steady nose poking rates in baseline, drug microinjections were conducted. Double determinations were made at each dosage in an ascending dose-effect function with baseline sessions occurring in between each drug tests. Determinations were established for mock-infusions (cannula without liquid were inserted into guides of subjects for 3 min), vehicle (artificial cerebral spinal fluid) and the D1-like receptor antagonist SCH-23390 (12 ng, 25 ng, and 50 ng) and the D2-like receptor antagonist sulpiride (12 ng, 25 ng, and 50 ng). All drugs were obtained from Sigma-Aldrich (St. Louis, MO). Each microinjection was 150 nl in volume and manually infused over 3 min using a microsyringe (Gilmont Instruments, Morgantown, PA). Microinjections were administered 15 min prior to behavioral contingency testing.

Videotaped scoring of locomotion/aggression

Each behavioral contingency test session was videotaped and scored for movement and aggression. Movement included time spent running/walking,

grooming and rearing during mock-infusion, vehicle and DA antagonist test conditions. Aggression included tail rattle, sideways threat, boxing, and biting during mock-infusion, vehicle and DA antagonist after the intruder mouse was introduced. Trained graduate students blinded to conditions scored videotaped sessions (see Miczek and O'Donnell 1978).

Open-field tests

Naïve cannulated mice were treated one time in each in each condition (mock-infusion, vehicle, 25 ng and 50 ng of SCH-23390 or sulpiride using a Latin square randomization design) 15 min prior to open-field test. Animals were then placed in a 43 cm X 43 cm open field chamber (ENV-515 test environment, MED Associates Inc., St. Albans, VT) for 15 min in a lit room. Total distance traveled was recorded and analyzed as the measure of locomotion using MED Associates SOF-811 Open Field Activity Software.

Histology

After completing behavioral contingency test, mice were deeply anesthetized with 800 mg/kg pentobarbital (Abbot Laboratories, Chicago, IL) and transcardially perfused with 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO). Brains were removed and cryoprotected by overnight submersion in 30% sucrose: 70% paraformaldehyde fixative. Tissue was frozen on dry ice and sliced at 50 µm using a microtome. Mounted tissue was then Nissel stained in order to verify cannula placement in the NAc. To verify size and spread of infusions, a

spread analysis was conducted. In this analysis, 15 min prior to perfusion, animals (n = 4) were infused with 150 nl of micro-ruby (Sigma-Aldrich, St. Louis, MO) mixed in vehicle. Fifteen min after the micro-ruby infusion, animals were perfused and tissue treated as described above.

Statistical analysis

Within-subjects, repeated measures Analysis of Variance (ANOVA) with Tukey-Kramer post-hoc analyses were used to analyze differences in behavioral response to drug doses in each test (i.e., behavioral contingency test, videotaped movement, open-field test). All comparisons of drug effects were in reference to vehicle.

Results

Histological verification of cannula placement

After perfusion of each cannulated animal, tissue was sliced and stained to verify placement. Twenty-five animals had cannula placed within the boundaries of the NAc. Fig. 15 shows a diagram depicting a coronal mouse section through the NAc (adapted from Paxinos and Watson, 2001) indicating cannula placement was accurate and consistent across all animals.

Infusion of micro-ruby prior to perfusion was conducted to quantify the spread of infusions. The infusions were tear-drop shaped with the mean length (dorsal to ventral) at the largest point being 475 µm ($\pm$120) and the mean width (medial to lateral) being 400 µm ($\pm$37).

Dopamine antagonist effects on the positively reinforcing properties of aggression

For cannula placements within the NAc, infusing SCH-23390 significantly reduced nose poking for aggression at the 50 ng dosage compared to vehicle ($F(3,49) = 6.26$; $p < .001$) (see Fig. 16a.). Infusing sulpiride into the NAc resulted in reduced nose poking for aggression at the 25 ng and 50 ng dosages of sulpiride compared to vehicle ($F(3,55) = 6.26$; $p < .001$) (see Fig. 16b). Table 2 (see appendix a.) shows high levels of agonistic behaviors during vehicle injections when intruder mice were present. Reductions in aggression occurred at the 50 ng dosage of SCH-23390 for biting ($F(3,56) = 5.06$; $p < .01$). Aggression was reduced at the 25 ng dosage of sulpiride for tail rattle ($F(3,56) = 11.04$; $p < .01$) and biting ($F(3,56) = 70.66$; $p < .01$) and for all aggressive behaviors at the 50 ng dosage of sulpiride ($p < .01$). For cannula placements outside the NAc, no dosage of SCH-23390 (Fig 17a.) or sulpiride (Fig. 17b.) affected nose poking for aggression.

Dopamine antagonist effects on movement during aggression tests

Each resident mouse was videotaped during each mock, vehicle and drug infusion while performing the behavioral contingency task and scored for time spent walking/running, grooming and rearing. In the SCH-23390 analysis, no

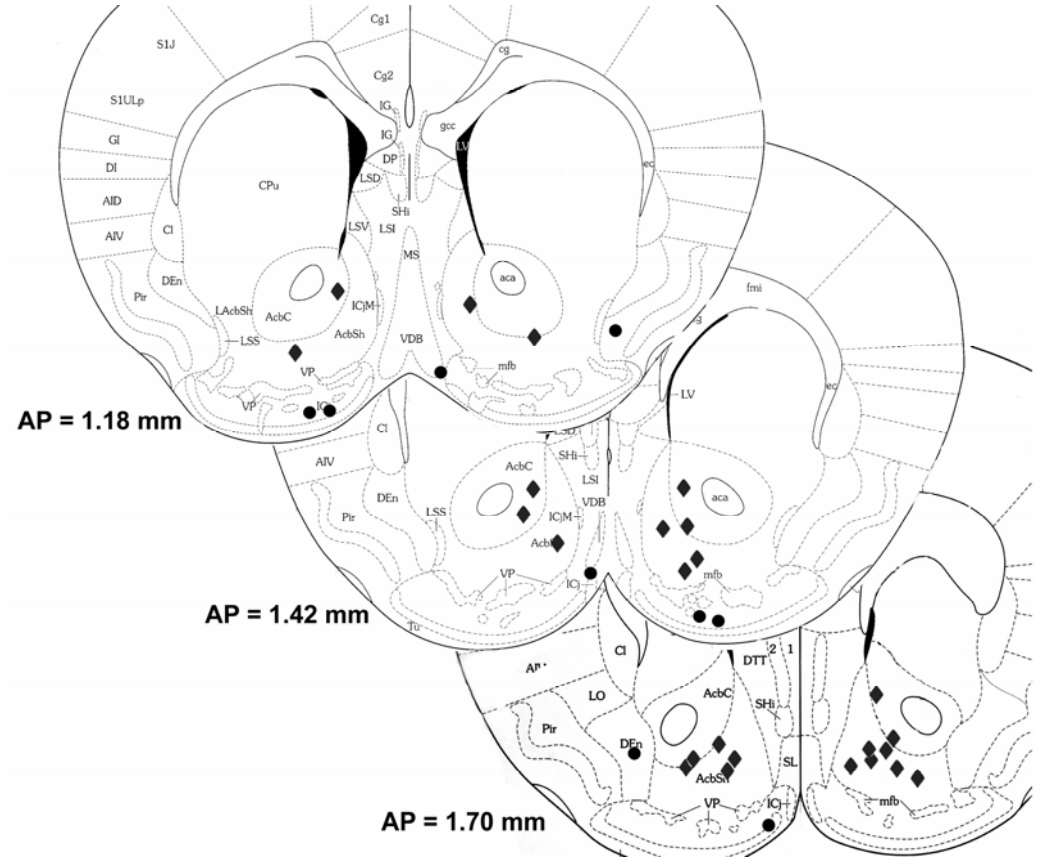

Figure 15. A diagram depicting coronal mouse sections at 3 different *AP* levels through the NAc (adapted from Paxinos & Watson, 1984). Each diamond represents the center point of an indwelling cannula that was included within the boundaries of the NAc (*n* = 25). Each circle represents the center point of an indwelling cannula that was considered outside the boundaries of the NAc (*n* = 9). *AP* measurements are mm from Bregma.

differences from vehicle were seen in total time spent moving. However, grooming, at both the 25 ng and 50 ng dosages was different from vehicle ($F_{(3,37)}$ = 46.76; p < .001). Rearing was also different between 50 ng SCH-23390 and vehicle ($F_{(3,37)}$ = 4.62; p < .01) (see Table 1; appendix a.). For the sulpiride analysis, total movement differed from vehicle only at the 50 ng dosage ($F_{(3,44)}$ = 31.40; p < .0001). Walking, grooming and rearing were different from vehicle only at the 50 ng dosage ($F_{(3,44)}$ = 27.35; p < .001; $F_{(3,44)}$ = 6.44; p < .001; $F_{(3,44)}$ = 32.72; p < .001; respectively).

Dopamine antagonist effects on movement during aggression tests

Each resident mouse was videotaped during each mock, vehicle and drug infusion while performing the behavioral contingency task and scored for time spent walking/running, grooming and rearing. In the SCH-23390 analysis, no differences from vehicle were seen in total time spent moving. However, grooming, at both the 25 ng and 50 ng dosages was different from vehicle ($F_{(3,37)}$ = 46.76; p < .001). Rearing was also different between 50 ng SCH-23390 and vehicle ($F_{(3,37)}$ = 4.62; p < .01) (see Table 1; appendix a.). For the sulpiride analysis, total movement differed from vehicle only at the 50 ng dosage ($F_{(3,44)}$ = 31.40; p < .0001). Walking, grooming and rearing were different from vehicle only at the 50 ng dosage ($F_{(3,44)}$ = 27.35; p < .001; $F_{(3,44)}$ = 6.44; p < .001; $F_{(3,44)}$ = 32.72; p < .001; respectively).

Table 1. Effects of SCH-23390 and Sulpiride on Walking, Grooming, Rearing and Total Movement
Data for each behavior are means +/- SEM. Values that are significantly different from average vehicle are printed in **boldface** ($p < 0.01$).

	Mock-Injection	Vehicle	12 ng SCH-23390	25 ng SCH-23390	50 ng SCH-23390	12 ng Sulpiride	25 ng Sulpiride	50 ng Sulpiride
Walking	8.91 +/- .47	8.58 +/- .40	7.9 +/-.39	6.35 +/-.41	6.64 +/- .49	8.13 +/- .45	7.67 +/- .48	**3.65 +/- .47**
Grooming	.84 +/- .09	.83 +/- .10	.79 +/-.14	**2.87 +/-.15**	**1.98 +/- .15**	0.82 +/- .05	.73 +/-.03	**.58 +/-.05**
Rearing	3.32 +/-.27	2.65 +/-.25	3.06 +/-.22	2.20 +/-.26	**1.73 +/- .19**	2.89 +/- .28	2.01 +/- .26	**.54 +/-.24**
Total	12.90 +/- .52	11.88 +/- .50	11.71 +/- .44	11.43 +/- .42	10.42 +/- .37	11.85 +/- .61	10.41 +/- .59	**4.77 +/- .63**

Table 2. Mean % of Earned Reinforcement Time Spent Performing Aggressive Behaviors.
Data for each behavior are means expressed as % +/- SEM. Values that are significantly different from vehicle are printed in **boldface** ($p < 0.01$). Note that for the 50 ng sulpiride condition, most subjects did not earn any reinforcement time. Scores for 50 ng sulpiride are base on a small amount of earned reinforcement time.

	Vehicle 1	12 ng Sul	25 ng Sul	50 ng Sul	Vehicle 2	12 ng SCH	25 ng SCH	50 ng SCH
Tail-Rattle	8.5 +/- 1.86	7.9 +/- 1.28	**5.3 +/- 1.04**	0	8 +/- 1.30	**9.97 +/- 1.04**	8.21 +/- 1.16	9.33 +/- 1.04
Sideways Threats	12.3 +/- 2.09	13+/- 2.10	12.6 +/- 1.00	11.02 +/- 1.04	11.9 +/- 1.35	10.08 +/- 2.70	12.33 +/- 1.27	15.23 +/- 3.27
Boxing	24.06+/- 1.55	24.6+/- 1.75	26.15 +/- 1.66	**19.43 +/- 1.46**	22.23 +/- 2.68	24.3 +/- 2.42	20 +/- 2.33	20.22 +/- 1.7
Biting	53.17+/- 1.56	51.7+/- 1.86	**46.89 +/- 1.46**	**25.22 +/- 1.88**	55.68 +/- 1.20	53.5 +/- 1.80	53.01 +/- 1.95	**48.12 +/- 1.95**
Total	98.03 +/- 4.09	97.2 +/- 4.39	**90.94 +/- 3.05**	**55.67 +/- 3.0**	97.81 +/- 3.80	97.85 +/- 4.30	93.55 +/- 4.92	92.9 +/- 5.35

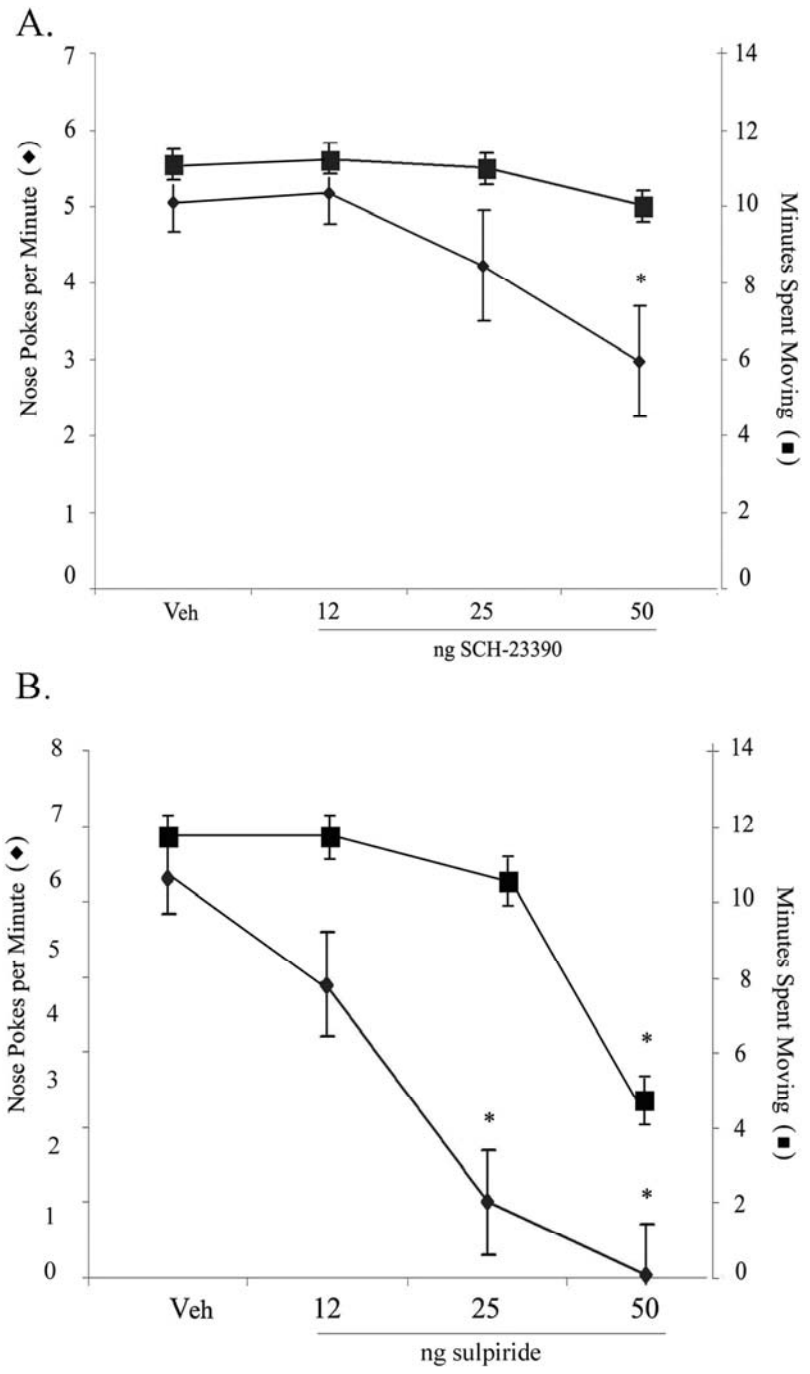

Figure 16. SCH-23390 (16a.) and sulpiride (16b.) dose-response curve (diamonds) and total minutes spent moving (squares) as scored from video taped sessions for animals with cannula placed within the boundaries of the NAc (*N* = 5; *N* = 6, respectively). Data is presented as means +/- *SEM* (vertical lines). Asterisks denote statistically significant differences relative to vehicle ($p < 0.05$)

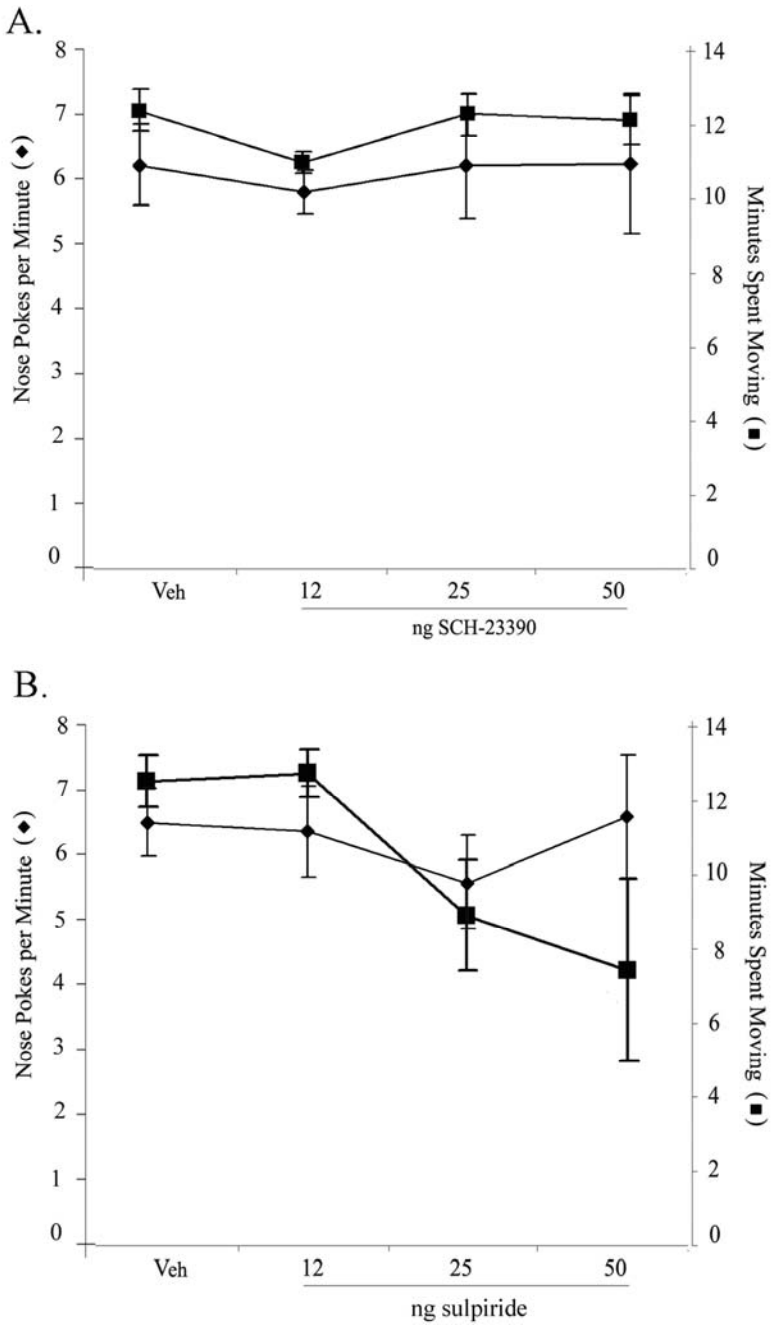

Figure 17. SCH-23390 (17a.) and sulpiride (17b.) dose-response curves (diamonds) and total minutes spent moving (squares) as scored from video taped sessions for animals with cannula placed outside the boundaries of the NAc (*N* = 3; *N* = 4, respectively). Data is presented as means +/- *SEM* (error bars). * denotes significant difference from vehicle ($p < 0.05$).

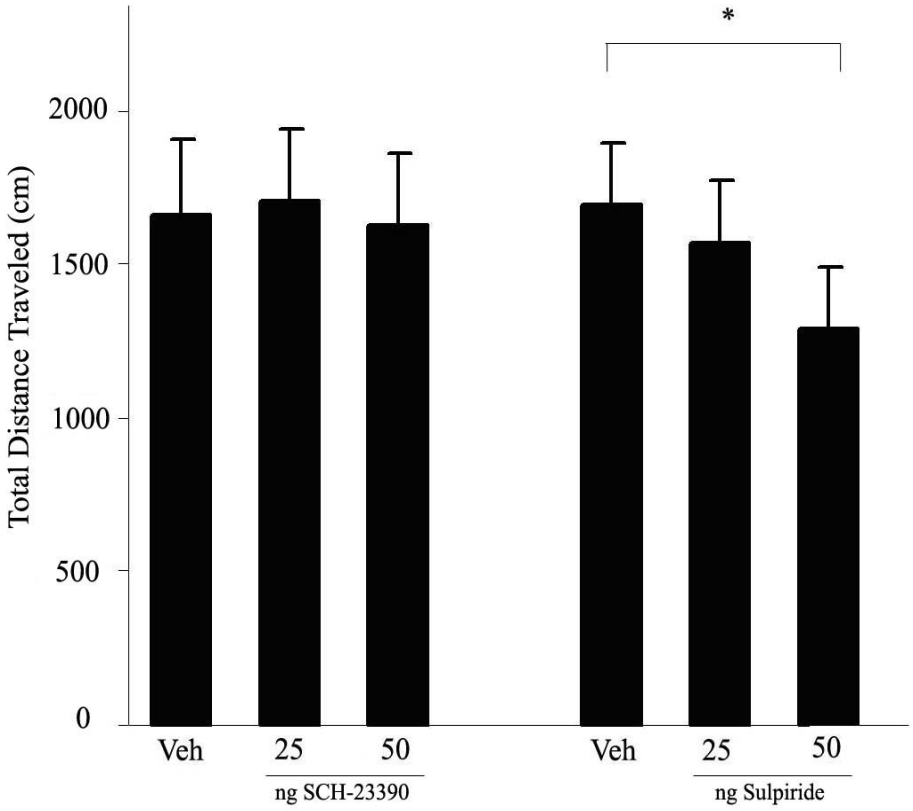

Figure 18. Effect of SCH-23390 and sulpiride on total distance traveled in an open field. Data is presented as means +/- *SEM* (vertical lines). Asterisks denote statistically significant differences relative to vehicle ($p < 0.05$).

Dopamine antagonist effects on movement in open-field tests

The distance traveled in the SCH-23390 open-field test yielded an overall significant effect ($F_{(3,37)}$ = 3.67, p < .05*)*. The mean distance traveled in the SCH-23390 groups was 1903 cm for mock-injections (*SEM* = 213), 1662 cm for vehicle (*SEM* = 189), 1701 cm for 25 ng (*SEM* = 195) and 1630 cm for 50 ng (*SEM* = 200). Tukey-Kramer post-hoc analyses revealed that there were no significant differences between drug dosages and vehicle (see Fig. 18). The distance traveled in the sulpiride open-field test yielded an overall significant effect ($F_{(5,54)}$ = 4.37, p < .05). The mean distance traveled in the sulpiride group was 2246 cm for mock-injections (*SEM* = 220), 1691 cm for vehicle (*SEM* = 223), 1573 cm for 25 ng (*SEM* = 184 cm), and 1291 cm for 50 ng (*SEM* = 207). Results of the Tukey-Kramer post-hoc analyses revealed that the 50 ng dosage differed from vehicle (p < .05) (see Fig. 18).

Discussion

We established contingent access to aggression as a positively reinforcing stimulus for male CFW mice. Localized administration into the NAc of a D1-like DA receptor antagonist (SCH-23390) or a D2-like DA receptor antagonist (sulpiride) decreased aggression at dosages that did not disrupt general motor behavior. Additional open-field tests were conducted demonstrating that SCH-23390 and sulpiride dosages that reduced aggression in the operant conditioning task did not impair general motor movement in the open-field test. These

findings suggest that mesocorticolimbic DA is involved in the mediation of the positively reinforcing effects of aggression in mice. In addition, we have demonstrated a technique for local administration of DAergic antagonists into the NAc that avoids previous confounds in regard to general motor suppression.

Previous experiments have established the viability of the operant conditioning task used in this experiment to study aggression as positive reinforcement. De Almeida and Miczek (2002), Fish et al. (2002), and May et al. (2007) have used contingent access to aggression under a range of response- and time-based positive reinforcement Schedules. An important aspect of this method is the separation of ethologically evoked aggression elicited from the introduction of a conspecific into the resident cage from the motivation of the resident mouse to earn access to aggression as a stimulus event by emitting instrumental behavior. This paradigm allowed us to analyze the motivational properties of aggression as a positively reinforcing stimulus separate from other behavioral processes evoked by agonistic encounters (Michael, 1982; Laraway et al., 2003).

Several previous studies have implicated mesocorticolimbic DA in relation to the positively reinforcing properties aggression. The most direct evidence for the involvement of the "reward pathway" comes from microdialysis experiments showing that extracellular levels of DA increase in the NAc after agonistic encounters, a finding that parallels microdialysis studies of other positively reinforcing stimuli (Ferrari et al., 2003; Van Erp et al., 2000). In our experiment we were able to further the microdialysis findings by directly suppressing DAergic

activity in the NAc. This NAc DAergic antagonism resulted in mice no longer engaging in instrumental behavior to earn access to aggression, further implicating NAc DA in the positively reinforcing properties of agonistic encounters.

The results of our experiments indicate a role for D1-like and D2-like DA receptors in the reinforcing properties of aggression. However, to what extent each DA receptor subtype serves in aggression and positive reinforcement is yet to be determined. Experiments administering apomorphine (a *SEM*I-selective D2-like receptor agonist) and N-n-Propyl-Norapomorphine (a potent D2-like receptor agonist), showed a facilitative agonistic effect under predatory, foot-shock and isolation-induced aggression paradigms (Baggio & Ferrari, 1980; Miczek et al., 2002; Siegel et al., 1999). Complimenting the findings of these experiments are studies using haloperidol and raclopride (D2-like receptor antagonists) which decreased aggression in rodents and humans, although the findings are problematic due to undesired motor side effects (Miczek et al.., 2002; Siegel et al., 1999). Experiments demonstrating a role for D1-like receptors in the modulation of aggression are also present in the literature. SCH-23390 and SKF-38393 (a selective D1-like receptor agonist) have been reported to reduce aggression in rodents, although movement confounds limit interpretation of previous studies (Miczek et al., 2002; Rodriguez-Arias et al., 1998) and interspecies replication has been limited (Siegel et al., 1999).

In other positively reinforcing behaviors, DA is the most strongly implicated neurotransmitter (Wise and Rompre´, 1989; Wise, 2004). Drugs of abuse, including cocaine, amphetamine, heroine and nicotine, are all associated with elevated DA and are thought to be addictive because of this elevated brain DA. Blockade of mesocorticolimbic DA receptors by mixed DA antagonists results in significantly reduced self-administration of these drugs of abuse (Di Chiara and Imperato, 1988; Yokel and Wise, 1975; de Wit and Wise, 1977; Corrigall et al., 1992; Franklin, 1978). Positively reinforcing behaviors besides the administration of DA-altering pharmacological agents have also been consistently attributed to mesocorticolimbic DA. For instance, studies with food/drink positive reinforcement and Intra-Cranial Self-Stimulation (ICSS) demonstrate attenuated responding with the administration of pimozide (potent mixed DA antagonist) in the rat (Fouriezos and Wise, 1976 Fouriezos et al., 1978; Zarevics and Setler, 1979; Franklin and McCoy, 1979; Gallistel et al., 1982; Gallistel and Karras, 1984; Gallistel and Freyd, 1987; McFarland and Ettenberg, 1995; Geary & Smith; 1985). Each of the above effects were seen in absence of motoric side effects.

Though the current literature clearly indicates that dopamine is involved in positive reinforcement, the relative roles of D1-like vs. D2-like receptor activation still remains controversial. Conflicting data supports D1 activation, D2 activation and activation of both. For instance, studies in human subjects report two D2 genetic polymorphisms, a TaqIA allele variant and a D2 receptor haplotype, associated with food and nicotine addictions (Morton et al., 2006). Supporting the role for D2-like activation in positive reinforcement is work demonstrating NAc

D2-like control over responding for food, cocaine, nicotine and ICSS (Pezze et al., 2007; Esposito et al., 1979; Seeger TF & Gardner, 1979; Gál K & Gyertyán I, 2006; Ross et al., 2007). Coupling these data with anatomical data demonstrating a high concentration of D3 receptors in the NAc shell; an area commonly associated with positive reinforcement, there is substantial support for a role of D2-like receptor activation in positive reinforcement (Scwartz et al.., 1994). In contrast to these data are those that demonstrate a role for D1 activation. Studies administering D1-like agonists into the NAc report reduced responding for food positive reinforcement (Katz et al., 2006; Caine, 2000; Bratcher et al.). However, it is interesting to note that antagonism of these behavioral effects by the D1 receptor antagonist, SCH-23390, was only seen for SKF 82958 infusions. This point indicates that the behavioral effects of the other D1-like receptor agonists used in these studies may be due to activation of receptors other than D1-like receptors and thus, must be considered carefully (Katz et al., 2006). In compliment to the work demonstrating the role of D1-like receptors in food positive reinforcement are studies showing that D1-like agonists also attenuate cocaine self-administration and D1-like antagonists potentiate cocaine self-administration (Caine et al., 1995, Barrett et al., 2004; Bari et al., 2005). In each of these pharmaco-behavioral studies implicating D1-like activation in positive reinforcement (both those that use food positive reinforcement and cocaine positive reinforcement paradigms), results regarding the effect of D2 agonism/antagonism vary with a tendency towards demonstrating little to no effect of D2 manipulation on positive reinforcement-

based responding (Katz et al., 2006; Bratcher et al.; Bari et al., 2005; Caine et al., 2005; Cheer, 2007). In direct opposition to the data demonstrating that D1-like agonism attenuates responding for food and cocaine but still supporting the exclusive role of D1-like receptors in positive reinforcement is work demonstrating that D1-like antagonists, but not D2-like antagonists, decreases responding for ICSS (Cheer et al., 2007).

Though there is still ongoing debate over an exclusive role for one or the other DA receptor subtype in various types of positive reinforcement, a majority of the literature does not support this. Instead, the abundance of studies are in accord with our findings; that both the D1 and D2 receptors are involved in positive reinforcement-based responding. It appears from the distinct D1-like and D2-like dose-response curves yielded from studies investigating food, cocaine, amphetamine, nicotine and ICSS positive reinforcement in species ranging from monkey to rat, that both D1-like and D2-like receptors play important roles in positive reinforcement; albeit very different ones that are still widely unknown (Ikemoto et al., 1997; Wolterink et al., 1993; White, Hu & Henry , 1993; Singh et al., 1997; Ranaldi & Beninger, 1994; Eiler et al., 1997; Caine et al., 2000; Barrett et al., 2004; SCHmidt et al., 2006; Phillips et al., 1994; Spealman, et al., 1991).

In addition to the lack of conclusive evidence on the nature of receptor subtype contributions in positive reinforcement, there is no literature, excluding our study, describing possible contributions of DAergic postsynaptic mechanisms in reinforcing aggression. In order to extend the findings of our study,

experiments utilizing antagonists exclusively specific for individual DA receptors 1-5, should be used in follow-up behavioral pharmacological work. Though there are specific antagonists for the D1, D2, D4 and D5 receptors, there has been little success at deriving a DA antagonist with exclusive affinity for the D3 receptor (Heidbreder et al., 2005). This becomes a reasonable problem in the search for DA receptor contributions to aggression as a reinforcer due to the fact that D3r's are most highly concentrated in the islands of Calleja and NAc; thus making it a likely contributor to the reinforcing properties of aggression. In attempts to identify possible presynaptic mechanisms acting in the reinforcing properties of aggression, experiments targeting DA production, release and turnover would be an asset

In our experiments described above, we were not able to isolate the role of nucleus accumbens core v. shell. Even though our histological data demonstrated consistent, isolated micro-injections not exceeding the boundaries of the NAc, it was not possible to isolate core vs. shell injections due to the extremely small size of the mouse ventral striatum. However, research with other species with other reinforcers suggests that the medial shell is strongly associated with the positively reinforcing effects of a stimulus, whereas the core contributes to behavioral activation. This hypothesis is strongly supported by a variety of evidence ranging from c-fos mRNA expression studies to studies utilizing 6-Hydroxydopmaine lesions in conjunction with a variety of positive reinforcement-response analysis paradigms (Sham et al., 2007; Floresco et al.,

2006; Selling's & Clark, 2006; Hara & Pickle, 2005; Kimono et al., 2005; Selling's & Clark, 2003).

Recent evidence has highlighted a role of serotonin (5-HT) and serotonin receptors (5-HTr) in aggression. Though the 5-HTr subtype most implicated in male aggression has been the 5-HT1_Br, some data has been offered implicating 5-HT 2 and 1_C as well (de Almeida & Miczek; 2002; Ferrari et al., 2005; De Almeida et al., 2006; Bannai et al., 2007; Olivier & Mos, 1992; Olivier et al., 1995; de Boer & Koolhaas, 2005). In light of this recent evidence regarding the role of 5-HTrs in aggression, it is important to acknowledge the possibility that the drugs used in our study, specifically SCH-23390, have some affinity at 5-HT 2r and 1_Cr sites. Studies conducted by Nicklaus et al.. 1988 demonstrate that at high concentrations, unlike what was used in our study, SCH-23390 can bind to 5-HT1_Cr (Nicklaus et al., 1988). However, studies to follow addressed the issue of SCH-23390 D1 receptor specificity by demonstrating that in vivo, the K_d for D1r is approximately .2nM whereas for 5-HT1_Cr, it is approximately 24nM (Bourne, 2001; Kaufman et al.. 1996). Most importantly, it was demonstrated that doses of SCH-23390 required to produce similar response at either the 5-HT1_C or 2r in vivo are greater than 10-fold higher than those required to induce a D1-mediated response (Bourne, 2001). It is also critical to note that most of the rational for 5-HTr involvement in aggression comes from regions of interest outside the NAc and in our study, we targeted only the NAc. Thus, at the doses we used in our study, we believe that it is highly unlikely that the decreased response rates following local NAc SCH-23390 infusions were mediated through 5-HT receptors.

CHAPTER IV

SPECIFIC AIM 3: *ARE THERE ANY ENDOGENOUS DIFFERENCES IN MESOLIMBIC DA BETWEEN AGGRESSIVE AND NON-AGGRESSIVE MICE?*

Rational

It is often asked about violent offenders weather the reason they are aggressive is primarily influenced by 'nature' or 'nurture'. The former idea suggests that there are innate biological circuits that enable a propensity towards aggression. The latter idea suggests that the individual's environment was such that the individual learned to be aggressive. While there has been substantial evidence demonstrating the role of environment in the production of aggressive individuals, the question: 'is there endogenous differences between aggressive and non-aggressive individuals', still remains unresolved.

Individual differences in aggression have both genetic and environmental determinants, and neurobiological experiments suggest that there is conservation across species in the neurochemical and anatomical systems that are activated during aggressive behavior in humans and non-human animals (for review, see Nelson & Trainor, 2007). Strong evidence exists implicating roles for both DA and 5-HT in the modulation of aggression. Specifically, increased mesocorticolimbic dopamine and decreased cortical and systemic serotonin have

been linked with aggressive phenotypes (Mann et al., 1995; Mann, 1999; Ryan, 2000; Couppis & Kennedy, 2007; Wallace et al., 2006)

Though these findings have been relatively consistent in neurobiology of aggression literature, it remains, however, unclear if the mesocorticolimbic DA system modulates reinforcing aggression as a result of plasticity from learned aggressive behavior or if endogenous, pre-association differences between aggressive and non-aggressive mesocorticolimbic dopamine systems exist.

As an initial attempt to address this issue, two strains of mice that differ significantly in presentation of aggressive behavior, A/J and BALB/cJ, were chosen for examination. A/J mice have been noted for their docile behavior, while BALB/cJ mice are so aggressive that they will attack on almost every encounter (Roubertoux et al., 2005; Roubertoux et al., 1999 Kessler et al., 1977). While these mice differ greatly in aggressive behavior, they share important behavioral characteristics including relatively low locomotor behavior, high emotional reactivity and low ethanol consumptive behavior (Whalsten et al., 2006; Crawley et al., 1997). These shared behavioral characteristics are of interest due to the fact that DA and 5-HT have also been strongly associated with their modulation (Gendreau et al., 2000; Wood et al.., 2007; Short et al., 2006; Izco et al., 2007; Waddington & O'Boyle, 1989; Kuczenski & Segal, 1989; Boyce-Rustay JM et al., 2006; Griebel G et al., 1994). As a result of the fact that BALB/cJ and A/J mice are similar in these DAergic and 5-HTergic mediated behaviors, but are very different in aggressive behavior make BALB/cJ and A/J mice ideal for isolating the endogenous neurobiology of aggression.

Three experiments were conducted to address the question of 'Are there any endogenous differences between aggressive and non aggressive mice'. First, a behavioral assessment was conducted to ascertain each strain of mice's level of aggression. Next, tissues extracted from the nucleus accumbens (NAc) and PFC of each strain of mice were compared for contents of biogenic amines including DA, 5-HT and their respective metabolites. Finally, quantitative receptor autoradiography was conducted to compare the expression levels and patterns of DA D1 and D2 receptors in the NAc.

Aggression Screening Rational

Prior to comparing the endogenous biology of A/J mice and BALB/cJ based on their reported differences in level of aggression, it is important to gain a more complete view of how these mice differ in aggression levels. Due to this, an initial behavioral assessment of aggression in each strain of mice was conducted. In addition to evaluating the differences in aggression between A/J and BALB/cJ mice, a brief comparison of the % of aggressive subjects in each strain was made to the % of aggressive CFW mice.

Aggression screening Materials and Methods

Subjects

6 male BALB/cJ and 6 male A/J mice were used in aggression screening tests. Each mouse was approximately 4 months old and housed with female mate 2 weeks prior to testing. Mice were maintained on *ad libitum* food and

water and 12:12 hr light/dark cycle. Mice were housed in standard 12 in X 8 in plastic cages with paper bedding.

For the comparison to CFW mice, male CFW mice (n = 39) approximately 4 months old were used. These mice were housed identically to the A/J and BALB/cJ, as described above.

Behavioral Test

Aggression screening consisted of 3 test sessions that were each separated by 3 days and is similar to the aggression screening method used in Couppis and Kennedy, 2007. Briefly, prior to each session, the female mate was removed from home cage for the duration of the session. The session consisted of a male intruder mouse of the same strain being placed in the home cage. The intruder mouse was placed at a consistent distance and orientation in relation to resident mouse; approximately 3 in away from resident's snout. The latency to social approach and attack the intruder mouse was measured by an observer's stopwatch. The observer was the same observer for all trials. If the resident attacked the intruder, the session ended after first attack in order to limit the resident's experience with aggression. If there was no attack, the session ended after 10 min. Social approach was defined by the resident contacting the intruder with either the snout or a paw. An attack was defined as the resident biting or hitting the intruder. If the resident attacked the intruder without prior social approach, the attack was also counted as social approach. Latencies for social approach and to attack were compared using two-tailed Students t-test.

Statistics

Latency to attack and latency to social approach values were compared between genotypes using t-tests.

Aggression Screening Results

In the aggression screening, 100% of BALB/cJ mice were aggressive on all three trials. None of the A/J mice were aggressive on any of the three trials. When latency to social approach was compared between strains, there were no significant differences between BALB/cJ and A/J mice on any of the three trials (Fig. 19). On each of the three trials, all subjects approached the intruder within 1 min (see fig. 19). All BALB/cJ mice attacked the intruder within 45 sec on each of the three trials, whereas every session for A/J mice was terminated by investigator at 10 min due to no aggressive activity (Fig. 20). As expected, latency to attack by BALB/cJ mice decreased during their second and third trials, as compared to the first exposure, although this failed to reach statistical significance, $p = .11$

When tallying the % of CFW mice qualifying as aggressive, it was determined that 86.35% of subjects were aggressive (see table 3).

HPLC Rational

HPLC followed by fluorescent and/or electrochemical detection is the method of choice for the quantitative assessment of amino acids, amino acid neurotransmitters, biogenic amines and metabolites (Cohen & Michaud, 1993;

Lindley et al.; 1998). When used with internal standards, the techniques have the ability to detect fentiomole to picomole quantities of amino acids (all 20 naturally occurring plus GABA) and major amine neurotransmitters (DA, NE, 5-HT) and metabolites with high accuracy and reproducibility. Although dialysates can be reasonably argued to be more informative of the releasable pools of these substances, dialysis approaches are clearly not suited for high-throughput analyses and are best left for investigations exploring in detail. Tissue levels are an indicator of the integrity of neurotransmitter systems and the general metabolic health of the tissue. For example, deficits in GABA, dopamine or 5-HT synthesis or storage will be evident via analyses of tissue levels and metabolites and then are likely to impact release and signaling. These measures may also suggest structural deficits in neurochemically defined pathways that can be explored in the anatomy core. Amino acid measures may point to insults to the nervous system in nutritional supply to the brain or metabolic disorders that are underlying behavioral or physiologic abnormalities.

HPLC Materials and Methods

Aggression screening

Subjects

6 male BALB/cJ and 6 male A/J mice were used in aggression screening tests. Screenings occurred between 1200 hr and 1400 hr on each testing day, using the resident-intruder model (DeAlmedia et al., 1997). Each mouse was

approximately 4 months old and housed with a female mate 2 weeks prior to testing. Mice were maintained on *ad libitum* food and water and 12:12 hr light/dark cycle. Mice were housed in standard 30 cm X 20 cm plastic cages with paper bedding.

Behavioral Test

Aggression screening consisted of 3 test sessions that were each separated by 3 days and is similar to the aggression screening method used in Couppis and Kennedy (2007). Briefly, prior to each session, the female mate was removed from home cage for the duration of the session. The session consisted of a male intruder mouse of the same strain being placed in the home cage. The intruder mouse was placed at a consistent distance and orientation in relation to resident mouse; approximately 7.5 cm away from resident's snout. The latency to social approach and attack the intruder mouse was measured by an observer's stopwatch. The observer was the same observer for all trials. If the resident attacked the intruder, the session ended after first attack in order to limit the resident's experience with aggression. If there was no attack, the session ended after 10 min. Social approach was defined by the resident contacting the intruder with either the snout or a paw. An attack was defined as the resident biting or hitting the intruder. If the resident attacked the intruder without prior social approach, the attack was also counted as social approach. Latencies for social approach and to attack were compared using two-tailed Students t-test.

Table 3. % of Aggressive A/J and BALB/cJ Subjects on Each Aggression Screening Trial.

	Trial 1	Trial 2	Trial 3
% BALB/cJ to Attack	100	100	100
% A/J to Attack	0	0	0

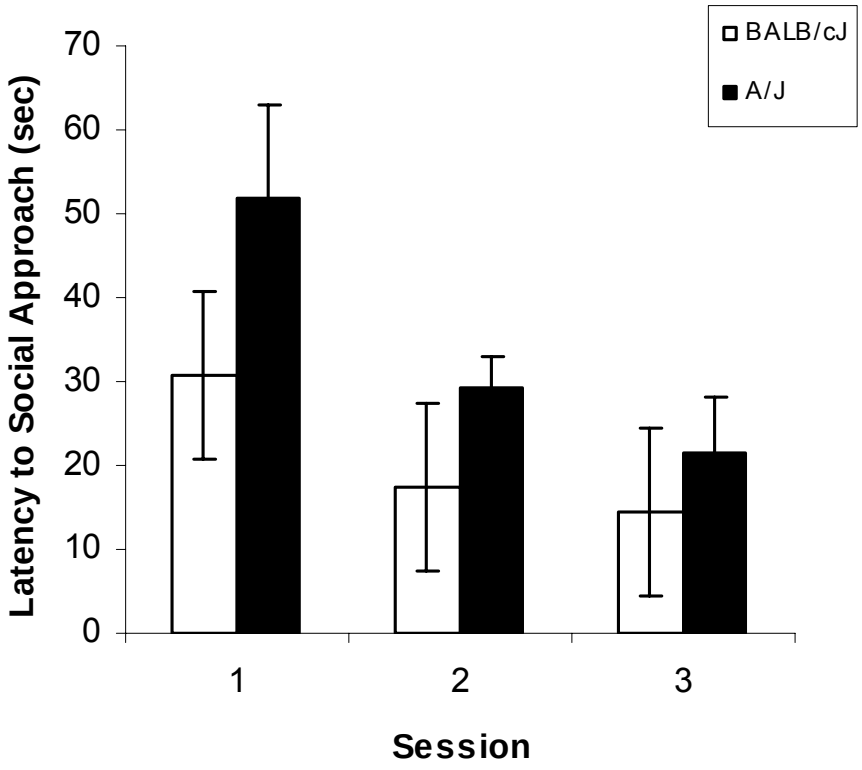

Figure 19. Mean time in sec for each strain of resident mice to socially approach an intruder mouse across 3 trials. Error bars denote *SEM*. Asterisks denote significant differences between strains. A/J mice are depicted in black. BALB/cJ are depicted in white.

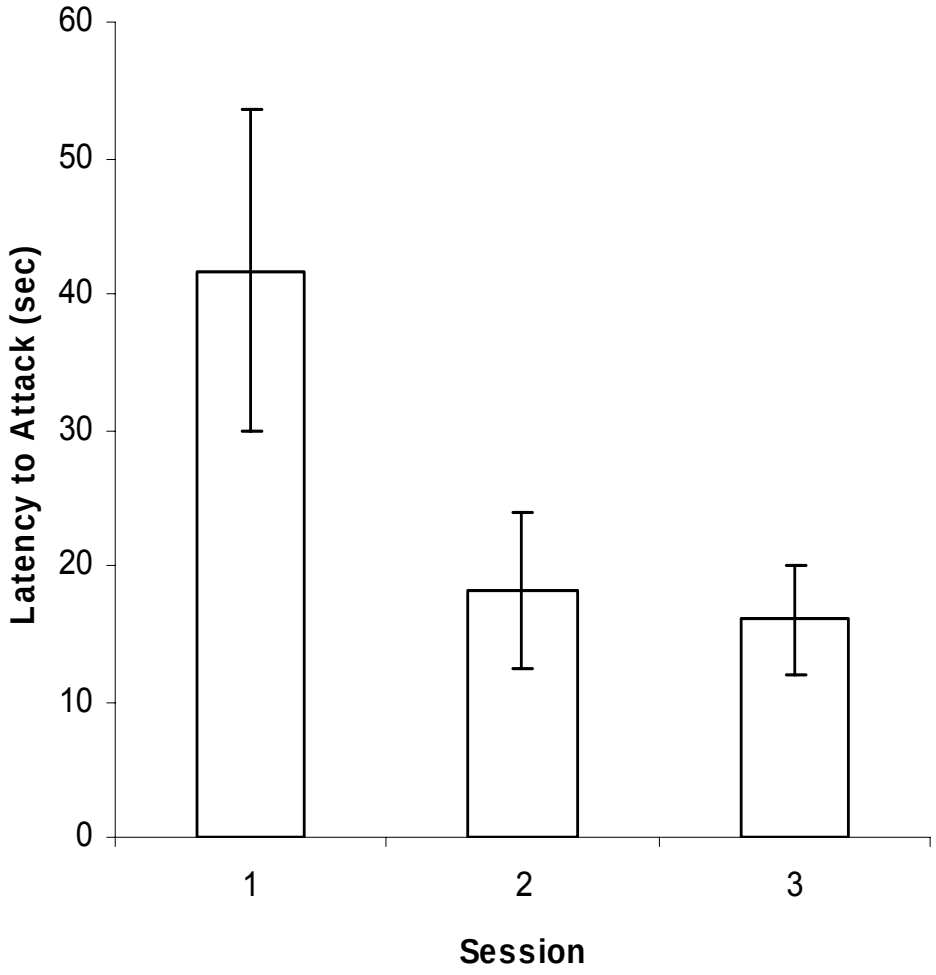

Figure 20. Mean time in sec for resident BALB/cJ mice to attack intruder mice across 3 trials. Error bars denote *SEM*

Neurochemistry

Subjects

13 BALB/cJ and 14 A/J male mice that were approximately 4 months old were used for the HPLC analysis. Mice were group-housed with 3-4 per cage, fed ad libitum and maintained on a 12:12 hr light/dark cycle. Mice were housed in standard 30 cm X 20 cm plastic cages with paper bedding.

Tissue Dissection

Animals were quickly decapitated without anesthesia using surgical grade shears. Skull cavity was peeled back to remove fresh brain tissue. Whole brain was removed and sliced at approximately *AP* = 2.8 mm and *AP* = 1.7 mm. Small samples of tissue were removed from pre-frontal cortex and nucleus accumbens. The samples were immediately frozen in centrifuge tubes on dry ice.

Biogenic Amine Analysis by HPLC

Tissue samples were transferred to Vanderbilt University's Center for Molecular Neuroscience HPLC core facility for processing using methods similar to those previously described (Gale & Perkel, 2005; Hackler et al., 2006; Perez and Palmiter, 2005). The brain sections are homogenized in 100-750 μl of 0.1M TCA, which contains 10^{-2} M sodium acetate, 10^{-4} M EDTA and 10.5 % methanol (pH 3.8). Samples are spun in a microcentrifuge at 10000 g for 20 min. The supernatant is removed and stored at –80 ˚C. The pellet is saved for protein

analysis. Supernatant is then thawed and spun for 20 min. Samples of the supernatant were then analyzed for biogenic monoamines

Three HPLC systems are employed in the Vanderbilt Neurochemistry Core Lab. The HPLC systems used for biogenic amine measurement consist of a Waters Model 515 pump, Waters 717+ Autosampler and an Antec Decade II Electrochemical Detector. The model 515 pump is a high quality HPLC pump that delivered solvent at a constant flow at 0.1 to 9.9 ml/min. The Waters 717+ Autosampler automatically injected 1-1000 μl samples from 96-sample trays to the HPLC column, and maintained samples at temperatures selectable from 5-37 ˚C (Cransac et al. 1998). The Antec Decade II Electrochemical Detector is equipped with a HPLC column oven and electronic shield. The HPLC instruments are controlled by the central Compaq Computer equipped with Millennium 32 software. This software allows for system control, data acquisition and processing, and results reporting.

Biogenic amines are determined by a specific HPLC assay utilizing an Antec Decade II (oxidation: 0.5) electrochemical detector operated at 33° C. Twenty μl samples of the supernatant are injected using a Water 717+ autosampler onto a Phenomenex Nucleosil (5u, 100A) C18 HPLC column (150 mm x 4.60 mm). Biogenic amines are eluted with a mobile phase consisting of 89.5% 0.1M TCA, 10-2 M sodium acetate, 10-4 M EDTA and 10.5% methanol (pH 3.8). Solvent is delivered at 0.6 ml/min using a Waters 515 HPLC pump. Using this HPLC solvent the following biogenic amines elute in the following order: dihydroxyphenylacetic acid (DOPAC), dopamine, 5-Hydroxyindoleacetic

acid (5-HIAA), homovanillic acid (HVA) and 5-HT. HPLC control and data acquisition are managed by Millennium 32 software.

Total protein concentration of the brain extracts are determined using BCA Protein Assay Kit purchase from Pierce Chemical Company (Rockford, IL). The frozen pellets are allowed to thaw and reconstituted in a volume of 0.5 N HCl that equals that previously used for tissue homogenization. On hundred μl of this solution is combined with 2 ml of color reagent and allowed to develop for 2 hours. A standard curve of bovine serum albumin is run at the same time spanning the concentration range of 20-2000 μg/ml. Absorbances of standard and samples are measured at 562 nm.

Statistical analyses of μg of compound per mg of protein values between genotypes were conducted using t-tests. Prior to t-test, outliers were determined using Grubb's analysis.

HPLC Results

Significant differences between BALB/cJ and A/J mice using independent groups t-test were seen both in the NAc and the PFC. In the NAc, NAc dopamine ($t_{(24)}$ = 4.2, p = .0003), NAc DOPAC/DA ($t_{(19)}$ = -3.5, p = .002), NAc HVA ($t_{(24)}$ = 3.57, p = .001), NAc 5-HIAA ($t_{(24)}$ = -2.56, p = .02), and NAc 5-HIAA/5-HT ($t_{(16)}$ = -3.01, p = .008) differed between strains (see Fig. 21 & 22). In addition to differences seen in the NAc, differences between A/J and BALB/cJ mice were also measured in the PFC. In the PFC, these two strains of mice differed in PFC DA ($t_{(17)}$ = 2.94, p = .01), PFC DOPAC/DA ($t_{(17)}$ = -2.55, p = .01),

PFC HVA/DA ($t_{(14)}$ = -2.06, p = .03), PFC 5-HT ($t_{(17)}$ = -2.8, p = .01) and PFC 5-HIAA ($t_{(17)}$ = -2.12, p = .04) (see Fig. 23 &24).

Autoradiography Rational

Autoradiography is the localization of radiolabel within a solid specimen. This localization is detected by covering decaying radioactive material (such as 14C, 3H, 35S, 125I) with photographic emulsified film. When radioactive isotopes decay, they emit a beta particle (an energetic electron). If a layer of photographic emulsion such as that found on x-ray film, is placed over a cell that contains 3H, a chemical reaction takes place involving silver halide ions, wherever a beta particle strikes the emulsion. The emulsion can then be developed like a photographic print so that the emission path of the beta particle appears as a black spot or silver grain. In effect, autoradiography is a process in which radioactively labeled molecules imprint themselves on film.

Autoradiography can be performed on a wide variety of solid specimens from whole organisms to ultra-thin tissue sections. Scientists use autoradiography to investigate a wide variety of biological specimen from DNA to receptors. Rather than being a single technique, autoradiography is a collection of methods that utilize the imprint decaying radioisotopes make on film. Between them, these techniques can supply information at the macroscopic, microscopic, and ultrastructural levels.

Autoradiography Materials and Methods

Subjects

11 BALB/cJ and 11 A/J male mice approximately 4 months old were used for the autoradiography analysis. Mice were group-housed with 3-4 per cage, fed *ad libitum* and maintained on a 12:12 hr light/dark cycle. Mice were housed in standard 12 in X 8 in plastic cages with paper bedding.

Tissue Dissection and Histology

Animals were quickly decapitated without anesthesia using surgical grade shears. Skull cavity was peeled back to remove fresh brain tissue. Whole brains were immediately frozen in ice cold 2-methyl-butene. Tissue was stored long term in at -78ºC. Brains were then sliced at 20 µm on a cryostat at -32ºC and mounted in triplicate on *probe-on plus* (VWR International) during slicing. After slicing and mounting, sections were left at room temperature for 15 min to allow for maximum drying and adhesion to the slide. After this time, slides were transferred back to -78ºC.

Radioligand Binding

We used methods similar to those described previously (Stanwood et al.., 2001a, 2001b, Stanwood & Levitt 2007). For D1-like receptor labeling (D1 + D5), brain sections were pre-incubated at room temperature for 20 min in a buffer solution containing 50 mM Tris HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 100 mM EDTA and 100 mM $MgCl_2$. Sections were then transferred into

one of two treatments; specific binding or non-specific binding. For specific binding, three slides (with three sections on each) from approximately A*P* = 1.80 mm-1.54 mm; 1.42 mm-1.18 mm; 1.10 mm-.90 mm were incubated in the above described buffer solution with the addition of 3 nM [^{3}H]-SCH-23390 for 90 min. For non-specific binding, two slides (with three sections on each) from approximately A*P* = 1.80 mm-1.54 mm and 1.10 mm-.90 mm were incubated in the above described radioactive buffer solution with the addition of 2 mM butaclamol for 90 min. After the 90 min incubation of specific and non-specific bound sections, slides were washed in ice-cold wash buffer containing 50 mM Tris and distilled water, 2 X 20 min. Slides were then rapidly dried using industrial dryer.

For D2/D3 labeling brain sections were pre-incubated at room temperature for 20 min in a buffer solution containing 50 mM Tris HCl buffer (pH 7.4) 120 mM NAcl, 5 mM KCl, 100 mM EDTA and 100 mM $MgCl_2$. Sections were then transferred into one of two treatments; specific binding or non-specific binding. For specific binding, three slides (with three sections on each) from approximately A*P* = 1.80 mm-1.54 mm; 1.42 mm-1.18 mm; 1.10 mm-.90 mm were incubated in the above described buffer solution with the addition of 3 nM [^{3}H]-raclopride for 90 min. For non-specific binding, two slides (with three sections on each) from approximately A*P* = 1.80 mm-1.54 mm and 1.10 mm-.90 mm were incubated in the above described radioactive buffer solution with the addition of 2 mM butaclamol for 90 min. After the 90 min incubation of specific and non-specific bound sections, slides were washed in ice-cold wash buffer

containing 50 mM Tris and distilled water, 2 X 20 min. Slides were then rapidly dried using industrial dryer.

Autoradiography

Radio-labeled and dried slides were laid into light-tight 10 X 12 cassettes with 4 subjects per cassette. Subjects' positioning in cassette, top to bottom, was counterbalanced across cassettes based on subject genotype. For each group (D1 and D2) of slides, 1 standard slide was placed in a cassette. This standard slide contained a spectrum of swatches of known radioactive content. After slides were arranged in light-tight cassettes, slides were secured with clear office-grade tape. In a dark room, Amersham Hyperfilm™ ^{3}H (GE Healthcare, USA) was placed in cassettes with emulsion side in contact with the radioactive slides. For the D1 experiment, cassettes were kept sealed for 21 days. For the D2 experiment, cassettes were kept sealed for 6 weeks. After image-film processing time, film was developed in a dark room using Kodak GBX developer and fixer diluted to 160ml solution: 420 ml distilled water. To develop, film was placed in developer for 3 min, and then washed in distilled water for 30 sec. Next, film was placed in fixer for 5 min and washed for 15 min in distilled water. After completed development, films were hanged to dry.

Analysis of Receptor Density

Dried film was scanned using an Epson scanner at 2400 dpi. Scanned film was analyzed using the free software, ImageJ. Prior to film analysis, ImageJ

was calibrated using the standard slide for each experiment. Regions of interest (ROI) in each section were outlined individually by a blinded researcher. ROI's were dorsal-medial dorsal striatum, dorsal-lateral dorsal striatum, ventral-lateral dorsal striatum, ventral-medial dorsal striatum and core, shell, rostral pole and total area of NAc. In sections where the complete ROI was not present, a measurement was excluded. Since no counter-staining was completed, ROI's were approximate. Measurements from non-specific binding were subtracted from specific binding sections to obtain the total binding. Total binding measurements at each separate AP level were averaged together in each strain of mice and compared to each other using t-tests.

Autoradiography Results

As described above in the methods section, we analyzed three distinct anterior-posterior levels of the NAc in coronal sections. For simplicity, we refer to these as "Anterior": A*P* = 1.80 mm-1.54 mm, "Mid": 1.42 mm-1.18 mm, and "Posterior": 1.10 mm-0.90 mm.

D1: There were no differences seen between A/J and BALB/cJ mice in [^{3}H]-SCH-23390 binding in the Mid or Posterior levels of the NAc. However, there was a significant increase in [^{3}H]-SCH-23390 binding in the Anterior region (i.e. rostral pole) of the NAc in A/J as compared to BALB/cJ mice ($t_{(2.70, 15.88)}$ = 2.67; $p < .02$, Fig 3). The mean µCi/g for A/J in the rostral pole was 74.86 with a SEM of 4.38. The mean µCi/g for BALB/cJ in the rostral pole was 54.62 with a SEM of 6.15 (see Fig. 25 & 27) At this level it was not possible to distinguish

shell v. core. At each of the other levels where it was possible to distinguish between shell and core, there were no differences in the mean µCi/g between strains.

D2: There were significant differences seen in [^{3}H]-raclopride labeling between A/J and BALB/cJ mice at all AP levels of the NAc ($t_{(2, 20.9)}$ = 5.01; $t_{(2,21.97)}$ = 5.26; $t_{(2,19.3)}$ = 5.31; respectively p < .001, Fig. 4 and 6). The mean µCi/g for A/J was1.63 (±0.17)), 1.75 (±0.18), and 1.7 (±0.16) at Anterior, Mid, and Posterior levels, respectively. In contrast the mean [^{3}H]-raclopride binding for BALB/cJ mice was 2.83 (±0.17), 3.12 (±0.19), and 3.2 (±0.23) (see Fig. 26 & 28). At the mid and posterior levels, where it was possible to distinguish between core and shell, there were differences in both core ($t_{(2,17)}$ = 3.90, p = .006; $t_{(2,21)}$ = 3.71, p = .001; respectively) and shell ($t_{(2, 15.14)}$ = 2.96, p = .005; $t_{(2,16.7)}$ = 4.75, p = .0002; respectively) between A/J and BALB/cJ mice.

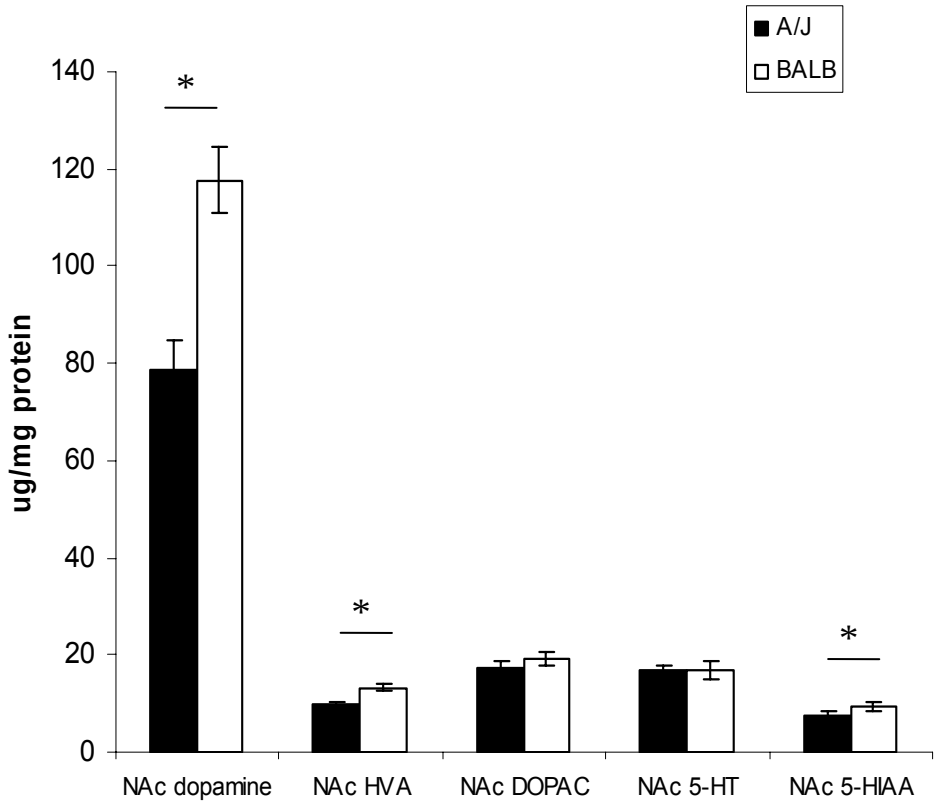

Figure 21. Mean monoamine content and monoamine metabolite content (expressed in ug/mg protein) in the NAc of A/J and BALB/cJ mice. Error bars denote *SEM*. Asterisks denote significant differences between strains. A/J mice are depicted in black. BALB/cJ are depicted in white.

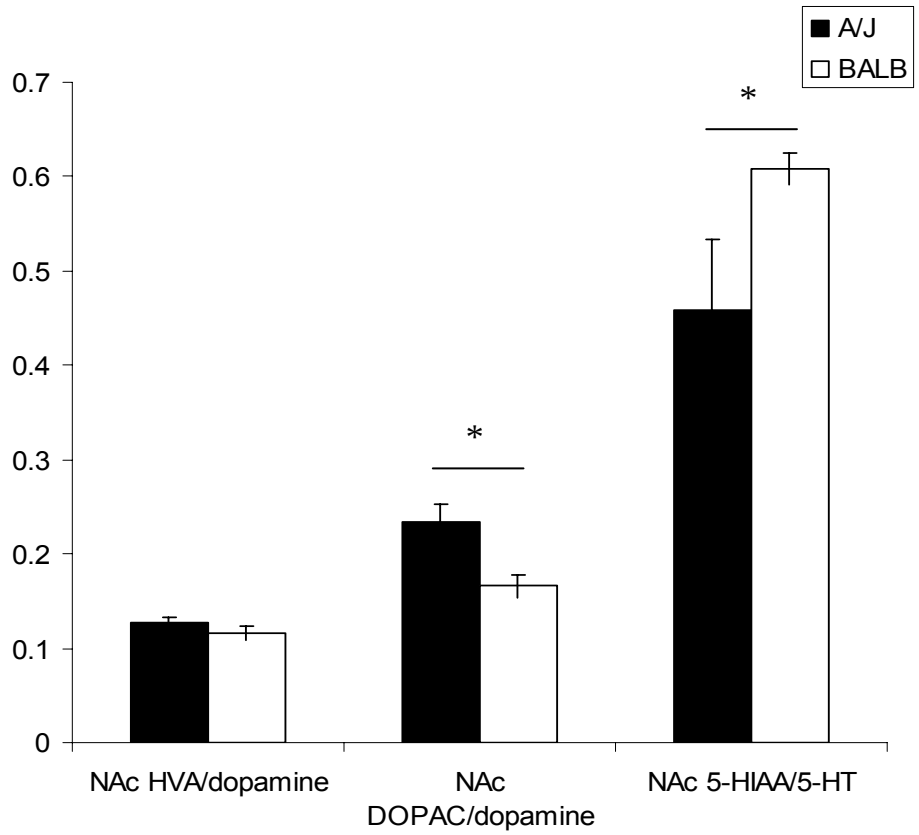

Figure 22. Mean metabolite/monoamine ratios in the NAc for A/J and BALB/cJ mice. Error bars denote *SEM*. Asterisks denote significant differences between strains. A/J mice are depicted in black. BALB/cJ are depicted in white.

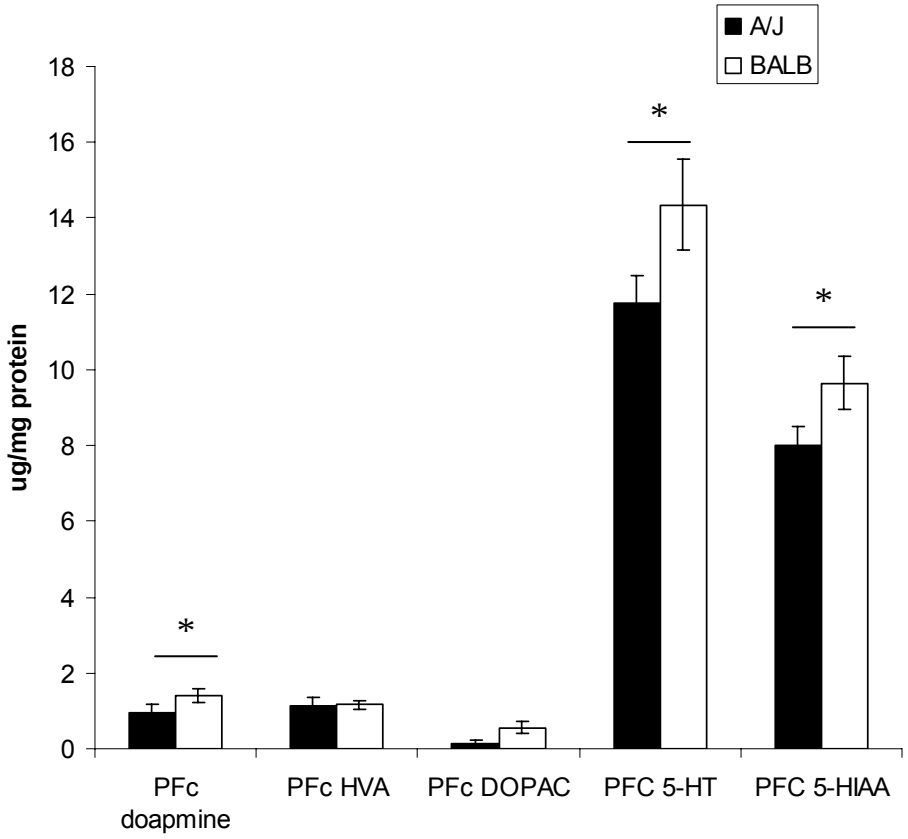

Figure 23. Mean monoamine content and monoamine metabolite content (expressed in ug/mg protein) in the PFC of A/J and BALB/cJ mice. Error bars denote *SEM*. Asterisks denote significant differences between strains. A/J mice are depicted in black. BALB/cJ are depicted in white.

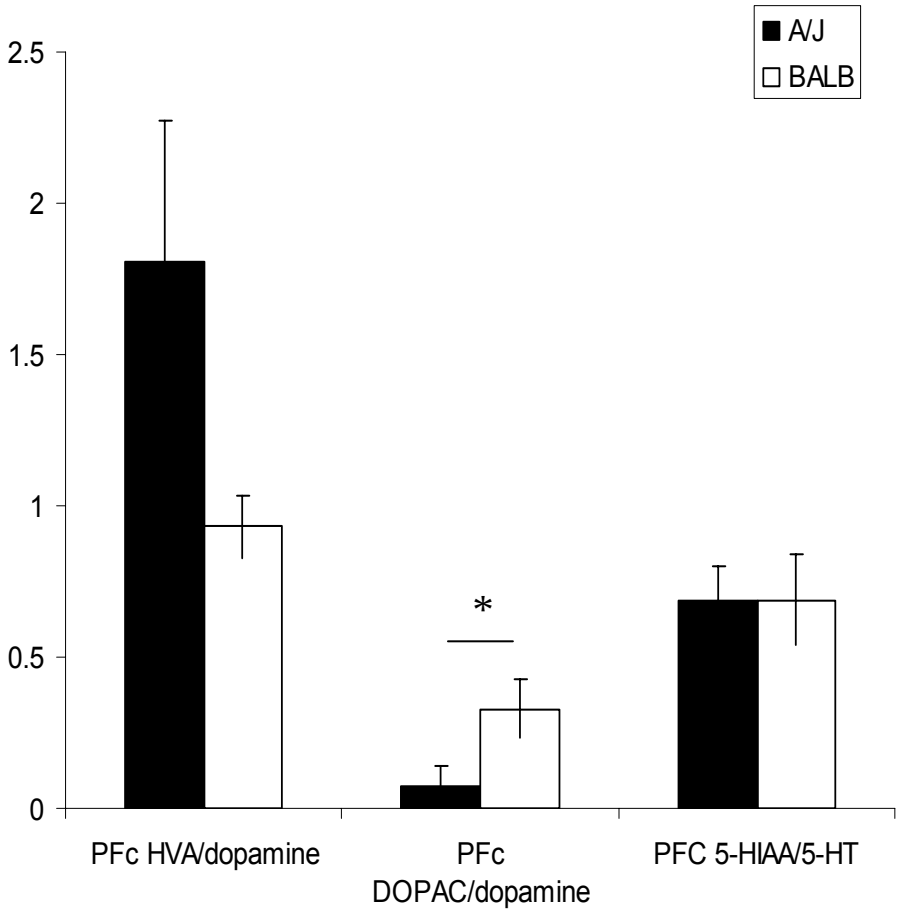

Figure 24. Mean metabolite/monoamine ratios in the PFC for A/J and BALB/cJ mice. Error bars denote *SEM*. Asterisks denote significant differences between strains. A/J mice are depicted in black. BALB/cJ are depicted in white.

Specific Aim 3 Discussion

Our behavioral screening confirmed previous studies suggesting that A/J mice are far less aggressive towards each other than are mice of BALB/cJ strain (Roubertoux et al., 2005; Roubertoux et al., 1999 Kessler et al., 1977). Comparing two very behaviorally different strains of mice provides several inherent advantages. As with any complex behavior, the development of animal models is crucial due to the high level of experimental control that model organisms afford. Due to the powerful methods available in studying mouse genetics, using mice for initial exploratory studies offers the advantage of future analysis utilizing a vast array of genetic tools. Finally and most importantly, comparing two strains of mice allows us to view two systems that naturally vary in aggression. This is a unique advantage as opposed to externally manipulating aggression in one strain; which offers several extraneous experimental variables and does not offer any insight about the 'nature' component of aggressive behavior.

In the above described HPLC experiments there were significant differences in biogenic amine levels both in the NAc and the PFC between A/J and BALB/cJ mice. It was determined that in the NAc, A/J mice had reduced DA levels compared to BALB/cJ. Also in the NAc, A/J mice displayed elevated DOPAC/DA ratios, 5-HIAA, 5-HIAA/5-HT ratios as compared to BALB/cJ mice.

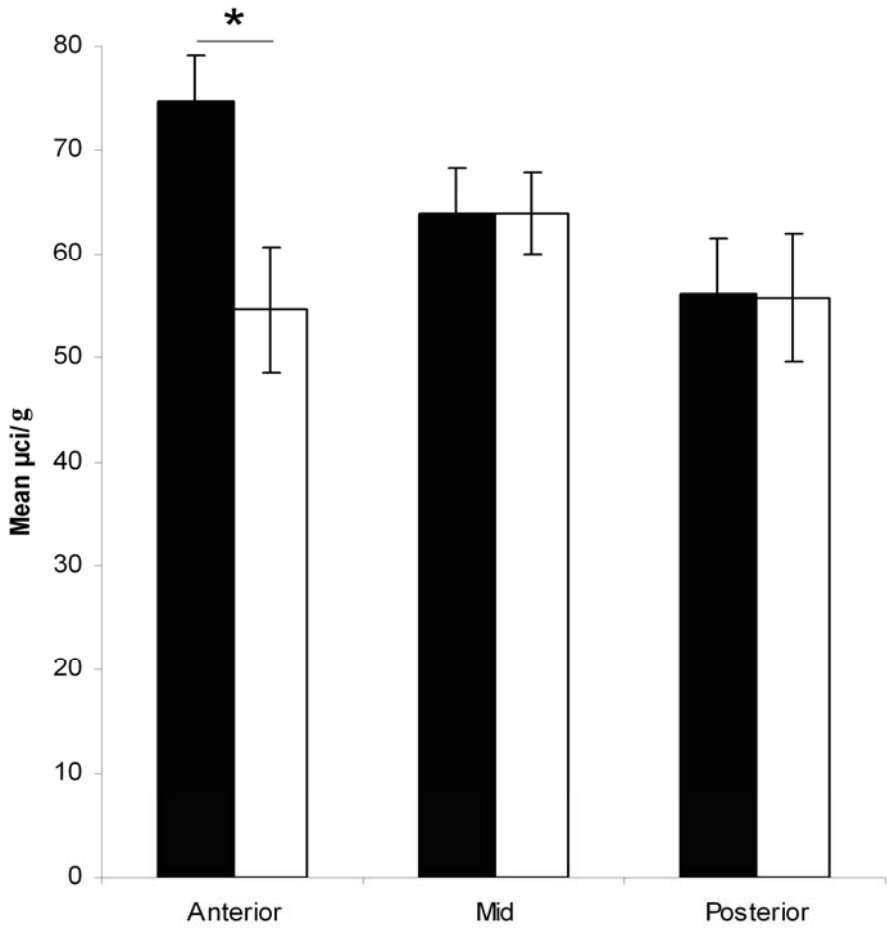

Figure 25. Mean µCi/gin the NAc for [H]-3-SCH-23390 binding in A/J and BALB/cJ mice at three different anterior-posterior regions. * Denotes significant differences between strains. Error bars denote +/- SEM. A/J means are depicted in black, BALB/cJ means are depicted in white. Please note that no alterations in brightness, contrast, or other variables were applied before quantitative analysis

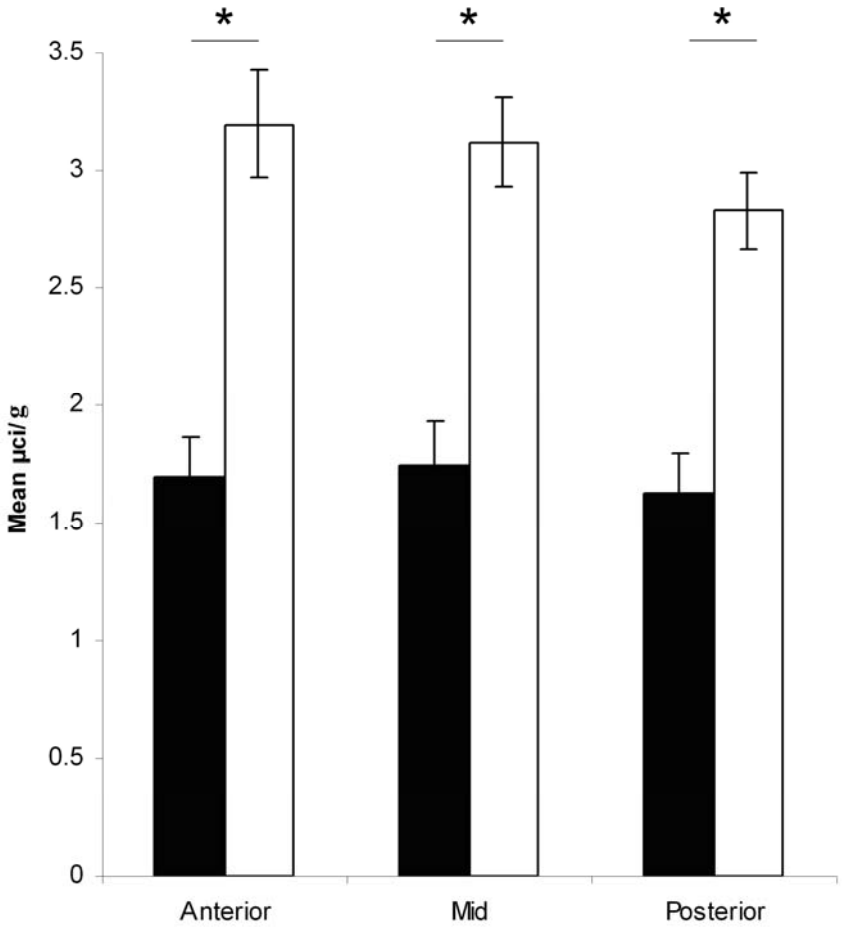

Figure 26. Mean µci/g in the NAc for [H]-3-Raclopride binding in A/J and BALB/cJ mice at three different anterior-posterior regions. * Denotes significant differences between strains. Error bars denote +/- SEM. A/J means are depicted in black, BALB/cJ means are depicted in white. Please note that no alterations in brightness, contrast, or other variables were applied before quantitative analysis.

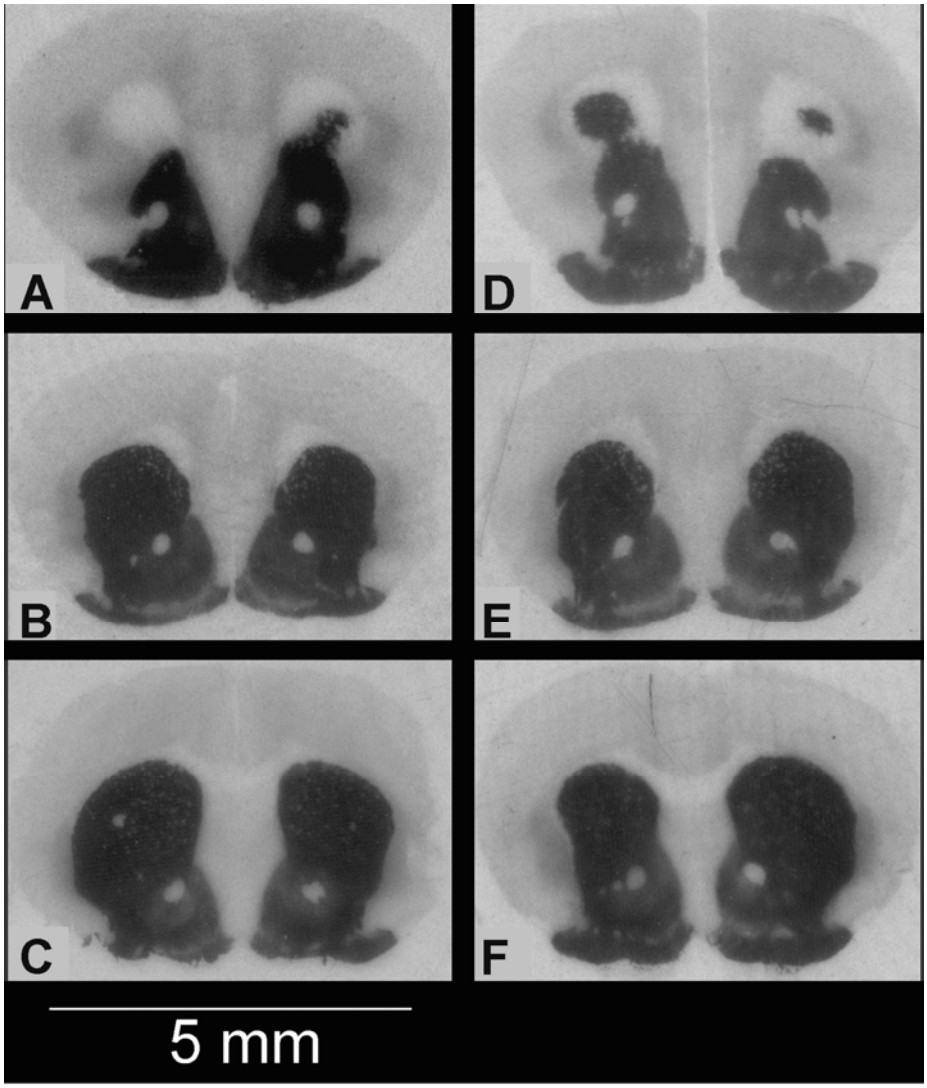

Figure 27. Representative autoradiographs reflecting [^{3}H]-SCH-23390 binding. A-C are Anterior, Mid and Posterior autoradiographs from non-aggressive A/J mice (respectively). D-F are Anterior, Mid and Posterior autoradiographs from aggressive BALB/cJ mice (respectively). Images were enhanced for illustration purposes only using Photoshop CS™.

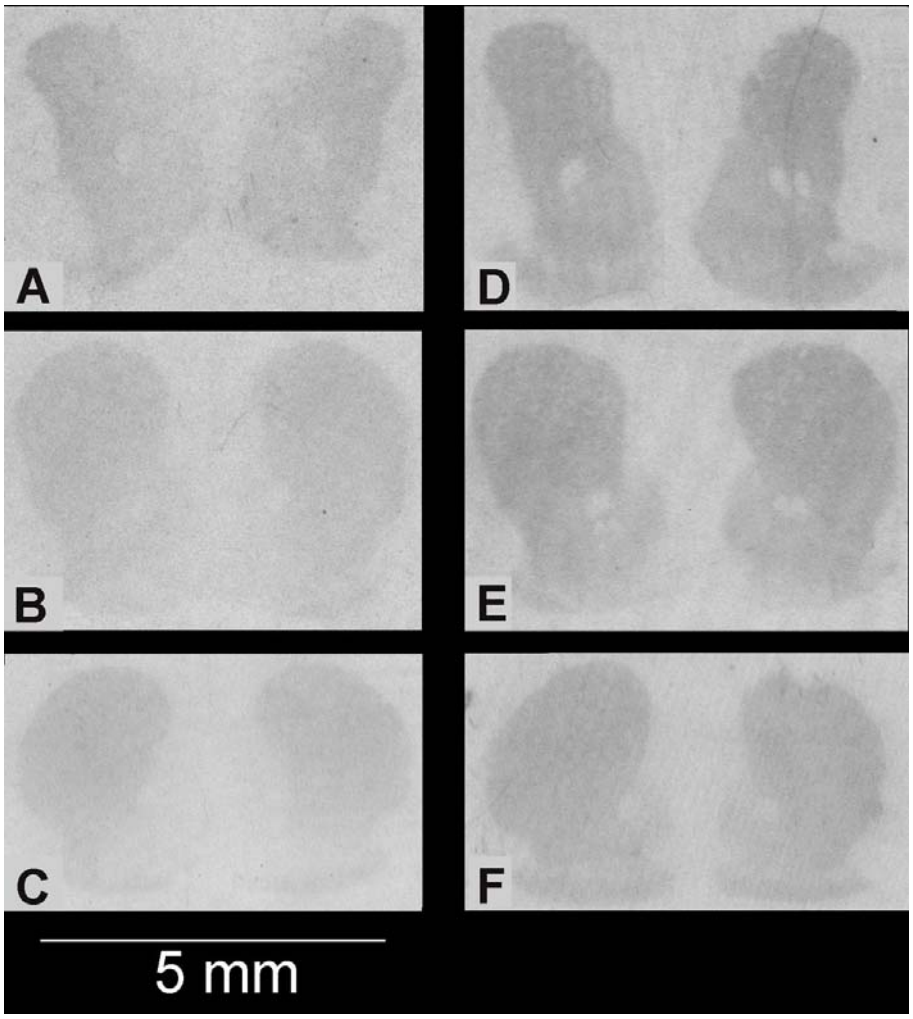

Figure 28. Representative autoradiographs reflecting [^{3}H]-raclopride labeling. A-C are Anterior, Mid and Posterior autoradiographs from non-aggressive A/J mice (respectively). D-F are Anterior, Mid and Posterior autoradiographs from aggressive BALB/cJ mice (respectively). Images were enhanced for illustration purposes only using Photoshop CS™.

In the PFC, A/J mice had reduced DA level as compared to BALB/cJ mice. However, in the PFC, A/J mice displayed elevated DOPAC, DOPAC/DA ratio, 5-HT and 5-HIAA as compared to BALB/cJ mice. Taken together, it is apparent that A/J mice have reduced DA, elevated DOPAC/DA ratios and altered 5-HT systems as compared to BALB/cJ mice across the sampled brain regions.

The results of our neurochemical study suggests that the non-aggressive A/J mice display characteristics of a highly efficient 5-HT system and increased 5-HT tone in the NAc and PFC, as compared to aggressive BALB/cJ mice. This parallels several lines of research suggesting anti-aggressive effects of 5-HT activation and increased aggression associated with 5-HT deficient systems. Mann et al. first brought attention to a 5-HT deficiency as a trait marker for aggressive behavior in humans, a claim later substantiated by many groups (Mann et al., 1995; Mann, 1999, Brown et al., 1982; Linnoila et al., 1983; Kruesi et al., 1990; Coccaro, 1992; Virkkunen et al., 1996; Kavoussi et al., 1997). In compliment to these data are data from juvenile monkeys demonstrating lowered CSF 5-HIAA content in individuals with high risk-taking behavior (Higley et al.., 1992, 1994; Mehlman, 1994). Also, in rodents, aggression is effectively reduced with 5-HT agonists (5-HT1A and 5-HT1B) and is increased in 5-HT1B receptor knockout mice (Olivier and Mos, 1986; Olivier et al., 1987; Saudou et al., 1994; De Almeida & Lucion, 1997; Miczek et al., 1978; Simon et al., 1998; de Boer et al., 1999; Ferris et al., 1999; Fish et al., 1999).

There is also a rich body of literature exploring the role of mesocorticolimbic DA in aggression. Increased dopaminergic tone in aggression

has been suggested by the clinical efficacy of DA antagonists to reduce aggressive outbursts in some disorders (Ryan, 2000). DA antagonists have also been shown to reduce the positively reinforcing properties of aggression in animal models (Couppis & Kennedy, 2007; Wallace et al., 2006). Further, amphetamine and cocaine (indirect DA agonists) commonly induce aggression in a wide array of mammals (Steensland, 2005; Darmani et al., 1990; Mickzek & O'Donnell, 1978).

In contrast to the 5-HT data provided from the HPLC experiment, the DA-ergic system data provided by the above experiments appear to be more complicated. The non-aggressive A/J mice exhibited higher NAc and PFC DA utilization, higher [^{3}H]-SCH-23390 binding in the rostral pole of the NAc, and lower [^{3}H]-raclopride binding across all of the NAc as compared to aggressive BALB/cJ mice. The functional consequences of this combination of greater DA turnover with higher D1-like receptor expression and lower D2/D3 receptor expression is unclear, but these data produce testable hypotheses. Microdialysis or *in vivo* voltammetry probes directed to accumbal subregions could provide intriguing data regarding anatomical heterogeneity in the regulation of extracellular dopamine by aggressive tendencies or behavior.

Moreover, our data on [^{3}H]-SCH-23390 binding differences between the two strains suggests the presence of an anterior/posterior heterogeneity on D1 receptor-mediated responses. We observed a significant decrease in D1 receptor expression in aggressive BALB/CJ mice only in the rostral pole of the NAc. Recent evidence by Berridge and colleagues support our finding that

biologically based reinforcers may utilize an AP functional heterogeneity as well as traditional shell/core anatomical heterogeneity within the NAc. In a unique series of studies, these authors have utilized microinjection of glutamatergic and GABAergic drugs into select subregions of the NAc to determine that the rostral shell (as opposed to caudal shell) preferentially channel appetitive information about stimulus valence (Reynolds and Berridge, 2001; 2003). There are also several differences between rostral and caudal shell with regard to anatomical connectivity and neurochemical composition (Van Dogen et al., 2005; Delfs et al., 1998; Groenewegen et al., 1999).

Differences seen in DA turnover and D2 receptor densities also offer information about possible mechanistic differences between these two strains of mice. A/J mice, along with their increased DA turnover, have lower D2 receptor levels throughout the NAc. This might result in greater D2 receptor occupancy in A/J mice as compared to BALB/cJ. Due to the fact that we do not see behaviors indicative of a highly efficient/functioning DA system (for example, hyperlocomotion), it is possible that this greater ratio of DA:D2 receptor binding in A/J mice as compared to the aggressive BALB/cJ mice may result in some mechanism of receptor desensitization in A/J mice. This functional outcome is not only consistent with what is seen in A/J motor behavior (i.e. A/J mice are slightly less motorically active than BALB/c lines; Wahlsten et al., 2006) but is also consistent with what we know about the role DA plays in aggression.

In stark contrast to what is seen in the non-aggressive A/J mice, the aggressive BALB/cJ mice have an abundance of DA2rs in combination with low

baseline DA turnover. This is the opposite neurobiological framework from what was seen in A/J mice. In reflection of this opposing neurobiological framework between A/J and BALB/cJ mice, we might also see in the BALB/cJ, a functional opposition to the proposed desensitization in A/J mice. Based on what is known about the relation between aggression and DA, it is plausible that the abundant DA2r's in the aggressive BALB/cJ mice might become sensitized to DA, due to its low turnover in these mice.

Weather the receptors in A/J mice become desensitized and receptors in BALB/cJ mice become sensitized is not able to be concluded from our experiment. However, what can be concluded from our studies is that these mice displaying opposite aggressive phenotypes have very different underlying biology of mesocorticolimbic DA and 5-HT systems. Taken in conjunction with the body of literature on DA, 5-HT and aggression, our neurochemistry and receptor expression data allow us to generate plausible hypotheses regarding the complicated interdependencies of neurobiology and behavior that may underlie the contrasting behavioral phenotypes seen in these animals and are congruent with a role of 'pre-wired' system in the exhibition of aggressive behavior.

However, there is one substantial caveat to the above discussed conclusions. The experiments conducted above were conducted on naïve animals with no experience with aggression. There is a very good possibility that the data harvested from these animals has no connection to aggressive behaviors but instead might reflect basal differences between two strains of mice.

The fact that our data is in accord with the greater body of literature on biogenic amines and aggression adds a significant contribution to the literature, but in no way conclusively demonstrates endogenous differences in DA and 5-HT systems between individuals who engage in aggressive behavior and those who do not. In order to demonstrate this, further investigation of the subject matter is necessary. A good way to approach the topic of endogenous differences between aggressive and non-aggressive individuals is to repeat the experiment conducted above but include several mouse strains that vary on a continuum of aggression. If it could be demonstrated that aggression co-varies in the appropriate direction with basal biogenic amine levels it would add great support for the idea that there is a significant 'nature' component to aggressive individuals.

Our data are generally in accord with the greater body of literature on biogenic amines and aggression, but clearly is correlational in nature. In order to demonstrate causal links, further investigation of the subject matter using functional assays is necessary. Finally, there are of course many other brain circuits that contribute to aggression, including (but not limited to) amygdalar, hypothalamic, and hippocampal pathways (for review, see Nelson & Trainor, 2007). Whether similar differences in activation or patterning are present in those systems is still an open question.

CHAPTER V

FINAL CONCLUSIONS

In specific aim 1 of this project, it was determined that aggression as a reinforcer can be brought under stimulus control. This finding is important for several reasons. First, this phenomena has never been demonstrated using classical definitions of stimulus control. While aggression has been used as a positively reinforcing stimuli in several studies, it has never been demonstrated that a) an aggressive encounter is positively reinforcing (i.e. demonstrating that upon removal of the reinforcing contingency, the trained response stops) and b) what part of the positively reinforcing event is the positively reinforcing stimuli.

In specific aim 1, we have clearly shown that upon the removal of the response-reinforcement contingency, the subjects extinguish responding. In addition, when the response-reinforcement contingency is re-established, subjects begin robustly responding.

In addition to demonstrating by classical definitions the positively reinforcing efficacy of an aggressive encounter, specific aim 1 demonstrated that the actual physical aggression in an aggressive encounter was the positively reinforcing stimuli. As any social interaction is a complex scenario, diverse in olfactory, visual, activity and novel cues, it is important for the study of aggression to determine which of these cues results in hedonic positive reinforcement. By careful isolation of the physical aggression component of the

aggressive encounter, it was able to be concluded that in these subjects, physical aggression was necessary to maintain the response-reinforcement contingency.

Implications of this work are far-reaching. The most salient and important implication of this work is that physical aggression is a primary reinforcer, much like food, water and sex. Like these other primary reinforcers, physical aggression serves a biological and evolutionally important purpose. Due to this, it is likely that the mechanism behind aggression's conservation across generations and species is through its innately reinforcing properties. While these primary reinforcing behaviors serve biologically necessary needs, they can also lead to pathological addictions such as eating addictions resulting in obesity and sex additions which result in a host of personal and societal problems. Due to the enlightenment that pathological aggression should also be viewed in such a way, we must consider treatment for addicted individuals in the same manner as we do other addicted individuals.

Currently, the heath care and legal systems deal with aggression and other addictions much differently. Medically, aggression is viewed as a symptom to another, more pervasive mental or physical disease as opposed to a disorder in its own right. Thus medications and behavior/environmental therapies are not aimed at the aggression, but instead at this more pervasive medical condition. In the treatment of other addictions such as addictions to food or drugs, the medical treatments prescribed to these individuals are designed specifically to eliminate these problems, not some other condition that may be interacting with the

problem addiction. For example, when an obese person seeks medical council, the doctor will prescribe daily exercise, a healthy diet and in extreme cases, medication that will reduce the amount of fat absorbed from food. When an aggressive individual is under the care of a physician, the physician might diagnose them with depression or bi-polar disorder or any number of mental disorders and prescribe to them medication to treat said disorder. In this situation, the actual aggression never gets targeted, but may simultaneously go down due to therapies that might coincidently lower aggression. In the case of the obese person, the problem of excessive caloric intake is targeted directly.

By recognizing aggression as an addiction, or being innately positively reinforcing, our criminal justice system may rethink correctional efforts. Currently, aggression is punished by a graduated system; whereby on your first offense the punishment is less sever and becomes more severe upon repeated offenses. These punishments usually consist of community service all the way up to lifetime prison sentences and the death penalty. When viewing pathological aggression as an addiction, these graduated punishments might be changed to reflect more of what is done in drug addicts. While there is still a graduated system of punishment for drug offenders, the punishments often include an element of personal behavioral rehabilitation; such admittance into a clinic specifically designed to give the individual medical and behavioral support aimed at decreasing the problem behavior. An important quality of the legal processes behind punishing drug offenders is that this rehabilitation is initialized early in the process of graduated punishment; a quality that has proven useful in

the reduction of all addictions, and would likely do so in pathological aggression as well.

In specific aim 2 of this project, we extended the finding from aim 1 to investigate the biological system that modulates the positively reinforcing properties of aggression. In these experiments, two DA antagonists, both D1-like and D2-like specific, reduced motivation to gain access to aggression. This finding is of great merit to the understanding of the biology behind aggression for several reasons. First, until this study, experiments examining dopamine and aggression were always hindered by generalized movement side effects. In these studies, we were not only able to demonstrate a reduced responding for the opportunity to aggress, but we were also able to demonstrate that aggressive motivation can be modified without generalized movement effects. The next, and possibly the most interesting implication of specific aim 2, is that while both DA antagonists reduced responding for aggression with out effecting movement, they did not reduce the actual level of aggression during an aggressive encounter. This demonstrates that not only have we dissociated the effects of DA antagonists on responding for aggression, but we have also been able to dissociate the motivation to engage in aggression with the actual reduction in the performance of aggressive behavior. This point becomes important when one thinks about the practical application of behavioral and medical therapies in human societies. Currently in the science of aggression, the focus has been on serenics (5-HT1 agonists); agents that reduce the subject's level of emitted aggression during an aggressive confrontation. However, in human society,

there are not artificial situations set up to test one's level of aggression or amount of aggressive behaviors emitted during an aggressive bout. Thus, creating medical therapies aimed at reducing the frequency of emitted aggressive behavior during an aggressive bout is neither reasonable nor useful. For example, reducing how much a person punches during a fight does not equal a successful behavior modification plan, nor is it ethologically adaptive not to fully engage in aggression once a bout has begun. Instead, what we see in human interactions, are aggressive individuals instigating aggressive encounters; i.e. demonstrating a motivation to engage in aggression. Most aggressive encounters that people have would not occur unless the instigator was motivated to engage in aggression. Thus, in a real-life scenario, reducing motivation to engage in aggression offers practical therapeutic potential.

Possible strategies utilizing the ability to differentiate the motivation to engage in aggression from the actual emission of aggressive behavior comes in the form therapies akin to those used to treat heroin and alcohol addictions. When an individual is addicted to heroine, he/she is prescribed methadone. Similarly, when one is addicted to alcohol, he/she is prescribed some form of Antabuse. Each of these strategies is aimed at reducing the positively reinforcing effects of the addictive substance. The studies outlined in aim 2 offer the infant stages of research for developing such therapies for aggression addiction; both behavioral and pharmacological. Demonstrating that the reinforcing properties of aggression are modulated by the same systems as other addictions offers numerous avenues of research governed by the hypothesis that

what has helped reduce other DA modulated addictions, may also help reduce pathological aggression.

The studies in specific aim three demonstrate that there is a significant 'nature' component to aggressive behavior. These studies were important due to the fact that all of the neurochemical/biological assays, until those described in aim 3, were completed in subjects with some experience of aggression. Thus, in all the previous biology of aggression literature, conclusions about biological states were massively effected by experience; in effect, the differences in the biology of these animals could be due to how each aggressive experience shaped their biology. In aim 3, we gain evidence congruent with the idea that there are neurochemical differences in individuals who are likely to become aggressive as compared to those who are not. These data in conjunction with data collected on humans that reach the same conclusion, could lead to early medical/genetic screening exams, much like what has been proposed for other diseases such as cancer. Though like with these other diseases, early genetic screening in order to identify 'high-risk' individuals suffers from an enormous amount of ethical issues. If these ethical issues can be resolved and the early genetic screening could identify with some respectable accuracy those who may have aggression problems later in life, it would prove to be a great asset to both the future victims of aggression and the aggressors themselves.

Regardless of the feasibility and practicality of early genetic screenings, the ability for science to demonstrate biologically based differences between those who are pathologically aggressive and those who are not would lead to a

paradigm shift in the medical and legal bodies surrounding aggression. If clear neurochemical substrates are characteristic of pathologically aggressive individuals, it would likely lead to viewing aggression not as a symptom common among several diseases, but a disorder unto itself that is co morbid with several other disorders. As outlined above, this philosophy of aggression offers many practical rehabilitation advantages over the current philosophy of aggression.

In synthesis, the data gleaned from specific aims 1, 2 and 3 suggest that aggression is understandable and charaterizable. From these experiments, we can conclude that physical aggression is a positively reinforcing stimuli, that both D1 and D2 receptors can modulate these positively reinforcing effects and that there may be biological differences between aggressive individuals and those that are not. These findings suggest problematic aggression has the potential to be controlled, as other behavioral addictions have demonstrated an ability to be controlled. Finally, and possibly the most striking implication of the above experiments on species atypical aggression, is that they reflect a growing need for both the legal and medical fields to change the way they think about managing aggression.

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