UNRAVELING THE ROLE OF G-PROTEINS IN HALLUCINOGENIC DRUG ACTION

By

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Dedicated to my mother

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LIST OF ABBREVIATIONS

5-HT	Serotonin, 5-hyroxytryptamine
cGMP	Guanosine-3′,5′-cyclic monophosphate
CNS	Central nervous system
DAG	Diacyl glycerol
DMEM	Dulbecco's modified Eagle's medium
DOI	(±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane
EC_{50}	Concentration of drug that gives 50% of the maximal response
EPM	Elevated plus maze
EtOH	Ethanol
GDP	Guanosine 5′-diphosphate
GFAP	Glial fibrillary acidic protein
G-protein	Guanine nucleotide binding protein
G_qCT	G_q protein blocking peptide
GPCR	G-protein coupled receptor
GTP	Guanosine 5′-triphosphate
H&E	Hematoxylin & Eosin
HBSS	Hank's balanced salt solution
HIV-1	Human Immunodeficiency virus 1
HTR	Head-twitch response
IP_3	Inositol 1,4,5-triphosphate
LSD	Lysergic acid diethylamide

mCPP	m-chlorophenylpiperazine
PI hydrolysis	Phosphatidylinositol hydrolysis
PIP_2	Phosphatidylinositol 4,5-bisphosphate
PLA_2	Phospholipase A_2
PLC	Phospholipase C
PLD	Phospholipase D
PKC	Protein kinase C

CHAPTER I

INTRODUCTION

Serotonin

Serotonin is an indoleamine neurotransmitter that was first isolated by Maurice Rappaport in 1948 from blood serum (Rapport, 1948), and initially characterized as a vasoconstrictor, which led to its descriptive name, serotonin (from a serum agent that affects vascular tone). It was later identified as 5-hydroxytryptamine (5-HT) and found extensively in the gastrointestinal tract, where it is made in enterochromaffin cells, followed by platelets and the nervous system. 5-HT containing neurons originate in the midline raphe nuclei of the brain stem and project to portions of the hypothalamus, the limbic system, the neocortex, and the spinal cord.

The initial step in the synthesis of 5-HT is the facilitated transport of the amino acid L-tryptophan from blood into brain. The biochemical pathway for 5-HT synthesis initially involves the conversion of L-tryptophan to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase, which serves as the rate limiting step. The subsequent and final metabolic step in the synthesis involves the decarboxylation of 5-HTP into 5-HT by the enzyme aromatic L-amino acid decarboxylase.

5-HT appears to have been conserved throughout evolution. 5-HT and its receptors can be found in lower organisms including *Caenorhabditis elegans*

(nematodes), *Plathyhelminths* (flatworms), *Aplysia californica* (mollusks), and *Drosophila melanogaster* (Weiger, 1997). In these organisms, 5-HT modulates various behaviors such as feeding, biting, escape swimming, and egg laying. The study of these lower organisms has enhanced our understanding of 5-HT functions in both invertebrates and vertebrates.

The pharmacology of 5-HT is quite complex, and its actions are largely mediated by a diverse family of 5-HT receptors. Serotonergic action is controlled by its reuptake into the presynaptic terminal by the 5-HT transporter (SERT) (Schloss and Williams, 1998). The huge body of ongoing research on this neurotransmitter, its receptors and transporter has led to the practical application of controlling, modulating, and normalizing 5-HT through pharmaceutical agents. Selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), and tricyclic antidepressants (TCAs) have successfully been used for the treatment of depression. Also, there has been much scientific interest in other mental health areas, particularly concerning schizophrenia and psychedelic drugs. 5-HT receptors have been associated with the mechanism of action of atypical antipsychotic drugs as well as hallucinogens. This underscores the importance of 5-HT and its pathways for the normal function of the brain.

Serotonin Receptors

5-HT interacts with a wide array of receptor subtypes, mediating a diverse range of physiological functions. Seven distinct families of 5-HT receptors have been identified (5-HT1-5-HT7). These subfamilies are characterized by common

gene organization, pharmacological properties, and second messenger signaling pathways (for review, see Hoyer et al., 1994; Hoyer et al., 2002). All 5-HT receptors, with the exception of 5-HT_3, belong to the G-protein-coupled receptor (GPCR) superfamily. 5-HT_3 receptors, on the other hand, are ligand-gated ion channels (Table 1).

The 5-HT_1 receptor subfamily is the largest subclass of 5-HT receptors, consisting of five receptor subtypes, termed, 5-$HT_{1A, 1B, 1D, 1E, 1F}$. Members of the 5-HT1 receptor family share 40-63% homology and couple primarily to $G_{i/o}$ to negatively regulate adenylyl cyclase thus inhibiting (cAMP) formation.

5-HT4, 5-HT6, and 5-HT7 receptors couple predominantly to G_s to positively activate adenylyl cyclase. However, due to their difference in sequence (<35%) and pharmacological action, they are classified in different subfamilies.

5-HT5 receptors were first identified in 1992 (Plassat et al., 1992) and are still being characterized. There are two subtypes of 5-HT5 receptors, 5-HT_{5A} and 5-HT_{5B}. Both subtypes have been cloned from the rat and the mouse; however, only the 5-HT_{5A} receptor has been cloned from the human (Rees et al., 1994). Recently, the human 5-HT_{5A} receptor has been shown to couple to both $G_{i/o}$ and $G_{q/11}$ (Noda et al., 2003). In addition, Waeber and colleagues have shown that 5-HT_{5A} receptors have high affinity for Lysergic Acid Diethylamide (LSD) (Waeber et al., 1998)

Table 1

Serotonin Receptor Family

Receptor Family	Receptor subtype	Signal Transduction
$5\text{-}HT_1$	$5\text{-}HT_{1A}$	Inhibition of Adenylyl Cyclase
	$5\text{-}HT1_{B/D}$	
	$5\text{-}HT_{1D}$	
	$5\text{-}HT_{1E}$	
	$5\text{-}HT_{1F}$	
$5\text{-}HT_2$	$5\text{-}HT_{2A}$	Activation of Phospholipase Cβ
	$5\text{-}HT_{2B}$	
	$5\text{-}HT_{2C}$	
$5\text{-}HT_3$		Ligand Gated Ion Channel
$5\text{-}HT_4$		Activation of Adenylyl Cycalse
$5\text{-}HT_5$	$5\text{-}HT_{5A}$	Inhibition of Adenylyl Cyclase Activation of PLCβ
	$5\text{-}HT_{5B}$	Unknown
$5\text{-}HT_6$		Activation of Adenylyl Cyclase
$5\text{-}HT_7$		Activation of Adenylyl Cyclase

5-HT2 receptor family

The 5-HT2 receptor family consists of three subtypes: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} (Hoyer et al., 1994). 5-HT2 receptors share a high level of sequence homology (~50%) and pharmacological profiles, and preferentially couple to PLCβ activation via $G_{q/11}$ (Conn and Sanders-Bush, 1984). The 5-HT_{2A} receptor was first identified as the D receptor by Gaddum and Picarelli (1957), named because of the sensitivity of this receptor subtype to the blocker dibenzyline. The 5-HT_{2B} receptor was initially referred as the 5-HT_{2F} receptor [or serotonin receptor like (SRL)] (Foguet et al., 1992), and the 5-HT_{2C} receptor was previously referred as the 5-HT_{1C} receptor (Pazos et al., 1984); however, cloning of the mouse, rat, and human 5-HT_{2C} receptor led to the conclusion that the 5-HT_{2C} receptor is more closely related to the 5-HT2 than the 5-HT1 subfamily (Humphrey et al., 1993).

5-HT_{2A} receptors

5-HT_{2A} receptors have been implicated in many peripheral as well as central functions including smooth muscle contraction, platelet aggregation, regulation of sleep, control of sexual activity, motor behavior, and psychiatric disorders such as epilepsy, anxiety, depression, and schizophrenia.

The 5-HT_{2A} receptor was first cloned from the rat brain by Princhett et al., (1988), and its sequence was later corrected by Julius et al., (1990). 5-HT_{2A} receptors have also been cloned from human (Saltzman et al., 1991), mouse

(Foguet et al., 1992), hamster (Chambard et al., 1990), and rhesus monkey (Johnson et al., 1995) with the rat and the human gene sharing 87% homology.

Receptor Distribution

Peripherally, 5-HT_{2A} receptors are found on platelets and throughout the gut and vasculature of smooth muscle. Centrally, 5-HT_{2A} receptors are highly expressed in the cortex, caudate nucleus, olfactory tubercle, nucleus accumbens, and hippocampus (Pazos et al., 1985). Cortical pyramidal neurons constitute the major source of 5-HT_{2A} receptor-expressing neurons, where the receptors are located both presynaptically and postsynaptically. However, in the prelimbic prefrontal cortex of the rat, 5-HT_{2A} receptors are predominantly expressed on postsynaptic structures (Xia et al., 2003). These receptors have been implicated as heteroreceptors in the regulation of glutamatergic signaling (Aghajanian and Marek, 1999b; Scruggs et al., 2000; Zhang et al., 2001; Boothman et al., 2003)

Signal transduction

5-HT_{2A} receptors activate a variety of signaling pathways by interacting with multiple G-proteins. The classical pathway associated with 5-HT_{2A} receptor signaling is the stimulation of phospholipase Cβ via G$\alpha_{q/11}$ in most tissues and cells in which it is expressed (Conn and Sanders-Bush, 1984; Grotewiel and Sanders-Bush, 1999; Chang et al., 2000a). The cloned rat and human receptors also stimulate PLC when expressed heterologously in mammalian cells

(Wainscott et al., 1993; Kursar et al., 1994; Kellermann et al., 1998). PLCβ then promotes the hydrolysis of the membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). DAG then activates protein kinase C (PKC) while IP_3 binds its intracellular receptor to promote intracellular calcium release.

In addition to PLC activation, 5-HT_{2A} receptors have been shown to couple to additional signaling pathways including activation of PLD via $G\alpha_{13}$ (Kurrasch-Orbaugh et al., 2003; Robertson et al., 2003) and activation of PLA_2 leading to arachidonic acid release (Berg et al., 1998; Tournois et al., 1998) (Figure 1).

5-HT_{2A} receptors have also been implicated with the regulation of L-type Ca^{2+} channels in some cell types (Eberle-Wang et al., 1994; Jalonen et al., 1997; Watts, 1998). In addition, increases in Ca^{2+} levels evoked by 5-HT_{2A} receptors have been linked to subsequent openings of K^+ channels in C6 glial cells (Bartrup and Newberry, 1994), and to an inward current mediated by Ca^{2+}-activated Cl^- channels in Xenopus oocytes (Montiel et al., 1997).

5-HT_{2A} receptors regulation occurs in response to both antagonists and agonists. Peroutka and Snyder (1980) were the first to demonstrate that chronic treatment with 5-HT_{2A} antagonists led to receptor desensitization (for review, see Gray and Roth, 2001). This phenomenon seems unique to 5-HT2 receptors, and has made them the focus of a variety of investigations into the desensitization process. In addition, this feature may play important roles in 5-HT_{2A} receptor signaling and in the mechanism of action of certain antipsychotic medications. For some time, it has been clear that 5-HT_{2A} receptors are desensitized primarily

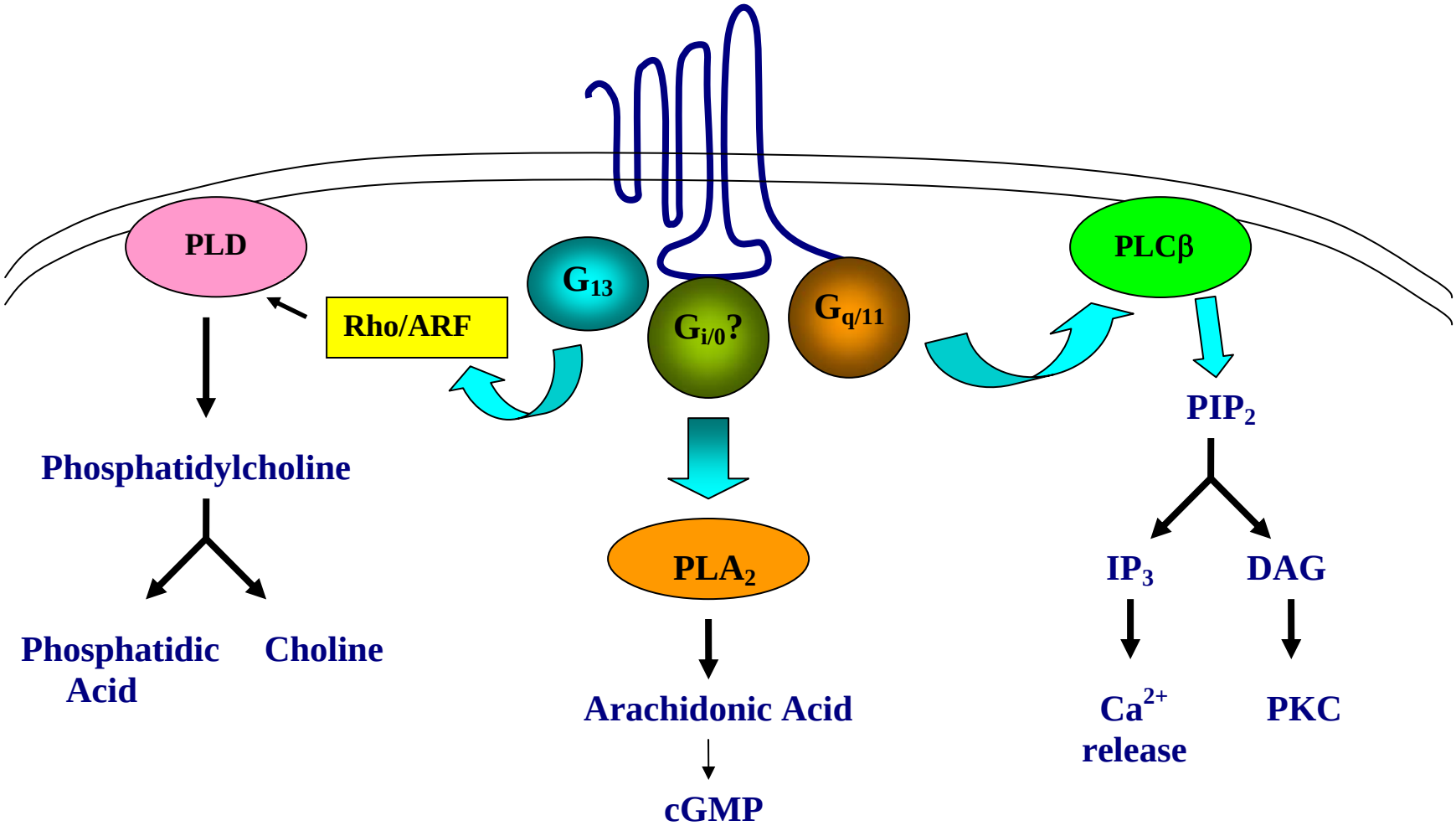


Figure 1: 5-HT$_{2A/2C}$ Singaling Pathways

5-HT$_{2A/2C}$ receptors activate various G-proteins to stimulate downstream effectors. PLC, phospholipase C; PIP$_2$, phosphatidylinositol 4,5-bisphosphate; DAG, diacyl glycerol; IP$_3$, inositol 1,4,5-trisphosphate; PKC, protein kinase C; cGMP, guanosine-3',5'-cyclic monophosphate; PLD phospholipase D; PLA$_2$, phospholipase A$_2$

following PKC activation (Kagaya et al., 1990; Kagaya et al., 1993; Rahimian and Hrdina, 1995), though cell-type specific effects have been noted. Furthermore, although arrestins are apparently involved in the short-term regulation of many GPCRs, studies from Bhatnagar and colleagues (2001) demonstrated that 5-HT_{2A} agonists and antagonists induce internalization via an arrestin-independent pathway; however, the dominant negative dynamin appears necessary for receptor internalization and increased rate of receptor resensitization.

Electrophysiological Responses

There is evidence for 5-HT_{2A}-mediated excitation of neurons in certain brain regions, although, in rat piriform cortex pyramidal cells, (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and LSD induce inhibitory postsynaptic potentials (IPSPs) by directly exciting GABAergic interneurons (Marek and Aghajanian, 1996). However, 5-HT_{2A} receptor activation causes different effects in other parts of the brain. For instance, in medial prefrontal cortex (mPFC) pyramidal cells, 5-HT_{2A} receptor activation causes some IPSPs, but primarily causes an increase in the amplitude and especially in frequency of spontaneous excitatory postsynaptic potentials (EPSPs). (Figure 2; Aghajanian and Marek, 1997; Aghajanian and Marek, 1999b; Marek and Aghajanian, 1999; Marek and Aghajanian, 1998). In addition, *in vivo* microdialysis studies of Scruggs and colleagues (2003) supporting a role for glutamate demonstrate that DOI induces an increase in extracellular glutamate in the medial prefrontal cortex of awake rats. The increase in glutamate levels elicited by intracortical DOI were

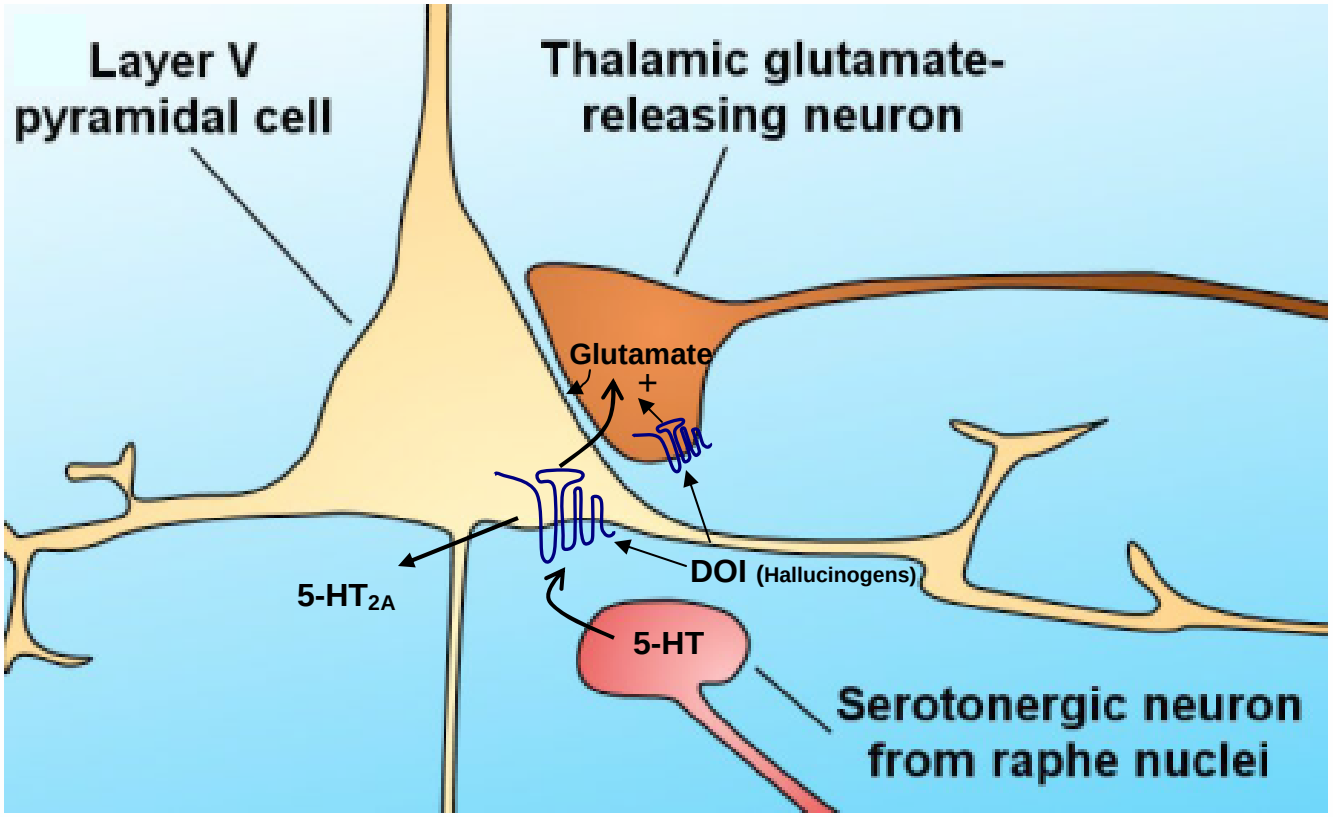


Figure 2: Schematic representation of 5-HT_{2A}-mediated glutamate release
Systemic and intracortical administration of the hallucinogen DOI increases extracellular glutamate levels in the cortex, consistent with the hypothesis that hallucinogens activate the cortex by increasing glutamate release from thalamocortical neurons. The ability of DOI to increase glutamate levels is blocked by pretreatment with MDL 100907, indicating a 5-HT_{2A}-mediated effect.

blocked by treatment with the selective 5-HT_{2A} antagonist MDL 100,907.

5-HT_{2B} receptors

5-HT_{2B} receptors exhibit about 70% homology to 5-HT_{2A} and 5-HT_{2C} receptors, and also appear to couple functionally to phosphoinositide hydrolysis. The presence of 5-HT_{2B} receptors in the brain has been controversial, but limited amounts are believed to be found in mouse and human brains (Loric et al., 1992; Bonhaus et al., 1995). However, their expression appears to be restricted to the cerebellum, lateral septum, medial amygdala, and dorsal hypothalamus. 5-HT_{2B} receptors have been implicated in the mediation of the mitogenic effects of 5-HT during neural development.

5-HT_{2C} receptors

5-HT_{2C} receptors are almost exclusively expressed in the central nervous system. High levels of 5-HT_{2C} receptor are found in the choroid plexus, with lower expression in the cortex, amygdala, hippocampus, nucleus accumbens, and the substantia nigra (Pazos et al., 1984; Conn et al., 1986; Hoyer et al., 1994; Abramowski et al., 1995). Human 5-HT_{2C} receptors have been cloned and display high homology with 5-HT_{2A} receptors (57%). In response to agonists, 5-HT_{2C} receptors activate PLCβ (Conn et al., 1986), and this activation was definitely demonstrated to occur via $G\alpha_q$ by Chang et al. (2000). 5-HT_{2C} receptors have also been associated with PLA_2-mediated arachidonic acid

release (Berg et al., 1996), and activation of PLD via Rho/ARF proteins (McGrew et al., 2002) (Figure 1)

5-HT_{2C} receptors undergo a unique process of post-transcriptional modification called RNA editing. This process leads to the generation of multiple receptor variants (for review, see Niswender et al., 1998). The variant edited receptors exhibit profound functional consequences including altered pattern of activation of heterotrimeric G-proteins and differential abilities to bind various ligands thus affecting downstream processes such as mobilization of intracellular calcium and stimulation of inositol phosphates (Fitzgerald et al., 1999; Price et al., 2001; McGrew et al., 2004; Niswender et al., 1998).

Pharmacology of 5-HT_{2A} vs 5-HT_{2C} receptors

Ligands

No truly selective 5-HT_{2A} or 5-HT_{2C} agonist has yet been found. 5-HT acts as a full agonist at both receptors; however, 5-HT binds 5-HT_{2A} and 5-HT_{2C} receptors with quite different affinities (63-250 nM for 5-HT_{2A} and 2-56 nM for 5-HT_{2C}). There are agonists that selectively activate 5-HT_{2A} and 5-HT_{2C} receptors over other 5-HT receptors (e.g., DOI, DOM, DOB), but these agents do not discriminate between these two receptor subtypes.

To address the lack of agonist selectivity in the 5-HT2 receptor family, selective antagonists have been synthesized (Table 2). One of the most selective classes of 5-HT_{2A} receptor ligands is the N-alkylpiperidines. The most commonly

Table 2

pKi of various ligands for 5-HT2 receptors

	5-HT$_{2A}$	**5-HT$_{2B}$**	**5-HT$_{2C}$**
5-HT$_{2A}$ receptor			
MDL 100907	9.4	5.5	5.9
Spiperone	8.8	Not Determined	6.9
Ketanserin	8.9	5.4	7.0
5-HT$_{2B}$ receptor			
5-MeOT	7.4	8.8	6.2
a-Methyl-5-HT	6.1	8.4	7.3
SB 204741	< 5.3	7.8	< 6.0
BW 723C86	< 5.4	7.9	< 6.9
5-HT$_{2C}$ receptor			
SB 242084	6.8	7.0	9.0
RS 10221	6.0	6.1	8.4
Ro 60-0175	6.0	5.8	8.8
5-HT$_{2B/2C}$ receptor			
SB 200646A	5.2	7.5	6.9
mCPP	6.7	7.4	7.8
SB 206553	5.8	8.9	7.9
Non-selective			
LY 53857	7.3	8.2	8.1
ICI 170809	9.1	Not Determined	8.3
Ritanserin	8.8	8.3	8.9
Mianserin	8.1	7.3	8.0
DOI	7.3	7.4	7.8

used drug of this class is ketanserin, which possesses a 100-fold higher affinity for the 5-HT_{2A} receptor over the 5-HT_{2C} receptor. Another common 5-HT2 antagonist is spiperone, which displays about 500-2000 fold selectivity for the 5-HT_{2A} receptor versus the 5-HT_{2C} receptor. However, a major drawback for spiperone is that it is also a potent dopamine D_2, 5-HT_{1A}, and 5-HT7 antagonist (Metwally et al., 1998). Various tricyclic agents (e.g., tricyclic neuroleptics and tricyclic antidepressants) also bind 5-HT_{2A} receptors. To date, one of the most selective 5-HT_{2A} receptor antagonists is MDL 100907 with 300-600 fold higher affinity for the 5-HT_{2A} receptor over the 5-HT_{2C} receptor (Marek and Aghajanian, 1994). In the mid-1990s, MDL 100907 was tested in clinical trials as a possible non-dopaminergic antipsychotic drug for patients with schizophrenia. However, due to its insufficient efficacy, these studies were terminated.

At this time, no 5-HT_{2C}-selective agents have been identified. MK212 and Ro 600175 are considered moderately selective 5-HT_{2C} agonists. mCPP is often used as a non-selective 5-HT_{2C} agonist for behavioral studies, and it has been suggested that the anxiogenic effects of mCPP are mediated by post-synaptic 5-HT_{2C} receptors (Gibson et al., 1994). SB 200646A was initially reported to be a selective 5-HT_{2C} receptor antagonist with about 50-fold selectivity over the 5-HT_{2A} receptor, but it was later found to have similar affinity for the 5-HT_{2B} receptor. SB 206553 is also an antagonist at 5-$HT_{2B/2C}$ receptors. To date, SB 242084 is the most selective antagonist at 5-HT_{2C} receptors, and it has been shown to display anxiolytic-like properties in animal models of anxiety (Kennett et al., 1997).

Hallucinogenic Drugs

Hallucinogenic drugs (psychedelics) are among some of the oldest drugs known to mankind, and they have a long history of traditional use in native medicine and religion, where they are prized for their perceived ability to promote physical and mental healing. Hallucinogens such as mescaline, a naturally occurring psychedelic found in several cactus species (i.e., Peyote and San Pedro), have been used in Native American ritual ceremonies since the 1800's. The earliest known depiction of San Pedro cactus is on a stone tablet found in Peru dating to 1300 B.C. In addition, ritual objects containing images of Peyote have been found dating back to 500 B.C. In the 1960's and 70's there was extensive usage of hallucinogens as part of the counter-culture hippie movement. During the "acid tests" of this era, hallucinogenic drugs were used for mind exploration.

The best definition of what is considered a *classic* psychedelic is the following -- "a psychedelic drug is one which, without causing physical addiction, craving, major physiological disturbances, delirium, disorientation, or amnesia, more or less reliably produces thought, mood, and perceptual changes otherwise rarely experienced except in dreams, contemplative and religious exaltation, flashes of vivid involuntary memory and acute psychoses" (Grinspoon ,1979). Despite their high degree of psychological safety and low addiction potential, hallucinogenic drugs have been placed in the most restrictive category (schedule I of the Controlled Substances Act) by law enforcement officials.

Hallucinogenic drugs are known to alter the subjective qualities of perception, thought or emotion, leading to altered states of consciousness or hallucinations. However, despite the name, at typical doses very few hallucinogens trigger true hallucinations; rather the effects are more akin to delusion. In addition, the nature of the hallucinations produced is dependent on the specific compound. Some of the physiological effects of hallucinogens include dilation of pupils, appetite loss, increases in blood pressure and body temperature, and excitation of the sympathetic nervous system.

Scientific interest in hallucinogenic drugs developed slowly. Mescaline was first extracted and isolated in the late 1800's by Arthur Heffter, and in 1919 it became the first psychedelic to be synthesized. For the next 35 years it remained a somewhat obscure compound known primarily to the psychiatric community. Then, in 1953 the popular novelist Aldous Huxley was introduced to mescaline by the psychiatrist Humphry Osmond. Soon thereafter Huxley became a pioneer of self-directed psychedelic drug use "in a search for enlightenment", famously taking 100 micrograms of LSD as he lay dying. His psychedelic drug experiences are described in the essays *The Doors of Perception* and *Heaven and Hell*. The title of the former became the inspiration for the naming of the rock band, *The Doors*. Some of his writings on psychedelics became frequent reading among early hippies.

In 1938, the Swiss chemist Dr. Albert Hofmann at the Sandoz Laboratories in Basel fist synthesized LSD as part of a large research program searching for medically useful ergot alkaloid derivatives. Its psychedelic

properties were unknown for 5 years until Hofmann, acting on what he has called a "peculiar presentiment," returned to work on the chemical. He attributed the discovery of the compound's psychoactive effects to the accidental absorption of a tiny amount through his skin in 1943, which led to him testing a larger amount (250 µg) on himself for psychoactivity (Hofmann, 1979). For the first few years following the discovery of LSD, it was used for purely scientific purposes; however, in the 1950s some psychotherapists began to utilize LSD as an adjunct to psychotherapy. Several mental health professionals involved in LSD research, most notably Harvard psychology professors Drs. Timothy Leary and Richard Alpert, became convinced of LSD's potential as a tool for spiritual growth. In 1961, Dr. Timothy Leary received a grant from Harvard University to study the effects of LSD on humans. 3,500 doses were given to over 400 people. Of those tested, 90% said they would like to repeat the experience, 83% said they had "learned something or had insight," and 62% said it had changed their life for the better. Recreational LSD use in the USA peaked in the 1970s, but its use decreased sharply as cocaine became the most common substance of choice among drug users. Underground recreational and therapeutic LSD use has continued in many countries, supported by a black market and demand for the drug. Legal, academic research experiments on the effects and mechanisms of LSD are also conducted, but rarely involve human subjects.

Psychedelic (mind manifesting) hallucinogens are classified as drugs whose primary function is to alter thought processes. The term is derived from Greek *psyche* (mind) and *delein* (to manifest), or *delos* (beautiful). The chemical

structures of psychedelics are classified into two categories: the tryptamines and the phenethylamines (Figure 3; for review, see Nichols, 2004). Many of the tryptamines and phenethylamines cause remarkably similar effects, despite their different chemical structure. However, most users report that the two families have subjectively different qualities in the "feel" of the experience which are difficult to describe.

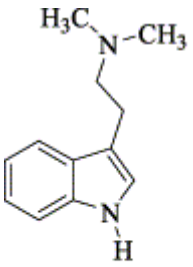
Substitutions to the tryptamine molecule give rise to a group of compounds collectively known as tryptamines. Tryptamine alkaloids found in fungi, plants and animals are commonly used by humans for their psychotropic effects. Prominent examples include 5-methoxy-DMT (5-MeO-DMT), psilocybin (from "magic mushrooms"), and N,N-dimethyltryptamine (DMT; from numerous plant sources, e.g. chacruna, often used in ayahuasca brews). The tryptamine backbone can also be identified as part of the structure of some more complex compounds, for example: ergoline alkaloids like LSD and related compunds.

Substituted phenethylamines are a broad and diverse class of compounds that include stimulants, hallucinogens, entactogens, bronchodilators, and antidepressants. The phenethylamines hallucinogens include the natural occurring compound mescaline, as well as the highly popular club drug 3,4-methylenedioxy-n-methylamphetamine (MDMA), better known as ecstasy. Amphetamine derivatives and selective 5-HT2 agonists including DOI, 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB), and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) also belong to this category.

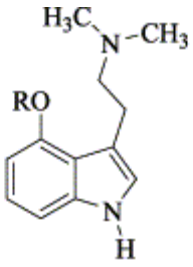
Figure 3

The Chemical Classes of Hallucinogens

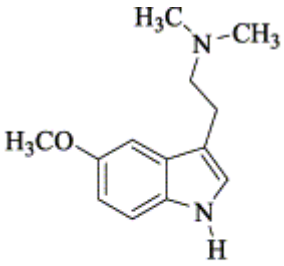
Tryptamines



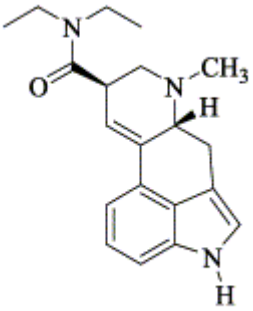
DMT



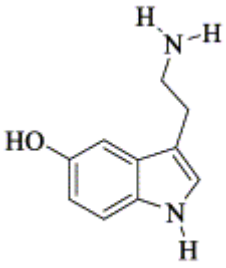
R = H; Psilocin
R = PO_3H; Psilocybin



5-Methoxy-DMT

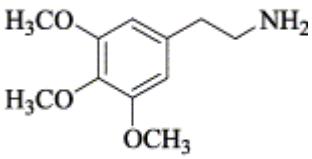


LSD

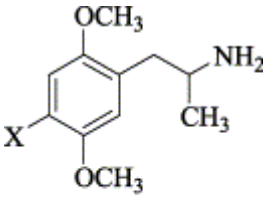


Serotonin; 5-HT

Phenethylamines



Mescaline



X = CH_3; DOM
X = Br; DOB
X = I; DOI

5-HT2 receptors: Clinical implications

5-HT_{2A} receptors play a role in many peripheral as well as central nervous system functions including smooth muscle contraction, platelet aggregation, appetite control, thermoregulation, sleep, cognition, and mood. They are also involved in various neuropsychiatric disorders such as depression and schizophrenia. Over fifty years ago, based on the behavioral abnormalities observed in normal individuals following LSD administration, along with the similarities in chemical structures between LSD and 5-HT, Wooley and Shaw (1954) suggested that 5-HT mediated certain mental processes, and that psychosis was caused by a disturbance in the serotoninergic system in the brain. More recently, it was suggested that the "positive" hallucination-like symptoms observed in acute schizophrenia may be due to a dysfunctional 5-HT_{2A} receptor signaling system in apical dendrites of pyramidal cells (Jakab and Goldman-Rakic, 1998). In addition, clozapine, the classical atypical antipsychotic drug (Meltzer et al., 1989), and a large number of new antipsychotic agents, such as olanzapine and risperidone, have 5-HT_{2A} receptor antagonism properties. These agents have been evaluated in patients with schizophrenia, providing evidence for the importance of this receptor in antipsychotic drug action.

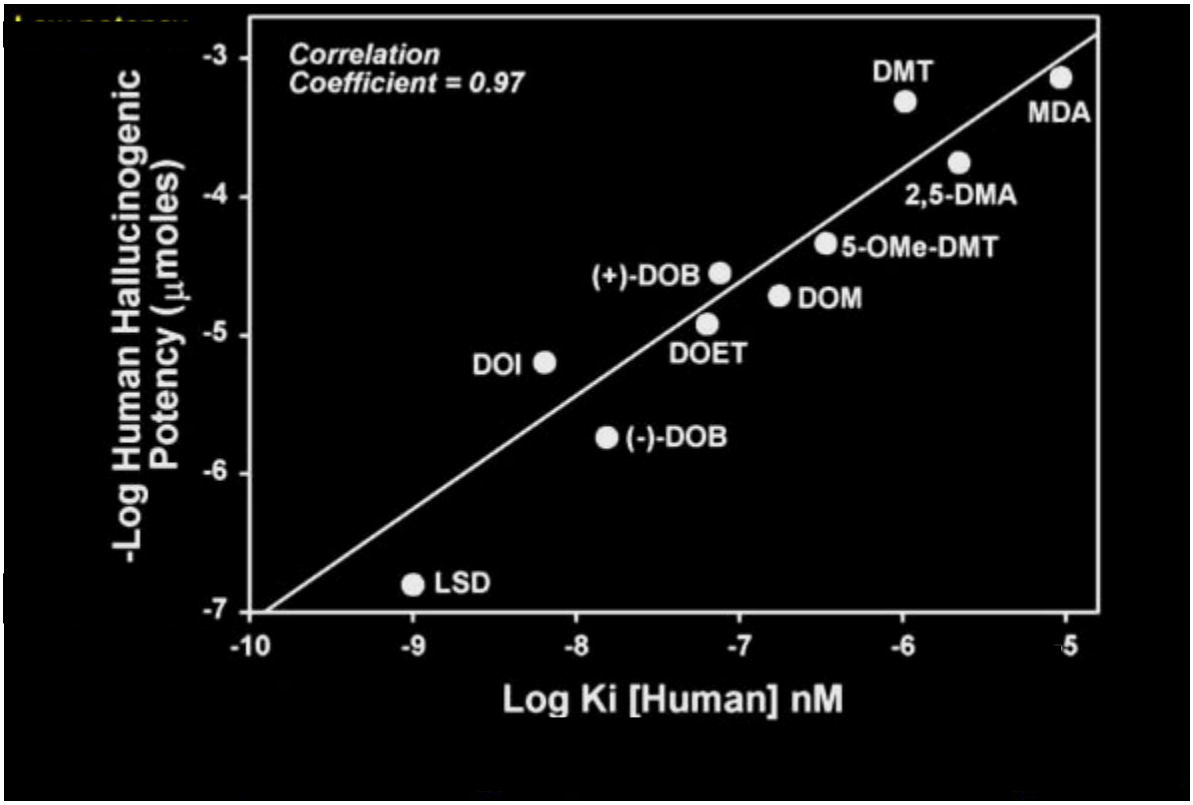
Since the early stages of hallucinogenic drug research, the serotonergic system has been closely associated with neuropsychiatric disorders as well as with the mechanism of action of hallucinogenic drugs. The first evidence that hallucinogens act through 5-HT2 receptors came from Glennon's group in 1983. In this study, rats were trained to discriminate the hallucinogen DOM from saline.

The authors showed that pretreatment with the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, blocked the DOM stimulus. Sadzot et al. (1989) then reported that there is a direct correlation between human hallucinogenic potency of classical hallucinogens and 5-HT_{2A} receptor affinities (Figure 4).

Given that 5-HT2 receptors are the only shared targets between phenethylamine hallucinogens and LSD (as well as other indolamine hallucinogens), it is believed that 5-$HT_{2A/2C}$ receptors are the main sites of action for hallucinogenic drugs since the 5-HT_{2B} receptor is weakly expressed in the brain (Table 3). Consistent with this idea, there is now vast evidence from biochemical, electrophysiological, and behavioral studies that hallucinogens, such as LSD, have a key site of action as agonists at 5-HT_{2A} receptors in the brain (for review, see Marek and Aghajanian, 1996; Aghajanian and Marek, 1999a; Nichols, 2004). Moreover, some hallucinogenic drugs, such as DOI, bind only 5-HT2 receptors, and are known to exert their behavior mainly through 5-HT_{2A} receptors (Johnson et al., 1987). 5-HT_{2A} receptors are considered the primary site of action for hallucinogenic drugs for various reasons. First, there is clear predominance of 5-HT_{2A} receptors over 5-HT_{2C} receptors in the cortex, where hallucinogens are thought to exert their main action (Pompeiano et al., 1994; Wright et al., 1995). More importantly, Ismaiel et al. (2003) showed that spiperone, with about 2000-fold selectivity for the 5-HT_{2A} receptor over the 5-HT_{2C} receptor, blocks the discriminative stimulus induced by the hallucinogen DOM. In addition, the highly selective 5-HT_{2A} antagonist MDL100907 was shown

Figure 4

Relationship between human hallucinogenic potency and 5-HT_{2A} receptor binding



The affinities of various hallucinogens for the 5-HT_{2A} receptor correlate with the behavioral effects produced in drug discrimination experiments

Adapted from Sadzot et. al., (1989)

Table 3

A comparison of human doses of selected hallucinogens with their potency using drug discrimination tests in LSD-trained rats

Drug	Ki $5HT_{2A}$ (nM)	Ki $5HT_{2C}$ (nM)	Drug discrimination ED_{50} (μM/kg)	Potency relative to LSD* (rat drug discrimination)	Human dose (mg)	Potency relative to LSD (human)
EthLAD	—	—	0.02	185	0.04-0.15	140
AllyLAD	—	—	0.013	285	0.08-0.16	110
LSD	2-4	3-6	0.037	100	0.06-0.20	100
ProLAD	—	—	0.037	100	0.10-0.20	90
DOB	0.6	1.3	1.06	2.3	1-3	7
DOI	0.7	2.4	0.28	9.2	1.5-3	6
DOM	19	—	0.89	3.3	3-10	2
Psilocin	15-25	10	1.0	2.6	10-15	1
DMCPA	—	—	0.66	4.5	15-20	0.7
MEM	73	124	12	0.2	20-50	0.4
MMDA-2	—	—	7	0.4	25-50	0.4
Mescaline	550	300	34	0.08	200-400	0.04

(* Where potency of LSD=100. (ED50 of LSD x 100) / (ED50 of compound X) = Potency relative to LSD)

Adapted from Nichols (2004)

to abolish the discriminative cue induced by DOI in rat drug discrimination studies (Schreiber et al., 1994), whereas the selective 5-HT_{2C} antagonist SB 200,646 failed to block the discriminative stimulus effect of DOI (Kennett et al., 1994). Interestingly, in mouse drug discrimination studies, 5-HT_{2C} receptors appear to have a small, but significant, role in mediating the discriminative stimulus induced by DOI (Smith et al., 2003). Furthermore, in human studies, Vollenweider et al. (1998) showed that psychosis-like symptoms induced by psilocybin treatment were blocked by ketanserin, which in humans has 10-30 fold higher affinity for the 5-HT_{2A} receptor over the 5-HT_{2C} receptor, leading the authors to conclude that psilocybin-induced psychosis is due to 5-HT_{2A} receptor activation, independent of dopamine stimulation.

Density and distribution of 5-HT_{2A} receptors have also provided insight into the role of the serotonergic system in various psychiatric disorders. For instance, 5-HT_{2A} receptor density decreases have been demonstrated in patients with autism (McBride et al., 1989), whereas increases in 5-HT_{2A} receptor density have been found in schizophrenic (Arranz et al., 2003), major depressive disorder (Hrdina and Vu, 1993), and suicidal patients (Mann et al., 2001).

Altered 5-HT_{2A} receptor density, observed in major depressive disorder patients, has been shown to be “corrected” by various serotonergic drugs with anti-depressive properties. For instance administration of either tricyclic antidepressants or selective serotonin reuptake inhibitors (SSIRs) leads to a decrease in 5-HT_{2A} receptor density (Yatham et al., 1999; Meyer et al., 2001). Likewise, treatment with the 5-HT_{2A} antagonist nefazodone not only leads to

decreases 5-HT_{2A} receptor density (Eison et al., 1990; Meyer et al., 1999), but it is as efficacious as SSRIs for the treatment of depression (Baldwin et al., 1996).

These data suggests that 5-HT_{2A} receptors are important mediators of the behavioral effects induced by hallucinogenic drugs, as well as the pathogenesis, treatment, and behavioral abnormalities observed in psychiatric disorders.

Role of 5-HT2 receptors in hallucinogenic behavior

Sensorimotor gating

Sensorimotor gating refers to the state-dependent regulation of transmission of sensory information to the motor system. An operational measure of sensorimotor gating is prepulse inbition of the startle response (PPI). PPI is a normal modulation of the startle reflex exhibited by both humans (Graham, 1975) and animals (Hoffman and Ison, 1980). In brief, PPI refers to the normal decrease in a startle response to a sudden stimulus, when that startling stimulus is preceded by a much weaker stimulus ("prepulse"). Patients with schizophrenia exhibit deficits in PPI (Kumari et al., 2000), in which prepulses do not diminish the startle reflex to the extent that they do in non-schizophrenics (Braff et al., 1978). In addition, pharmacological agents with antipsychotic properties have an ability to reverse drug-induced PPI disruptions (Swerdlow et al., 1992). The finding that PPI could be disrupted in rats by NMDA antagonists and certain serotonergic agents, including hallucinogenic drugs, led to an interest in PPI as a

possible model for the sensorimotor gating deficits observed in schizophrenic patients (Markou et al., 1994).

Prepulse inhibition: In the PPI procedure, a rodent is placed in a small chamber or inside a tube where it is exposed to a brief pulse of noise. The test is used to assess the animal's ability to filter environmental information (gating). In the prepulse inhibition of the startle response, a weak acoustic stimulus (prepulse) decreases the reflexive response (startle) produced by a second, more intense, stimulus (the pulse).

DOI and DOB have extensively been shown to disrupt PPI in the rat (Sipes and Geyer, 1994; Johansson et al., 1995; Sipes and Geyer, 1995; Padich et al., 1996; Wadenberg et al., 2001), while the preferential 5-HT_{2C} agonist mCPP showed no effects on PPI, despite producing dose-dependent decreases in startle reactivity (Sipes and Geyer, 1994). The effect of DOI on PPI was shown to be mediated by 5-HT_{2A} receptors as MDL 100907, but not SDZ SER 082 (5-HT_{2C} antagonist) antagonized DOI's effect on PPI (Sipes and Geyer, 1995). It was later found by Sipes and Geyer (1997) that direct infusion of DOI into the ventral pallidum disrupted PPI without having effects on startle reactivity, leading the authors to conclude that 5-HT_{2A} receptors within the ventral pallidum are key players for the modulation of PPI. In addition, LSD-induced disruption of PPI appears to also be mediated by 5-HT_{2A} receptors. In rat PPI experiments, LSD effects were completely reversed by pretreatment with the selective 5-HT_{2A} antagonist MDL 100907. In contrast, pretreatment with antagonists at 5-HT_{2C} (SB

242084), 5-$HT_{2B/2C}$ (SDZ SER 082), 5-HT_{1A} (WAY 100135), and 5-HT_6 receptors (RO 04-6790) all failed to influence LSD-induced disruption of PPI (Ouagazzal et al., 2001). Interestingly, there appear to be species differences in the hallucinogen-induced effects on PPI between rats and mice. 5-HT2 agonists, such as DOM and DOI, do not appear to alter PPI in either inbred or outbred mouse strains (Dulawa and Geyer, 2000), even though such drugs have consistently been shown to disrupt PPI in rats (for review, see Geyer et al., 2001). In addition, 5-HT_{1A} agonists appear to have opposing effects on PPI depending on the species studied as 5-HT_{1A} agonists, including 8-OH DPAT and flesinoxan, impair PPI in rats, but increase PPI in mice (Nanry and Tilson, 1989; Rigdon and Weatherspoon, 1992; Dulawa et al., 1998).

Operant behavior

Operant behavior is characterized by a form of learning in which a spontaneous behavior is followed by a stimulus that changes the probability that the behavior will occur again. Operant drug discrimination is the animal model with the most predictive validity and specificity for investigation of the psychoactive effects of drugs, and it plays an important role in drug discovery and investigations of drug abuse. Drug discrimination has proven to be a useful tool to study the neural mechanisms that mediate the action of hallucinogens.

Drug Discrimination: The drug discrimination method is a way for training experimental subjects to recognize drug effects and to measure the effects in a

precise, reliable and quantitative manner. In this task, there are typically two response options available in an operant chamber. When an animal is pretreated with a drug, one response option (i.e., left lever press) is food (or water) reinforced. The other response option (right lever press) is not reinforced. When the animal is treated with saline (no drug), the reinforcer relation is switched; i.e., right lever presses are reinforced, whereas left lever presses are not. Rodents (like humans) can learn to discriminate drug versus no drug, ultimately pressing the food-associated lever with over 90% accuracy.

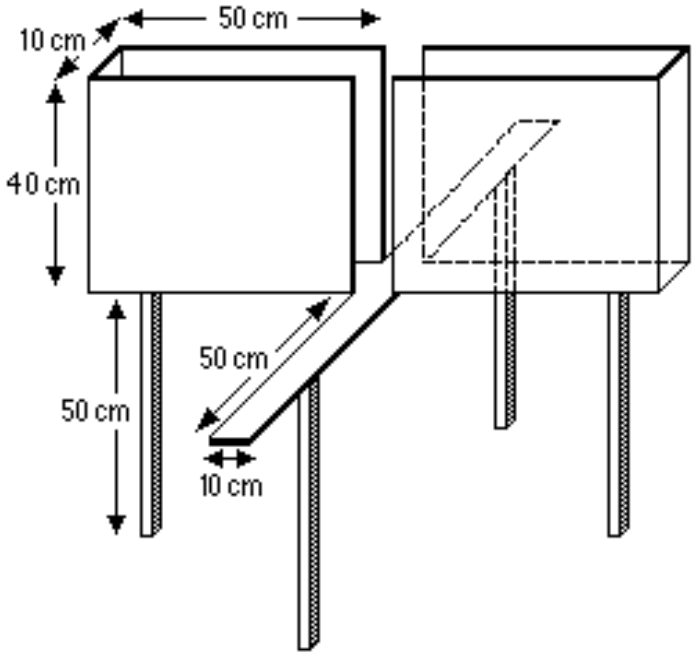
5-HT_{2A} receptors are considered to be key mediators of the discriminative stimulus induced by hallucinogens. Although hallucinogens are shown to activate various serotonin receptors, particularly 5-HT_{2A} and 5-HT_{2C}, behavioral studies using antagonists selective for each receptor subtype have shown that the 5-HT_{2A} receptor is the main mediator of the discriminative stimulus effects of hallucinogenic drugs including LSD, DOI, and DOM (Ismaiel et al., 1993; Schreiber et al., 1994; Smith et al., 1998; Smith et al., 1999). However, it was recently shown by Smith et. al., (2003) that in mouse drug discrimination studies, 5-HT_{2C} receptors appear to have a small, but significant, role in mediating the discriminative stimulus induced by DOI (Smith et al., 2003). Moreover, Benneyworth et.al., (2005) have shown that in mice, the stimulus effects of LSD have both a 5-HT_{2A} and a 5-HT_{1A} receptor component.

Anxiety-like behaviors

The corticolimbic pattern of 5-HT_{2A} receptor distribution in the brain suggests that activation of these receptors may be implicated in the neuropathology, regulation, and treatment of a variety psychiatric disorders including anxiety. DOI, and other selective 5-HT2 agonists, have been shown to effects have been suggested to be 5-HT_{2A} receptor-mediated.

Elevated plus maze: This is a standard test of fear and anxiety in which the animal is placed in the center of an elevated 4-arm maze in which 2 arms are open and 2 are enclosed (Figure 5). The maze is equipped with infrared photobeams, and the rodent's behavior is recorded and analyzed automatically by a computer. In addition to recording other behaviors that may reflect general motor activity, the number of times the animal enters each of the arms and the time spent in each arm is noted.

DOI has been shown to act as an anxiolytic-like drug in the elevated plus maze test with potency comparable to that of benzodiazepines (Onaivi et al., 1995; Nic Dhonnchadha et al., 2003a); 5-HT_{2A} receptors appear to be predominantly involved in the mediation of these effects. Antagonist studies indicate that anxiolytic-like effects in the elevated plus maze are blocked only by selective 5-HT_{2A} receptor antagonists. On the other hand, selective antagonists for 5-HT_{2C} and 5-$HT_{2C/2B}$ receptors did not have an effect on DOI-induced anxiety-like behavior (Nic Dhonnchadha et al., 2003b).



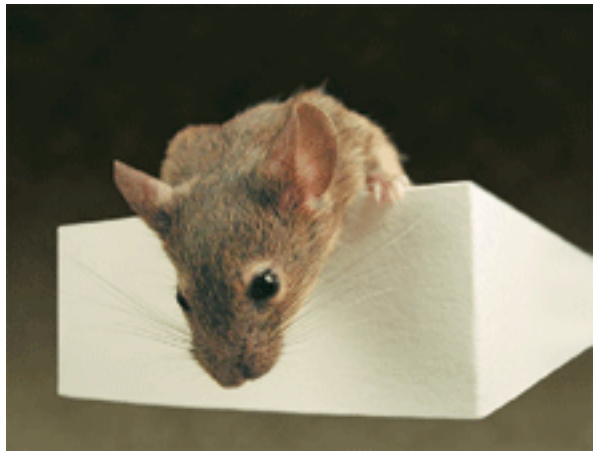


Figure 5: Elevated Plus Maze

The elevated plus maze consists of two open arms and two closed arms emanating from a central platform to form a plus shape. The maze is built from black Plexiglas, and equipped with infrared photobeams. The entire maze is elevated 50 cm above the floor. Light beam breaks are recorded and analyzed automatically by Motor Monitor software

Four Plates Test: The four plates test is an animal model of anxiety in which the exploration of a novel surrounding is suppressed by the delivery of a mild electric foot shock. This test involves the animals' conditioned response to stressful events, and anxiolytic-like activity is reflected by a compound's ability to induce an anti-punishment effect.

DOI has been shown to produce a dose-dependent induction of accepted punished passes in the four plates test. This effect was not observed with preferential 5-HT_{2C} agonists including mCPP and RO 60-0175. Furthermore, the anti-punishment action of DOI was abolished by pretreatment with selective 5-HT_{2A} receptor antagonists, while 5-HT_{2C} and 5-$HT_{2B/2C}$ antagonists failed to have an effect (Nic Dhonnchadha et al., 2003b).

Stereotypical behaviors

Stereotypical behavior or stereotypy is repetitive motor behavior without obvious purpose or function often seen in captive animals, particularly those held in small enclosures with little opportunity to engage in more normal behaviors. Stereotypical behaviors are also observed following treatment with 5-HT2 agonists including certain hallucinogens.

Head-twitch response: The head-twitch response is a stereotypical behavior characterized by a very rapid rotational movement of the head. The drug-elicited head-twitch response is a selective behavioral model for 5-HT2 agonist activity in the rodent, and several previous studies have established that direct and indirect

5-HT agonists induce this effect (Peroutka et al., 1981; Green et al., 1983) (Darmani et al., 1990a; Darmani et al., 1990b; Darmani et al., 1996). Moreover, induction of a head-twitch response in rodents appears to be a common property of hallucinogenic drugs, and studies employing antagonists selective for 5-HT_{2A} (SR 46349B, MDL 100907) and 5-HT_{2C} (SB 200,646A) receptors have shown that DOI-induced head-twitch response is mediated by 5-HT_{2A} receptors (Schreiber et al., 1995; Dursun and Handley, 1996; Dave et al., 2002). This action appears to be mediated by 5-HT_{2A} receptors located in the medial prefrontal cortex. Direct bilateral administration of DOI into the medial prefrontal cortex of rats produces a dose–dependent induction of head-twitches which is blocked by the 5-HT_{2A} antagonist MDL 100907 but not with the 5-$HT_{2C/2B}$ antagonist SDZ SER 082 (Willins and Meltzer, 1997).

Ear-scratch response: A less investigated stereotypical behavior induced by hallucinogens is a rapid scratching movement of the head and/or neck with a hindlimb. This effect, alternatively referred as scratch reflex, scratch-reflex stereotypy or the ear-scratch response, was first observed following mescaline administration by Deagan and Cook (1958). Pharmacological studies to determine the mechanism of action of hallucinogen-induced ear-scratch response are still needed. However, a study from Darmani et al., (1994) suggests that this effect may be due primarily to 5-HT_{2C} receptor activation.

Heterotrimeric G-proteins

Heterotrimeric G-proteins represent an ancient protein family highly conserved throughout evolution. G-proteins tranduce signals from a wide array of G-protein coupled receptors (GPCRs), initiating a plethora of second messenger cascades. Alfred Gilman and Martin Rodbell were awarded the Nobel Prize in Physiology and Medicine in 1994 for their colaborative research on the discovery of G-proteins. G-protein-mediated signal transduction has been widely studied and reviewed (for example, see Birnbaumer, 1992; Neer, 1995; Wettschureck and Offermanns, 2005). Heterotrimeric G-proteins, consisiting of three subunits, Gα, Gβ, and Gγ, act as molecular switches between their active and inactive states in response to guanine nucleotides [exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP)] (Figure 6). To date, multiple isoforms of each subunit have been identified in mammalian cells, 17 α subunits, 5 β subunits and 12 γ subunits (Table 4; Hamm, 1998; Wettschureck and Offermanns, 2005). There are also "small" G proteins or small GTPases, like Ras, that are monomeric, but also bind GTP and GDP and are involved in signal transduction (Macara et al., 1996).

Receptor-activated G-proteins are bound predominantly to the intracellular face of the plasma membrane, where they directly interact with the receptor and with effector components of the signaling pathway. Ligand binding to the GPCR induces a conformation change in the receptor that allows the G-protein to bind to the receptor. The G-protein then releases its bound GDP from the Gα subunit, and binds a new molecule of GTP. GTP binding leads to a conformational

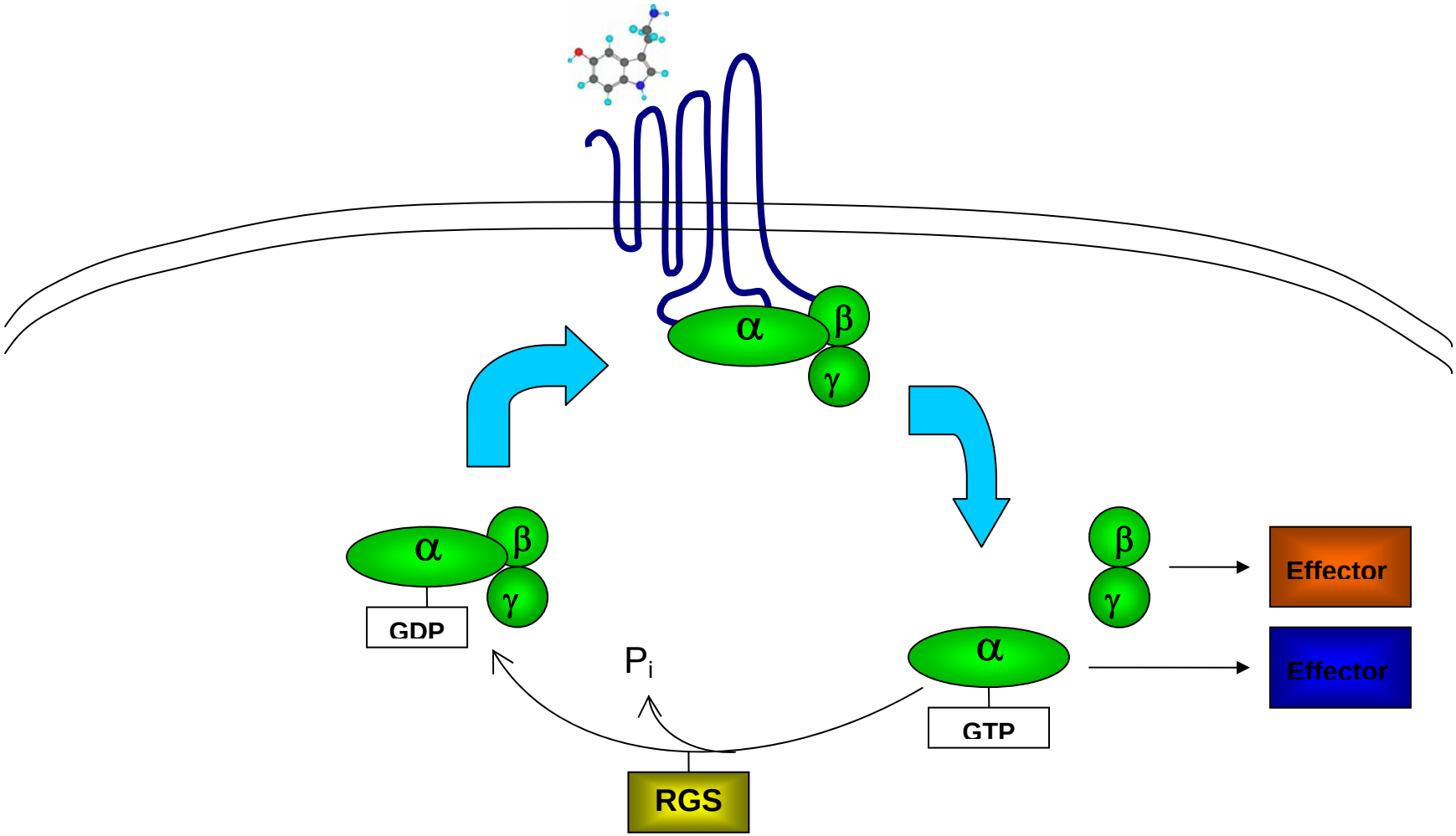


Figure 6: Heterotrimeric G-protein activation cycle
RGS, Regulators of G-proteins; GDP, guanosine diphosphate; GTP, guanosine triphosphate

change in Gα, which decreases its affinity for both the receptor and Gβγ subunits. This exchange triggers the dissociation of the Gα subunit, the Gβγ dimer, and the receptor. In its active state, the receptor has decreased affinity for its agonist, which leads to ligand release. Both Gα-GTP and Gβγ can then activate different effector pathways to modulate different signaling cascades and intracellular processes. The Gα subunit eventually hydrolyzes the attached GTP to GDP by its intrinsic GTPase activity, allowing it to reassociate with Gβγ to start a new cycle. However, by itself, the intrinsic level of GTP hydrolysis by the α subunit is often too slow for the efficient cycling of G-proteins. Another mechanism of GTP hydrolysis is carried out by regulators of G-protein signaling (RGS), which markedly accelerate α subunit GTP hydrolysis thus terminating its signaling, and indirectly terminating Gβγ signaling as well.

GPCRs Structure and Function

All G-protein-coupled receptors share different structural features, including a central core domain consisting of seven transmembrane α-helices connected by three intracellular and three extracellular loops of varying length with an intracellular carboxyl terminus and an extracellular amino terminus. The 5-HT_{2A} receptor belongs to the rhodopsin-like, or Class A, family of GPCRs. Class A GPCRs are characterized by their sequence homology, which includes shared cysteine residues in extracellular groups 1 and 2, a DRY motif in intracellular group 2, and a NPXXY motif in transmembrane 7 (Figure 7; Bockaert and Pin, 1999). While in other types of receptor ligands bind externally

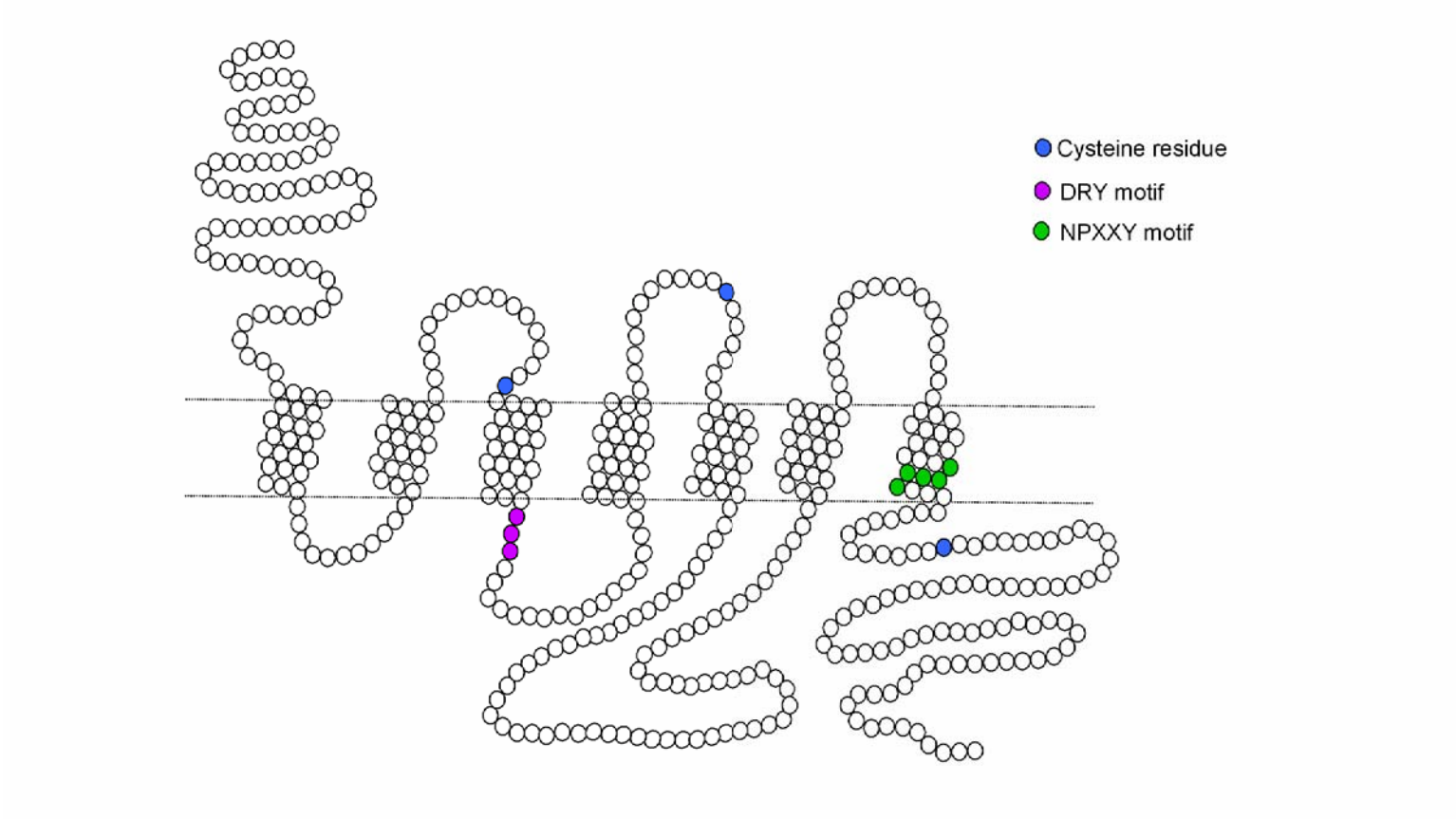


Figure 7: Class A GPCR conserved domains and residues

to the membrane, the ligands of GPCRs typically bind within the transmembrane domain. Once the ligand is recognized, the receptor shifts conformation and thus mechanically activates the G-protein, which detaches from the receptor to induce an intracellular response. When receptors are exposed to ligands for prolonged periods of time, they undergo a receptor-regulation process named desensitization. There are two recognized forms of desensitization: 1. Homologous desensitization, in which the activated GPCR is "turned off" or desensitized and 2. Heterologous desensitization, where the activated GPCR causes the inhibition of a different GPCR to signal. The key reaction of this desensitization is the phosphorylation of the intracellular receptor domain by protein kinases (Ferguson, 2001).

Classification of G-proteins

G-protein classification was historically determined by the functional interaction of the $G\alpha$ subunit with specific effector molecules. Both $G\alpha$ and $G\beta\gamma$ regulate various effectors including $PLC\beta$ (Katz et al., 1992) and adenylyl cyclase isoforms (Tang et al., 1992). Despite this, heterotrimeric G-proteins are classified into four families based on the sequence alignment, biochemical, and functional criteria of their unique α-subunits: $G\alpha_s$, $G\alpha_i/G\alpha_o$, $G\alpha_q/G\alpha_{11}$, and $G\alpha_{12}/G\alpha_{13}$ (Table 4).

The widely-expressed G-proteins of the $G_{i/o}$ family mediate receptor-dependent inhibition of adenylyl cyclase (Sunahara et al., 1996). The function of members of the G_i/G_o family has often been studied using a toxin from

Table 4
G-Protein Families

G-protein family	Subtype	Consequence of Activation	Tissue Distribution	Comments
G_s	$G\alpha_s$	↑ Adenylyl Cyclase	Ubiquitous	Cholera Toxin Activation
	$G\alpha_{olf}$		Brain/ Olfactory	
$G_{i/o}$	$G\alpha_{oA,B}$	To be determined	Brain	Pertussis Toxin inhibition
	$G\alpha_{i1,2,3}$	↓ Adenylyl cyclase ↑ K Channels ↓ Ca2+ channels	Wide	
	$G\alpha_{t1,2}$		Retina	
	$G\alpha_z$	↓ Adenylyl cyclase	Brain/Retina	
G_q	$G\alpha_q$	↑ Phopholipase Cβ	Ubiquitous	
	$G\alpha_{11}$		Ubiquitous	
	$G\alpha_{14}$		Stroma/ Epithelial	
	$G\alpha_{15}$		Myeloid	
	$G\alpha_{16}$		Myeloid	
G_{12}	$G\alpha_{12}$	Rho GTP exchange Catalyst	Ubiquitous	
	$G\alpha_{13}$		Ubiquitous	
Gβ	β1-β4, β6		Ubiquitous	80% homology
	β5			53% homology
Gγ	γ1-γ12		Ubiquitous	

Clostridium botulinum (pertussis toxin; PTX) which is able to ADP-ribosylate most of the members of the $G\alpha_{i/o}$ family close to their carboxy termini, which results in the inhibition of coupling of the G-protein to the receptor. Unlike most G-proteins, members of the G_o family appear to regulate effector activation via $G\beta\gamma$. Whether $G\alpha_o$ can regulate effectors directly is currently not clear (Wettschureck and Offermanns, 2005). A PTX-insensitive member of the $G_{i/o}$ family is the less widely expressed G_z. G_z is mainly expressed in the brain, retina and platelets (Gagnon et al., 1991). Members of the G_s family mediate receptor-dependent adenylyl cyclase activation which results in increases in intracellular cAMP concentration. G_s subunits contain sites for cholera toxin (CTX)-dependent ADP ribosylation, which leads to the inhibition of GTPase activity and reassembly with $G\beta\gamma$ subunits.

The G_{12}/G_{13} family is often activated by receptors that couple to $G_{q/11}$ and are expressed ubiquitously. The study of $G_{12/13}$ signaling has been challenging since, at this time, there are no specific inhibitors of these G-proteins available. Furthermore, receptors coupling to $G_{12/13}$ usually activate other G-proteins as well. Most of the information about the $G_{12/13}$ family has been obtained with the use of constitutively active $G\alpha_{12}$ and $G\alpha_{13}$ mutants. These studies showed that G_{12} and G_{13} induce a variety of signaling pathways linked to the activation of several downstream effectors including phospholipase A_2 (PLA_2), Na^+/H^+ exchanger, and c-*jun* NH_2-terminal kinase (Dhanasekaran and Dermott, 1996; Hooley et al., 1996; Fromm et al., 1997). Members of the $G_{q/11}$ family will be the focus for the rest of this discussion.

$G_{q/11}$ Family

Receptors coupled to members of the $G_{q/11}$ family mediate a wide range of cellular responses including cell growth and proliferation, neuronal signaling, hematopoietic cell differentiation, leukocyte activation, platelet aggregation, glucose secretion, and smooth muscle contraction among other physiological responses. Within this family, $G\alpha_q$ and $G\alpha_{11}$ signaling are the most widely studied. $G\alpha_{14}$ and $G\alpha_{15}$ signaling are less understood; however, based on their common abilities for PLCβ activation, they are generally assumed to be functionally similar to $G\alpha_q$ and $G\alpha_{11}$ (for review, see Hubbard and Hepler, 2006)

Distribution

Members of the $G_{q/11}$ family have very different tissue and cell expression patterns (Table 5). The α-subunits of G_q and G_{11} are ubiquitously distributed among tissues. (Wilkie et al., 1991). They share 88% amino acid sequence identity and are expressed together in almost every cell type (Strathmann and Simon, 1990; Wilkie et al., 1991) with the exception of platelets which selectively express $G\alpha_q$ but not $G\alpha_{11}$ (Milligan et al., 1993; Ushikubi et al., 1994). Quantitative measurements of regional distribution of $G\alpha_q$ and $G\alpha_{11}$ in the rat brain showed that $G\alpha_q$ is more widely expressed than $G\alpha_{11}$ with ratios levels of $G\alpha_q$ to $G\alpha_{11}$ varying from 5:1 to 2:1 (Milligan, 1993). $G\alpha_{14}$, $G\alpha_{15}$, and $G\alpha_{16}$ distribution patterns are more limited. $G\alpha_{14}$ expression has been found in spleen, lung, kidney, pancreas, liver, testis, and bone marrow (Wilkie et al., 1991), whereas $G\alpha_{15}/\alpha_{16}$ expression is found exclusively in tissues rich in hematopoietic

Table 5

Biochemical and Cell Signaling Diversity of $G\alpha_q$ family members

$G\alpha$ property	$G\alpha_q$	$G\alpha_{11}$	$G\alpha_{14}$	$G\alpha_{15/16}$
Effector coupling	PLCβ	PLCβ	PLCβ	PLCβ
Receptor coupling	Selective	Selective	Limited selectivity	Non-Selective
Tissue distribution	Ubiquitous	Ubiquitous	Kidney,liver, lung	Hematopoietic cells
Amino acid sequence identity with $G\alpha_q$	100%	90%	80%	57%
N-terminal AA sequence identity (first 40 AA)	100%	83%	65%	35%

cells (Amatruda et al., 1991; Wilkie et al., 1991; Tenailleau et al., 1997). Since the $G_{q/11}$ signaling pathway is the classical pathway associated with 5-HT_{2A} receptor signaling, I will focus on these G-proteins for the remainder of this discussion.

$G_{q/11}$ signaling pathways

The G_q/G_{11} family of G-proteins couples membrane receptors to isoforms of phospholipase Cβ (for review, see Exton, 1996; Rhee, 2001). Receptors that are able to couple to the G_q/G_{11} family do not appear to discriminate between $G\alpha_q$ and $G\alpha_{11}$ (Wange et al., 1991; Wu et al., 1993; Offermanns et al., 1994; Xu et al., 1998). In general, receptors that activate inositol lipid signaling and calcium release in a PTX-insensitive manner are considered to be linked to $G\alpha_q$ family members.

As illustrated in figure 8, activated $G\alpha_q$ family members stimulate all four isoforms of PLCβ (β1-β4) to hydrolyze phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) into inositol trisphosphate (IP_3) and diacylglycerol (DAG) (Rhee, 2001). IP_3 binds IP_3 receptors and promotes calcium release from intracellular calcium stores, while DAG activates protein kinase C (PKC) enzymes. In addition, several studies have linked G_q activation to the mitogen-activated protein kinase (MAPK) cascade in cultured cells, although it is believed that this occurs downstream from PLC activation (Buhl et al., 1995; Wan et al., 1996).

$G\alpha_q$ family members have been shown to not only regulate kinase pathways, but they themselves are regulated by phosphorylation. Both $G\alpha_q$ and

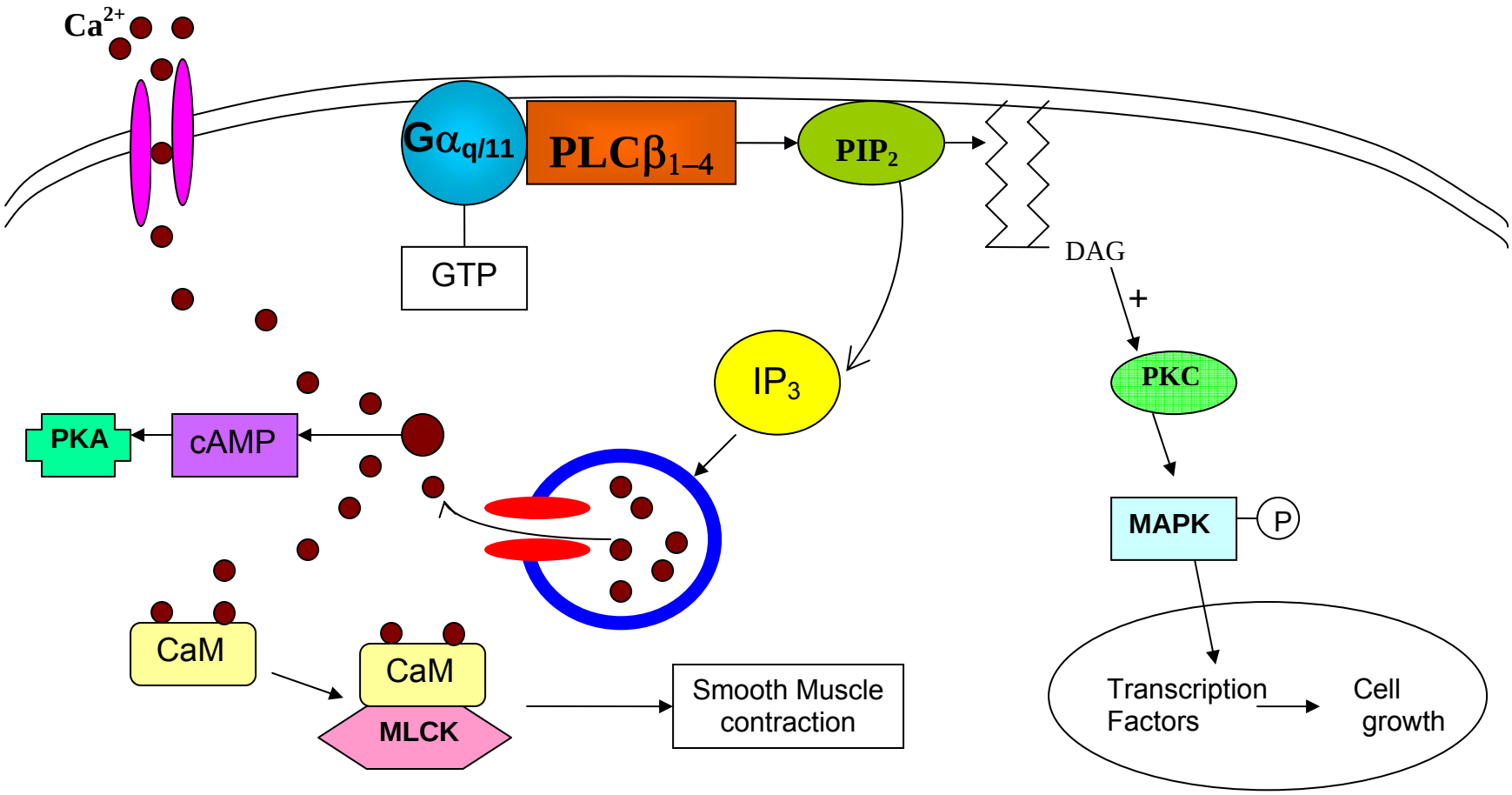


Figure 8: G_q signaling pathway

PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacyl glycerol; IP_3, inositol 1,4,5-trisphosphate; PKC, protein kinase C; MAPK, mitogen-activated protein Kinase; cAMP cyclic adenosine monophosphate; PKA, protein Kinase A; CaM, calmodulin-dependent protein; MLCK, myosin light-chain kinase

$G\alpha_{11}$ are tyrosine phosphorylated at Y356 *in vitro* and *in vivo* (Umemori et al., 1997). This event appears to be necessary for full PLC stimulation by $G\alpha_q$ and $G\alpha_{11}$ following carbachol treatment, but is also tightly regulated by phosphatases. Moreover, various studies indicate that $G\alpha_q$ negatively affects phosphatidylinositol 3-kinase (PI3K) and its downstream effector Akt, which areimportant regulators of cell survival signaling pathways and growth response mechanisms (Hampoelz and Knoblich, 2004). In addition, apoptosis of cardiomyocytes has been associated with $G\alpha_q$-linked inhibition of the PI3K/Akt pathway (Hartmann et al., 2004)

Genetic manipulations for $G\alpha_q$ and $G\alpha_{11}$

Early genetic investigations studying $G\alpha_{q/11}$-dependent physiology with knockouts were characterized by embryonic lethality. Double homozygous mice with mutations for $G\alpha_q$ and $G\alpha_{11}$ die at embryonic day 10.5 due to cardiomyocyte hypoplasia, and mice with one intact allele of either of the two genes die shortly after birth due to cardiac malformation (Offermanns et al., 1998). However, single gene knockouts have been proven to be successful alternatives for studying the physiological roles of G_q and G_{11} *in vivo*.

Selective disruption of $G\alpha_q$ leads to the most pronounced phenotype, and their generation is depicted in figure 9. In brief, a targeting vector containing a 10.4-kb $G\alpha_q$ genomic DNA fragment disrupted by a neomycin phototranferase gene was constructed. Three independently targeted embryonic stem cells generated by homologous recombination carried the targeted mutation of the

$G\alpha_q$ gene through the germ line. Western blot analyses from whole brain and cellular membranes demonstrated that there was no detectable $G\alpha_q$ protein in homozygous mutant [$G\alpha_q$(-/-)] mice.

Mice heterozygous for the $G\alpha_q$ mutation [$G\alpha_q$(+/-)] appear normal and show no abnormalities over a 20-month period. On the other hand, homozygous knockout mice have increased bleeding and deficits in platelet aggregation. $G\alpha_q$(-/-) mice show increased mortality during the first postnatal day, and the mortality appears to result from internal bleeding occurring during birth trauma (60-70% of homozygous mutants survive the postnatal period) (Offermanns et al., 1997c). Mice lacking $G\alpha_q$ exhibit defective lung function due to a loss of response to allergen changes (Borchers et al., 2003). These animals also exhibit slowed growth rates possibly due to impaired $G\alpha_q$-dependent signaling in the hypothalamic/pituitary axis (Wettschureck et al., 2005). These symptoms are less pronounced in the mixed C57BL6/129Sv background (Melvin Simon, personal communication)

$G\alpha_q$ knockout mice display awkward jerky movements and loss of balance during rearing. They also exhibit signs of ataxia with typical wobbling and tottering steps. Mutant mice cannot walk in a straight line and tend to drag their feet. Despite motor coordination and signs of ataxia, $G\alpha_q$ knockouts showed no obvious morphological defects in their peripheral and central nervous system. In addition, extensive examination of the cerebral cortex by histological, immunohistochemical, and electron microscopic techniques indicated that

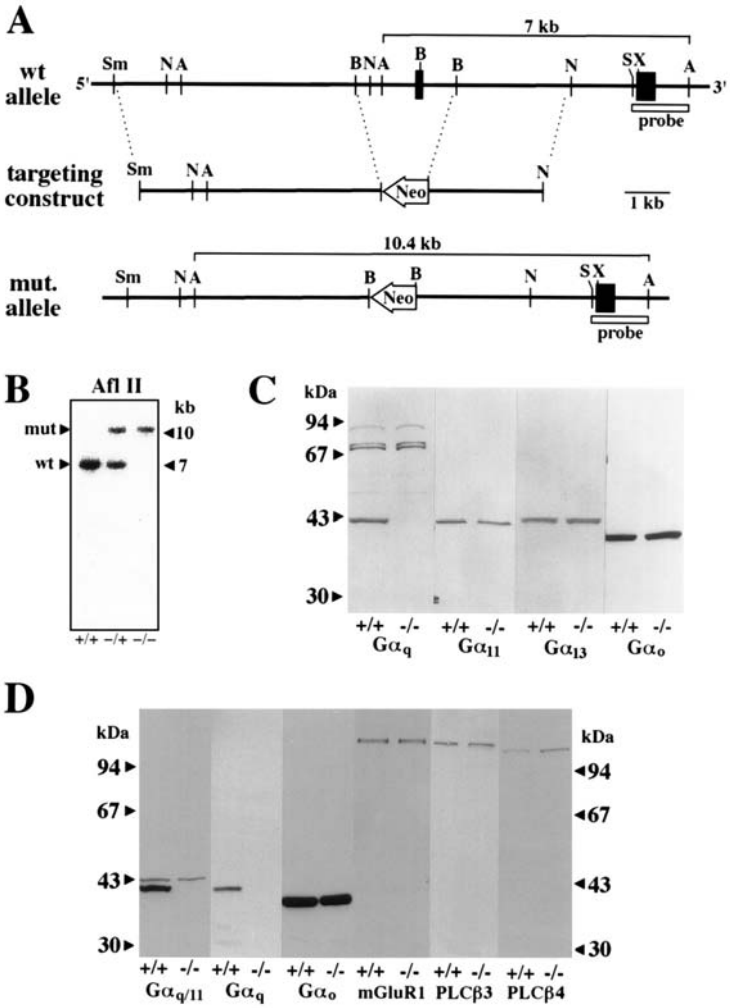
deletion of the $G\alpha_q$ gene does not affect gross development of cerebellar anatomy, cell production, and cytodifferentiation (Offermanns et al., 1997a).

Mice genetically deficient in $G\alpha_{11}$ exhibit normal growth characteristics and do not display any apparent physiological deficiencies (Offermanns et al., 1998). On the other hand, mice lacking $G\alpha_q$ and one of the $G\alpha_{11}$ alleles display craniofacial defects. These defects were absent in $G\alpha_{11}$ knockouts with one intact allele for $G\alpha_q$, indicating that the craniofacial phenotype is a result of selective loss of $G\alpha_q$ (Offermanns et al., 1998). In addition, Wettschureck and colleagues (2004) showed that selective disruption of G_q and G_{11} in the forebrain disrupts maternal behavior in mice with absent or markedly blunted nest building, pup retrieving, crouching, and nursing. However, their olfaction, motor behavior, and mammary gland function remained undisturbed leading the authors to conclude that heterotrimeric G proteins of the $G_{q/11}$ family are indispensable for the neuronal circuit that connects the perception of neonates to the activation of the medial proptic area, which in turn controls maternal behavior.

These studies indicate that genetic deletion of a single $G\alpha$ of the $G_{q/11}$ family often leads to targeted rather than global physiological changes in adult animals. This may be reflective of the ability of G_q family members to functionally compensate for one another in cells where they are naturally coexpressed.

Figure 9

Generation of $G\alpha_q$ knockout mice



(Offermanns et.al., 1997)

Targeted disruption of the murine $G\alpha_q$ gene. (*A*) Part of the wild-type $G\alpha_q$ locus containing the two last exons (wt allele), the targeting construct, and the targeted locus (mut. allele) are shown. Neo, neomycin resistance gene. The sizes of the *Afl*II fragments predicted to hybridize to the indicated diagnostic probe are shown. Restriction endonucleases: A, *Afl*II; B, *Bgl*II; N, *Nde*I; S, *Sac*I; Sm, *Sma*I; and X, *Xho*I. (*B*) Southern blot analysis of *Afl*II-digested genomic DNA from wild-type (+/+), hemizygous (−/+), and homozygous mutant mice (−/−) with the diagnostic probe indicated in *A*. (*C*) Western blot analysis of whole brain cholate extracts from wild-type (+/+) and homozygous $G\alpha_q$ mutant mice (−/−) with antibodies recognizing the α-subunits of G_q ($G\alpha_q$), G_{11} ($G\alpha_{11}$), G_{13} ($G\alpha_{13}$), and G_o ($G\alpha_o$). (*D*) Western blot analysis of cerebellar membrane fractions from wild-type (+/+) and homozygous $G\alpha_q$ mutant animals (−/−) with antibodies recognizing the α-subunits of G_q and G_{11} ($G\alpha_q$/$G\alpha_{11}$), G_o ($G\alpha_o$), type 1 metabotropic glutamate receptor (mGluR1), phospholipase C-β3 (PLC-β3), and phospholipase C-β4 (PLC-β4).

Specific Aims

5-HT_{2A} receptors are known to be a key site of action for hallucinogenic drug action in both humans and laboratory animals. There is now vast evidence from biochemical, electrophysiological, and behavioral studies that hallucinogens, such as LSD and DOI, have a key site of action as agonists at 5-HT_{2A} receptors in the brain. 5-HT_{2A} receptors are known to couple to various G-proteins to mediate a wide array of second messenger signaling pathways; however, the classical pathway associated with 5-HT_{2A} receptor signaling is stimulation of PLCβ via Gα_q protein.

The precise signal transduction pathway involved in the *in vivo* effects of hallucinogenic drugs is not known. The objective of the present study is to evaluate the role of G_q proteins in hallucinogen-induced biochemical and behavioral effects. As a tool to assess the role of the G_q signaling pathway, we have utilized of a lentiviral delivery system of G_q blocking peptides as well as genetically modified mice in which the gene encoding for the α-subunit of G_q protein has been eliminated [Gα_q(-/-)].

The following specific aims were formulated to better understand the role of Gα_q protein in hallucinogenic drug action.

1. To evaluate the role of G_q in hallucinogen-induced biochemical assays
2. To evaluate the role of G_q in hallucinogen-induced behaviors
3. To establish a viral gene transfer system to block G_q-signaling

CHAPTER II

MANIPULATION OF 5-HT_{2A} SIGNALING BY LENTIVIRAL DELIVERY OF G_q BLOCKING PEPTIDES

Introduction

Many scientific advances have taken place with the advent of knockout mice; however, there still are many disadvantages and limitations to this approach. One of the main concerns with knockout technology is that a gene is deleted from the earliest moments of embryonic development, which may compromise the animal's survival even to later embryonic stages. Moreover, many of the phenotypic outcomes observed in knockout animals may be related to developmental deficits. In other words, the absence of important signaling molecules might have a critical role in development that is different from their role in adult plasticity; consequently, the deletion may interfere with further analyses and behavioral experimentation (Chapman, 2002). In order to address these issues, Alcino Silva's group made use of an innovative pharmacogenetic approach, which takes advantage of the synergism between pharmacological and genetic manipulations (Ohno et al., 2001). By combining a heterozygous genotype that does not itself produce a significant phenotype, with the application of a subthreshold concentration of a drug that acts upstream or downstream of the knockout gene, it is possible to create inducible manipulations with interesting and interpretable outcomes (Chapman, 2002). This approach integrates the high

temporal specificity of pharmacological manipulations with the molecular specificity of genetic disruptions.

Recent behavioral data obtained in our laboratory suggest that the anterior cingulate cortex is a key region for LSD discrimination (Gresch et al., 2006). These studies showed that direct microinjection of LSD into the anterior cingulate cortex reproduces the effects of systemic LSD administration in drug discrimination studies. Additionally, MDL 100907 injected into the anterior cingulate cortex blocks systemic LSD (Figure 10). Based on these findings, we have concluded that the anterior cingulate cortex is a crucial site in the neuronal pathway mediating LSD discrimination. Therefore, *in vivo* studies of the lentiviral delivery system targeted the anterior cingulate cortex, where 5-HT_{2A} receptors, expressed on pyramidal neurons, have been implicated in hallucinogenic drug action as well as in schizophrenia (Selemon et al., 1995; Willins et al., 1997; Jakab and Goldman-Rakic, 1998).

Viral-mediated gene transfer is a powerful way to deliver molecules to the CNS in a site-specific manner (Elliott and O'Hare, 1997; Neve et al., 1997; Palfi et al., 2002). I used a modification of the inducible pharmacogenetic approach, with a lentiviral-mediated gene transfer system as the “pharmacological” treatment, in mice heterozygous for the $G\alpha_q$ gene [$G\alpha_q$(+/-)]. To evaluate the effects of local manipulation of G_q levels in biochemical and behavioral assays, we used a recombinant replication-deficient HIV-1-based vector containing a minigene encoding the last 11 amino acids (LQLNLKEYNLV) of the carboxy terminus of the $G\alpha$ subunit of $G_{q/11}$ (G_qCT).

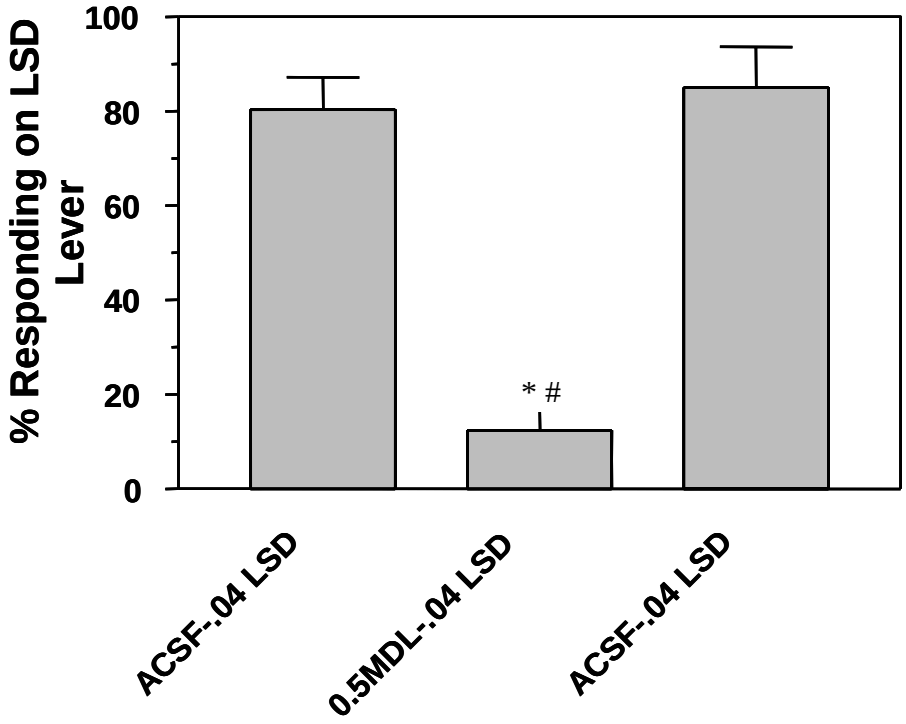


Figure 10: Intracerebral microinjections of MDL 100907 followed by systemic LSD

Rats were trained to discriminate 0.085 mg/kg LSD s.c. from saline. Following acquisition, bilateral canulae were implanted into the anterior cingulate cortex. Intra-ACC injection of M100907 blocks systemic LSD. The 5-HT_{2A} receptor antagonist, M100907 (M)(0.5 µg/µl/side) was injected bilaterally into the ACC 20 mins prior to systemically administered LSD (0.04 mg.kg s.c.). Intra-ACC injection ofM100907 significantly reduced the ability of rats to discriminate LSD, given systemically. * = $p < 0.001$ compared to intracerebral (ic) ACSF- sc LSD; # = $p < 0.001$ compared to retest ic ACSF- sc LSD; (n = 4-5).

Extensive evidence shows that peptides mimicking the last eleven amino acids of the Gα-carboxy terminus selectively block signal transduction activation mediated by a given G-protein. (Table 6; Gilchrist et al., 1999). The G_qCT peptide has been shown to effectively and specifically disrupt receptor coupling to the G_q heterotrimeric protein (Figure 11 and 12; Gilchrist et al., 1999; Chang et al., 2000b).

My proposed modification of the inducible pharmacogenetic approach, combining microinjection of a viral gene delivery system and mice hetereozygous for $G\alpha_q$, provides not only temporal but anatomical specificity. The objective of this study was to determine the biochemical and behavioral consequences following manipulations of $G_{q/11}$ signaling in mature mice in a site-specific manner.

Materials and Methods

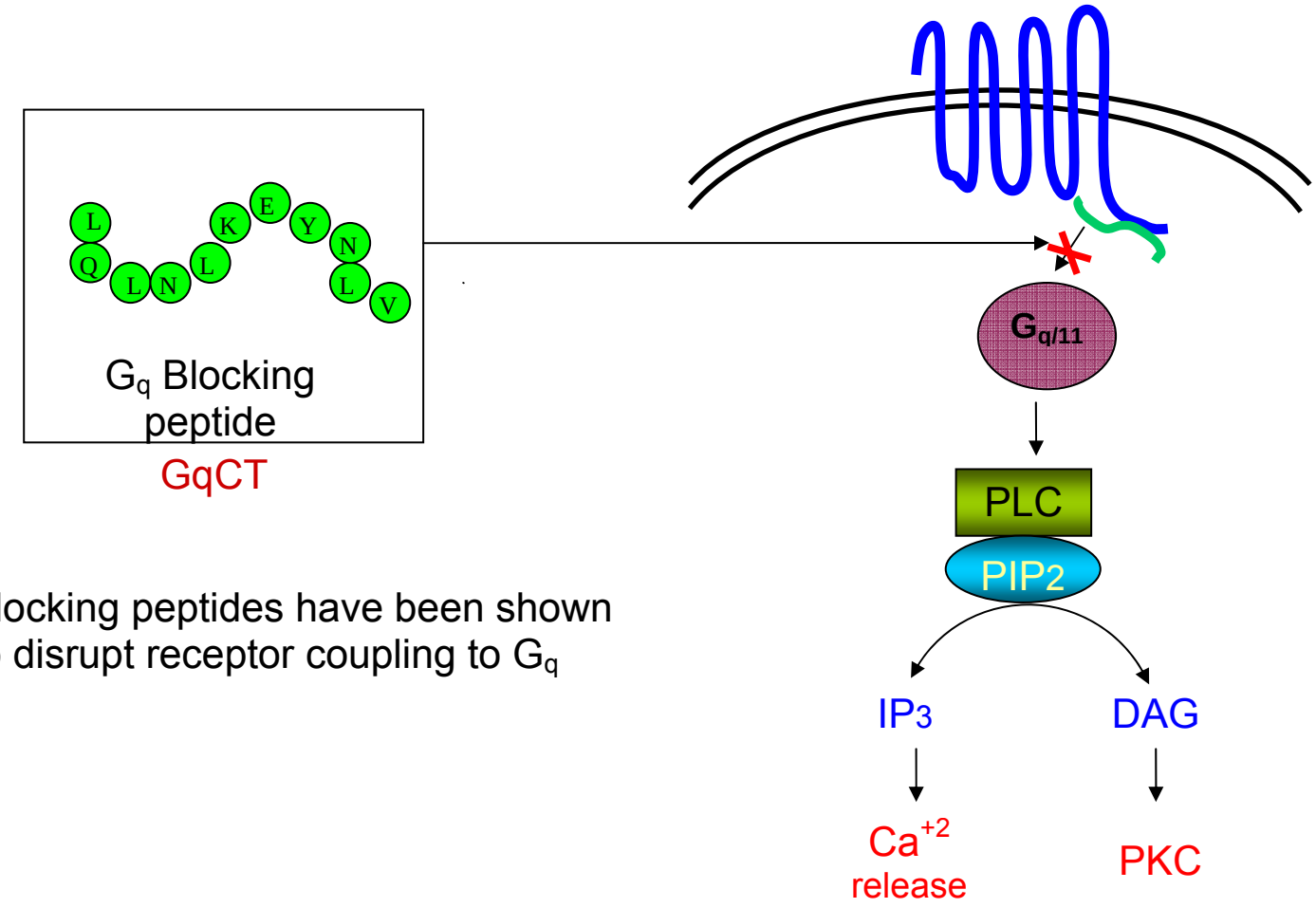
The Lentivirus Construct

Virus was produced in HEK 293T cells by transient transfection. For safety reasons, the putative packaging signaling of the HIV-1 virus (ψ), necessary for viral RNA packaging, was deleted; and a three-plasmid cotransfection strategy was used for vector production to reduce the potential risk of wild type recombination (Reiser et al., 1996)

Table 6

Carboxy Terminal Amino Acid Sequence of G-proteins

G-protein	Amino Acid Sequence										
α_t	I	K	E	N	L	K	D	C	G	L	F
$\alpha_{i1/2}$	I	K	N	N	L	K	D	C	G	L	F
α_{i3}	I	A	N	N	L	K	E	C	G	L	F
α_{o1}	I	A	N	N	L	R	G	C	G	L	Y
α_{o2}	I	A	K	N	L	R	G	C	G	L	Y
α_z	I	Q	N	N	L	K	Y	I	G	L	C
$\alpha_{q/11}$	L	Q	L	N	L	K	E	Y	N	L	V
α_{12}	L	Q	E	N	L	K	D	I	M	L	Q
α_{13}	L	H	D	N	L	K	Q	L	M	L	Q
α_{14}	L	Q	L	N	L	R	E	F	N	L	V
$\alpha_{15/16}$	L	A	R	Y	L	D	E	I	N	L	L
α_s	Q	R	M	H	L	R	Q	Y	E	L	L



G_q blocking peptides have been shown to disrupt receptor coupling to G_q

Figure 11: Inactivation of G_q signaling pathway by G_q blocking peptides

G_q blocking peptides (LQLNLKEYNLV) competitive bind the receptor preventing G_q protein activation thus inhibiting downstream signaling

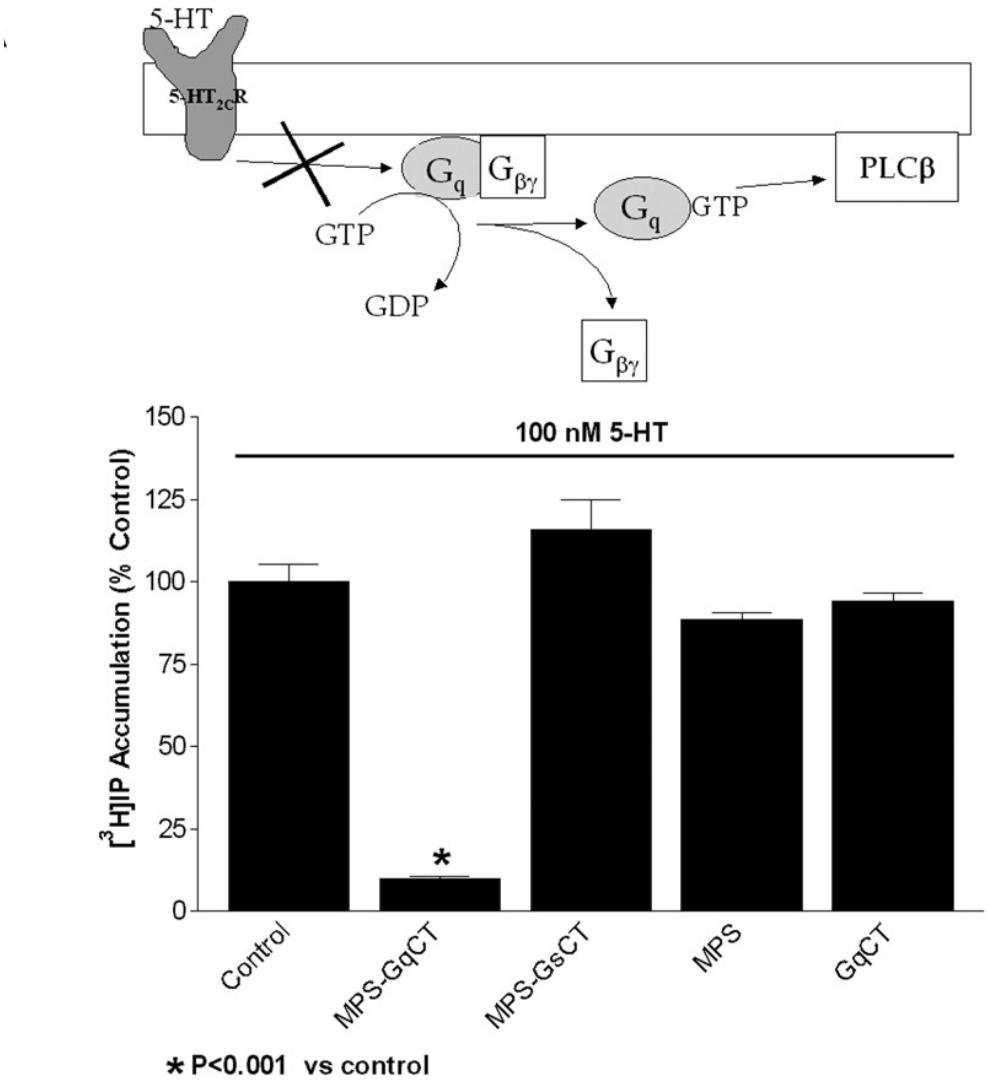


Figure 12: Effect of G_q carboxyl-terminal (MPS-G_qCT) Peptide.
Primary cultured CPE cells were stimulated with 100 nM 5-HT and assayed for PI hydrolysis in the absence or presence of blocking peptides. Schematic illustrating the designated point of blockade by MPS-GqCT peptide. MPS-GqCT inhibits PI hydrolysis in cultured CPE cells as compared with the untreated control. Individual responses were normalized to the average control value corresponding to that particular experiment and are plotted as mean ± S.E. Statistical analyses were performed using one-way analysis of variance (ANOVA) with a nonparametric TUKEY test.

These vector constructs were developed in collaboration with Dr. Zhennan Lai at the National Institutes of Health. The three-plasmid design (Figure 13; Reiser et al., 2000) consists of a helper (packaging), which encodes for *gag, pol*, and the poly-A site necessary for transduction. The transducing vector, which carries the minigene encoding for G_qCT. Transgene expression was driven by a neuron-specific enolase (NSE) promoter, which has been shown to be more effective in driving gene expression in the brain than the CMV promoter (Lai and Brady, 2002). This serves to increase efficiency and potency of gene delivery. The vector also carries an internal ribosomal entry site (IRES), which serves to drive expression of the enhanced green fluorescent protein (EGFP). Two regulatory genes *tat* and *rev*; mouse heat-stable antigen, and long terminal repeats (LTR) are also part of this construct and are essential for transducing vector function. Finally, the third component of the three plasmid design is an env expression construct encoding the vesicular stomatitis virus G protein (VSV-G). VSV-G recognizes a phospholipid that is present on all cell types, and thus can theoretically allow the efficient infection of any mitotic or non-mitotic cell. Its expression is driven by the HIV-1 LTR. In addition, a recombinant lentivirus expressing the herpes simplex virus type 1 tegument protein VP22 in tandem with G_qCT was also be generated. VP22 has been shown to exhibit a unique property of increasing intercellular spread. The protein is secreted by cells and imported into the nuclei and cytoplasm of adjacent cells (Lai et al., 2000).

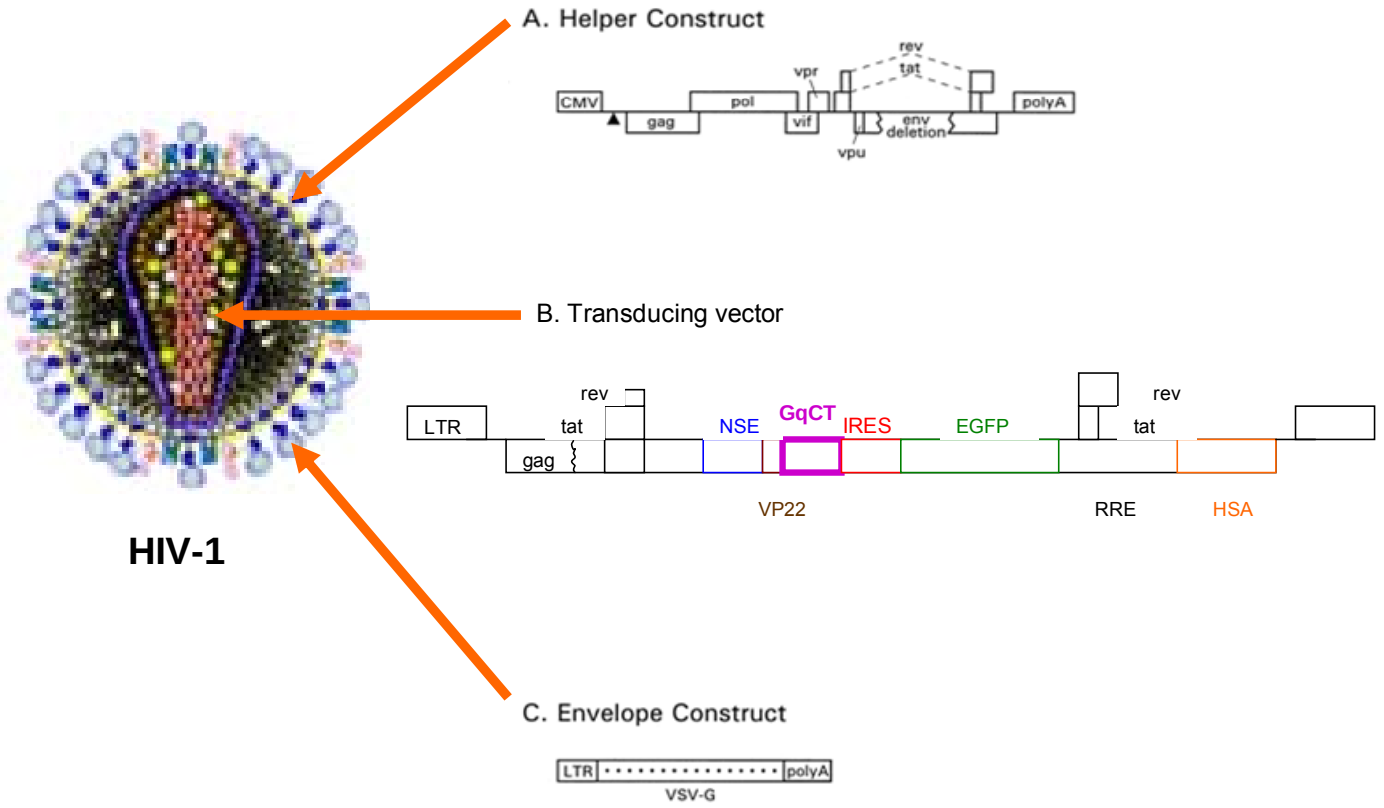


Figure 13: Recombinant HIV-1 lentiviral construct: Three plasmid design

A. Helper construct encodes for the viral outer capsid B. Tranducing vector contains the GqCT carxoxy-terminus blocking peptide and the enhanced green fluorescent protein (EGFP) reporter. Their expression is driven by the nuclear-specific enolase promoter (NSE). The internal ribosomal entry site (IRES) sequence was inserted to allow for the co-expression of individual proteins rather than a tagged GqCT protein. Mouse heat-stable antigen protein (HSA) is another reporter protein used to visualize transducing vector expression. C. Envelope construct encodes for the vesicular stomatitis virus G protein (VSV-G)

Transfection

Cells were plated the day prior to the transfection experiment in 12-well plates. Once cells reached 60-80% confluency, a 3:1 ratio of fugene-6 to DNA (transducing vector) was mixed in serum-free Opti-MEM media (Fugene-6 was added to the media prior to adding the DNA). Following a 20 min incubation period, 20 μl of Fugene-6/DNA solution was added dropwise onto the media in each well. Cells were then assayed 24-48 hours post-treatment.

Lentivirus Production

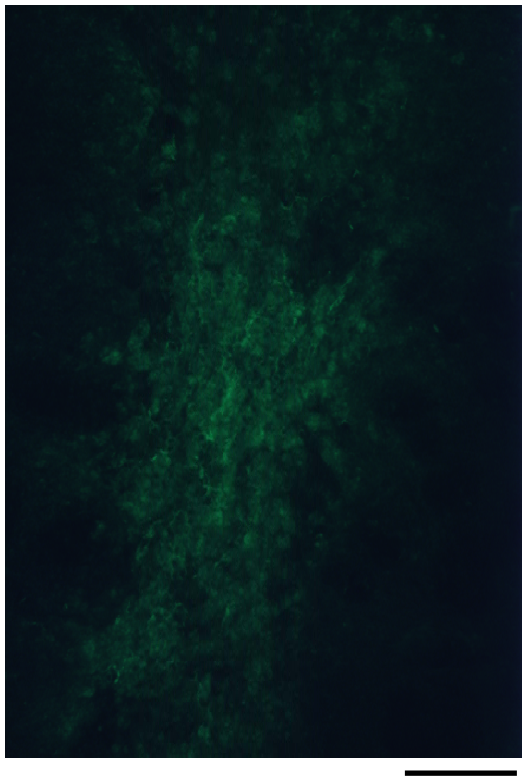
HIV-1 pseudotype helper plasmid DNA (4 μg), Env plasmid DNA (4 μg) and vector plasmid DNA (4 μg) were transfected into sub-confluent 293T cells using lipofectamine 2000 reagent (Invitrogen. Carlsbad, CA). Approximately $4x10^7$ cells were seeded into 10 cm petri-dishes 18-24 hours prior to transfection. The next day, medium containing the DNA-lipofectamine 2000 complexes were removed, and replaced by DMEM containing 10% FBS, 2mM L-glutamine, 0.1 mM MEM non-essential amino acids, and 1% penicillin/streptomycin. The virus stocks were harvested 48 hours post transfection and filtered through a 0.45 μm filter. Further vector concentration was achieved by centrifugation at 250,000 rpm in a Ti-55 Beckman rotor for 2 hours. After centrifugation, the pellet was resuspended in 60 μL of PBS.

Stereotaxic Microinjections of the Virus

The HIV-1-G_qCT construct was stereotaxically microinjected unilaterally into the anterior cingulate cortex (Figure 14). Mice were anesthetized with 80-100 mg/kg ketamine plus 10mg/kg xylacine and placed in a stereotaxic device for placement of the injection needle. The concentrated virus, in a volume of 3 μL, was injected at a rate of 0.5 μL/min), and the needle was left in place for an additional 5 mins. The skin was then closed, sutured, and animals were monitored until they recovered spontaneous movement. Animals were maintained in home cages, usually one to three week after surgery.

Phosphoinositide Hydrolysis

G_qCT expressing cells plated in 24 well plates were incubated with DMEM (-inositol) and ^{3}H-myoinositol (1μCi/mL) approximately 16 hours prior to assay. Following incubation, in order to prevent further metabolism of IP, 10mM lithium chloride and 10mM pargyline were added to each well. After 15 min, agonist was added. Incubations were continued for 30 min. Reactions were stopped by removal of media, and cells were fixed with methanol. Cells were solubilized with EBB/trypsin buffer and then sonicated for 5 seconds. Inositol phosphates were separated from other cellular components by organic extraction and centrifugation. [^{3}H] inositol monophosphates were isolated by anion exchange chromatography as previously described (Barker et al., 1994). 750 μL of the aqueous layer was removed from each extract and placed in columns. These were washed twice with 5mM myo-inositol to elute free inositol; and 5mL of



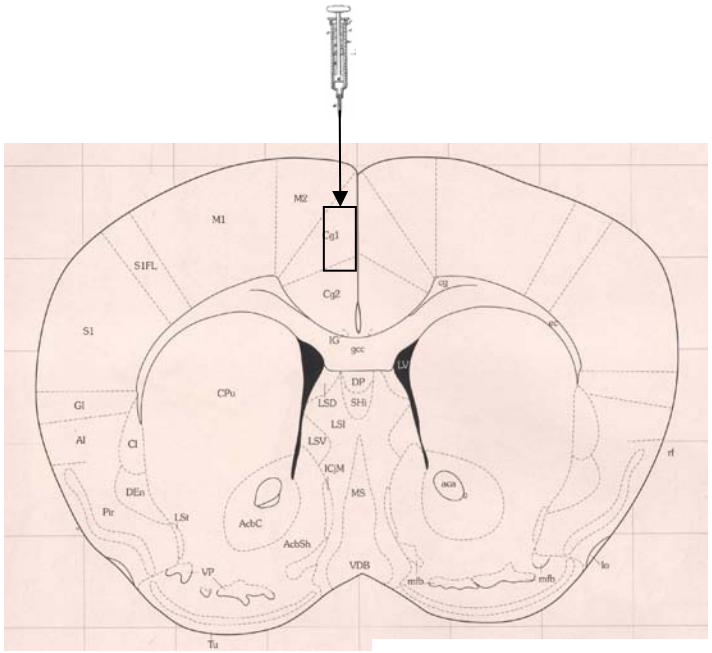


Figure 14: Lentiviral vector injection into the rat anterior cingulate cortex
Brain section shows a large number of EGFP positive cells transduced by (HIV1)-NSE-G_qCT-IRES-EGFP (Scale 60μm). Coordinates AP : +1.30, MD: 0.5, DV: -1.5

sodium tetraborate/60 mM amonium formate to elute glyceroinositol phosphate. Finally, [^{3}H] inositol monophosphate was eluted with 200 mM ammonium formate/0.1 formic acid and then counted in a liquid scintillation counter. Data was then analyzed using GraphPad Prism 3.0 software.

Virus titering

20,000 NIH 3T3 cells were seeded in a 24 well-plate on the day prior to infection. On day two, virus was diluted in serial dilutions in 300μl of DMEM media with 5μg/ml polybrene according to its p24 value and added to the cells overnight. Following incubation, fresh medium was added, and 48 hours after inoculation, cells were washed with 1X PBS, detached with trypsin, and fixed with 4% paraformaldehyde. The number of infected (GFP-positive) cells was then determined by fluorescence activated cell sorting (FACS)

Hematoxylin & Eosin Staining

Fixed coronal sections were cut at 40μm thickness on a vibratome. Sections were re-hydrated in two changes of absolute alcohol for 5 min, and then dipped into 95% and 70% ethanol solutions for 2 min each. Following re-hydration, sections were briefly washed in distilled water and stained in Harris hematoxylin solution (Sigma. St. Louis, MO) for 8 min. Sections were then washed by 5 min exposure to water, briefly submerged in 1% acid alcohol for 30 sec prior to treatment with saturated lithium carbonate for 1 min. Following a 5 min water wash and 10 dips in 95% ethanol, sections were counterstained in

eosin Y solution (Sigma. St. Louis, MO) for 1 min. Sections were then washed, mounted on slides, dehydrated through a series of increasing ethanol concentrations, treated with xylenes, and coverslipped with Mounting Medium (Richard-Allan Scientific. Kalamazoo, MI)

GFAP Immunohistochemistry

Mice were anesthetized with 150 mg/kg pentobarbital i.p. before transcardiac perfusion with 30 ml of 0.1M phosphate-buffered saline (PBS) followed by 30 ml of 4% paraformaldehyde in 0.1M PBS. Brains were removed immediately, post-fixed in paraformaldehyde overnight at 4^{o}C, and then transferred to increasing concentrations of sucrose. Coronal sections were cut at 40μm thickness on a vibratome and collected into buffer containing 30% ethylene glycol, 30% glycerol, 10% 0.1M PBS, and 30% water. Immunohistochemistry was performed on free floating sections. Sections were preincubated for 30 min in 0.3% hydrogen peroxide, washed 3 times with PBS, and then incubated for one hour in 5% goat serum/2% Triton X-100 to block non-specific binding. Sections were incubated for 48h at 4^{o}C in anti-GFAP primary antibody (Chemicon. Temecula, CA) diluted in blocking solution 1:2000. Following 3 washes in PBS, a Vectastain Elite ABC horseradish peroxidase kit (Vector Labs) was used for the secondary antibody and avidin–biotin complex steps. The colorimetric detection reaction produced 3-3'-diaminobenzidine tetrahydrochloride (DAB) as a brown chromagen product. Sections were then washed, mounted on slides, dehydrated through a series of solution with

increasing ethanol concentrations, treated with xylenes, and coverslipped with Mounting Medium (Richard-Allan Scientific. Kalamazoo, MI)

NeuN Immunohistochemistry

Brains were fixed and sliced on a vivatrome at 40μM thickness. Immunohistochemistry was performed in free floating sections. Sections were washed 3 times with 1μM PBS, and then incubated for one hour in blocking solution. Sections were then incubated overnight at 4^{o}C in NeuN (Chemicon. Temecula, CA) primary antibody diluted 1:2000 in blocking solution. Following 3 washes with PBS, sections were incubated in the dark at room temperature with cy-3 secondary antibody (Jackson Immunoresearch. West Groove, PA) for 2 hours. Sections were then washed 3 times with PBS, placed on slides, and allowed to air-dy.

Statistical Analyses

All data are presented as mean ± S.E.M. The effects of G_qCT expression on phosphoinositide hydrolysis were analyzed by a one-way ANOVA followed by Tukey's multiple comparison tests.

Results and Discussion

Lentiviruses have been shown to be a powerful way to deliver molecules to the brain in a site specific manner. In the present study, we used a replication-deficient HIV-1 virus to deliver $G_{q/11}$ blocking peptides into the anterior cingulate cortex of $G\alpha_q$(+/-) mice. This modification of the inducible pharmacogenetic approach (Ohno et al., 2001) combines the spatial and temporal specificity of a lentiviral construct (as the "drug treatment") with the molecular specificity of genetic disruptions [$G\alpha_q$ (+/-)].

Phosphoinositide (PI) hydrolysis assays were performed in cultured cells as an initial *in vitro* validation of the efficacy of the HIV-1-CMV-G_qCT-EGFP construct. When infecting cells in culture, it was challenging to reach high threshold levels of infection necessary to evaluate potential changes in $G_{q/11}$-mediated signaling induced by the expression of the viral construct. Following extensive consultation, I found that this is not an uncommon experience when working with lentiviruses *in vitro*. To address this limitation, I decided to transiently transfect the transducing vector into cell lines expressing endogenous $G_{q/11}$-coupled receptors, namely lysophosphatidic acid (LPA) receptor-expressing 293T cells (Figure 15), and histamine receptor-expressing COS and Hela cells (Figures 16 and 17). Transgene expression was, however, limited by transfection efficiency in the cell population, and intracellular expression levels were difficult to control. Consequently, expression of the G_qCT peptide led to variable results. First, LPA-induced PI hydrolysis in 293T cells was significantly decreased in cells transfected with the HIV-1-CMV-G_qCT-EGFP vector versus cells tranfected with

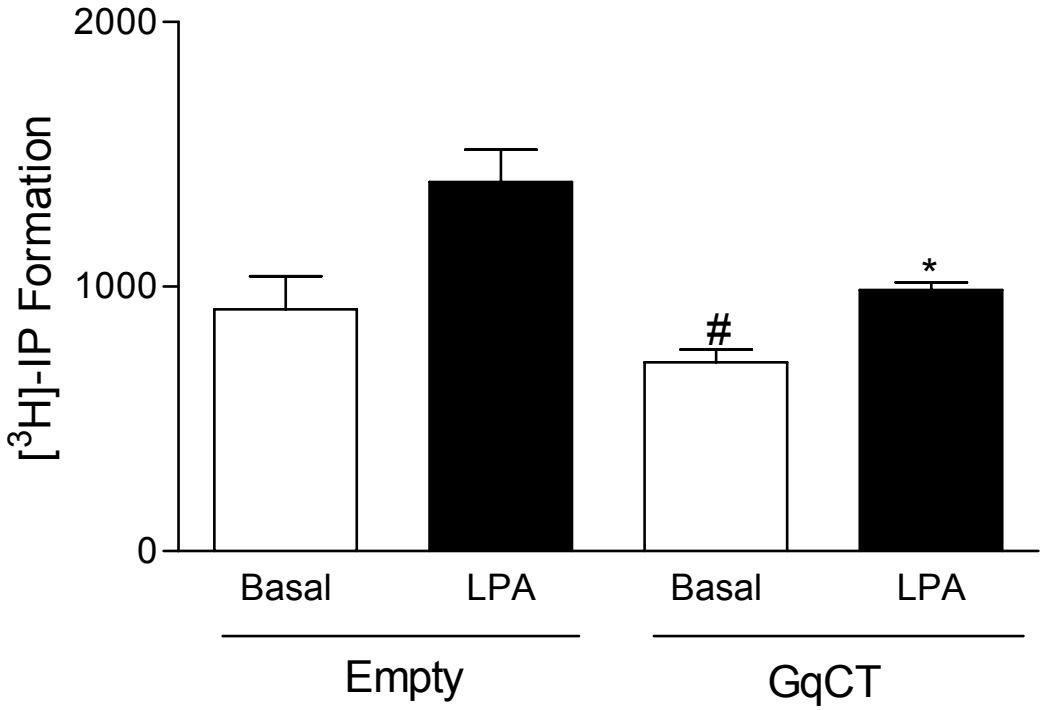


Figure 15: Lysophosphatidic acid-induced phosphoinositide hydrolysis in HEK 293Tcells

HEK 293T cells were incubated with inositol-free DMEM with 1μCi/ml [3H]-inositol for 16 hours prior to stimulation with 10 μM histamine. [3H]-inositol monophosphates were isolated by anion exchange chromatography. Basal and LPA-induced PI hydrolysis were decreased in cells expressing GqCT; $p<0.01$, $p<0.001$ respectively

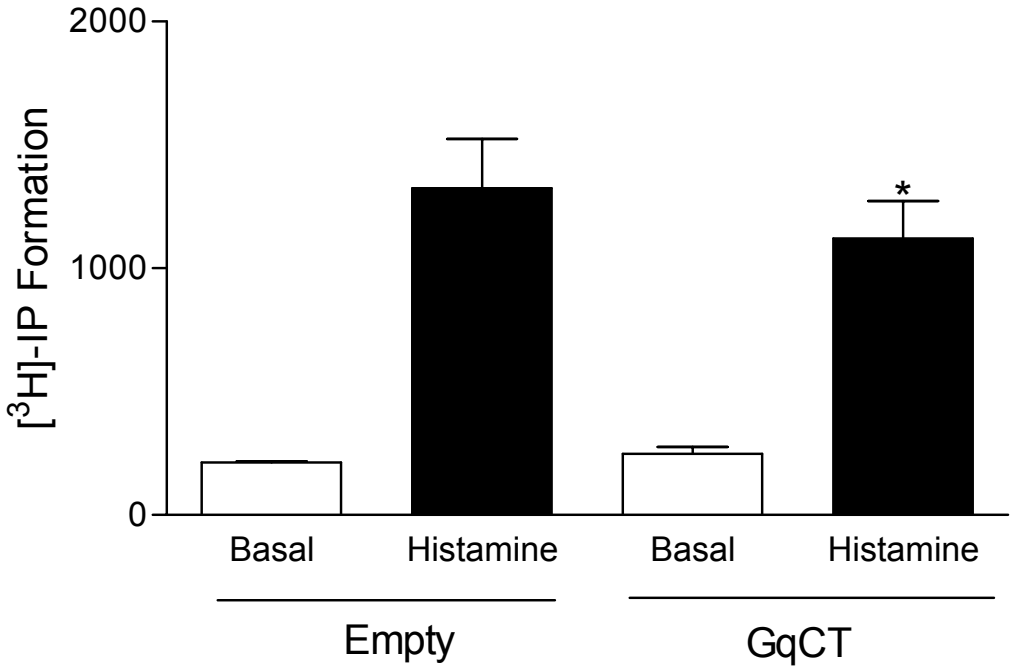


Figure 16: Histmaine-induced phosphoinositide hydrolysis in Hela cells

Hela cells were incubated with inositol-free DMEM with 1μCi/ml [3H]-inositol for 16 hours prior to stimulation with 100nM histamine. [3H]-inositol monophosphates were isolated by anion exchange chromatography. Histamine-induced PI hydrolysis was decreased in cells expressing G_qCT; $p<0.05$

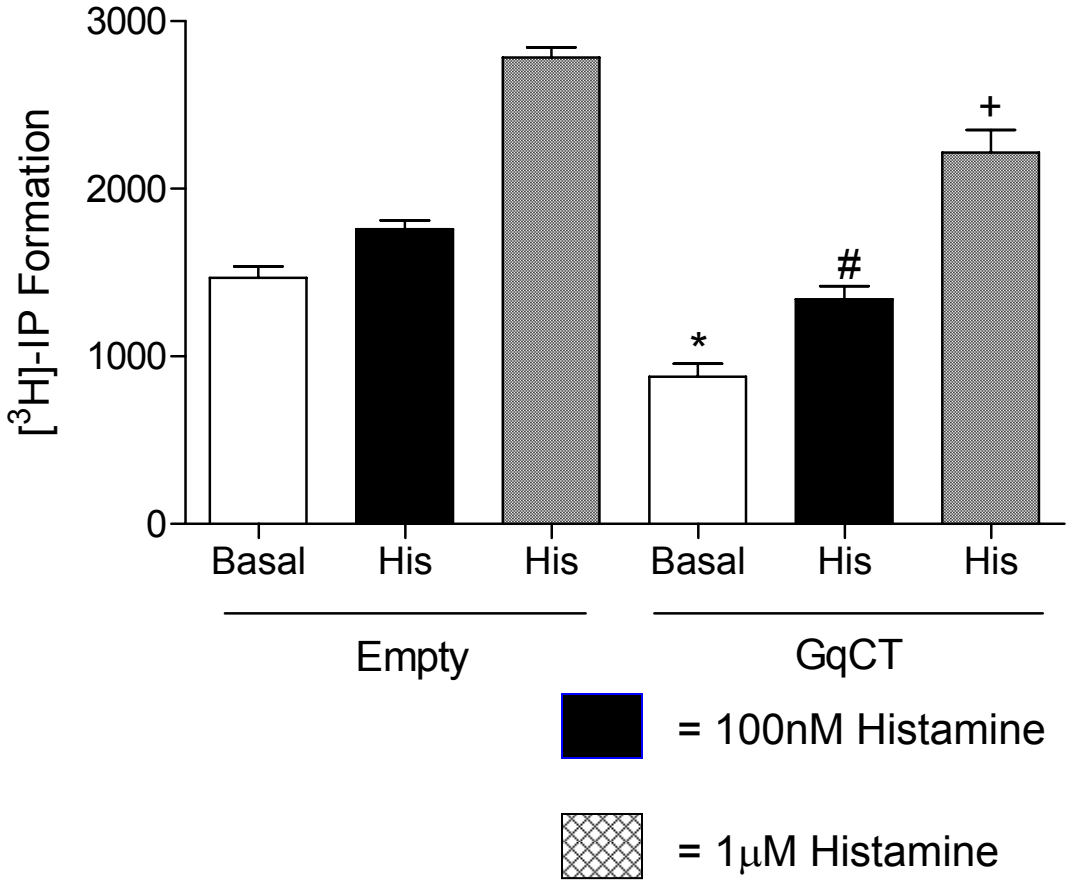


Figure 17: Histamine-induced phosphoinositide hydrolysis in COS cells

COS cells were incubated with inositol-free DMEM with 1μCi/ml [3H]-inositol for 16 hours prior to stimulation with 100nM and 1μM histamine. [3H]-inositol monophosphates were isolated by anion exchange chromatography. Basal and histamine-induced PI hydrolysis were decreased in cells expressing G_qCT; $p<0.01$ for basal, $p<0.05$ for 100nM histamine, $p<0.001$ for 1μM histamine

HIV-1-CMV-EGFP (empty vector) ($p<0.001$). In addition, basal [^{3}H]-IP formation was also reduced in G_qCT expressing cells ($p<0.001$) (Figure 15). Similarly, histamine-induced PI hydrolysis was decreased in COS ($p<0.05$ for 100nM; $p<0,001$ for 1uM) (Figure 16) and Hela ($p<0.05$) cells transfected with the transducing vector (Figure 17). Even though basal [^{3}H]-IP formation was reduced in COS cells expressing the G_qCT peptide ($p<0.01$), G_qCT induced no change on basal [^{3}H] IP expression in Hela cells (Figure 17).

However, results were highly variable, and they were often difficult to reproduce due to high cell death or low transfection efficiency. For instance, 293T cells showed the highest levels of transfection efficiency, but cells often detached from wells following tranfection with the viral construct. On the other hand, COS and Hela cells were resilient to viral transfections, but transfection efficiency lagged suboptimally between 30-40%.

To validate the lentiviral construct *in vivo*, I delivered a recombinant replication-deficient HIV-1-based vector containing a minigene encoding G_qCT into the anterior cingulate cortex of mice heterozygous for $G\alpha_q$ (Figure 18). Two weeks post-injection, virus infectivity was determined by GFP analyses, and assessed transgene expression and function was assessed by *c-fos* expression. GFP analyses showed the viral construct was effective at infecting cells *in vivo*. I consistently observed a large number of fluorescent (EGFP positive) cells along the needle track ipsilaterally; while no fluorescent cells were evident contralaterally (Figure 18). However, DOI-induced *c-fos* expression assays showed that our lentiviral construct (HIV1-NSE-G_qCT-EGFP), as well as virus

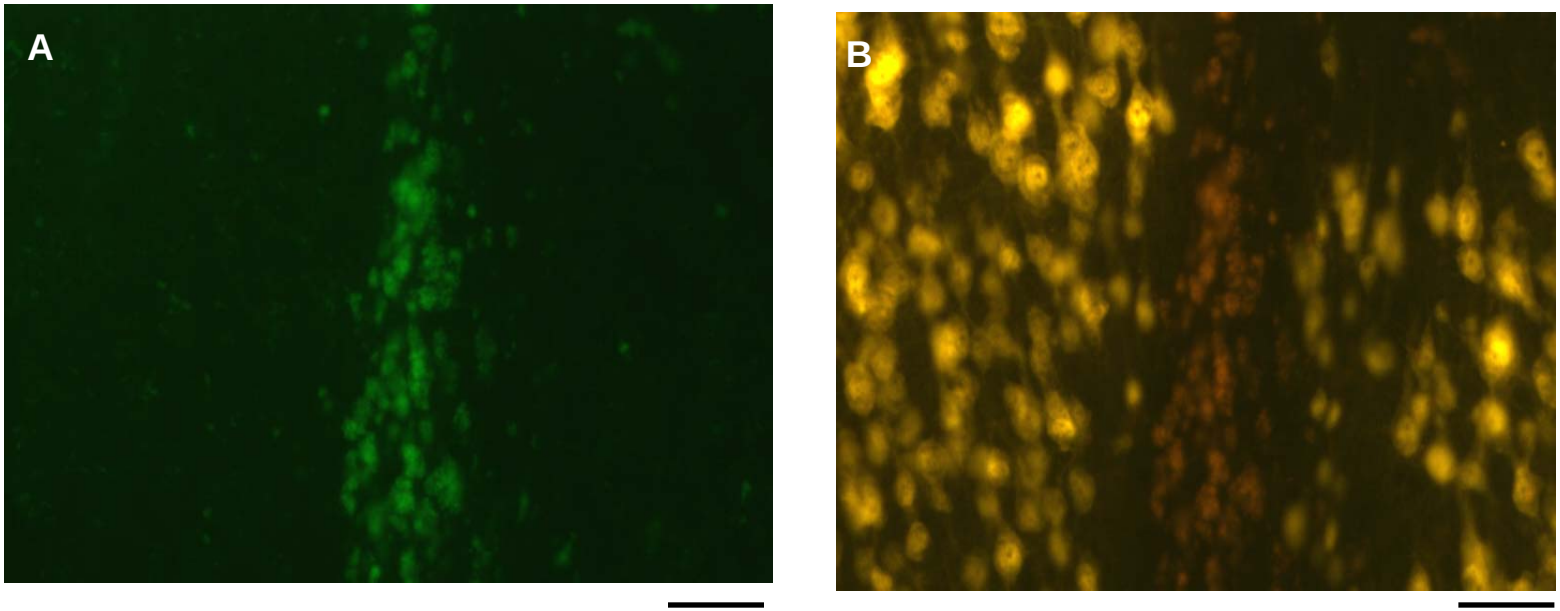


Figure 18: Lentiviral vector injection into the mouse anterior cingulate cortex
A Brain section shows a large number of EGFP-positive cells tranduced by the human immunodeficiency virus HIV-1-NSE-GqCT-IRES-EGFP. **B** Colocalization of EGFP positive cells with the neuronal marker NeuN. (scale 60 mm)

alone (HIV1-NSE-EGFP) completely abolished FOS expression in the area of infection (Figure 19). Due to this observation, I performed further H&E (Figure 20) and GFAP stains (Figure 21), which demonstrated that the virus induces severe gliosis accompanied by necrosis in the area of infection.

The cause of virus toxicity is not known, but it may be related to several factors. First, in order to obtain high levels of transgene expression, the lentivirus was highly concentrated (HIV1-NSE-EGFP= 75 μg/ml, HIV-1-NSE-G_qCT-EGFP= 143.2 μg/ml). It is possible that infection by high titers of virus could have caused some post-delivery tissue damage. However, lowering the virus titer was not a feasible alternative since it severely decreased the levels of GFP expression in target areas; given that G_q blocking peptides disrupt specific protein-protein interactions in a competitive manner, we assume that high levels of expression are needed. Another possible cause of the toxic effects observed may be lack of virus purity. Recently, Dr Wolfgang R. Dostmann (personal communication) informed me that impurities in the viral concentrate could be one of the causes leading to toxicity following lentiviral delivery. The severe toxicity I observed likely reflects a combination of these factors.

Alternatively, a new tool for systematically deciphering gene functions and interactions was recently developed with the advent of short hairpin RNAs. Hairpin RNAs are precursors to the short interfering RNAs (siRNAs) that are the powerful mediators of RNA interference (RNAi). In RNAi, genes homologous in sequence to the siRNA are silenced at the post-transcriptional state. Short

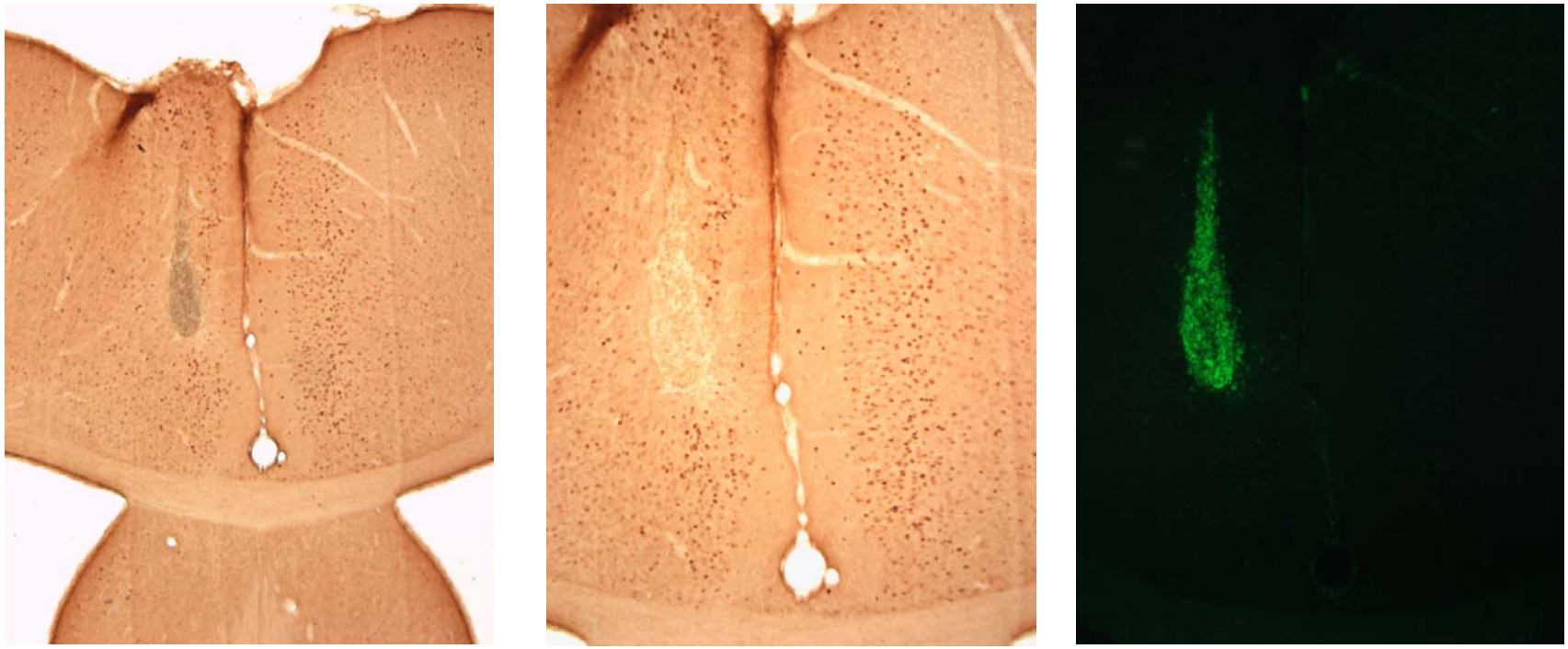


Figure 19: (HIV)-1-NSE-EGFP injection into the mouse anterior cingulate cortex.
(A), Representative image showing DOI-induced c-fos expression following treatment with 5mg/Kg DOI, (4X). Oval illustrates area of viral infection. (B), Previous image at a higher magnification (10X). (C), GFP fluorescence indicating area of infection.

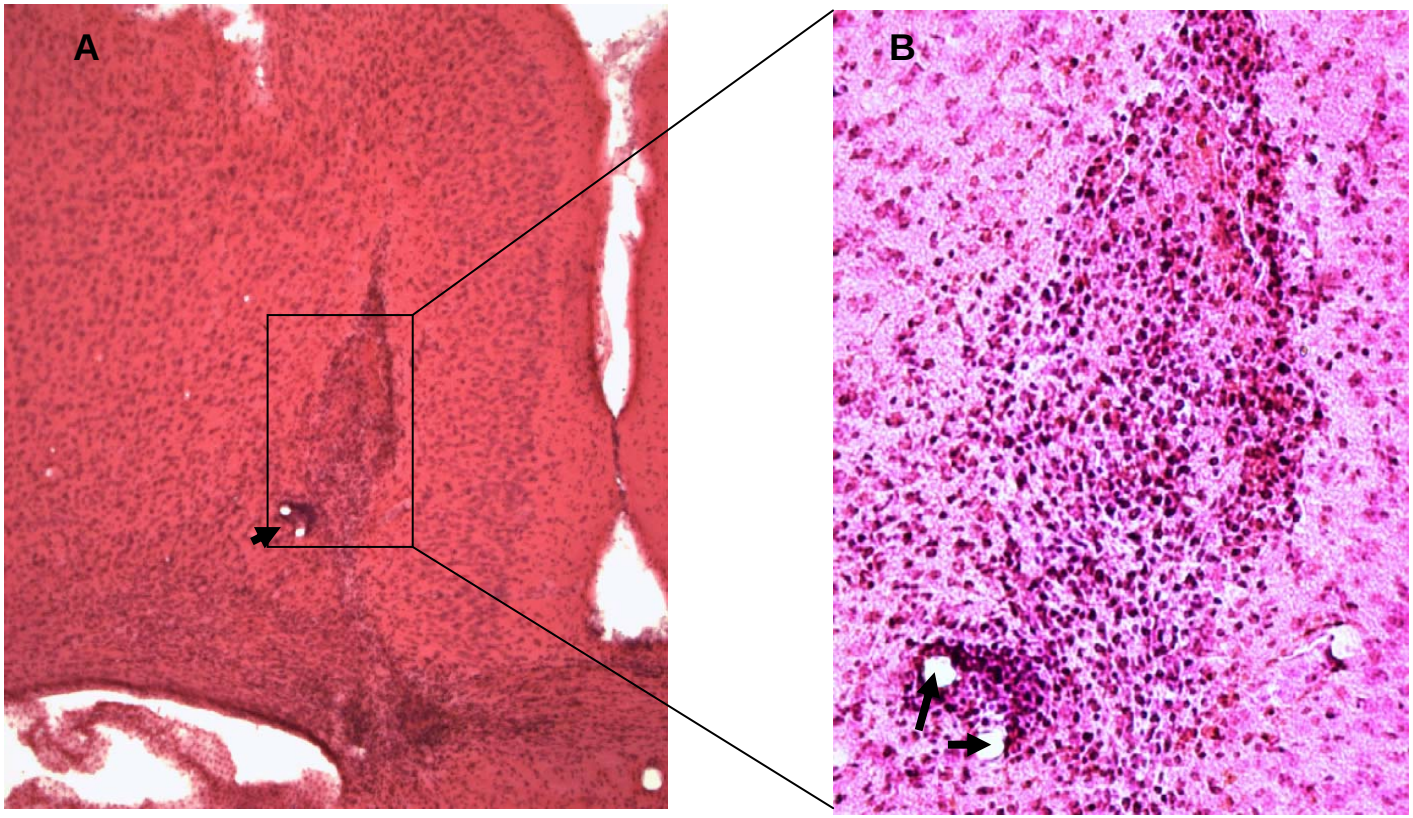


Figure 20: Hematoxylin and Eosin (H&E) staining
HIV-1-NSE-EGFP induces extensive gliosis, characterized by increased numbers of nuclei on the area of viral infection. Arrows point at necrosis in the tissue. (A), 5X. (B), 10X

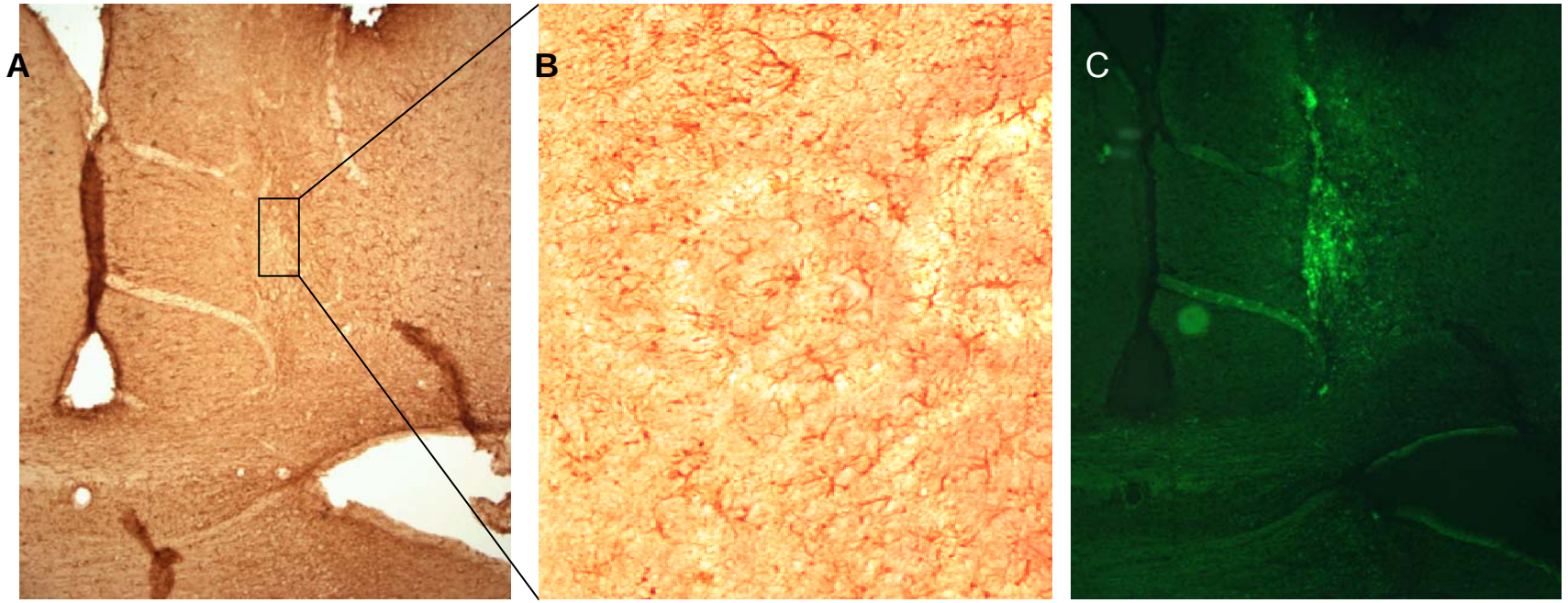


Figure 21: GFAP Immunostaining

(A), Expression of the lentivirus induces gliosis in the area surrounding the injection site (5X). (B), Higher magnification showing representative amounts of GFAP immunoreactivity immediately adjacent to the injection site (10X). (C), GFP fluorescence indicating area of infection.

hairpin RNAs can be expressed from lentiviruses, allowing for high efficiency transfection in a variety of cell types including neurons. This technique can be highly advantageous for studying the role of G_q-signaling blockade in a site-specific manner. By using a lentivirus expressing siRNA sequence homologous to $G\alpha_q$, we would be able to avoid one of the main limitations of the present study - the need for achieving high levels of transgene expression to overcome a competitive blockade by $G_{q/11}$ blocking peptides, thereby limiting potential viral toxicity induced by injection of high concentrations of lentivirus.

CHAPTER III

ROLE OF G_q IN HALLUCINOGENIC DRUG ACTION

Introduction

G-proteins transduce signals from a wide array of G-protein coupled receptors (GPCRs) initiating a plethora of second messenger cascades. The $G_{q/11}$ subfamily of G-proteins mediates the activation of inositol lipid signaling and calcium release in a PTX-insensitive manner. 5-Hydroxytryptamine$_{2A}$ $(5\text{-HT})_{2A}$ receptors are a subtype of the 5-HT_2 subfamily of receptors, known to stimulate the PLC pathway via $G\alpha_q$ (Chang et al., 2000a). There is now vast evidence from biochemical, electrophysiological, and behavioral studies that hallucinogens, such as lysergic acid diethylamide (LSD), have a key site of action as agonists at 5-HT_{2A} receptors in the brain (for review, see Marek and Aghajanian, 1996; Aghajanian and Marek, 1999a; Nichols, 2004).

The hallucinogen (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a highly selective 5-HT2 agonist (McClue et al., 1989), has been utilized to evaluate the role of 5-HT_{2A} receptors in many biochemical and behavioral responses in rodents (Darmani et al., 1990a; Darmani et al., 1990c; Darmani et al., 1990b; Leslie et al., 1993; Mazzola-Pomietto et al., 1995). Acute administration of DOI elicits a 5-HT_{2A} receptor-dependent induction of the immediate early gene *c-fos* and its protein FOS in the rat cortex (Leslie et al., 1993; Tilakaratne and Friedman, 1996; Scruggs et al., 2000). In mouse

behavioral assays, DOI has been shown to exhibit anxiolytic-like properties in anxiety paradigms such as the four plates test and the elevated plus maze (Onaivi et al., 1995; Nic Dhonnchadha et al., 2003a). Moreover, activation of 5-HT_{2A} receptors by DOI evokes a head-twitch response in both rats and mice (Schreiber et al., 1995; Dursun and Handley, 1996; Kleven et al., 1997).

The precise signal transduction pathway involved in the *in vivo* effects of DOI and other hallucinogenic drugs is unknown. The objective of the present study is to evaluate the role of G_q proteins in DOI-induced biochemical and behavioral effects. As a tool to assess the role of the G_q signaling pathway, we have made use of genetically modified mice in which the gene encoding for the α-subunit of G_q protein has been eliminated [$G\alpha_q(-/-)$].

Materials and Methods

Animals

$G\alpha_q(-/-)$ mice were generated by mating heterozygous [$G\alpha_q(+/-)$] males and heterozygous females to obtain wild-type and knock-out littermates. Mice were kept on a C57BL/6x129/Sv background, and their genotype was determined by PCR of genomic DNA from tail samples as previously described (Offermanns et al., 1997a). Although $G\alpha_q(-/-)$ mice exhibit signs of ataxia and motor incoordination, their peripheral and central nervous system morphology is largely undisturbed (Offermanns et al., 1997a). However, these ataxic characteristics are less severe in the C57BL/6x129/Sv hybrid background.

C57BL/6 mice (Harlan, Indianapolis, IN) were utilized for control experiments evaluating the role of 5-HT_{2A} receptors in the *in vivo* effects of DOI. Animals had free access to food and water, and were maintained on a 12:12 light/dark cycle. An equal number of male and female mice were used for experimental studies; the mice were 6-8 weeks of age at the time of testing. All experiments were done in compliance with the guide *Principles of Laboratory Animal Care* (NIH publication No. 85-23) and the Vanderbilt University Animal Care and Use Committee.

c-fos Immunohistochemistry

Mice were anesthetized with 150 mg/kg pentobarbital i.p. before transcardiac perfusion with 30 ml of 0.1 M phosphate-buffered saline (PBS) followed by 30 ml of 4% paraformaldehyde in 0.1M PBS. Brains were removed immediately, post fixed in paraformaldehyde overnight at 4^{o}C, and then transferred to increasing concentrations of sucrose. Coronal sections were cut at 40μm thickness on a vibratome and collected into buffer containing 30% ethylene glycol, 30% glycerol, 10% 0.1M PBS, and 30% water. Immunohistochemistry was performed on free floating sections. Sections were preincubated for 30 min in 0.3% hydrogen peroxide, washed 3 times with PBS, and then incubated for one hour in 5% goat serum/2% Triton X-100 to block non-specific binding. Sections were incubated for 48h at 4^{o}C in anti-FOS primary antibody (Oncogene Research Products) diluted in blocking solution 1:30,000. Following 3 washes in PBS, a Vectastain Elite ABC horseradish peroxidase kit (Vector Labs) was used

for the secondary antibody and avidin–biotin complex steps. The colorimetric detection reaction produced 3-3'-diaminobenzidine tetrahydrochloride (DAB) as a brown chromagen product. Sections were then washed, mounted on slides, dehydrated through a series of solution with increasing ethanol concentrations, treated with xylenes, and coverslipped with Mounting Medium (Richard-Allan Scientific. Kalamazoo, MI)

Analysis of FOS-Li Positive Nuclei

Brain sections containing medial prefrontal cortex were analyzed as described previously (Gresch et al., 2002). Briefly, bright field images were taken using Openlab 2.2.5 software (Improvision. Lexington, MA) with a Coolsnap cf camera (Photometrics. Tucson, AZ) mounted on a Zeiss Axioverts S100 microscope. All settings were kept constant throughout the image collection process. Analysis and quantification of images were performed using Image J 1.33u (Wayne Rasband, NIH) in mPFC sections that correspond to AP +1.34mm relative to bregma (Franklin and Paxinos, 1997). An image of 600μm x 450 μm area was analyzed for the number of FOS-Li positive nuclei. Cells with brown black nuclei were considered positive FOS-Li. These were determined by using the particle count macro in Image J 1.33u where the pixel density threshold had been set four times above background levels.

Radioligand Binding

Frontal cortex was dissected and homogenized in binding buffer (50mM

Tris and 10mM $MgCl_2$, pH 7.4). The homogenate was centrifuged at 20,000g for 20 min at 4°C, and the pellet was resuspended in binding buffer. Protein concentration was determined with Bio-Rad protein assay dye reagent (Hercules, CA). Membrane preparations (200μg/sample) were incubated with [^{3}H]-ketanserin (10nM) for 30 min at 37°C. Nonspecific binding was determined with 10 μM methysergide. Following incubation, free radioligand was separated from bound by vacuum filtration through Whatman GF/C glass filters (Brandel, Gaithersburg, MD). Filters were placed in vials and counted in a liquid scintillation counter.

Elevated Plus Maze

The elevated plus maze (Hamilton-Kinder. San Diego, CA) consisted of two open arms (37.5 x 5.0 x 0.25 cm) and two closed arms (37.5 x 5.0 x 15 cm) emanating from a central platform (5cm x 5cm) to form a plus shape. The maze was built from black Plexiglas, and equipped with infrared photobeams. The entire maze was elevated 45 cm above the floor. Light beam breaks were recorded and analyzed automatically by Motor Monitor software (Hamilton-Kinder).

Animals were transported to the experiment room, and following a habituation period of 15 min, they were injected i.p. with drug, and placed back into their home cages for 30 min after DOI or 5 min after ethanol. Animals were individually placed into the central platform of the plus maze and allowed 5 min of free exploration. Time and percent time spent in the open arms were used as an

index of anxiolytic-like effects, and total distance traveled was used as a measure of general activity. After each session, the plus maze was cleaned with 30% ethanol and allowed to completely air dry prior to placing the next animal for testing.

Head Twitch Response

The head twitch response is a distinctive behavior characterized by a rapid, rotational movement of the head, ears, and neck. Mice were injected i.p with drug and immediately following treatment, they were transferred to a 3000 mL glass beaker lined with pinedust bedding for observation. Head twitches were counted, in consecutive 5 min bins, for 30 minutes following drug administration by two observers (one of them blind to the treatment) with over 95% inter-rater reliability.

Drugs

Drugs were administered i.p. in an injection volume of 10 ml/kg. All drugs were diluted in 0.9% saline solution. Ethanol concentration, 15% (w/v), was obtained by diluting absolute ethanol (AAPER. Shelbyville, KY) with saline. DOI was purchased from Sigma-Aldrich, and MDL 100907 was a gift from Marion Merrell Dow (Cincinnati, Ohio).

Statistical Analyses

All data are presented as mean ± S.E.M. The effects of DOI and ethanol in

the elevated plus maze, and the effects of DOI in the head-twitch response test were compared by two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests. The effects of MDL100907 were analyzed by a one-way ANOVA followed by Tukey's multiple comparison tests.

Results

Role of 5-HT_{2A} receptors in DOI-induced behaviors

Consistent with earlier reports (Nic Dhonnchadha et al., 2003a), DOI significantly increased open arm activity in the elevated plus maze. Pretreatment with the highly selective 5-HT_{2A} receptor antagonist MDL 100907 abolished the anxiolytic-like effects of DOI on elevated plus maze behavior (Figure 22). Similarly, DOI induced a robust head-twitch response, which was completely blocked by MDL 100907 (Figure 23).

Characterization of DOI-induced FOS expression in mice

The effect of DOI on FOS expression has not been previously evaluated in mice. We therefore performed control pharmacological experiments to establish potency. DOI (0, 0.3, 3, 10 mg/kg, i.p. 3 hours pre-test) elicits a dose-dependent induction of FOS-Li (Figure 24) nuclei in the medial prefrontal cortex (mPFC) of mice (Figure 25). The EC_{50} value for FOS induction was 5.6 mg/kg. Pretreatment with the 5-HT_{2A} receptor antagonist MDL 100907 blocked the ability of DOI (5mg/kg) to induce FOS-Li expression in the mPFC (Figure 26).

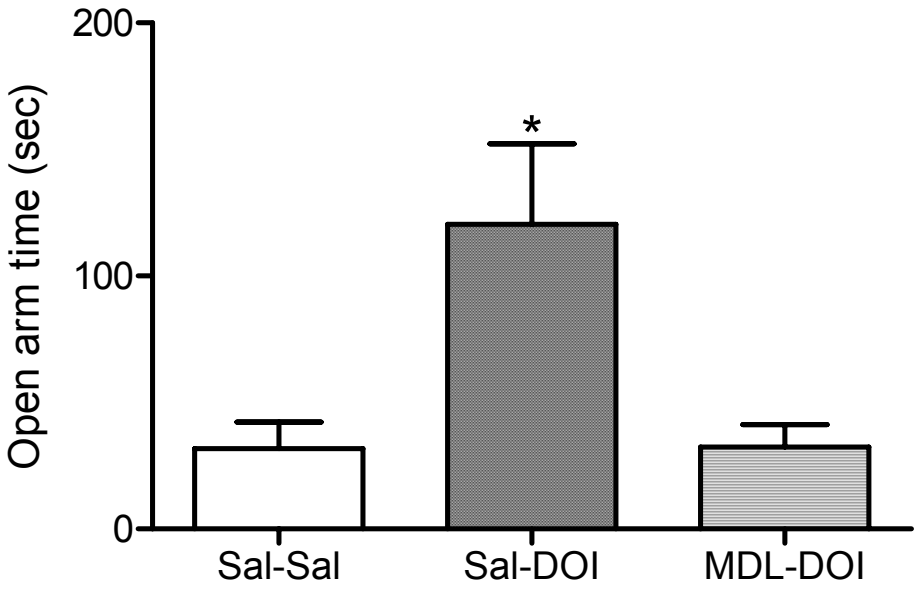


Figure 22: Effect of pretreatment with MDL100907 on the anxiolytic-like effects of DOI in the elevated plus maze.

A Anxiolytic-like effects of DOI (2.5 mg/Kg, i.p. 30 min pre-test) are prevented by pretreatment with the 5-HT_{2A} receptor antagonist MDL100907 (0.25 mg/Kg, i.p. 50 min pre-test). Data shown as means ± S.E.M., n= 6 per group. (*)$p<0.05$ relative to saline control group

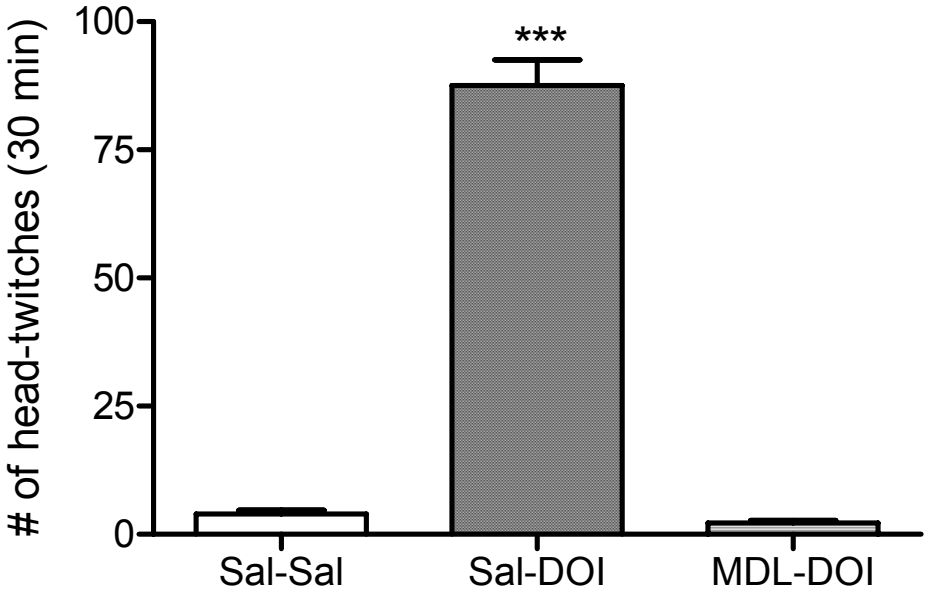


Figure 23: Effect of pretreatment with MDL100907 on DOI-induced head-twitch behavior.

DOI-induced head-twitches counted during a 30 min observation period were inhibited by pretreatment with MDL100907 (0.25 mg/Kg, i.p. 20 min pre-test). Data shown as means ± S.E.M., n= 6 per group. (***)$p<0.001$ relative to saline control group.

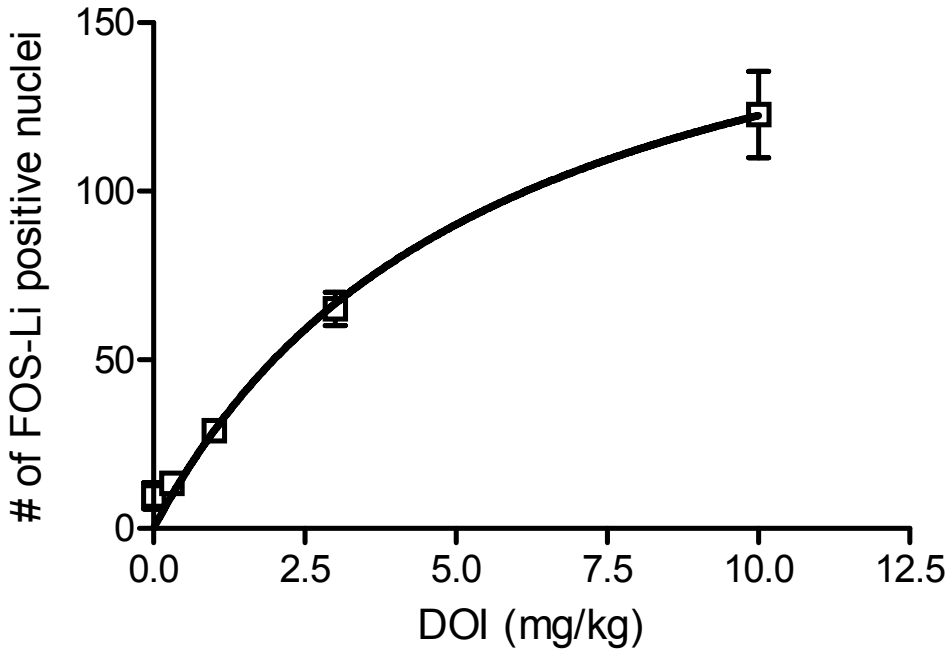


Figure 24: Quantitative analysis of FOS-Li positive nuclei in the medial prefrontal cortex of C57BL/6 mice.
Dose response of DOI-induced FOS expression in the mPFC. Data shown as means ± S.E.M., n=3.

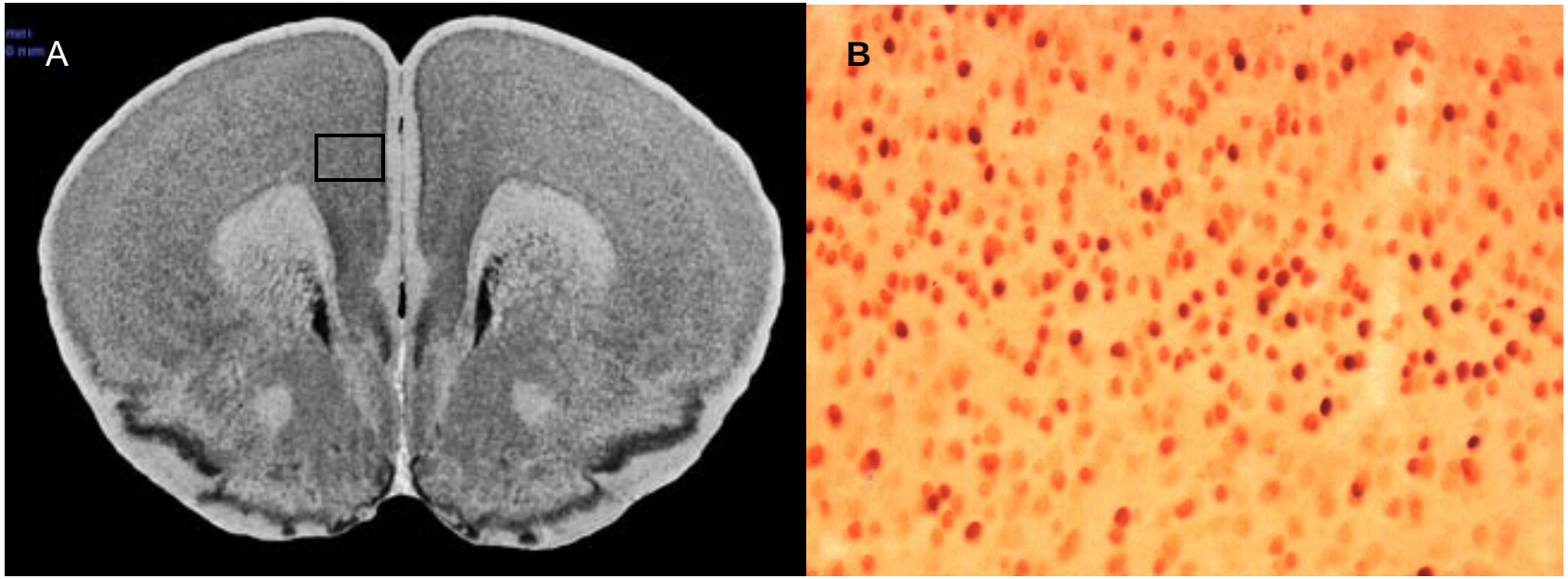


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Figure 25: A. Schematic representation of regional analysis; box highlights area of quantification B. Representative image showing Fos-li containing nuclei in mPFC of mice

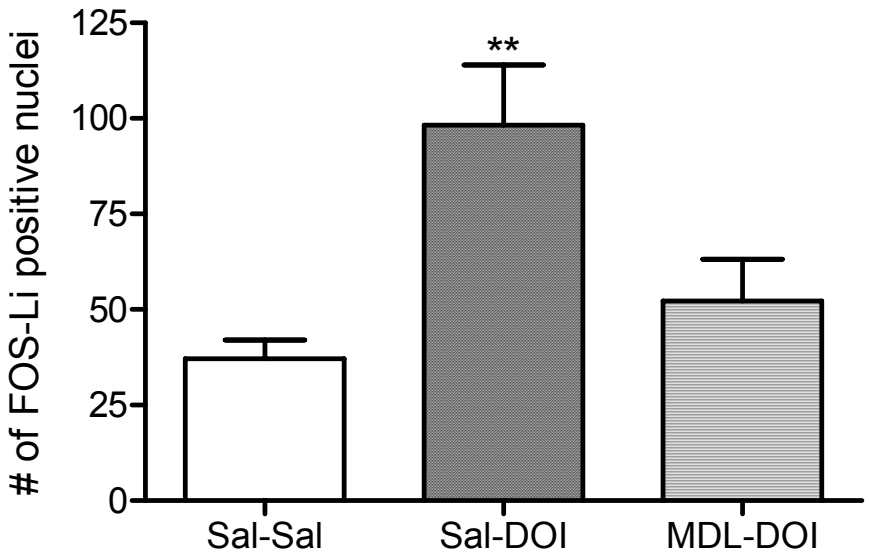


Figure 26: Quantitative analysis of FOS-Li positive nuclei in the medial prefrontal cortex of C57BL/6 mice.

DOI (2.5 mg/Kg, i.p.) induced FOS-Li expression is inhibited by pretreatment with MDL100907 (0.25mg/kg, i.p. 20 min pre-DOI). Data shown as means ± S.E.M., n= 6. (**) $p<0.01$ relative to saline (Sal-Sal).

$G\alpha_q$ is required for the anxiolytic-like effect of DOI in the elevated plus maze

The anxiolytic-like effect of DOI was compared in the elevated plus maze using wild-type and $G\alpha_q$(-/-) mice. Although DOI elicited an increase in both time spent in the open arms ($p<0.01$) (Figure 27A) and percent open arm time ($p<0.01$) (Figure 27B) in wild-type littermates, this effect of DOI was abolished in mice deficient for $G\alpha_q$. In contrast, ethanol (1.5 g/kg, i.p. 5 min pre-test) significantly increased the time spent in the open arms (Figure 27A) as well as percent open arm time (Figure 27B) in both wild-type and $G\alpha_q$ knockout mice. These effects were independent of activity changes; there was no difference in total distance traveled between $G\alpha_q$(-/-) and wild-type littermates following any of the treatment conditions (Figure 27C).

As an additional control, we compared [^{3}H]-ketanserin binding in wild-type and $G\alpha_q$(-/-) mice to determine if altered 5-HT_{2A} receptor expression could explain the behavioral difference. There was no significant difference in binding of a maximum concentration of [^{3}H]-ketanserin (10nM) between $G\alpha_q$(-/-) and wild-type littermates (500±40 fmole/mg protein for $G\alpha_q$(-/-) vs 486±64 fmole/mg protein for WT, n=6; $p>0.05$)

DOI-induced head-twitch response is decreased in $G\alpha_q$ knockouts

Intraperitoneal administration of DOI elicited a dose-dependent head-twitch response in wild-type mice which peaked at 10 minutes (Figure 28A). This response was significantly blunted in mice deficient for $G\alpha_q$ ($p<0.05$ for 1.0 mg/kg

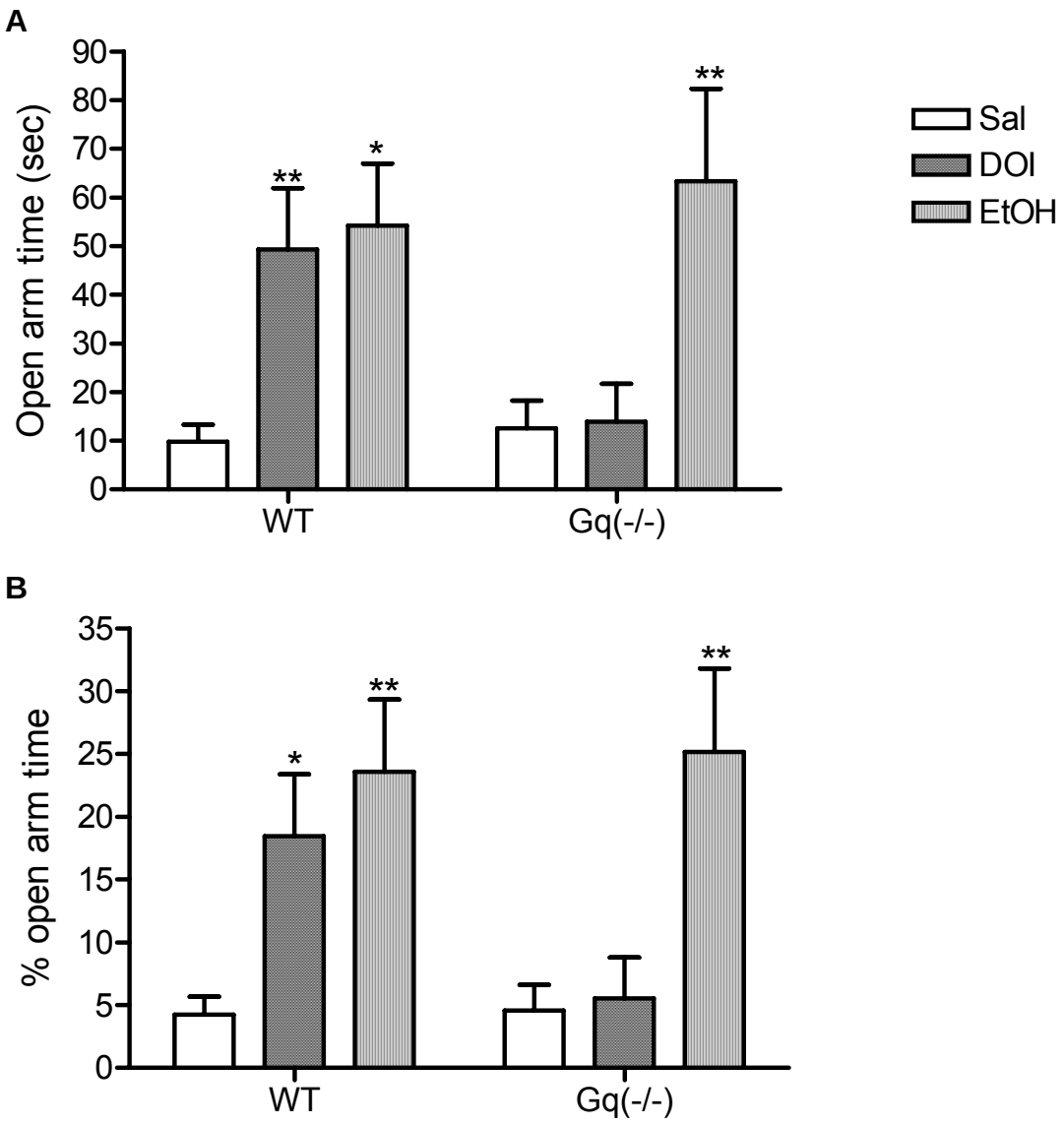
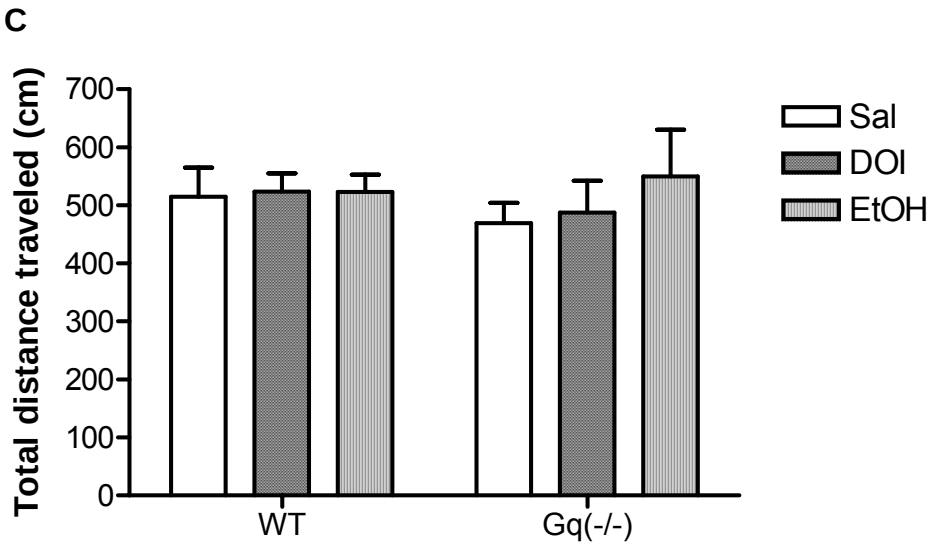


Figure 27: Anxiolytic-like activity of DOI on the elevated plus maze is absent in mice deficient for $G\alpha_q$.

A Time spent in the open arms, and **B** % time spent in the open arms, as a percentage of time spent in the open and close arms (center excluded) during the 5 min test session following administration of DOI (2.5 mg/kg, i.p. 30 min pre-test) or ethanol (1.5 g/kg, i.p. 5 min pre-test). **C** Exploratory activity measured in total distance traveled during the elevated plus maze test. Data shown as means ± S.E.M., n= 7-12 per group. (*) $p<0.05$, (**) $p<0.01$ relative to saline control group.



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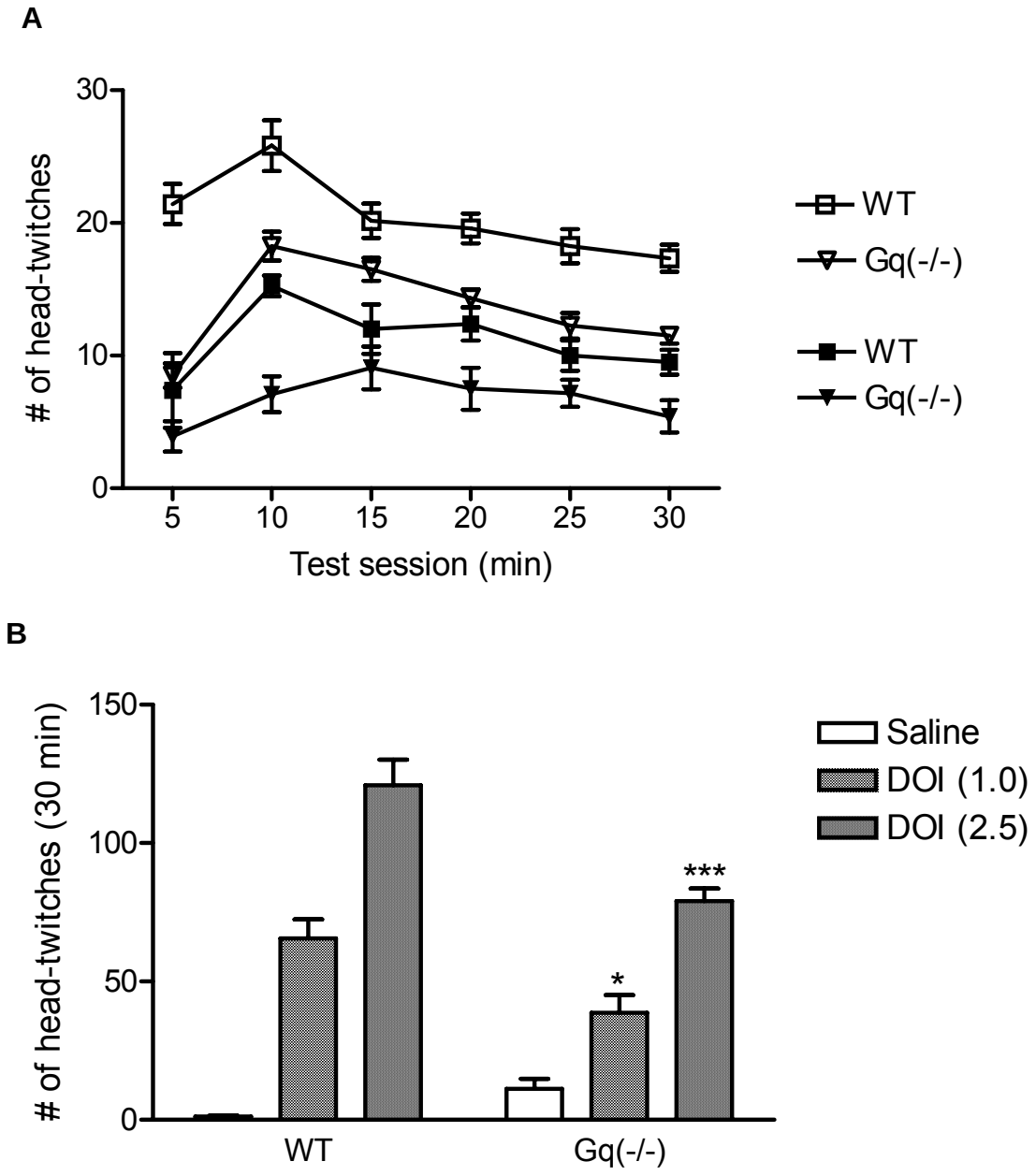

Figure 28: DOI-induced head-twitch response is reduced in $G\alpha_q$ knockouts

Mice were injected with DOI (2.5 mg/Kg, i.p.) prior to a 30 min observation period. **A** Number of head twitches in 5 min bins (open symbols DOI=2.5 mg/kg, closed symbols DOI=1.0 mg/kg). Main effect of genotype, $p<0.0001$. **B** Each column represents the total number of head-twitches counted during 30-min test time. Data shown as means ± S.E.M., n= 6-7 per group. (*) $p<0.05$, (***) $p<0.001$ relative to wild-type control groups.

DOI. $p<0.001$ for 2.5 mg/kg DOI) (Figure 28B). The percent decrease was comparable for the two doses of DOI (40% for 1mg/kg vs 35% for 2.5 mg/kg). Thus, the head-twitch response appears to be less sensitive to a loss of $G\alpha_q$ than is the elevated plus maze. This conclusion is supported in heterozygous $G\alpha_q(+/-)$ mice, in which $G\alpha_q$ protein is reduced by 50% (Offermanns et al., 1997a). The DOI-induced head-twitch response was reproduced in $G\alpha_q(+/-)$ mice; however, DOI effects in the elevated plus maze were completely eliminated in $G\alpha_q(+/-)$ knockouts (Figure 29).

Cortical FOS expression is reduced in $G\alpha_q(-/-)$ mice

Based on the previous dose response data, an EC_{50} dose of DOI (5 mg/kg) was utilized. DOI markedly increased the number of FOS-Li positive nuclei in the medial prefrontal cortex of wild-type mice ($p<0.001$). This increase was abolished in $G\alpha_q(-/-)$ mice (Figure 30).

Discussion

5-HT_{2A} receptors are known to be a key site of action for hallucinogenic drug action in both humans and laboratory animals. There is abundant evidence from biochemical, electrophysiological, and behavioral studies in rats that hallucinogens, including LSD and DOI, are potent partial agonists at 5-HT_{2A} receptors in the central nervous system (Aghajanian and Marek, 1999; Sanders-Bush et al., 1988; Aghajanian and Marek, 1999a). Furthermore, correlations

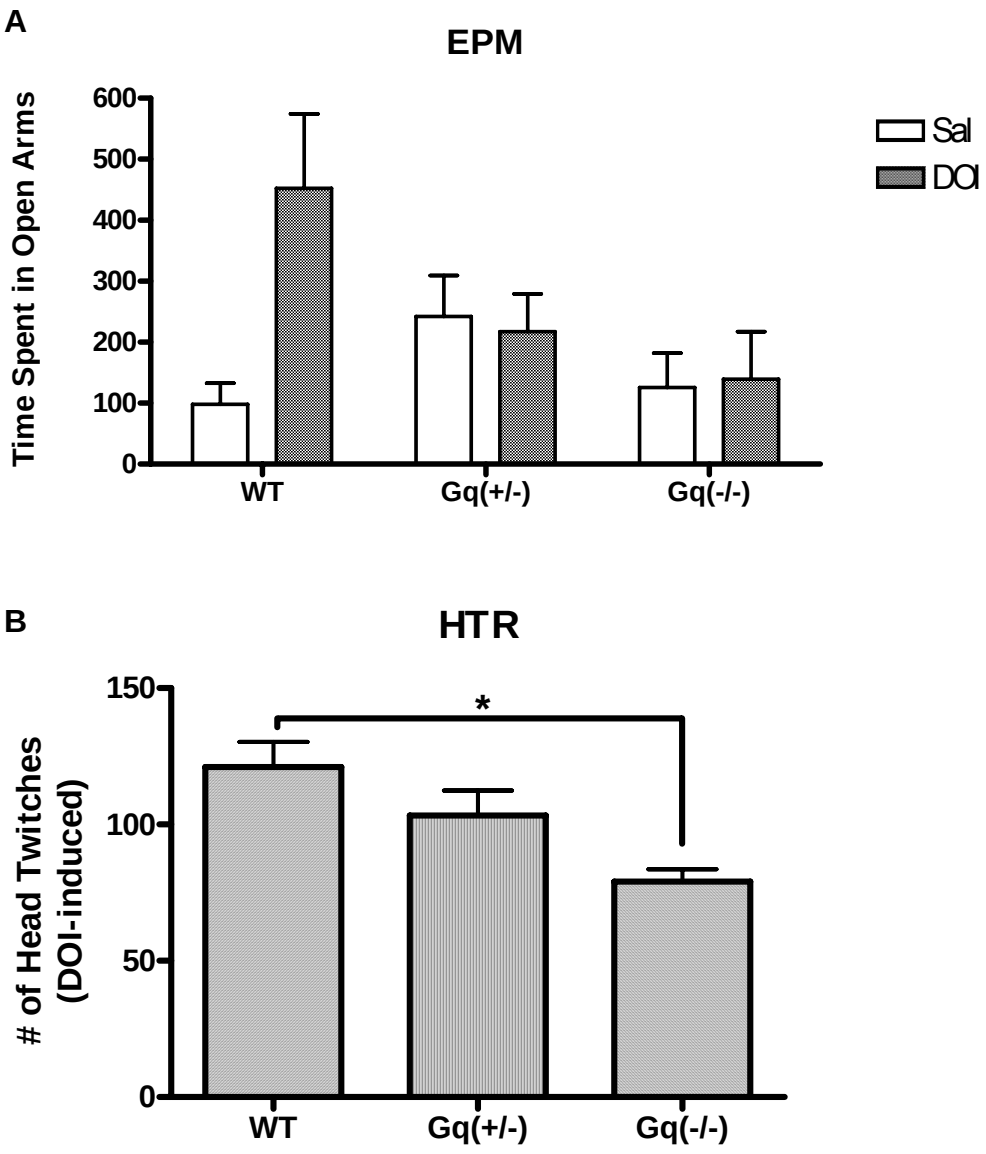

Figure 29: DOI-induced behavioral Effects on $G\alpha_q$(+/-) mice

A. Anxiolytic-like effects of DOI on the elevated plus maze are absent in $G\alpha_q$(+/-) and $G\alpha_q$(-/-).Time spent in the open arms during the 5 min test session following administration of DOI (2.5 mg/kg, i.p. 30 min pre-test) . **B** DOI-induced head-twitch response is unchanged in $G\alpha_q$ (+/-) mice. Mice were injected with DOI (2.5 mg/Kg, i.p.) prior to a 30 min observation period. Data shown as means ± S.E.M., n=6-12 for EPM and n=6-7 for HTR. (*) $p<0.05$, (**) $p<0.01$

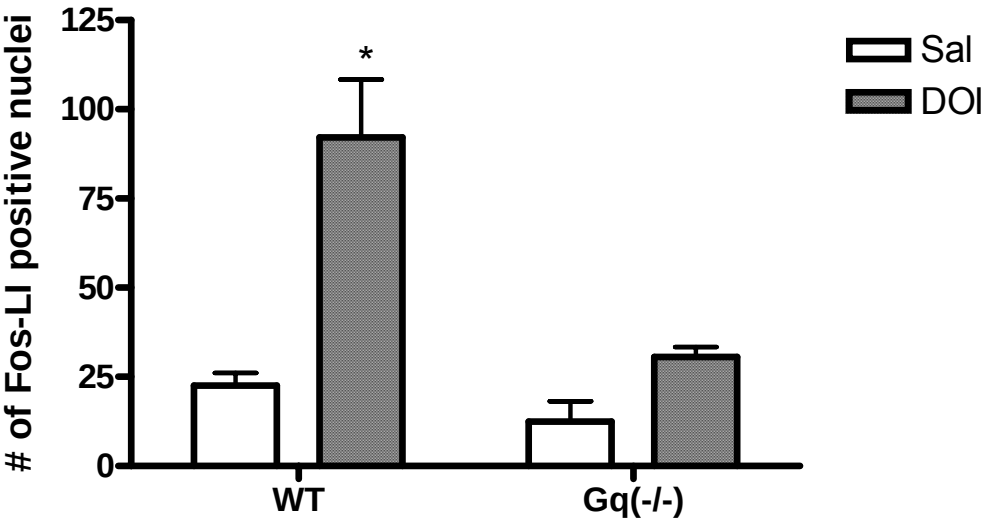

Figure 30: Quantitative analysis of number of FOS-Li positive nuclei in the medial prefrontal cortex of wild-type and Gα_q(-/-) mice.

Values are numbers of FOS-Li positive cells (mean ± S.E.M. within area of analysis; n=6). DOI-induced FOS expression in the medial prefrontal cortex is abolished in Gα_q(-/-) mice. (***) $p<0.001$ relative to saline control group

between human hallucinogenic potency and 5-HT_{2A} receptor binding affinity are consistent with the hypothesis that classical hallucinogens exert their hallucinogenic effects through 5-HT_{2A} receptors (Glennon et al., 1984; Arvanov et al., 1999). In addition, studies in our laboratory with DOI in mice suggest a major role for the 5-HT_{2A} receptor in the elevated plus maze and head-twitch response (present study), as well as in drug discrimination (Smith et al., 2003).

5-HT_{2A} receptors are known to couple to multiple G-proteins including G_q, G_{11}, and G_{13} to mediate a wide array of second messenger signaling pathways, including stimulation of PLCβ via Gα_q protein (Berg et al., 1998; Kurrasch-Orbaugh et al., 2003; Robertson et al., 2003). However, it is not known what intracellular signals mediate the *in vivo* actions of hallucinogens. In the present study, I evaluated the role of the Gα_q protein in several behavioral and biochemical assays, including the elevated plus maze, head-twitch response, 5-HT_{2A} receptor binding, and *c-fos* immunohistochemistry.

The current studies demonstrate that activation of the G_q signaling pathway is required for the mediation of the anxiolytic-like effects of DOI in the elevated plus maze. DOI significantly increased time spent in the open arms, as well as percent open arm time in wild-type animals; however, these effects were absent in Gα_q knockout mice. Baseline performance on the elevated plus maze and total exploratory activity did not differ between Gα_q knockout mice and wild-type controls suggesting that deletion of the α subunit of G_q does not produce alterations in overall anxiety-related behavior. As an additional, positive control to determine whether the absence of DOI effects in Gα_q knockouts was due to non-

specific, ectopic effects or developmental abnormalities altering anxiety-related behavior, we evaluated ethanol, known to exert its anxiolytic-like effects through the ionotropic GABA-A receptor system (Durcan and Lister, 1988; Prunell et al., 1994). The ability of ethanol treatment to increase open arm activity in the elevated plus maze for wild-type mice was reproduced in $G\alpha_q$ knockouts, suggesting that the behavioral deficits in $G\alpha_q$(-/-) mice are specifically related to the loss of the α-subunit of the G_q protein. Additional experiments using strategies for conditional knockout or knockdown of $G\alpha_q$ signaling are needed to entirely rule out developmental issues.

As with the elevated plus maze, DOI-induced head-twitch response was blunted in $G\alpha_q$(-/-) mice; however, unlike the elevated plus maze results, DOI still produced a significant head-twitch response in the knockout mice. Thus it appears that the G_q signaling pathway is not the sole mediator of the 5-HT_{2A} receptor-dependent behavioral effects of DOI. G_q and G_{11} proteins are close structural and functional analogs that substitute for each other in the intracellular signaling cascade. Although these two proteins coexist, G_q expression exceeds G_{11} throughout the brain (Milligan, 1993), including cortical and midbrain areas that mediate these behaviors. Compensatory changes, for instance, in G_{11} or in other components of the signaling complex, such as RGS proteins, may differ within the relevant brain sites, leading to the differential sensitivity of the two behaviors. Alternatively, some other signaling pathway, e.g., G_{13} activation of PLD (McGrew et al., 2002; Kurrasch-Orbaugh et al., 2003) or 5-HT_{2A} receptor–

mediated activation of PLA_2 (Berg et al., 1998; Tournois et al., 1998), may contribute to the head-twitch behavior.

Following DOI administration, wild-type mice exhibited a robust increase in the number of FOS-Li positive nuclei in the mPFC; however this increase was abolished in $G\alpha_q$(-/-) littermates. Intrestingly, Mackowiak et al. (2002) reported that activation of phospholipase A_2 via 5-HT_{2A} receptors is engaged in the mechanism of DOI-induced FOS expression in the rat cortex. Since DOI-induced FOS expression is essentially eliminated in $G\alpha_q$(-/-) mice, this suggests that phospholipase A_2 activation may be downstream of G_q activation in mice. Future characterizations of the role of the PLA_2 pathway in DOI-induced FOS expression and behavior in mice deficient for $G\alpha_q$ should lead to a better understanding of the molecular mechanisms responsible for DOI's effects.

In conclusion, the present study provides evidence that activation of the G_q protein pathway, downstream of 5-HT_{2A} receptors, is a key signaling mechanism involved in the mediation of hallucinogen-induced behavioral effects. One of the most striking findings is the difference in sensitivity of the two behaviors in $G\alpha_q$ null mice. DOI fails to elicit anxiolytic-like behavior in mutant mice, whereas the head-twitch response to DOI is reduced less than 50%, suggesting that other mechanisms are equally important for mediating this behavior. Given that different brain sites mediate these behaviors (Graeff et al., 1993; Willins and Meltzer, 1997), it is possible that 5-HT_{2A} receptors within these sites are differentially coupled to G protein signaling pathways or that different compensatory mechanisms exist. Importantly, our studies suggest that the G_q

signaling pathway is crucial for the full expression of hallucinogen-induced behaviors.

CHAPTER IV

SUMMARY AND FUTURE STUDIES

Despite a large body of evidence that points to 5-HT_{2A} receptors as mediators of the unique behavioral effects of hallucinogens (for review, see Marek and Aghajanian, 1996; Aghajanian and Marek, 1999a; Nichols, 2004), the intracellular mechanisms mediating these effects are still undetermined. Numerous studies have demonstrated that 5-HT_{2A} receptors couple to various G-proteins to activate different second messenger cascades. For instance, stimulation of 5-HT_{2A} receptors has been associated with PLD activation via $G\alpha_{13}$, and these receptors are likewise thought to be linked to activation of PLA_2 possibly via $G\alpha_{i/o}$. Nevertheless, the most prominent and well understood signaling pathway associated with 5-HT_{2A} receptor signaling is activation of PLCβ via $G\alpha_q$ (Conn and Sanders-Bush, 1984; Roth et al., 1984).

The current studies demonstrate that $G\alpha_q$ activation is crucial for the full expression of different hallucinogen-induced behaviors. For example, disrupting $G\alpha_q$ results in an abolition of DOI-induced anxiolytic-like responses in the elevated plus maze task, and likewise $G\alpha_q$ knockouts exhibit decreased levels of DOI-induced head twitch-responses. Furthermore, these studies demonstrated that there is a difference in sensitivity of the aforementioned behaviors in $G\alpha_q$ null mice, suggesting that some of the behavioral effects observed following 5-HT_{2A} receptor activation are not exclusively mediated by $G\alpha_q$. To illustrate, $G\alpha_q$

appears to be the main component mediating DOI-induced anxiolytic-like behavior in mice. On the other hand, DOI-induced head-twitch response appears to be mediated by various pathways suggesting that other mechanisms are equally important for the mediation of this behavior.

How this differential behavioral sensitivity in $G\alpha_q$ null mice relates to human hallucinogenic drug experience is not known. Recent studies of psilocybin in humans suggest that anxiety is a significant symptom (Griffiths et al., 2006), although presumably unrelated to the unique psychedelic experience. The head-twitch response is a stereotypical behavior that is a well accepted behavioral model of 5-HT_{2A} receptor activation in rodents (Schreiber et al., 1995; Dursun and Handley, 1996; Kleven et al., 1997), but difficult to relate to the human hallucinogenic experience. The introceptive cues, responsible for drug discrimination in rodents, are believed to model the subjective effects of drugs in humans. This is based largely on extensive studies showing that discriminative stimuli of psychoactive drugs in rats closely parallel the subjective effects reported by humans (Barry, 1974; Altman et al., 1976). Therefore, additional behavioral studies characterizing the hallucinogen-induced drug discriminative cue in $G\alpha_q$(-/-) mice are planned.

As with the elevated plus maze, activation of G_q signaling pathway appears to be necessary for DOI-induced FOS expression. Since the DOI-induced FOS expression is mediated by activation of 5-HT_{2A} receptors, and 5-HT_{2A} receptors couple to G_q protein, a logical conclusion is that these two events are directly related. However, G_q protein is coupled to many neurotransmitter

receptors, making it impossible to rule out the alternative interpretation that some other receptor's interaction with G_q is the relevant point of intervention leading to a disruption of the DOI-induced effects. Given that the 5-HT_{2A} receptor-G_q protein pathway is the likely signaling pathway, it is interesting that Mackowiak et al. (2002) reported that the arachidonic acid cascade, downstream from 5-HT_{2A} activation of PLA_2, is engaged in the mechanism of DOI-induced FOS expression in the rat cortex. Since DOI-induced FOS expression is essentially eliminated in $G\alpha_q$(-/-) mice, this suggests that phospholipase A_2 activation may be downstream of G_q activation in mice. Additional studies of the phospholipase C and phospholipase A_2 pathways in mice deficient for $G\alpha_q$ should enhance our understanding of the molecular mechanisms responsible for DOI's effects.

G_q and G_{11} are expressed almost ubiquitously throughout the central nervous system. One could argue that due to the high functional redundancy of $G\alpha_q$ and $G\alpha_{11}$, compensatory mechanisms in which $G\alpha_{11}$ substitutes for $G\alpha_q$ could be contributing to the effects of DOI on the head-twitch response. Given that $G\alpha_{q/11}$ knockouts die at day 10.5 of embryonic development (Offermanns et al., 1998), a Cre/*lox*P system (Orban et al., 1992) could be used to generate a mouse line which allows for tissue-specific conditional inactivation of $G\alpha_q$ in $G\alpha_{11}$-deficient mice. Unfortunately, there are several issues with this strategy including dwarfism accompanied by a high mortality rate (Wettschureck et al., 2005). Moreover, since levels of $G\alpha_q$ exceed those of $G\alpha_{11}$ by several fold in the CNS (Milligan, 1993), it is currently not clear whether $G\alpha_{11}$ levels would be too

low to compensate for the loss of $G\alpha_q$ or whether both proteins serve at least in part non-interchangeable functions.

Despite numerous controls, it is still debatable that the effects of genetic deletion of $G\alpha_q$, on the earliest stages of embryonic and postnatal development, may have affected the outcomes observed in the current studies. To address this concern, a modification of the lentiviral strategy to disrupt G-protein activation in adult animals should be considered in future experiments. First, after careful consideration of recent advances in the gene silencing field, I have decided that utilizing lentiviral delivery of G-protein blocking peptides may not be the best alternative to disrupt G-protein signaling activation in the mammalian brain. Given that blocking peptides disrupt receptor/G-protein signaling in a competitive manner, it is quite possible that achieving high enough levels of transgene expression following lentiviral delivery may not ultimately be feasible. On the other hand, lentiviral delivery of RNAi has successfully been shown to be a powerful aid to probe gene function *in vivo* (Van den Haute et al., 2003; Sapru et al., 2006; Szulc et al., 2006; for review, see Sandy et al., 2005). RNA interference, or RNAi, is an efficient and potent gene-specific silencing technique that uses double-stranded RNAs (dsRNA) to mark a particular transcript for degradation *in vivo* (Hanon, 2002). Key to the technique are dsRNAs 21-25 nucleotides long, called short interfering RNAs (siRNA), which are produced by degradation of long dsRNA. Once formed, the siRNAs associate with a multiprotein complex called RISC. This ribonucleoprotein complex then scans the mRNA cell-content to degrade the corresponding mRNA target in a highly

specific manner. RNAi would allow us to silence specific G-proteins without the concern of delivering sufficient quantities of blocking peptides to competitively inhibit endogenous $G\alpha_q$. An additional advantage of the RNAi strategy is that it allows us to specifically study the role of G-proteins in the adult animal, without potential complications in embryonic development commonly seen in genetically engineered knockouts. Furthermore, RNAi would allow us to evaluate the role of other G-proteins, downstream from 5-HT_{2A} receptors, in hallucinogenic drug action. For instance, 5-HT_{2A} receptor signaling has been shown to be linked to the activation of phospholipase D via $G\alpha_{13}$ (Mitchell et al., 1998; Robertson et al., 2003). Given that the knockout of $G\alpha_{13}$ in mice results in embryonic lethality at midgestation (Offermanns et al., 1997b), and that there are no available PLD inhibitors effective *in vivo*, RNAi appears to be the most viable alternative to explore the role of $G\alpha_{13}$ in hallucinogen-induced behaviors.

One caveat of these studies is that G_q protein couples to many other GPCRs in addition to the 5-HT_{2A} receptor, and these secondary targets might directly or indirectly influence the hallucinogen-induced effects observed in $G\alpha_q$ null mice. However, over the years, many studies of the pharmacological properties of hallucinogens have failed to implicate any other G_q coupled receptor except the 5-HT_{2C} receptor. To address this potential caveat in future experiments, we plan to utilize a modification of the pharmacogenetic approach described in chapter III, in which mice heterozygous for the 5-HT_{2A} receptor will be treated with a lentivirus expressing siRNA against $G\alpha_q$ mRNA in key brain regions known to mediate hallucinogen induced behaviors. We expect to observe

a synergistic effect of the genotype plus the lentiviral transgene delivery as the "drug treatment". With this strategy, we will be able to formulate more precise conclusions about the role of the G_q signaling pathway, downstream from 5-HT_{2A} receptors, in hallucinogen induced behaviors.

Future experiments, such as the ones outlined in this chapter, will further our understanding of the intracellular mechanisms underlying hallucinogenic drug action. Identifying the consequences of selective disruption 5-HT_{2A}-mediated pathways will bring us closer to elucidating both the mechanisms of action of hallucinogens, as well as their possible relevance to behavioral abnormalities observed in many psychiatric disorders.

REFERENCES

Abramowski D, Rigo M, Duc D, Hoyer D and Staufenbiel M (1995) Localization of the 5-hydroxytryptamine2C receptor protein in human and rat brain using specific antisera. *Neuropharmacology* **34**:1635-1645.

Aghajanian GK and Marek GJ (1997) Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. *Neuropharmacology* **36**:589-599.

Aghajanian GK and Marek GJ (1999a) Serotonin and hallucinogens. *Neuropsychopharmacology* **21**:16S-23S.

Aghajanian GK and Marek GJ (1999b) Serotonin, via 5-HT2A receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. *Brain Res* **825**:161-171.

Altman JL, Albert JM, Milstein SL and Greenberg I (1976) Drugs as the discriminative events in humans. *Psychopharmacol Commun* **2**:327-330.

Amatruda TT, 3rd, Steele DA, Slepak VZ and Simon MI (1991) G alpha 16, a G protein alpha subunit specifically expressed in hematopoietic cells. *Proc Natl Acad Sci U S A* **88**:5587-5591.

Appel, JB (1990) Drug discrimination as a tool in drug abuse research. *Psychopharmacology* **101**, S65

Arranz B, Rosel P, Sarro S, Ramirez N, Duenas R, Cano R, Maria Sanchez J and San L (2003) Altered platelet serotonin 5-HT2A receptor density but not second messenger inositol trisphosphate levels in drug-free schizophrenic patients. *Psychiatry Res* **118**:165-174.

Arvanov VL, Liang X, Russo A and Wang RY (1999) LSD and DOB: interaction with 5-HT2A receptors to inhibit NMDA receptor-mediated transmission in the rat prefrontal cortex. *Eur J Neurosci* **11**:3064-3072.

Baldwin DS, Hawley CJ, Abed RT, Maragakis BP, Cox J, Buckingham SA, Pover GH and Ascher A (1996) A multicenter double-blind comparison of nefazodone and paroxetine in the treatment of outpatients with moderate-to-severe depression. *J Clin Psychiatry* **57 Suppl 2**:46-52.

Barker EL, Westphal RS, Schmidt D and Sanders-Bush E (1994) Constitutively active 5-hydroxytryptamine2C receptors reveal novel inverse agonist activity of receptor ligands. *J Biol Chem* **269**:11687-11690.

Barry H, 3rd (1974) Classification of drugs according to their discriminable effects in rats. *Fed Proc* **33**:1814-1824.

Bartrup JT and Newberry NR (1994) 5-HT2A receptor-mediated outward current in C6 glioma cells is mimicked by intracellular IP3 release. *Neuroreport* **5**:1245-1248.

Berg KA, Maayani S and Clarke WP (1996) 5-hydroxytryptamine2C receptor activation inhibits 5-hydroxytryptamine1B-like receptor function via arachidonic acid metabolism. *Mol Pharmacol* **50**:1017-1023.

Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P and Clarke WP (1998) Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol Pharmacol* **54**:94-104.

Birnbaumer L (1992) Receptor-to-effector signaling through G proteins: roles for beta gamma dimers as well as alpha subunits. *Cell* **71**:1069-1072.

Bockaert J and Pin JP (1999) Molecular tinkering of G protein-coupled receptors: an evolutionary success. *Embo J* **18**:1723-1729.

Bonhaus DW, Bach C, DeSouza A, Salazar FH, Matsuoka BD, Zuppan P, Chan HW and Eglen RM (1995) The pharmacology and distribution of human 5-hydroxytryptamine2B (5-HT2B) receptor gene products: comparison with 5-HT2A and 5-HT2C receptors. *Br J Pharmacol* **115**:622-628.

Boothman LJ, Allers KA, Rasmussen K and Sharp T (2003) Evidence that central 5-HT2A and 5-HT2B/C receptors regulate 5-HT cell firing in the dorsal raphe nucleus of the anaesthetised rat. *Br J Pharmacol* **139**:998-1004.

Borchers MT, Biechele T, Justice JP, Ansay T, Cormier S, Mancino V, Wilkie TM, Simon MI, Lee NA and Lee JJ (2003) Methacholine-induced airway hyperresponsiveness is dependent on Galphaq signaling. *Am J Physiol Lung Cell Mol Physiol* **285**:L114-120.

Braff D, Stone C, Callaway E, Geyer M, Glick I and Bali L (1978) Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology* **15**:339-343.

Buhl AM, Osawa S and Johnson GL (1995) Mitogen-activated protein kinase activation requires two signal inputs from the human anaphylatoxin C5a receptor. *J Biol Chem* **270**:19828-19832.

Chambard JC, Van Obberghen-Schilling E, Haslam RJ, Vouret V and Pouyssegur J (1990) Chinese hamster serotonin (5-HT) type 2 receptor cDNA sequence. *Nucleic Acids Res* **18**:5282.

Chang M, Zhang L, Tam JP and Sanders-Bush E (2000a) Dissecting G protein-coupled receptor signaling pathways with membrane-permeable blocking peptides. Endogenous 5-HT(2C) receptors in choroid plexus epithelial cells. *J Biol Chem* **275**:7021-7029.

Chang MS, Tam JP and Sanders-Bush E (2000b) Dissecting intracellular signaling pathways with membrane-permeable peptides. *Sci STKE* **2000**:PL1.

Chapman PF (2002) Giving drugs to knockout mice: can they do that? *Trends Neurosci* **25**:277-279.

Conn PJ and Sanders-Bush E (1984) Selective 5HT-2 antagonists inhibit serotonin stimulated phosphatidylinositol metabolism in cerebral cortex. *Neuropharmacology* **23**:993-996.

Conn PJ, Sanders-Bush E, Hoffman BJ and Hartig PR (1986) A unique serotonin receptor in choroid plexus is linked to phosphatidylinositol turnover. *Proc Natl Acad Sci U S A* **83**:4086-4088.

Darmani NA, Martin BR and Glennon RA (1990a) Withdrawal from chronic treatment with (+/-)-DOI causes super-sensitivity to 5-HT2 receptor-induced head-twitch behaviour in mice. *Eur J Pharmacol* **186**:115-118.

Darmani NA, Martin BR, Pandey U and Glennon RA (1990b) Do functional relationships exist between 5-HT1A and 5-HT2 receptors? *Pharmacol Biochem Behav* **36**:901-906.

Darmani NA, Martin BR, Pandey U and Glennon RA (1990c) Pharmacological characterization of ear-scratch response in mice as a behavioral model for selective 5-HT2-receptor agonists and evidence for 5-HT1B- and 5-HT2-receptor interactions. *Pharmacol Biochem Behav* **37**:95-99.

Darmani NA, Shaddy J and Gerdes CF (1996) Differential ontogenesis of three DOI-induced behaviors in mice. *Physiol Behav* **60**:1495-1500.

Dave KD, Harvey JA and Aloyo VJ (2002) A novel behavioral model that discriminates between 5-HT2A and 5-HT2C receptor activation. *Pharmacol Biochem Behav* **72**:371-378.

Dhanasekaran N and Dermott JM (1996) Signaling by the G12 class of G proteins. *Cell Signal* **8**:235-245.

Dulawa SC and Geyer MA (2000) Effects of strain and serotonergic agents on prepulse inhibition and habituation in mice. *Neuropharmacology* **39**:2170-2179.

Dulawa SC, Hen R, Scearce-Levie K and Geyer MA (1998) 5-HT1B receptor modulation of prepulse inhibition: recent findings in wild-type and 5-HT1B knockout mice. *Ann N Y Acad Sci* **861**:79-84.

Durcan MJ and Lister RG (1988) Time course of ethanol's effects on locomotor activity, exploration and anxiety in mice. *Psychopharmacology (Berl)* **96**:67-72.

Dursun SM and Handley SL (1996) Similarities in the pharmacology of spontaneous and DOI-induced head-shakes suggest 5HT2A receptors are active under physiological conditions. *Psychopharmacology (Berl)* **128**:198-205.

Eberle-Wang K, Braun BT and Simansky KJ (1994) Serotonin contracts the isolated rat pylorus via a 5-HT2-like receptor. *Am J Physiol* **266**:R284-291.

Eison AS, Eison MS, Torrente JR, Wright RN and Yocca FD (1990) Nefazodone: preclinical pharmacology of a new antidepressant. *Psychopharmacol Bull* **26**:311-315.

Elliott G and O'Hare P (1997) Intercellular trafficking and protein delivery by a herpesvirus structural protein. *Cell* **88**:223-233.

Exton JH (1996) Regulation of phosphoinositide phospholipases by hormones, neurotransmitters, and other agonists linked to G proteins. *Annu Rev Pharmacol Toxicol* **36**:481-509.

Ferguson SS (2001) Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol Rev* **53**:1-24.

Fitzgerald LW, Iyer G, Conklin DS, Krause CM, Marshall A, Patterson JP, Tran DP, Jonak GJ and Hartig PR (1999) Messenger RNA editing of the human serotonin 5-HT2C receptor. *Neuropsychopharmacology* **21**:82S-90S.

Foguet M, Nguyen H, Le H and Lubbert H (1992) Structure of the mouse 5-HT1C, 5-HT2 and stomach fundus serotonin receptor genes. *Neuroreport* **3**:345-348.

Franklin KBJ. and Paxinos G., (1997) The mouse brain in stereotaxic coordinates. *Academic Press.* San Diego, CA.

Fromm C, Coso OA, Montaner S, Xu N and Gutkind JS (1997) The small GTP-binding protein Rho links G protein-coupled receptors and Galpha12 to the serum response element and to cellular transformation. *Proc Natl Acad Sci U S A* **94**:10098-10103.

Gagnon AW, Manning DR, Catani L, Gewirtz A, Poncz M and Brass LF (1991) Identification of Gz alpha as a pertussis toxin-insensitive G protein in human platelets and megakaryocytes. *Blood* **78**:1247-1253.

Geyer MA, Krebs-Thomson K, Braff DL and Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology (Berl)* **156**:117-154.

Gibson EL, Barnfield AM and Curzon G (1994) Evidence that mCPP-induced anxiety in the plus-maze is mediated by postsynaptic 5-HT2C receptors but not by sympathomimetic effects. *Neuropharmacology* **33**:457-465.

Gilchrist A, Bunemann M, Li A, Hosey MM and Hamm HE (1999) A dominant-negative strategy for studying roles of G proteins in vivo. *J Biol Chem* **274**:6610-6616.

Glennon RA, Titeler M and McKenney JD (1984) Evidence for 5-HT2 involvement in the mechanism of action of hallucinogenic agents. *Life Sci* **35**:2505-2511.

Graeff FG, Silveira MC, Nogueira RL, Audi EA and Oliveira RM (1993) Role of the amygdala and periaqueductal gray in anxiety and panic. *Behav Brain Res* **58**:123-131.

Graham FK (1975) Presidential Address, 1974. The more or less startling effects of weak prestimulation. *Psychophysiology* **12**:238-248.

Gray JA and Roth BL (2001) Paradoxical trafficking and regulation of 5-HT(2A) receptors by agonists and antagonists. *Brain Res Bull* **56**:441-451.

Green AR, O'Shaughnessy K, Hammond M, Schachter M and Grahame-Smith DG (1983) Inhibition of 5-hydroxytryptamine-mediated behaviour by the putative 5-HT2 antagonist pirenperone. *Neuropharmacology* **22**:573-578.

Gresch PJ, Barrett RJ, Sanders-Bush E and Smith RL (2006) Serotonin-2A Receptors in Rat Anterior Cingulate Cortex Mediate the Discriminative Stimulus Properties of Lysergic Acid Diethylamide. *J Pharmacol Exp Ther*.

Gresch PJ, Strickland LV and Sanders-Bush E (2002) Lysergic acid diethylamide-induced Fos expression in rat brain: role of serotonin-2A receptors. *Neuroscience* **114**:707-713.

Griffiths RR, Richards WA, McCann U and Jesse R (2006) Psilocybin can occasion mystical-type experiences having substantial and sustained personal meaning and spiritual significance. *Psychopharmacology (Berl)* **187**:268-283; discussion 284-292.

Grinspoon L, Bakalar J (1979), *Psychedelic Drugs Reconsidered*. p. 9.

Grotewiel MS and Sanders-Bush E (1999) Differences in agonist-independent activity of 5-Ht2A and 5-HT2c receptors revealed by heterologous expression. *Naunyn Schmiedebergs Arch Pharmacol* **359**:21-27.

Hamm HE (1998) The many faces of G protein signaling. *J Biol Chem* **273**:669-672.

Hampoelz B and Knoblich JA (2004) Heterotrimeric G proteins: new tricks for an old dog. *Cell* **119**:453-456.

Hartmann J, Blum R, Kovalchuk Y, Adelsberger H, Kuner R, Durand GM, Miyata M, Kano M, Offermanns S and Konnerth A (2004) Distinct roles of Galpha(q) and Galpha11 for Purkinje cell signaling and motor behavior. *J Neurosci* **24**:5119-5130.

Hoffman HS and Ison JR (1980) Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol Rev* **87**:175-189.

Hofmann A (1979) How LSD originated. *J Psychedelic Drugs* **11**:53-60.

Hooley R, Yu CY, Symons M and Barber DL (1996) G alpha 13 stimulates Na+-H+ exchange through distinct Cdc42-dependent and RhoA-dependent pathways. *J Biol Chem* **271**:6152-6158.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR and Humphrey PP (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev* **46**:157-203.

Hoyer D, Hannon JP and Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* **71**:533-554.

Hrdina PD and Vu TB (1993) Chronic fluoxetine treatment upregulates 5-HT uptake sites and 5-HT2 receptors in rat brain: an autoradiographic study. *Synapse* **14**:324-331.

Hubbard KB and Hepler JR (2006) Cell signalling diversity of the Gqalpha family of heterotrimeric G proteins. *Cell Signal* **18**:135-150.

Humphrey PP, Hartig P and Hoyer D (1993) A proposed new nomenclature for 5-HT receptors. *Trends Pharmacol Sci* **14**:233-236.

Ismaiel AM, De Los Angeles J, Teitler M, Ingher S and Glennon RA (1993) Antagonism of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane stimulus with a newly identified 5-HT2- versus 5-HT1C-selective antagonist. *J Med Chem* **36**:2519-2525.

Jakab RL and Goldman-Rakic PS (1998) 5-Hydroxytryptamine2A serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. *Proc Natl Acad Sci U S A* **95**:735-740.

Jalonen TO, Margraf RR, Wielt DB, Charniga CJ, Linne ML and Kimelberg HK (1997) Serotonin induces inward potassium and calcium currents in rat cortical astrocytes. *Brain Res* **758**:69-82.

Johansson C, Jackson DM, Zhang J and Svensson L (1995) Prepulse inhibition of acoustic startle, a measure of sensorimotor gating: effects of antipsychotics and other agents in rats. *Pharmacol Biochem Behav* **52**:649-654.

Johnson MP, Baez M, Kursar JD and Nelson DL (1995) Species differences in 5-HT2A receptors: cloned pig and rhesus monkey 5-HT2A receptors reveal conserved transmembrane homology to the human rather than rat sequence. *Biochim Biophys Acta* **1236**:201-206.

Johnson MP, Hoffman AJ, Nichols DE and Mathis CA (1987) Binding to the serotonin 5-HT2 receptor by the enantiomers of 125I-DOI. *Neuropharmacology* **26**:1803-1806.

Kagaya A, Mikuni M, Kusumi I, Yamamoto H and Takahashi K (1990) Serotonin-induced acute desensitization of serotonin2 receptors in human platelets via a mechanism involving protein kinase C. *J Pharmacol Exp Ther* **255**:305-311.

Kagaya A, Mikuni M, Muraoka S, Saitoh K, Ogawa T, Shinno H, Yamawaki S and Takahashi K (1993) Homologous desensitization of serotonin 5-HT2 receptor-stimulated intracellular calcium mobilization in C6BU-1 glioma cells via a mechanism involving a calmodulin pathway. *J Neurochem* **61**:1050-1056.

Katz A, Wu D and Simon MI (1992) Subunits beta gamma of heterotrimeric G protein activate beta 2 isoform of phospholipase C. *Nature* **360**:686-689.

Kellermann O, Tournois C, Richard S, Manivet P, Maroteaux L and Launay JM (1998) Signaling pathways and targets of the 5-HT2B receptor in the 1C11 serotonergic cell line. *Ann N Y Acad Sci* **861**:248.

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, Avenell KY, Stean T, Upton N, Bromidge S, Forbes IT, Brown AM, Middlemiss DN and Blackburn TP (1997) SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. *Neuropharmacology* **36**:609-620.

Kennett GA, Wood MD, Glen A, Grewal S, Forbes I, Gadre A and Blackburn TP (1994) In vivo properties of SB 200646A, a 5-HT2C/2B receptor antagonist. *Br J Pharmacol* **111**:797-802.

Kleven MS, Assie MB and Koek W (1997) Pharmacological characterization of in vivo properties of putative mixed 5-HT1A agonist/5-HT(2A/2C) antagonist anxiolytics. II. Drug discrimination and behavioral observation studies in rats. *J Pharmacol Exp Ther* **282**:747-759.

Kumari V, Soni W, Mathew VM and Sharma T (2000) Prepulse inhibition of the startle response in men with schizophrenia: effects of age of onset of illness, symptoms, and medication. *Arch Gen Psychiatry* **57**:609-614.

Kurrasch-Orbaugh DM, Parrish JC, Watts VJ and Nichols DE (2003) A complex signaling cascade links the serotonin2A receptor to phospholipase A2 activation: the involvement of MAP kinases. *J Neurochem* **86**:980-991.

Kursar JD, Nelson DL, Wainscott DB and Baez M (1994) Molecular cloning, functional expression, and mRNA tissue distribution of the human 5-hydroxytryptamine2B receptor. *Mol Pharmacol* **46**:227-234.

Lai Z and Brady RO (2002) Gene transfer into the central nervous system in vivo using a recombinanat lentivirus vector. *J Neurosci Res* **67**:363-371.

Lai Z, Han I, Zirzow G, Brady RO and Reiser J (2000) Intercellular delivery of a herpes simplex virus VP22 fusion protein from cells infected with lentiviral vectors. *Proc Natl Acad Sci U S A* **97**:11297-11302.

Leslie RA, Moorman JM and Grahame-Smith DG (1993) Lithium enhances 5-HT2A receptor-mediated c-fos expression in rat cerebral cortex. *Neuroreport* **5**:241-244.

Loric S, Launay JM, Colas JF and Maroteaux L (1992) New mouse 5-HT2-like receptor. Expression in brain, heart and intestine. *FEBS Lett* **312**:203-207.

Macara IG, Lounsbury KM, Richards SA, McKiernan C and Bar-Sagi D (1996) The Ras superfamily of GTPases. *Faseb J* **10**:625-630.

Mann JJ, Brent DA and Arango V (2001) The neurobiology and genetics of suicide and attempted suicide: a focus on the serotonergic system. *Neuropsychopharmacology* **24**:467-477.

Marek GJ and Aghajanian GK (1994) Excitation of interneurons in piriform cortex by 5-hydroxytryptamine: blockade by MDL 100,907, a highly selective 5-HT2A receptor antagonist. *Eur J Pharmacol* **259**:137-141.

Marek GJ and Aghajanian GK (1996) LSD and the phenethylamine hallucinogen DOI are potent partial agonists at 5-HT2A receptors on interneurons in rat piriform cortex. *J Pharmacol Exp Ther* **278**:1373-1382.

Marek GJ and Aghajanian GK (1998) 5-Hydroxytryptamine-induced excitatory postsynaptic currents in neocortical layer V pyramidal cells: suppression by mu-opiate receptor activation. *Neuroscience* **86**:485-497.

Marek GJ and Aghajanian GK (1999) 5-HT2A receptor or alpha1-adrenoceptor activation induces excitatory postsynaptic currents in layer V pyramidal cells of the medial prefrontal cortex. *Eur J Pharmacol* **367**:197-206.

Markou A, Matthews K, Overstreet DH, Koob GF and Geyer MA (1994) Flinders resistant hypocholinergic rats exhibit startle sensitization and reduced startle thresholds. *Biol Psychiatry* **36**:680-688.

Mazzola-Pomietto P, Aulakh CS, Wozniak KM, Hill JL and Murphy DL (1995) Evidence that 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced hyperthermia in rats is mediated by stimulation of 5-HT2A receptors. *Psychopharmacology (Berl)* **117**:193-199.

McBride PA, Anderson GM, Hertzig ME, Sweeney JA, Kream J, Cohen DJ and Mann JJ (1989) Serotonergic responsivity in male young adults with autistic disorder. Results of a pilot study. *Arch Gen Psychiatry* **46**:213-221.

McClue SJ, Brazell C and Stahl SM (1989) Hallucinogenic drugs are partial agonists of the human platelet shape change response: a physiological model of the 5-HT2 receptor. *Biol Psychiatry* **26**:297-302.

McGrew L, Chang MS and Sanders-Bush E (2002) Phospholipase D activation by endogenous 5-hydroxytryptamine 2C receptors is mediated by Galpha13 and pertussis toxin-insensitive Gbetagamma subunits. *Mol Pharmacol* **62**:1339-1343.

McGrew L, Price RD, Hackler E, Chang MS and Sanders-Bush E (2004) RNA editing of the human serotonin 5-HT2C receptor disrupts transactivation of the small G-protein RhoA. *Mol Pharmacol* **65**:252-256.

Meltzer HY, Matsubara S and Lee JC (1989) The ratios of serotonin2 and dopamine2 affinities differentiate atypical and typical antipsychotic drugs. *Psychopharmacol Bull* **25**:390-392.

Metwally KA, Dukat M, Egan CT, Smith C, DuPre A, Gauthier CB, Herrick-Davis K, Teitler M and Glennon RA (1998) Spiperone: influence of spiro ring substituents on 5-HT2A serotonin receptor binding. *J Med Chem* **41**:5084-5093.

Meyer JH, Cho R, Kennedy S and Kapur S (1999) The effects of single dose nefazodone and paroxetine upon 5-HT2A binding potential in humans using [18F]-setoperone PET. *Psychopharmacology (Berl)* **144**:279-281.

Meyer JH, Kapur S, Eisfeld B, Brown GM, Houle S, DaSilva J, Wilson AA, Rafi-Tari S, Mayberg HS and Kennedy SH (2001) The effect of paroxetine on 5-HT(2A) receptors in depression: an [(18)F]setoperone PET imaging study. *Am J Psychiatry* **158**:78-85.

Milligan G (1993) Regional distribution and quantitative measurement of the phosphoinositidase C-linked guanine nucleotide binding proteins G11 alpha and Gq alpha in rat brain. *J Neurochem* **61**:845-851.

Milligan G, Mullaney I and McCallum JF (1993) Distribution and relative levels of expression of the phosphoinositidase-C-linked G-proteins Gq alpha and G11 alpha: absence of G11 alpha in human platelets and haemopoietically derived cell lines. *Biochim Biophys Acta* **1179**:208-212.

Mitchell R, McCulloch D, Lutz E, Johnson M, MacKenzie C, Fennell M, Fink G, Zhou W and Sealfon SC (1998) Rhodopsin-family receptors associate with small G proteins to activate phospholipase D. *Nature* **392**:411-414.

Montiel C, Herrero CJ, Garcia-Palomero E, Renart J, Garcia AG and Lomax RB (1997) Serotonergic effects of dotarizine in coronary artery and in oocytes expressing 5-HT2 receptors. *Eur J Pharmacol* **332**:183-193.

Nanry KP and Tilson HA (1989) The role of 5HT1A receptors in the modulation of the acoustic startle reflex in rats. *Psychopharmacology (Berl)* **97**:507-513.

Neer EJ (1995) Heterotrimeric G proteins: organizers of transmembrane signals. *Cell* **80**:249-257.

Neve RL, Howe JR, Hong S and Kalb RG (1997) Introduction of the glutamate receptor subunit 1 into motor neurons in vitro and in vivo using a recombinant herpes simplex virus. *Neuroscience* **79**:435-447.

Nic Dhonnchadha BA, Bourin M and Hascoet M (2003a) Anxiolytic-like effects of 5-HT2 ligands on three mouse models of anxiety. *Behav Brain Res* **140**:203-214.

Nic Dhonnchadha BA, Hascoet M, Jolliet P and Bourin M (2003b) Evidence for a 5-HT2A receptor mode of action in the anxiolytic-like properties of DOI in mice. *Behav Brain Res* **147**:175-184.

Nichols DE (2004) Hallucinogens. *Pharmacol Ther* **101**:131-181.

Niswender CM, Sanders-Bush E and Emeson RB (1998) Identification and characterization of RNA editing events within the 5-HT2C receptor. *Ann N Y Acad Sci* **861**:38-48.

Noda M, Yasuda S, Okada M, Higashida H, Shimada A, Iwata N, Ozaki N, Nishikawa K, Shirasawa S, Uchida M, Aoki S and Wada K (2003) Recombinant human serotonin 5A receptors stably expressed in C6 glioma cells couple to multiple signal transduction pathways. *J Neurochem* **84**:222-232.

Offermanns S, Hashimoto K, Watanabe M, Sun W, Kurihara H, Thompson RF, Inoue Y, Kano M and Simon MI (1997a) Impaired motor coordination and persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking Galphaq. *Proc Natl Acad Sci U S A* **94**:14089-14094.

Offermanns S, Heiler E, Spicher K and Schultz G (1994) Gq and G11 are concurrently activated by bombesin and vasopressin in Swiss 3T3 cells. *FEBS Lett* **349**:201-204.

Offermanns S, Mancino V, Revel JP and Simon MI (1997b) Vascular system defects and impaired cell chemokinesis as a result of Galpha13 deficiency. *Science* **275**:533-536.

Offermanns S, Toombs CF, Hu YH and Simon MI (1997c) Defective platelet activation in G alpha(q)-deficient mice. *Nature* **389**:183-186.

Offermanns S, Zhao LP, Gohla A, Sarosi I, Simon MI and Wilkie TM (1998) Embryonic cardiomyocyte hypoplasia and craniofacial defects in G alpha q/G alpha 11-mutant mice. *Embo J* **17**:4304-4312.

Ohno M, Frankland PW, Chen AP, Costa RM and Silva AJ (2001) Inducible, pharmacogenetic approaches to the study of learning and memory. *Nat Neurosci* **4**:1238-1243.

Onaivi ES, Bishop-Robinson C, Darmani NA and Sanders-Bush E (1995) Behavioral effects of (+/-)-1-(2,5-dimethoxy-4-iodophenyl)-2-

aminopropane, (DOI) in the elevated plus-maze test. *Life Sci* **57**:2455-2466.

Orban PC, Chui D and Marth JD (1992) Tissue- and site-specific DNA recombination in transgenic mice. *Proc Natl Acad Sci U S A* **89**:6861-6865.

Ouagazzal A, Grottick AJ, Moreau J and Higgins GA (2001) Effect of LSD on prepulse inhibition and spontaneous behavior in the rat. A pharmacological analysis and comparison between two rat strains. *Neuropsychopharmacology* **25**:565-575.

Padich RA, McCloskey TC and Kehne JH (1996) 5-HT modulation of auditory and visual sensorimotor gating: II. Effects of the 5-HT2A antagonist MDL 100,907 on disruption of sound and light prepulse inhibition produced by 5-HT agonists in Wistar rats. *Psychopharmacology (Berl)* **124**:107-116.

Palfi S, Leventhal L, Chu Y, Ma SY, Emborg M, Bakay R, Deglon N, Hantraye P, Aebischer P and Kordower JH (2002) Lentivirally delivered glial cell line-derived neurotrophic factor increases the number of striatal dopaminergic neurons in primate models of nigrostriatal degeneration. *J Neurosci* **22**:4942-4954.

Pazos A, Cortes R and Palacios JM (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res* **346**:231-249.

Pazos A, Hoyer D and Palacios JM (1984) The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site. *Eur J Pharmacol* **106**:539-546.

Peroutka SJ, Lebovitz RM and Snyder SH (1981) Two distinct central serotonin receptors with different physiological functions. *Science* **212**:827-829.

Plassat JL, Boschert U, Amlaiky N and Hen R (1992) The mouse 5HT5 receptor reveals a remarkable heterogeneity within the 5HT1D receptor family. *Embo J* **11**:4779-4786.

Pompeiano M, Palacios JM and Mengod G (1994) Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. *Brain Res Mol Brain Res* **23**:163-178.

Price RD, Weiner DM, Chang MS and Sanders-Bush E (2001) RNA editing of the human serotonin 5-HT2C receptor alters receptor-mediated activation of G13 protein. *J Biol Chem* **276**:44663-44668.

Prunell M, Escorihuela RM, Fernandez-Teruel A, Nunez JF and Tobena A (1994) Anxiolytic profiles of alprazolam and ethanol in the elevated plus-maze test and the early acquisition of shuttlebox avoidance. *Pharmacol Res* **29**:37-46.

Rahimian R and Hrdina PD (1995) Possible role of protein kinase C in regulation of 5-hydroxytryptamine 2A receptors in rat brain. *Can J Physiol Pharmacol* **73**:1686-1691.

Rapport GA, Page IH (1948) Crystalline Serotonin. Science **108**: 329-330

Rees S, den Daas I, Foord S, Goodson S, Bull D, Kilpatrick G and Lee M (1994) Cloning and characterisation of the human 5-HT5A serotonin receptor. *FEBS Lett* **355**:242-246.

Reiser J, Harmison G, Kluepfel-Stahl S, Brady RO, Karlsson S and Schubert M (1996) Transduction of nondividing cells using pseudotyped defective high-titer HIV type 1 particles. *Proc Natl Acad Sci U S A* **93**:15266-15271.

Reiser J, Lai Z, Zhang XY and Brady RO (2000) Development of multigene and regulated lentivirus vectors. *J Virol* **74**:10589-10599.

Rhee SG (2001) Regulation of phosphoinositide-specific phospholipase C. *Annu Rev Biochem* **70**:281-312.

Rigdon GC and Weatherspoon JK (1992) 5-Hydroxytryptamine 1a receptor agonists block prepulse inhibition of acoustic startle reflex. *J Pharmacol Exp Ther* **263**:486-493.

Robertson DN, Johnson MS, Moggach LO, Holland PJ, Lutz EM and Mitchell R (2003) Selective interaction of ARF1 with the carboxy-terminal tail domain of the 5-HT2A receptor. *Mol Pharmacol* **64**:1239-1250.

Roth BL, Nakaki T, Chuang DM and Costa E (1984) Aortic recognition sites for serotonin (5HT) are coupled to phospholipase C and modulate phosphatidylinositol turnover. *Neuropharmacology* **23**:1223-1225.

Saltzman AG, Morse B, Whitman MM, Ivanshchenko Y, Jaye M and Felder S (1991) Cloning of the human serotonin 5-HT2 and 5-HT1C receptor subtypes. *Biochem Biophys Res Commun* **181**:1469-1478.

Sanders-Bush E, Burris KD and Knoth K (1988) Lysergic acid diethylamide and 2,5-dimethoxy-4-methylamphetamine are partial agonists at serotonin receptors linked to phosphoinositide hydrolysis. *J Pharmacol Exp Ther* **246**:924-928.

Sandy P, Ventura A and Jacks T (2005) Mammalian RNAi: a practical guide. *Biotechniques* **39**:215-224.

Sapru MK, Yates JW, Hogan S, Jiang L, Halter J and Bohn MC (2006) Silencing of human alpha-synuclein in vitro and in rat brain using lentiviral-mediated RNAi. *Exp Neurol* **198**:382-390.

Schloss P and Williams DC (1998) The serotonin transporter: a primary target for antidepressant drugs. *J Psychopharmacol* **12**:115-121.

Schreiber R, Brocco M, Audinot V, Gobert A, Veiga S and Millan MJ (1995) (1-(2,5-dimethoxy-4 iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT) 2A receptors: modulation by novel 5-HT2A/2C antagonists, D1 antagonists and 5-HT1A agonists. *J Pharmacol Exp Ther* **273**:101-112.

Schreiber R, Brocco M and Millan MJ (1994) Blockade of the discriminative stimulus effects of DOI by MDL 100,907 and the 'atypical' antipsychotics, clozapine and risperidone. *Eur J Pharmacol* **264**:99-102.

Scruggs JL, Patel S, Bubser M and Deutch AY (2000) DOI-Induced activation of the cortex: dependence on 5-HT2A heteroceptors on thalamocortical glutamatergic neurons. *J Neurosci* **20**:8846-8852.

Selemon LD, Rajkowska G and Goldman-Rakic PS (1995) Abnormally high neuronal density in the schizophrenic cortex. A morphometric analysis of prefrontal area 9 and occipital area 17. *Arch Gen Psychiatry* **52**:805-818; discussion 819-820.

Sipes TA and Geyer MA (1994) Multiple serotonin receptor subtypes modulate prepulse inhibition of the startle response in rats. *Neuropharmacology* **33**:441-448.

Sipes TE and Geyer MA (1995) DOI disruption of prepulse inhibition of startle in the rat is mediated by 5-HT(2A) and not by 5-HT(2C) receptors. *Behav Pharmacol* **6**:839-842.

Smith RL, Barrett RJ and Sanders-Bush E (1999) Mechanism of tolerance development to 2,5-dimethoxy-4-iodoamphetamine in rats: down-regulation of the 5-HT2A, but not 5-HT2C, receptor. *Psychopharmacology (Berl)* **144**:248-254.

Smith RL, Barrett RJ and Sanders-Bush E (2003) Discriminative stimulus properties of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane [(+/-)DOI] in C57BL/6J mice. *Psychopharmacology (Berl)* **166**:61-68.

Smith RL, Canton H, Barrett RJ and Sanders-Bush E (1998) Agonist properties of N,N-dimethyltryptamine at serotonin 5-HT2A and 5-HT2C receptors. *Pharmacol Biochem Behav* **61**:323-330.

Strathmann M and Simon MI (1990) G protein diversity: a distinct class of alpha subunits is present in vertebrates and invertebrates. *Proc Natl Acad Sci U S A* **87**:9113-9117.

Sunahara RK, Dessauer CW and Gilman AG (1996) Complexity and diversity of mammalian adenylyl cyclases. *Annu Rev Pharmacol Toxicol* **36**:461-480.

Swerdlow NR, Caine SB and Geyer MA (1992) Regionally selective effects of intracerebral dopamine infusion on sensorimotor gating of the startle reflex in rats. *Psychopharmacology (Berl)* **108**:189-195.

Szulc J, Wiznerowicz M, Sauvain MO, Trono D and Aebischer P (2006) A versatile tool for conditional gene expression and knockdown. *Nat Methods* **3**:109-116.

Tang WJ, Iniguez-Lluhi JA, Mumby S and Gilman AG (1992) Regulation of mammalian adenylyl cyclases by G-protein alpha and beta gamma subunits. *Cold Spring Harb Symp Quant Biol* **57**:135-144.

Tenailleau S, Corre I and Hermouet S (1997) Specific expression of heterotrimeric G proteins G12 and G16 during human myeloid differentiation. *Exp Hematol* **25**:927-934.

Tilakaratne N and Friedman E (1996) Genomic responses to 5-HT1A or 5-HT2A/2C receptor activation is differentially regulated in four regions of rat brain. *Eur J Pharmacol* **307**:211-217.

Tournois C, Mutel V, Manivet P, Launay JM and Kellermann O (1998) Cross-talk between 5-hydroxytryptamine receptors in a serotonergic cell line. Involvement of arachidonic acid metabolism. *J Biol Chem* **273**:17498-17503.

Umemori H, Inoue T, Kume S, Sekiyama N, Nagao M, Itoh H, Nakanishi S, Mikoshiba K and Yamamoto T (1997) Activation of the G protein Gq/11 through tyrosine phosphorylation of the alpha subunit. *Science* **276**:1878-1881.

Ushikubi F, Nakamura K and Narumiya S (1994) Functional reconstitution of platelet thromboxane A2 receptors with Gq and Gi2 in phospholipid vesicles. *Mol Pharmacol* **46**:808-816.

Van den Haute C, Eggermont K, Nuttin B, Debyser Z and Baekelandt V (2003) Lentiviral vector-mediated delivery of short hairpin RNA results in

persistent knockdown of gene expression in mouse brain. *Hum Gene Ther* **14**:1799-1807.

Wadenberg MG, Browning JL, Young KA and Hicks PB (2001) Antagonism at 5-HT(2A) receptors potentiates the effect of haloperidol in a conditioned avoidance response task in rats. *Pharmacol Biochem Behav* **68**:363-370.

Waeber C, Grailhe R, Yu XJ, Hen R and Moskowitz MA (1998) Putative 5-ht5 receptors: localization in the mouse CNS and lack of effect in the inhibition of dural protein extravasation. *Ann N Y Acad Sci* **861**:85-90.

Wainscott DB, Cohen ML, Schenck KW, Audia JE, Nissen JS, Baez M, Kursar JD, Lucaites VL and Nelson DL (1993) Pharmacological characteristics of the newly cloned rat 5-hydroxytryptamine2F receptor. *Mol Pharmacol* **43**:419-426.

Wan Y, Kurosaki T and Huang XY (1996) Tyrosine kinases in activation of the MAP kinase cascade by G-protein-coupled receptors. *Nature* **380**:541-544.

Wange RL, Smrcka AV, Sternweis PC and Exton JH (1991) Photoaffinity labeling of two rat liver plasma membrane proteins with [32P]gamma-azidoanilido GTP in response to vasopressin. Immunologic identification as alpha subunits of the Gq class of G proteins. *J Biol Chem* **266**:11409-11412.

Watts SW (1998) Activation of the mitogen-activated protein kinase pathway via the 5-HT2A receptor. *Ann N Y Acad Sci* **861**:162-168.

Wettschureck N, Moers A, Wallenwein B, Parlow AF, Maser-Gluth C and Offermanns S (2005) Loss of Gq/11 family G proteins in the nervous system causes pituitary somatotroph hypoplasia and dwarfism in mice. *Mol Cell Biol* **25**:1942-1948.

Wettschureck N and Offermanns S (2005) Mammalian G proteins and their cell type specific functions. *Physiol Rev* **85**:1159-1204.

Wilkie TM, Scherle PA, Strathmann MP, Slepak VZ and Simon MI (1991) Characterization of G-protein alpha subunits in the Gq class: expression in murine tissues and in stromal and hematopoietic cell lines. *Proc Natl Acad Sci U S A* **88**:10049-10053.

Willins DL, Deutch AY and Roth BL (1997) Serotonin 5-HT2A receptors are expressed on pyramidal cells and interneurons in the rat cortex. *Synapse* **27**:79-82.

Willins DL and Meltzer HY (1997) Direct injection of 5-HT2A receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. *J Pharmacol Exp Ther* **282**:699-706.

Wright DE, Seroogy KB, Lundgren KH, Davis BM and Jennes L (1995) Comparative localization of serotonin1A, 1C, and 2 receptor subtype mRNAs in rat brain. *J Comp Neurol* **351**:357-373.

Wu D, Katz A and Simon MI (1993) Activation of phospholipase C beta 2 by the alpha and beta gamma subunits of trimeric GTP-binding protein. *Proc Natl Acad Sci U S A* **90**:5297-5301.

Xia Z, Hufeisen SJ, Gray JA and Roth BL (2003) The PDZ-binding domain is essential for the dendritic targeting of 5-HT2A serotonin receptors in cortical pyramidal neurons in vitro. *Neuroscience* **122**:907-920.

Xu X, Croy JT, Zeng W, Zhao L, Davignon I, Popov S, Yu K, Jiang H, Offermanns S, Muallem S and Wilkie TM (1998) Promiscuous coupling of receptors to Gq class alpha subunits and effector proteins in pancreatic and submandibular gland cells. *J Biol Chem* **273**:27275-27279.

Yatham LN, Liddle PF, Dennie J, Shiah IS, Adam MJ, Lane CJ, Lam RW and Ruth TJ (1999) Decrease in brain serotonin 2 receptor binding in patients with major depression following desipramine treatment: a positron emission tomography study with fluorine-18-labeled setoperone. *Arch Gen Psychiatry* **56**:705-711.

Zhang Y, D'Souza D, Raap DK, Garcia F, Battaglia G, Muma NA and Van de Kar LD (2001) Characterization of the functional heterologous desensitization of hypothalamic 5-HT(1A) receptors after 5-HT(2A) receptor activation. *J Neurosci* **21**:7919-7927.