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Matthew Brodsky

Striatal 5-HT₆ receptors:
Roles in cocaine reinforcement
and neuronal primary cilia

Matthew Brodsky

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Reading Committee:
John F. Neumaier, Chair
Jane M. Sullivan
Charles Chavkin

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Abstract

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Matthew Brodsky

Chair of the Supervisory Committee:
Professor John F. Neumaier
Psychiatry and Behavioral Sciences

Serotonin (5-HT) is neurotransmitter that modulates a range of emotional behaviors and is involved in mediating behaviors related to reinforcement learning. Disturbances of the 5-HT system are implicated in psychiatric conditions such as post-traumatic stress disorder, depression, and anxiety. Of the 14 described 5-HT receptors, 5-HT₆ receptors have been implicated in a variety of cognitive processes and have generated interest due to increasing evidence for their role in feeding, obesity, anxiety, depression and cognition. The 5-HT₆ receptor is an excitatory G_s coupled receptor that is found almost exclusively in the brain and is localized abundantly on the primary cilia of neurons, especially in the striatum. Primary cilia are sensory organelles found on most neurons that receive both chemical and mechanical signals from the surrounding environment. The striatum is a brain region of the basal ganglia that mediates a

variety of neural functions including movement, learning, and cognition. From their subcellular localization on primary cilia to their pathway specific role in the striatum, this thesis dissertation will describe my work on understanding the underlying function of 5-HT₆ receptors.

My work shows 5-HT₆ receptors in the striatum can alter normal cocaine reward conditioning, and explores the effect of 5-HT₆ receptors on neuronal primary cilia. In particular, I show that increased expression of 5-HT₆ receptors in the indirect pathway medium spiny neurons (MSNs) but not direct pathway MSNs of the ventral striatum lead to an increase in the sensitivity to the reinforcing properties of cocaine. I also show that the activity of 5-HT₆ receptors affects the morphology of primary cilia in striatal neurons and that the amount of heterologous receptor expressed affects the subsequent subcellular receptor localization. Together my results continue to implicate an important role of 5-HT₆ receptors in animal behavior and indicate a need for further analysis of 5-HT₆ receptors in both a sub region and subcellular-specific manner.

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DEDICATION

This doctoral dissertation is dedicated to my family with particular regard to my late grandfather Dr. Anatol Brodsky.

CHAPTER I: INTRODUCTION

A. 5-HT

Serotonin (5-hydroxytryptamine, 5-HT) action was first observed in 1912 when researchers studying vasoconstriction by blood serum found that serum caused vasoconstriction in the intestine (O'Connor, 1912). The theory at the time was that adrenaline caused all vasoconstriction, however these researchers saw adrenaline relaxing intestine, implicating a substance in the serum other than adrenaline responsible for vasoconstriction (Göthert, 2013; O'Connor, 1912). 5-HT was isolated and crystallized some 35 years later and named “serotonin” indicating, “that its source is serum and its activity is one of causing constriction” (Rapport *et al*, 1948). Upon crystallization from blood, the chemical structure of serotonin was determined to be an indolealkylamine and identified as 5-hydroxytryptamine yet the dual nomenclature exists to this day (Göthert, 2013; Rapport, 1949). A few years later 5-HT was identified in the brain (Twarog and Page, 1953) and hypothesized to function as a possible neurotransmitter because lysergic acid diethylamide (LSD) suppressed action of 5-HT on the central nervous system of mice (Brodie *et al*, 1955; Shore *et al*, 1955). In the nearly 70 years since its discovery, 5-HT has been a major pharmacological target in brain research and found to be involved in many physiological functions in the brain.

The biochemical mechanism underlying the synthesis of 5-HT involves the hydroxylation of the essential amino acid, L-tryptophan, by tryptophan hydroxylase (TPH). L-tryptophan is taken up by neurons by a plasma membrane transporter (Boado *et al*, 1999), hydroxylation is catalyzed by the enzyme tryptophan hydroxylase to form 5-hydroxytryptophan (5-HTP), which then is rapid decarboxylated, by the enzyme L-amino acid decarboxylase, to form 5-HT (Berger

et al, 2009; Côté *et al*, 2003; Kandel, 2000; Walther *et al*, 2003) (Figure 1.1). After the biochemical synthesis of 5-HT it is stored in presynaptic nerve terminal vesicles with a serotonin binding protein, where it remains until synaptic vesicle release (Tamir and Kuhar, 1975).

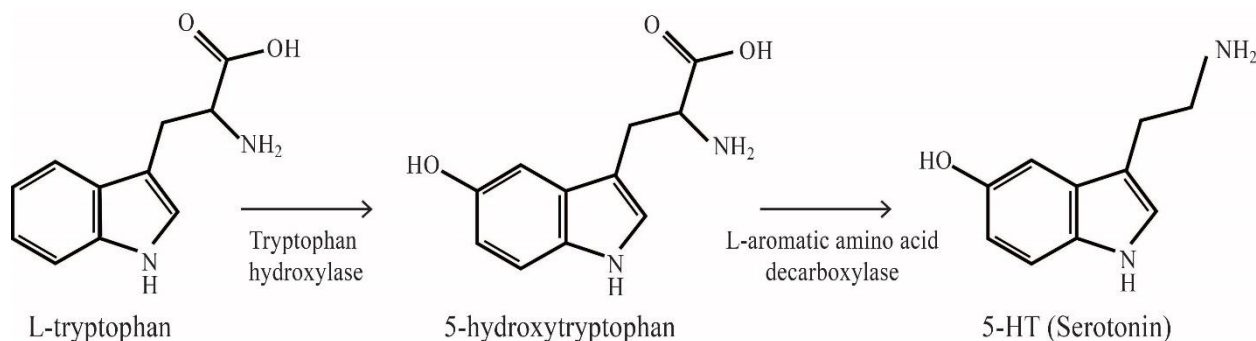


Figure 1.1 Synthesis of Serotonin (5-HT)

Although much of current research on 5-HT involves studying the monoamine's function in the brain, 5-HT is also found throughout the periphery, including the gastrointestinal tract, the cardiovascular system and immune cells (Barnes and Neumaier, 2011; Chamba *et al*, 2008; Lucki, 1998). 95% of peripheral 5-HT is found in the enteric nervous system in the lining of the gastrointestinal tract (Berger *et al*, 2009; Côté *et al*, 2003; Göthert, 2013). 5-HT in the gastrointestinal tract has been found to mediate responses as varied as nausea, intestinal secretion, and peristalsis and has even been implicated in irritable bowel syndrome (Berger *et al*, 2009; Hoyer *et al*, 2002). 5-HT's function throughout the central nervous system (CNS) is of primary concern regarding its implications in the neuromodulation of a broad array of behaviors,

including sleep, locomotion, feeding, vomiting, addiction, aggression, sexual activity and affect (Heath and Hen, 1995).

In the mammalian CNS, 5-HT is manufactured by a cluster of neurons located in the brainstem. These neuronal clusters are part of the raphe nuclei (Lucki, 1998; McDevitt *et al*, 2014; Tamir and Kuhar, 1975). Of the raphe nuclei, most of serotonergic neurons have their cell bodies in the dorsal and median nuclei, and supply the 5-HT throughout the forebrain. These serotonergic neurons in the dorsal and median raphe project extensively to nearly every brain region and are characteristically slow and rhythmic with regards to firing patterns (Allers and Sharp, 2003; Schweimer *et al*, 2011; Wang and Aghajanian, 1982). This slow, often tonic, discharge pattern provides electrophysiologists with a unique electrical signature for discerning the serotonergic neurons of the dorsal raphe nucleus (DRN) from other neurons in the region (Andrade and Haj-Dahmane, 2013; Wang and Aghajanian, 1982). Even though, the number of serotonergic neurons from the raphe nuclei is a relatively small number (~ 20,000 neurons in rat brain)(Andrade and Haj-Dahmane, 2013) as compared to the number of glutamatergic or dopaminergic neurons, the raphe nuclei send projections throughout the CNS (Jacobs and Azmitia, 1992; Whitaker-Azmitia, 1999). These relatively few projections have been found to influence a broad range of physiological systems.

5-HT projections have been implicated in a variety of physiological functions and multiple behaviors, including circadian rhythm entrainment, appetite, aggression, sexual behavior, sensorimotor reactivity, and pain sensitivity (Lucki, 1998; Prosser *et al*, 2014). More recently, a growing body of work has established that the serotonergic system affects multiple types of associative learning in mammals (Cowen and Lucki, 2011; Eskenazi and Neumaier, 2011a; Eskenazi *et al*, 2015; Mitchell *et al*, 2007). The neural circuitry responsible for each of

these 5-HT-mediated behavioral processes is not well understood, yet there is much research implicating specific nuclei, projections, and systems that are modulated by these 5-HT projections.

Dysfunction of the serotonergic system is implicated in multiple neuropsychiatric illnesses and impairments (Berger *et al*, 2009; Kirby *et al*, 2011). Among some of the disorders and diseases associated with 5-HT are depression, post-traumatic stress disorder, and anxiety (Carr and Lucki, 2012; Lucki, 1998). Due to the prevalence of 5-HT in all of the aforementioned disorders, serotonergic neurons, 5-HT receptors, and the serotonin transporter (SERT) have become major targets for pharmaceutical research. In particular, selective serotonin reuptake inhibitors (SSRIs) have become a ubiquitous treatment for affective disorders including depression and anxiety disorders. SSRIs target SERT function which manipulates the endogenous levels of 5-HT in the brain (Carr and Lucki, 2012; Carr *et al*, 2011; Fuller and Wong, 1990; Martinowich and Lu, 2008). Along with drugs targeting SERT function, many pharmaceuticals target the various 5-HT receptors. Research into depression and anxiety disorders has uncovered much about system and function of the 5-HT receptors.

B. 5-HT Receptors

The cellular effects of 5-HT are mediated by several pharmacologically and structurally distinct 5-HT receptors. These 5-HT receptors are found both in the central and peripheral nervous system (Barnes and Sharp, 1999; Göthert, 2013). The initial studies that identified the existence of more than one 5-HT receptor for serotonin were performed in the guinea pig ileum, where 5-HT mediated contractions were partially blocked by the indirect action of morphine and further blocked by dibenzylamine. They found that this additive blockade by two different drugs

represented two different mechanisms, and thus two different 5-HT receptors (Gaddum and Picarelli, 1957). It was not until 20 years later that radioligand binding of different radiolabeled ligands, [³H]5-HT, [³H]LSD or [³H]spiperone, demonstrated the presence of more than two distinct 5-HT receptor binding sites (Bennett and Snyder, 1976; Peroutka *et al*, 1981). Since these original receptor differentiation experiments many more 5-HT receptors have been identified.

Currently, 14 distinct serotonin receptors have been identified (Barnes and Neumaier, 2011; Barnes and Sharp, 1999; Hannon and Hoyer, 2008). All of the known 5-HT receptors are G protein-coupled receptors (GPCRs) with the exception of the ionotropic 5-HT₃ receptor (Barnes and Sharp, 1999; Hoyer *et al*, 2002). In the 1990s, a system for naming the receptors was developed by the International Union of Pharmacology; this new system included the operational, phylogenetic, and structural characteristics of each receptor (Hoyer and Martin, 1996; Hoyer *et al*, 1994). This wide range of 5-HT receptors allows 5-HT to mediate a wide range of actions via a variety of signal-transduction pathways. Each 5-HT receptor has a distinct and varied distribution throughout the body. Many experimental approaches have been used to evaluate and determine varied 5-HT receptor expression at the neuroanatomical level (Hannon and Hoyer, 2008).

In mammalian brain, the function of many 5-HT receptors are now associated with specific physiological responses, extending from modulation of neuronal activity to effects on animal behavior (Barnes and Neumaier, 2011). Studies on animal behavior in response to selective 5-HT receptor activity have led to the development and clinical use of drugs with 5-HT receptor-subtype selectivity (Barnes and Sharp, 1999). Research on the potential therapeutic effects of these receptors in treating anxiety, depression, pain, and cognition is ongoing. Based

on known differences in receptor sequences and function, the 13 different 5-HT GPCRs developed differential coupling and signaling cascades through evolution (Figure 1.2).

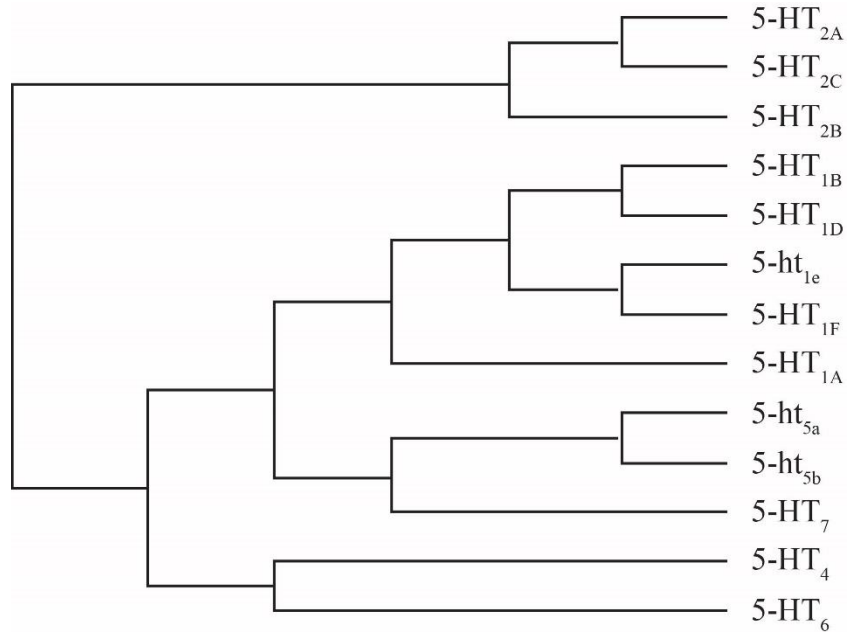


Figure 1.2 Phylogeny of 5-HT G-Protein Coupled Receptors (GPCRs) Divergent evolution of the 13 5-HT GPCRs. Nomenclature follows that of the International Union of Pharmacology guidelines (upper case used only when *in vivo* functional data are available for a receptor; lower case describes newly described recombinant receptors).

The 14 distinct mammalian 5-HT receptor subtypes (Table 1.1) can be divided into seven sub-families (5-HT₁ to 5-HT₇) based on their amino acid sequence, function, and structural characteristics (Hoyer and Martin, 1996; Hoyer *et al*, 2002). The 5-HT₁ receptor class is comprised of five receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}), which all signal through G $\alpha_{i/o}$ to inhibit cAMP formation (Hoyer *et al*, 1994) typically leading to decreases in cellular activity. The 5-HT₂ receptor class is comprised of three receptors (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}), which couple preferentially to G α_q to increase the hydrolysis of inositol phosphates and elevate cytosolic [Ca²⁺], typically leading to increased neuronal excitability (Cunningham *et*

al, 2011; Heath and Hen, 1995). The 5-HT₃ receptor is the only 5-HT receptor that is a ligand-gated ion channel, a non-selective cation channel that modulate fast neurotransmission (Barnes *et al*, 2009; Sugita *et al*, 1992). The 5-HT₄, 5-HT₆ and 5-HT₇ receptor families all preferentially couple to G α_s and typically elicit increases in neuronal activity through the increase of cAMP formation and the regulation of both calcium and potassium channels (Andrade and Chaput, 1991; Barnes and Sharp, 1999; Kohen *et al*, 2001; Sleight *et al*, 1998). The 5-HT₅ receptor family (5-HT_{5A} and 5-HT_{5B}) are not as well understood as the other 5-HT receptors as their preferential coupling remains unclear, but is hypothesized to be to either G $\alpha_{i/o}$ or possibly G α_s (Matthes *et al*, 1993; Waeber *et al*, 1998).

5-HT Receptor	Location in CNS	G-Protein Effector	Transduction System	Downstream Effects on Ion Channels
5-HT _{1A}	Hippocampus (hipp), raphé nuclei (RN), and cortex (Cor)	G $\alpha_{i/o}$	↓cAMP	↑Potassium channel
5-HT _{1B}	Striatum, RN, hipp	G $\alpha_{i/o}$	↓cAMP	↑Potassium channel
5-HT _{1D}	Hipp, Cor, DRN, spinal cord and periaqueductal grey	G $\alpha_{i/o}$	↓cAMP	↑Potassium channel
5-ht _{1E}	Cor, hipp, amygdala, hypothalamus (hypo)	G $\alpha_{i/o}$	↓cAMP	↑Potassium channel
5-ht _{1F}	Hipp, cortex, DRN, striatum (stri), hypo and thalamus (thal)	G $\alpha_{i/o}$	↓cAMP	↑Potassium channel
5-HT _{2A}	Cortex, hipp, olfactory tubercle and stri	G α_q	↑PLC	↓Leak potassium channel ↑Non-selective cation channel
5-HT _{2B}	Cerebellum, lateral septum, hypo, and medial amygdala	G α_q	↑PLC	↓Leak potassium channel ↑Non-selective cation channel
5-HT _{2C}	Choroid plexus, cor, hipp, nucleus accumbens (NAc), amygdala and basal ganglia	G α_q	↑PLC	↓Leak potassium channel ↑Non-selective cation channel
5-HT ₃	Hipp, area postrema, dorsal motor nucleus of the solitary tract, and stri	N/A	Ion conductance (K ⁺ , Na ⁺ , Ca ²⁺)	↑Non-selective cation channel
5-HT ₄	Stri, hipp, amygdala, olfactory tubercle	G α_s	↑cAMP	↓Leak potassium channel ↑Non-selective cation channel
5-ht _{5A}	Cerebellum, hipp, hypo, stri, cerebral cortex	?	?	Unknown
5-ht _{5B}	Cor, hipp, DRN, olfactory tubercle	?	?	Unknown
5-HT ₆	Stri, NAc, hipp, amygdala, cor, hypo, thal, caudate and putamen	G α_s	↑cAMP	↓Leak potassium channel ↑Non-selective cation channel
5-HT ₇	Thal, hypo, hipp	G α_s	↑cAMP	↓Leak potassium channel ↑Non-selective cation channel

Table 1.1 Summary of known 5-HT receptors. ↑, Activation; ↓, Inhibition (Barnes and Neumaier, 2011; Hannon and Hoyer, 2008; Hoyer and Martin, 1996; Noda *et al*, 2004)

C. 5-HT₆ receptors

One of the more recently identified 5-HT receptors is the 5-HT₆ receptor. Two independent groups simultaneously isolated and identified the 5-HT₆ receptor in the rat brain using newer techniques in molecular biology rather than the traditional pharmacological profiling (Monsma Jr. *et al*, 1993; Ruat *et al*, 1993). The groups utilized PCR probes based on known receptors to screen cDNA libraries from rat striatum and identified a new 5-HT receptor with GPCR structure and high affinity for both adenylyl cyclase and tricyclic psychotropic drugs (Monsma Jr. *et al*, 1993; Ruat *et al*, 1993).

Some 5-HT receptors have species-specific differences in drug affinities and interest in 5-HT₆ receptors as a tool for psychopharmacology led to the cloning and characterization of human 5-HT₆ receptor using PCR amplification from the human caudate cDNA library (Kohen *et al*, 1996). The human 5-HT₆ receptor protein was found to consist of 440 amino acids and a molecular mass of 46.96 kDa. When comparing the different 5-HT₆ receptors to each other the human 5-HT₆ had 89% amino acid sequence homology with the rat 5-HT₆ receptor (Branchek and Blackburn, 2000; Kohen *et al*, 1996). Studies of the mouse 5-HT₆ receptor found the nucleic acid sequence to have 94% and 84% homology to rat and human respectively (Hirst *et al*, 2003; Kohen *et al*, 2001). In humans, there is one described single nucleotide polymorphism in the coding region for the 5-HT₆ receptor as well as a nonfunctional truncated splice variant (Lee *et al*, 2005; Masellis *et al*, 2001; Olsen *et al*, 1999); these receptor variants have not be associated with any human disease.

5-HT₆ receptors are almost exclusively found in brain and 5-HT₆ receptor mRNA is detectable during development starting at embryonic day 12 (Boess and Martin, 1994; Grimaldi *et al*, 1998). A variety of methods including: northern blot analysis, *in situ* hybridization histochemistry, immunohistochemistry, and RT-PCR have localized 5-HT₆ receptors in striatum, nucleus accumbens, hippocampus, amygdala, cerebral cortex, hypothalamus, thalamus, olfactory tubercle, caudate nucleus and putamen of both rat and human brain samples (Gerard *et al*, 1997; Marazziti *et al*, 2013; Monsma Jr. *et al*, 1993; Ruat *et al*, 1993; Schechter *et al*, 2008; Sleight *et al*, 1998) The majority of the brain's 5-HT₆ receptors are found in striatum, where the 5-HT₆ receptors are detected in both direct and indirect pathway medium spiny neurons (MSNs) (Helboe *et al*, 2015; Tassone *et al*, 2011; Ward *et al*, 1995).

The subcellular localization of 5-HT₆ receptors is particularly interesting. 5-HT₆ receptors were originally visualized in the neuropil and electron microscopy suggested that localization to dendrites (Gerard *et al*, 1997). Further electron microscopy studies revealed that the 5-HT₆ receptor not only associated with dendrites, but also with primary neuronal cilia (Brailov *et al*, 2000; Hamon *et al*, 1999). 5-HT₆ receptors are the only serotonin receptors that localize to primary cilia (Berbari *et al*, 2008a; Guadiana *et al*, 2013). Localization to the primary cilia is thought to involve unique ciliary trafficking mechanisms which may require the presence of an amino acid consensus sequence in the third intracellular (i3) loop of the receptor (Berbari *et al*, 2008a; Nachury *et al*, 2010; Nagata *et al*, 2013). This primary cilia localization may have profound implications for interactions with other primary cilia-specific proteins, including adenylyl cyclase 3 (AC3), which is exclusively localized in primary cilia (Berbari *et al*, 2007; Ou *et al*, 2009; Wang *et al*, 2009).

5-HT₆ receptors are thought to signal as traditional G α _s-coupled GPCRs, but they also engage more subtle signaling pathways suggesting a more complex role (Dayer *et al*, 2015; Jacobshagen *et al*, 2014). As with other G α _s-coupled GPCRs, 5-HT₆ receptors activate adenylylate cyclase leading to intracellular increases of cAMP and thus activating protein kinase A activity (Hirst *et al*, 2006; Marazziti *et al*, 2013; Monsma Jr. *et al*, 1993; Ruat *et al*, 1993; Sebben *et al*, 1994; Yun and Rhim, 2011). G α _s activation by 5-HT₆ receptor agonism can activate several adenylylate cyclase isoforms, including AC3, AC5, and AC8 but not AC1 or AC8 (Baker *et al*, 1998; Kohen *et al*, 1996). 5-HT₆ receptor activation in striatum has also been shown to activate DARPP₃₂, which in turn activates Fyn kinase and Jun activation domain-binding protein-1. Fyn is a Src tyrosine kinase family member and when phosphorylated, Fyn is thought to activate Erk1/Erk2 kinases via the Ras-Raf-MEK pathway (Fienberg *et al*, 1998; Riccioni *et al*, 2011; Yun and Rhim, 2011; Yun *et al*, 2007). Recently, 5-HT₆ receptors were shown to independently associate with both the mammalian target of rapamycin (mTOR) and cyclin-dependent kinase 5 (Cdk5) (Dayer *et al*, 2015; Duhr *et al*, 2014; Jacobshagen *et al*, 2014; Meffre *et al*, 2012). The fact that 5-HT₆ receptors activate a plethora of signaling cascades (PKA, Erk, Cdk5, Fyn, and mTor) is indicative of how complex signaling through the receptor may be and underlie some of the unique roles 5-HT₆ receptors have in animal behavior,

Although the story behind 5-HT₆ receptor signaling is intricate, its impact on cognition has been repeatedly demonstrated, particularly on the role of 5-HT₆ receptors on learning and memory tasks and with reward and reinforcement learning (Ferguson *et al*, 2008; Mitchell and Neumaier, 2005; Svenningsson *et al*, 2007). Early studies on rats showed that 5-HT₆ receptor antagonism improved memory consolidation on instrumental learning and memory tasks (Meneses, 1999, 2001). Further work showed that 5-HT₆ antagonists have a restorative function

for cognition in memory deficit models (Foley *et al*, 2004; Mitchell and Neumaier, 2008; Mitchell *et al*, 2006). 5-HT₆ receptor antagonists elicit pro-cognitive effects in several other behavioral including novel-object recognition (King *et al*, 2004; Woods *et al*, 2012). Since blocking 5-HT₆ receptor is pro-cognitive, it seemed the normal function of the receptor must be impair cognition and memory. However, potent selective 5-HT₆ receptor agonists have also been shown to enhance learning and memory in cognitive tasks (Burnham *et al*, 2010; Kendall *et al*, 2011; Woods *et al*, 2012). Thus, both 5-HT₆ receptor agonist and antagonist compounds show promise as pro-cognitive therapeutics but an explanation for their paradoxical effects has not been forthcoming; however, one possible explanation is that 5-HT₆ activation may be pro-cognitive in some brain regions while reducing 5-HT₆ activation in other brain regions may be pro-cognitive (Fone, 2008).

There is also some research on the role of 5-HT₆ receptors with regard to reward and reinforcement learning. Antagonism of 5-HT₆ receptors resulted in dose-dependent increases in locomotion and self-administration of amphetamine (Frantz *et al*, 2002). Some reports found no effect of a systemic 5-HT₆ agonist or antagonist on cocaine self-administration (cocaine SA) (Fijal *et al*, 2010; Frantz *et al*, 2002; Valentini *et al*, 2012), while other studies suggested that cocaine reinforcement or reinstatement was increased by 5-HT₆ receptor activity (van Gaalen *et al*, 2010; Valentini *et al*, 2012). More recently, studies indicated that increasing expression of 5-HT₆ receptors in the dorsomedial striatum interfered with the acquisition of a simple operant task but did not affect learning or recall in the Morris Water Maze task (Mitchell *et al*, 2007). Analogously, increased 5-HT₆ receptor expression in nucleus accumbens (NAc) blocked the acquisition of conditioned-place preference (CPP) to cocaine without altering psychomotor sensitization (Ferguson *et al*, 2008). 5-HT₆ receptors also regulate reward-motivated learning and

the expression of habitual actions (Eskenazi and Neumaier, 2011a, 2011b). In both of these studies, 5-HT₆ receptors were increased in medium spiny neurons (MSNs) generally and were not targeted at the direct (dMSNs) or indirect (iMSNs) pathway specifically. It was also observed that increased striatal 5-HT₆ receptor activity has different effects on reward motivated learning using sucrose pellets as the reinforcer when expression is restricted to either the dMSNs or iMSNs (Eskenazi *et al*, 2015), suggesting that the distribution of the receptors is a key determinant of the behavioral impact of 5-HT₆ receptor signaling.

D. The Striatum

The basal ganglia are a group of four subcortical brain regions that are associated with a variety of neural functions including movement, learning, motivation, and cognition (Graybiel and Ragsdale, 1978; Kandel, 2000). Comprised of the striatum, the globus pallidus, the substantia nigra, and the subthalamic nucleus, the basal ganglia are some of the most widely researched nuclei in neuroscience (Bornstein and Daw, 2011; Graybiel and Ragsdale, 1978). Receiving input from cerebral cortex, thalamus, and brainstem, the striatum is known as the main input structure of the basal ganglia (Graybiel, 2005; Gruber and McDonald, 2012). Most (~95%) of the neurons in striatum are GABAergic MSNs (de Rover *et al*, 2002; Ward *et al*, 1995; Witten *et al*, 2010), which project through two distinct pathways, known as the direct (striatonigral) or indirect (striatopallidal) pathways (Gerfen *et al*, 1990, 1991; Surmeier *et al*, 2007). The dMSNs express mainly D₁ dopamine receptors and dynorphin (Dyn) whereas the iMSNs express mainly D₂ dopamine receptors and enkephalin (Enk) (Gerfen *et al*, 1990, 1991; Hikida *et al*, 2010). Enhanced output via the direct pathway is thought to facilitate motivated behaviors and other simple and complex actions while activation of the indirect pathway is thought to inhibit actions

(Chandra *et al*, 2015; Macpherson *et al*, 2014; Yager *et al*, 2015). Although the striatum has long been associated with the control of movement, current evidence suggests the striatum also contributes directly to many other executive functions including decision making (Balleine *et al*, 2007; Ferguson *et al*, 2013; Macpherson *et al*, 2014).

In rodents, the striatum can be separated into distinct anatomically and functionally diverse sub regions. Dorsal striatum is comprised of two major nuclei, dorsomedial striatum (caudate nucleus) and dorsolateral striatum (the putamen). In humans these nuclei are separated by the internal capsule. Ventral striatum is also comprised of two separate regions: the nucleus accumbens (NAc) and the olfactory tubercle (Graybiel and Ragsdale, 1978; Graybiel, 2005; Holt *et al*, 1997). Many functional differences exist between these different regions in dorsal and ventral striatum, which are thought to arise from differences in associated input and output circuitry. Dorsomedial striatum (DMS) is thought to be involved in processes relating to more flexible goal-directed or reward sensitive behaviors. The DMS is thought to be crucial for rapidly switching plans of actions based on current task conditions. This has been repeatedly tested in rats using dynamic changes in contingencies of skill learning and operant conditioning (Eskenazi *et al*, 2015; Gruber and McDonald, 2012; Kimchi and Laubach, 2009). Dorsolateral striatum (DLS) differs from DMS in that it is thought to be involved in cue-associated learning and habit formation. Under certain conditions, goal-directed actions eventually transform into a reflexive habitual response (Corbit *et al*, 2012; Eskenazi and Neumaier, 2011b; Gruber and McDonald, 2012). The NAc is the main part of ventral striatum and can also be divided into two separate structures: the NAc core and the NAc shell (Macpherson *et al*, 2014; Stuber *et al*, 2005; Valentini *et al*, 2012). These two subdivisions differ in that the NAc shell receives more dopaminergic input from the VTA than the core and may have slightly different implications in

reward mediated behaviors (McDevitt *et al*, 2014; Neumaier *et al*, 2002; Packard and Knowlton, 2002). NAc MSNs fire in patterns corresponding to and in anticipation of external events and cues. This temporal responsiveness in the NAc is likely encoding the relative value of cues and anticipated value of an action (Everitt *et al*, 1999; Wise *et al*, 1995; Yager *et al*, 2015). It is for this reason that the NAc is a common target of research for therapeutics regarding irregularities in the perceived relative valence of rewards, as seen in drug addiction (Stuber *et al*, 2005).

E. Primary Cilia

5-HT₆ receptors are thought to primarily exist on neuronal primary cilia. Every mammalian cell, including neurons, express a primary cilium at some point during their life cycle (Bishop *et al*, 2007; Singla and Reiter, 2006; Wheatley *et al*, 1996). Primary cilia are microtubule-supported organelles that stem from the basal body and extend beyond the plasma membrane and outside the surface of a cell, essentially turning each primary cilium into a separate antennae-like subcellular compartment (Arellano *et al*, 2012; Avasthi and Marshall, 2012; Louvi and Grove, 2011). Originally identified by the Swiss anatomist K.W. Zimmerman in 1898, primary cilia were ignored in biological research for many years and even misclassified as a vestigial component of the invertebrate nerve net (Praetorius and Spring, 2003; Whitfield, 2004). Often referred to as “non-motile” cilia, due to not being involved in generating motion, these receptor-rich structures receive both chemical and mechanical signals from the surrounding environment (Davenport and Yoder, 2005; Pazour and Witman, 2003; Praetorius and Spring, 2003). These signals are transduced by a discrete set of membrane-bound receptors specifically localized to the primary cilium. The large diversity and specificity of primary cilia-localized

receptors suggest that primary cilia have a great capacity for complex cellular signaling, particularly on neurons. The role of neuronal primary cilia in normal cognitive functions is not yet understood, but there is evidence that impairments of ciliary signaling produce cognitive deficits (Berbari *et al*, 2008a; Davenport and Yoder, 2005).

The unique structure of the primary cilium is comprised of multiple components. The stem-like core of primary cilia is known as the ciliary axoneme, which contains nine microtubule doublets as opposed to the 11 (9+2 arrangement) found in motile cilia (Domire and Mykytyn, 2009; Wheatley, 2005). The ciliary axoneme stems from a ciliary basal body containing the mother centriole of the cell (Arellano *et al*, 2012; Avasthi and Marshall, 2012). The basal body and associated structures function as a selectively permeable barrier between the primary cilium and the rest of the cell, essentially turning the primary cilium into a separate subcellular compartment (Louvi and Grove, 2011). Proteins which are present in primary cilia must be selectively trafficked across this barrier (Jin *et al*, 2010; Kulaga *et al*, 2004; Pazour and Witman, 2003). Once designated proteins cross the basal body barrier, they are carried along the ciliary axoneme by intraflagellar transport (IFT). IFT utilizes kinesin and dynein motor proteins to transport proteins along the axoneme to distal portions of primary cilia (Nachury *et al*, 2010). A select group of GPCRs are among the proteins that traffic to primary cilia in neurons. These GPCRs include: 5-HT₆ receptors, somatostatin receptor 3 (Sstr3), melanin-concentrating hormone receptor 1 (Mchr1), and D₁ dopamine receptors (Domire *et al*, 2011; Green *et al*, 2012; Nagata *et al*, 2013). Some of these primary cilia localizing receptors express exclusively to primary cilia while others may have a broader expression pattern; all of the receptors utilize the established mechanisms to reach primary cilia (Berbari *et al*, 2008b; Nachury *et al*, 2010; Ross *et al*, 2005). These ciliary localizing GPCRs are thought to have conserved amino acid sequences

on the third intracellular (i3) loop which are required for ciliary localization (Berbari *et al*, 2008a), as well as possible associated chaperone proteins which are needed for ciliary uptake (Berbari *et al*, 2008b). The large diversity and specificity of ciliary localizing receptors suggest primary cilia indicate that modulating these receptors may exploit a novel strategy for altering cellular activity in neurons without directly affecting synaptic neurotransmission.

Recently, there is increasing interest in the function and significance of primary cilia, especially with regards to their role in a variety of disorders now known as “ciliopathies” (Ainsworth, 2007; Novarino *et al*, 2011). Some of the best characterized ciliopathies arise from the mutation or complete loss of genes responsible for formation and function of primary cilia (Kim *et al*, 2010; Lee and Gleeson, 2011). Physical malformations of primary cilia include: Primary cilia shortening, elongation, and ablation. These physical changes are often associated with ciliopathies (Avasthi *et al*, 2012; Marley and von Zastrow, 2012; Ou *et al*, 2009). Although, little is known about the function of primary cilia on neurons, common symptoms of ciliopathies include brain deformation and cognitive impairments (Green *et al*, 2012; Louvi and Grove, 2011). The presence of primary cilia on neurons and the study of ciliopathy symptoms has led to a convergence of evidence that primary cilia may play an important role in the pathology of various neuropsychiatric disorders not yet classified as ciliopathies (Marley and von Zastrow, 2012; Ruat *et al*, 2012). Screens of genes linked to diseases such as schizophrenia, bipolar affective disorder, autism spectrum disorder, and Alzheimer’s have identified upwards of 100 genes that are also related to the function, regulation, and formation of primary cilia neurons (Armato *et al*, 2013; Marley and von Zastrow, 2012; Massinen *et al*, 2011). Despite the phenotypic diversity of these neuropsychiatric disorders, a common component of their pathologies may include the primary cilia as a cellular “node” (Marley and von Zastrow, 2012).

In addition to possible roles in the pathology of neuropsychiatric disorders, disruption of neuronal primary cilia lead to an adverse effect on the migration and differentiation of developing neurons (Guadiana *et al*, 2013). Since both neuronal development and genetic neuropsychiatric disorders seem to rely on some genes associated with primary cilia, genes coding for proteins present on neuronal primary cilia, including GPCRs, seem to be a logical target of comprehensive studies.

F. Summary

From the subcellular localization of 5-HT₆ receptors in primary cilia to their striatal pathway specific role in the reinforcing properties of cocaine, in this thesis I will describe my work on understanding the underlying function of 5-HT₆ receptors. My work in the Neumaier lab both expanded on previous work from the lab showing that increased expression of 5-HT₆ receptors in the striatum can alter normal cocaine instrumental reward mechanisms, and opened new doors regarding the effect of 5-HT₆ receptors on neuronal primary cilia. In chapter II, I describe how increased expression of 5-HT₆ receptors in the indirect pathway MSNs but not direct pathway MSNs of the NAc shell leads to an increase in the sensitivity to the reinforcing properties of cocaine. This finding further implicates the importance of location-specific 5-HT₆ receptor activation in reward learning and furthers the notion that differential activation of the striatal pathways affects cocaine reinforcement. In chapter III, I address the unique subcellular localization of 5-HT₆ receptors to neuronal primary cilia. I found that the activity of 5-HT₆ receptors affects the morphology of primary cilia in striatal cultures and that the amount of exogenous receptor you express affects the subsequent localization of the heterologous receptor. These findings are some the first to implicate a role for endogenous 5-HT₆ receptors on primary

cilia function or structure. These findings also challenge conventional approaches of exogenously increasing receptor expression by showing that the amount of heterologous receptor expressed directly effects how it is expressed in the system. The implications of these studies are further considered in Chapter IV, along with an outline of the future direction of studies of striatal 5-HT₆ receptors.

CHAPTER II:
**Striatal 5-HT₆ receptors regulate cocaine reinforcement in a
pathway-selective manner**

* This chapter has currently been resubmitted with revisions as an article with the same title to *Neuropsychopharmacology*. Alec W. Gibson, Sunila Nair, Denis Smirnov, and John F. Neumaier are coauthors.

A.W.G., S.N., J.F.N. and I contributed to experimental design. A.W.G. and I collected all the experimental data with assistance from D.S. I carried out data analysis. I prepared the manuscript with A.W.G. and J.F.N.

A. Summary

The nucleus accumbens (NAc) in the ventral striatum integrates many neurochemical inputs including dopamine and serotonin projections from midbrain nuclei to modulate drug reward. While D₁ and D₂ dopamine receptors are differentially expressed in the direct and indirect pathway medium spiny neurons (dMSNs and iMSNs, respectively), 5-HT₆ receptors are expressed in both pathways, more strongly than anywhere else in the brain, and are an intriguing target for neuropsychiatric disorders. In the present study, we used viral vectors utilizing dynorphin- or enkephalin promoters to drive expression of 5-HT₆ receptors or green fluorescent protein (GFP) selectively in the dMSNs or iMSNs of the NAc shell (NAcSh). Rats were then trained to self-administer cocaine. Increased 5-HT₆ receptor expression in dMSNs did not change any parameter of cocaine self-administration measured. However, increasing 5-HT₆ receptors in iMSNs reduced the amount of cocaine self-administered under fixed-ratio schedules, especially at low doses, increased the time to the first response and the length of the inter-infusion interval, but did not alter motivation as measured by progressive ratio “break point” analysis. Modeling of cocaine pharmacokinetics in NAc showed that increased 5-HT₆ receptors in iMSNs reduced the rat’s preferred tissue cocaine concentration at each dose. Finally, increased 5-HT₆ receptors in iMSNs facilitated conditioned place preference for a low dose of cocaine. We conclude that 5-HT₆ receptors in iMSNs of NAcSh increase the sensitivity to the reinforcing properties of cocaine, particularly at low doses, suggesting that these receptors may be a therapeutic target for the treatment of cocaine addiction.

B. Introduction

Substance use disorders are prevalent neuropsychiatric conditions that are costly on personal and societal levels. A recent estimate holds substance addiction accountable for over 500 billion dollars spent annually within the United States alone (Rehm *et al*, 2009; U.S. Department of Justice National Drug Intelligence Center, 2011; United States Department of Health and Human Services, 2014). Addiction is thought to involve maladaptations of the neural processes that normally mediate reward learning in neural circuitry converging in the dorsal and ventral striatum (Hyman *et al*, 2006), which lead to compulsive substance taking. In particular, the nucleus accumbens (NAc) of ventral striatum is crucial to drug reward and may be an appropriate target for future addiction therapies (Stuber *et al*, 2011). The NAc is comprised of mostly GABAergic medium spiny neurons (MSNs) which project through two distinct pathways, known as the direct (striatonigral) and indirect (striatopallidal) pathways (Gerfen *et al*, 1990, 1991; Surmeier *et al*, 2007). Output via the direct pathway is thought to facilitate actions while the indirect pathway is thought to inhibit actions (Chandra *et al*, 2015; Macpherson *et al*, 2014; Yager *et al*, 2015). The direct pathway MSNs (dMSNs) express mainly D₁ dopamine receptors and dynorphin (Dyn) whereas the indirect pathway MSNs (iMSNs) express mainly D₂ dopamine receptors and enkephalin (Enk) (Gerfen *et al*, 1990, 1991; Hikida *et al*, 2010). The specific functions and mechanisms underlying drug seeking within these pathways is not well understood, but the relative activity in these pathways is likely to play a role in the progression of addiction (Bock *et al*, 2013; Hikida *et al*, 2010).

Cocaine's reinforcing properties involve direct interactions with monoamine transporters including the serotonin transporter (Sora *et al*, 2001; Uchimura and North, 1990). Serotonin

neurons strongly innervate ventral striatum (Dölen *et al*, 2013; McDevitt *et al*, 2014; Parsons and Justice, 1993a) and cocaine increases extracellular serotonin in NAc (Andrews and Lucki, 2001; Parsons and Justice, 1993b). By increasing extracellular 5-HT, cocaine enhances signaling at multiple 5-HT receptor subtypes, several of which have been implicated in mediating the reinforcing properties of cocaine in NAc (Müller and Homberg, 2015).

Among the 14 identified serotonin receptors, 5-HT₆ receptors are notable for their abundance in striatum, where they are expressed in both dMSNs and iMSNs (Hirst *et al*, 2003; Tassone *et al*, 2011; Ward *et al*, 1995). Some reports found no effect of a systemic 5-HT₆ agonist or antagonist on cocaine self-administration (cocaine SA) (Fijal *et al*, 2010; Frantz *et al*, 2002; Valentini *et al*, 2012), while other studies suggested that cocaine reinforcement or reinstatement was regulated by 5-HT₆ receptor activity (van Gaalen *et al*, 2010; Valentini *et al*, 2012). We previously showed that viral-mediated increases in 5-HT₆ receptor expression in NAcSh blocked the acquisition of conditioned place preference to cocaine without altering psychomotor sensitization (Ferguson *et al*, 2008). 5-HT₆ receptors also regulate reward motivated learning and the expression of habitual actions (Eskenazi and Neumaier, 2011a, 2011b). In both these studies, 5-HT₆ receptors were increased in MSNs generally and were not specifically targeted at the direct or indirect pathway. Recently, we observed that increased striatal 5-HT₆ receptor activity had different effects on reward motivated learning using sucrose reinforcers when expression was restricted to either the dMSNs or iMSNs (Eskenazi *et al*, 2015), suggesting that the distribution of the receptors is a key determinant of the behavioral impact of 5-HT₆ receptor signaling.

In general, striatal 5-HT₆ receptors tend to oppose dopamine's effects on drug reward (Eskenazi *et al*, 2015). Because this might involve opposing the differential activation of both

pathways by dopamine, we used phenotype-specific viral vectors to increase expression of 5-HT₆ receptors or GFP selectively in the dMSNs or iMSNs in the NAc shell (NAcSh) of rats. Our original hypothesis was that 5-HT₆ receptors in dMSNs and iMSNs would increase and decrease cocaine reinforcement, respectively. However, upon examination of several parameters of cocaine reinforcement and reward, we conclude that increasing 5-HT₆ receptors only in iMSNs increases the sensitivity to the reinforcing properties of cocaine, particularly at low doses, suggesting that these receptors may be a target for pharmacological manipulation in the treatment of cocaine addiction.

C. Materials and Methods

Animals

For all experiments, male Long-Evans rats (Charles River, Raleigh, NC) weighing 350-450 grams were used. Rats were double-housed for one week to acclimate them to the temperature- and humidity-controlled vivarium prior to the experiment, and were kept under a 12-hour light–dark cycle. All experiments were carried out during the light period. Following implantation of intravenous catheters, the rats were housed individually. Food and water were freely available at all times except during the cocaine SA sessions. All experimental procedures were approved by the University of Washington Institutional Animal Care and Use Committee and were conducted in accordance to the guidelines of the “Principles of Laboratory Animal Care” (NIH publication no. 86–23, 1996). A total of 76 rats were used for these experiments, of which 4 were excluded due to failure to learn to self-administer cocaine, 5 were excluded because viral-mediated gene expression was outside the target brain region, and 11 were removed from the study due to health issues (such as lost IV access).

Intravenous catheter placement and intracranial virus-mediated gene transfer

Jugular catheters were implanted as previously described (Nair *et al*, 2013). Rats were allowed to recover for 10 days before cocaine SA training. During the recovery and training phases, catheters were flushed every 48 hours using sterile gentamicin (0.08 mg/mL). We used replication-deficient herpes-simplex viral (HSV) vectors to increase 5-HT₆ receptor expression in either dMSNs or iMSNs of the NAcSh. The experiment utilized four different viral cassettes for this manipulation: two that express fully functional hemagglutinin-tagged (HA) 5-HT₆ receptors under either the proenkephalin promoter (Enk-5-HT₆) or the prodynorphin promoter (Dyn-5-HT₆), and two control viruses that express GFP alone under either the proenkephalin promoter (Enk-GFP) or the prodynorphin promoter (Dyn-GFP). We have previously confirmed that these viral vectors produce HA-tagged 5-HT₆ receptors in either enkephalin or dynorphin containing neurons and not glia (Eskenazi *et al*, 2015; Ferguson *et al*, 2011, 2013; Michaelides *et al*, 2013). HSV vectors were injected at 400 nl/min using surgical procedures previously described (Eskenazi *et al*, 2015; Ferguson *et al*, 2013). The volume of viral vector (2 µl) was chosen based on previous studies in our laboratory to induce discrete infection in the target region (Mitchell and Neumaier, 2008; Mitchell *et al*, 2007). Using a 10° angle of approach, the NAcSh was targeted using the coordinates relative to bregma: +1.7 mm (anterior-posterior), ± 2.3 mm (medial-lateral), and -7.6 mm (dorsal-ventral). To confirm the injection site, rats were perfused as previously described (Eskenazi and Neumaier, 2011a), brains were dissected and post-fixed in 2.5% paraformaldehyde for 6 hours, after which they were placed 30% sucrose in PBS. Tissue sections were made on a Leica Jung CM 3000 cryostat and mounted on slides at 40 µm thickness. Accuracy of injection coordinates was confirmed by visualization of the injection

needle tracts. Rats with injection sites outside of the targeted brain region were excluded from the experiments.

Behavioral testing

Apparatus

The rats were trained and tested in standard Med Associates operant chambers (Med Associates, Georgia, VT). Each chamber was equipped with two levers located 9 cm above the grid floor. Lever-presses on the active lever activated the infusion pump whereas lever-presses on the inactive lever had no programmed response. All chambers were kept in sound-attenuating boxes equipped with fans for temperature regulation and to provide white noise. All chambers were connected to a Med Associates interface, and experimental data were collected using Med-PC software.

Behavioral Procedures

The procedure consisted of four phases: fixed-ratio cocaine SA (11 days), progressive ratio (4 days), low-dose responding (3 days) and high-dose responding (3 days).

Cocaine SA

Rats were trained to self-administer cocaine for 2 hours/day (2 one hour sessions with a 5 minute interval between sessions) for 11 days. Cocaine hydrochloride (National Institute on Drug Abuse, Bethesda, MD) was dissolved in sterile injectable 0.9% saline and infused in a volume of 0.1 ml at a dose of 0.75 mg/kg/infusion. Each session started with the turning on of a white house-light and introduction of the levers into the operant chamber. During training, cocaine infusions were earned under a fixed-ratio-1 (FR1), 20 second timeout reinforcement schedule and were accompanied by a compound tone-light cue for 5 seconds. During the 20 second timeout period, lever presses were recorded but did not result in cocaine delivery. A maximum of 20 cocaine infusions/hour was set to prevent cocaine overdose. At the end of each session, the house-light was turned off and the levers retracted.

Progressive ratio

Following the FR1 sessions, rats were trained to SA cocaine for four days on a progressive ratio (PR) reinforcement schedule, during which the response requirement to earn a cocaine infusion (0.75 mg/kg/infusion) increased after each infusion earned. The response requirement increased incrementally in accordance with the following equation: Response ratio = $(5e^{[\text{injection number} \times 0.2]} - 5)$ (Richardson and Roberts, 1996). The PR sessions were terminated when the rat failed to receive a cocaine infusion within one hour.

Cocaine dose-response experiments

Following cocaine SA on FR1 and PR schedules, behavioral responding was measured for 3 days each on a low and a high dose of cocaine. The low-dose testing was done during a 2 hour FR1 SA session where each response yielded a cocaine infusion (0.375 mg/kg/infusion). Three days of high-dose (1.5 mg/kg/infusion) FR1 sessions followed immediately after the low dose period.

Cocaine pharmacokinetic modeling

Whole-brain levels of cocaine were modeled using a two-compartment mathematical model for rats receiving iv cocaine as previously described (Zimmer *et al*, 2011), based on an original report that measured extracellular cocaine concentration in the NAc measured by microdialysis (Pan *et al*, 1991). The average estimated cocaine concentration for each session was calculated between 10-120 minutes after the initiation of the session using the equation:

$$c = \frac{dk}{v(\alpha - \beta)} (e^{-\beta t} - e^{-\alpha t})$$

Equation 2.1 Estimated cocaine brain concentration

which gives the estimated cocaine brain concentration (c) by accounting for the dose of cocaine (d), the transport of cocaine between the blood and brain ($k = 0.233 \text{ min}^{-1}$), the brain volume ($v = 0.151 \text{ kg}^{-1}$), and the removal of cocaine from circulation via redistribution ($\alpha = 0.642 \text{ min}^{-1}$) and

elimination ($\beta = 0.097\text{min}^{-1}$). Group means for each unit dose were calculated from the average values for each animal over the three testing sessions.

Locomotor Activity

On the day following the completion of all cocaine SA behavior, locomotor activity was measured in infrared beam break activity boxes (22 x 45 x 23 cm; San Diego Instruments, San Diego, California) for 30 minutes in a dimly lit room. Locomotor activity was analyzed in 3 minute bins.

Conditioned place preference

A separate cohort of rats began place conditioning trials on the eleventh day after viral infusion using a three-chamber conditioned place preference (CPP) apparatus (Medical Associates, St Albans, VT, USA) comprised of two large side chambers (24 x 21 x 21 cm) separated by a small central chamber (12 x 21 x 21 cm). The three chambers differed in lighting (dim, medium or bright), wall color (white, black or gray) and floor texture (grid, rod or solid). Before the onset of the study, light intensities were adjusted so that there was no overall preference by a separate test group of animals for any chamber. We did not use a pre-exposure trial to avoid latent inhibition of associations between the drug effect and the chamber cues (Barot *et al*, 2007; Tzschentke, 1998). Chamber pairing with drug was randomly assigned in a counterbalanced fashion so any preexisting individual preferences would be randomly distributed.

The first trial of each conditioning day began at 09.00–10.00 am, with each animal receiving an injection of isotonic saline (1 mL/kg, i.p.). After the injection, animals were

confined to one of the side chambers of the CPP apparatus for 15 minutes. Three hours after the morning trial, animals received cocaine hydrochloride (NIDA 5 mg/kg in 1 ml saline, i.p.), after which they were placed into the other side chamber for 15 minutes. Conditioning trials were repeated in the same manner for the following 3 days. The day following the three conditioning trials (Day 14), animals were tested for CPP. The animal was placed into the central chamber, and after a 3 minute habituation period the doors were raised and the animal was allowed to explore the entire apparatus for 15 minutes. Time spent in each compartment was recorded automatically.

Immunohistochemistry

Floating sections (40 μm) were washed in 0.5% Triton-X/PBS for 10 minutes, then blocked in 10% normal goat serum (NGS)-Triton-X/PBS for 1 hour. Sections were then incubated in 5% NGS-Triton-X/PBS containing HA (1:400, rabbit, Cell Signaling) with gentle agitation at 4°C overnight. Next, sections were rinsed 4 times in PBS and incubated with Alexa 488-conjugated, goat anti-rabbit secondary antibody (1:250, Invitrogen, Carlsbad, CA) for 2 hours. Sections were washed 3 times in PBS, mounted on slides and cover-slipped with ProLong Gold Antifade mounting medium (Life Technologies). Images were captured with a Nikon fluorescence microscope and associated ZEN software.

Statistical analyses

Data from the cocaine SA sessions were collected using Med PC IV software. Analysis was performed using GraphPad Prism (Version 5.01). Significance for all cocaine SA data was

tested with two-way analyses of variance (ANOVAs; with or without repeated measures, as warranted) followed by Bonferroni post hoc tests (Supplemental Table 1). For analysis involving only two samples we used a two-tailed t-test. For all comparisons, we used an alpha value of 0.05.

D. Results

Pathway Specific Targeting of 5-HT₆ Receptors in NAcSh

To increase expression of 5-HT₆ receptors in either the direct or indirect pathway neurons selectively, we used HSV viral vectors that have been described (Figure 2.1a) (Eskenazi *et al*, 2015; Ferguson *et al*, 2011, 2013; Michaelides *et al*, 2013). These vectors utilize the rat prodynorphin (Dyn) or the rat proenkephalin (Enk) promoter to induce transgene expression of either hemagglutinin (HA) tagged 5-HT₆ receptors or GFP in direct and indirect pathway medium spiny neurons (dMSNs and iMSNs), respectively. The dMSN and iMSN manipulations were performed on separate cohorts of animals run at different times and were hence analyzed separately. The medial NAcSh was targeted and accurate injections were confirmed histologically (Figure 2.1b); five animals were excluded due to inaccurate injections. Transgene expression using these the Enk and Dyn promoters has been shown by our lab to be present up to two months post-injection (Ferguson *et al*, 2008) and was confirmed in NAcSh 40 days post-injection using immunohistochemistry (Figure 2.1c). The experimental timeline is illustrated in Figure 2.1d.

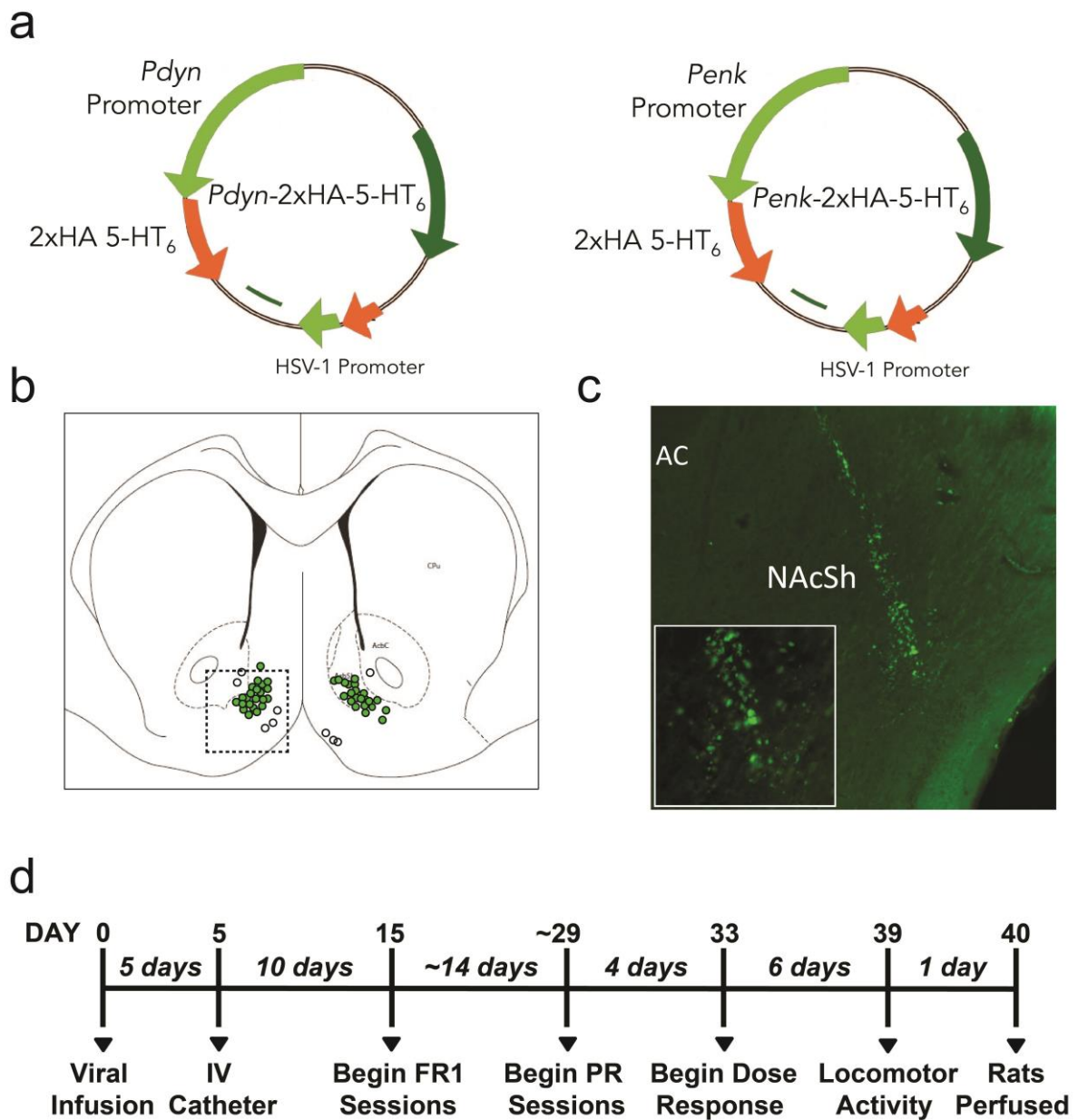


Figure 2.1 Pathway Specific Targeting of 5-HT₆ Receptors in NAcSh. (a) Viral vector plasmid maps: plasmid maps for both experimental vectors expressing hemagglutinin (HA)-tagged 5-HT₆ receptors via either the *Pdyn* promoter (Dyn-5-HT₆) or the *Penk* promoter (Enk-5-HT₆). (b) Diagram depicting locations of viral injections in NAcSh in experimental animals. Hits are shown in green and misses are shown as empty circles. Inset dashed square shows area imaged for fig 1c. (c) Photomicrograph at 5X magnification depicting the immunostaining of cells in the NAcSh infected with experimental virus Enk-5-HT₆ more than 40 days past infection. Anti-HA antibody is shown in green. Inset shows same area at 20X magnification. (d) Timeline of experimental procedure.

Increasing 5-HT₆ receptors in NAcSh iMSNs but not the dMSNs decreased operant cocaine SA on a FR1 schedule.

In rats treated with Dyn-GFP or Dyn-5-HT₆, there was no significant difference between viral vector treatment groups on the number of cocaine infusions (Figure 2.2a, $F_{(1,13)} = 0.87$, $p = 0.37$), indicating that increased expression of 5-HT₆ receptors in dMSNs did not alter cocaine reinforcement at a moderate unit dosage (0.75 mg/kg/infusion). The rats had established stable patterns of cocaine self-administration after the first 8 days; as a result, we examined the final three days of FR1 responding but there was no significant difference in average cocaine infusions between these treatment groups (Figure 2.2b, t-test, $p = 0.87$). In contrast, in rats expressing either Enk-5-HT₆ or Enk-GFP in medial NAcSh there was a significant difference between viral vector treatment groups on the number of cocaine infusions (Figure 2.2c, $F_{(1,24)} = 5.32$, $p = 0.03$) indicating that increased expression of 5-HT₆ receptors in iMSNs decreased cocaine SA on a FR1 reinforcement schedule. We examined the final three days of FR1 responding and the Enk-5-HT₆-expressing rats took significantly less cocaine (Figure 2.2d, t-test, $p = 0.015$). There was a significant difference between active and inactive lever presses in both the 5-HT₆ receptor and GFP groups in the dMSNs (Supplemental Figure 2.1a, 1-Way ANOVA, $p < 0.001$), as well as the 5-HT₆ receptor and GFP in the iMSNs (Supplemental Figure 2.1b, 1-Way ANOVA, $p < 0.001$) across all days of FR1 training.

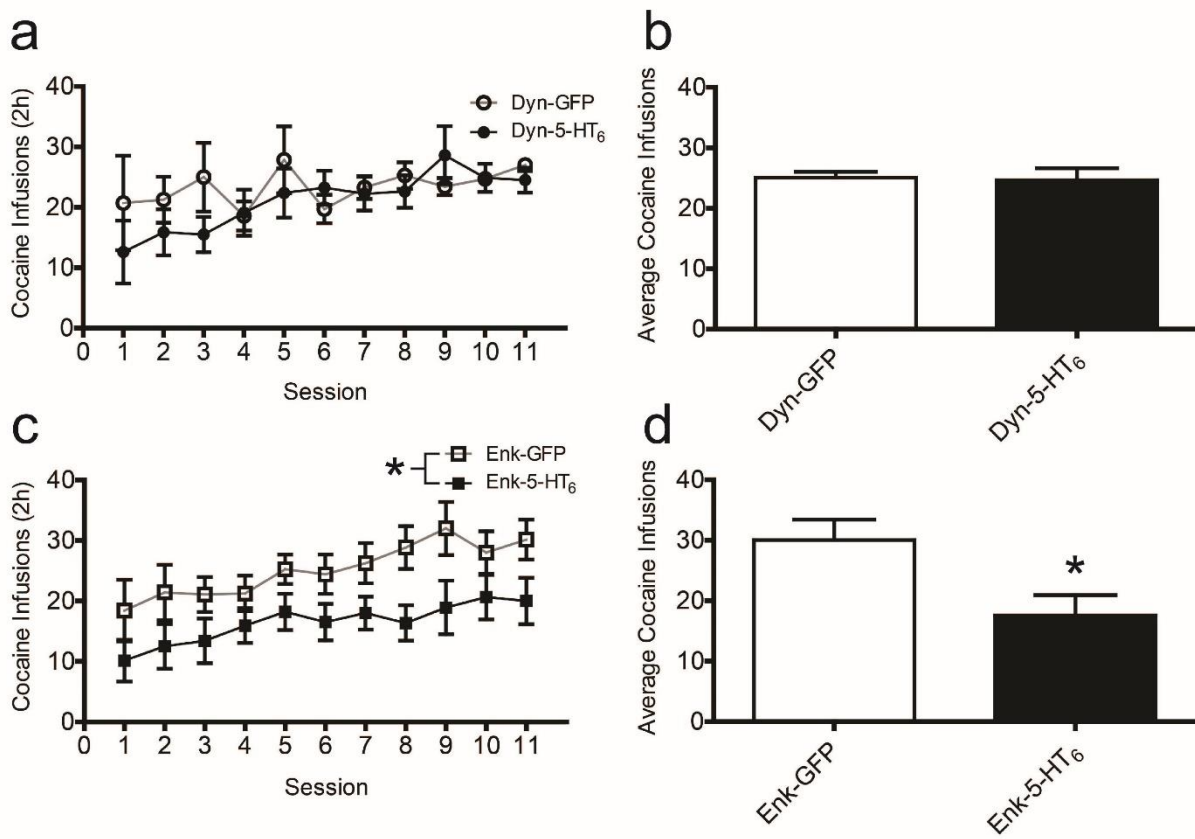
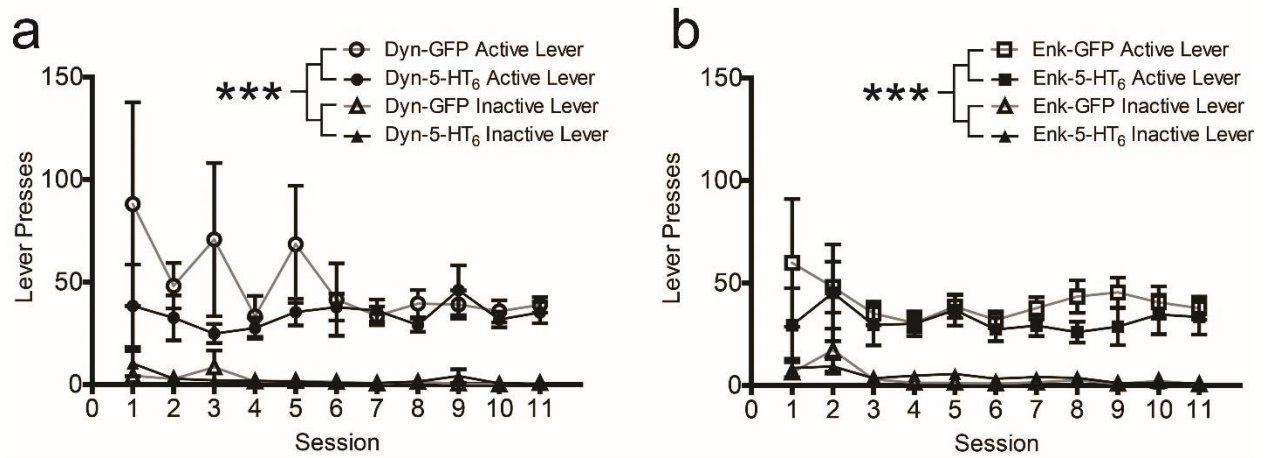


Figure 2.2 Increasing 5-HT₆ receptors in NAcSh iMSNs but not the dMSNs decreased operant cocaine SA on a FR1 schedule.

(a) Average cocaine infusions (Mean \pm SEM) for Dyn-GFP (n = 7) and Dyn-5-HT₆ (n = 8) ($F_{(1,13)}=0.87$; $p=0.37$) from 2 hour FR1 sessions. (b) Average FR1 cocaine infusions during the last 3 days of training (Mean \pm SEM) for Dyn-GFP (n = 7) and Dyn-5-HT₆ (n = 8) (t-test $p=0.87$). (c) Daily FR1 cocaine infusions (Mean \pm SEM) for Enk-GFP (n = 12) and Enk-5-HT₆ (n = 13) (2-Way ANOVA with repeated measures $F_{(1,24)}=5.32$; $p=0.03$). (d) Average cocaine infusions during the last 3 days of training (Mean \pm SEM) for Enk-GFP (n = 12) and Enk-5-HT₆ (n = 13) (t-test $p=0.016$) from 2 hour FR1 sessions. * = $P < 0.05$, ** = $P < 0.001$, *** = $P < 0.0001$.



Supplemental Figure 2.1 Active and inactive lever presses shown for both increased 5-HT₆ receptors and GFP in the dMSNs and iMSNs averaged across all days of FR1 training.

(a) Daily FR1 cocaine active and inactive lever presses (Mean ± SEM) for Dyn-GFP (n = 7) and Dyn-5-HT₆ (n = 8) (one way ANOVA; p<0.001). (b) Daily FR1 cocaine active and inactive lever presses (Mean ± SEM) for Enk-GFP (n = 12) and Enk-5-HT₆ (n = 13) (one way ANOVA p<0.001) * = P < 0.05, ** = P < 0.001, *** = P < 0.0001.

Increased expression of 5-HT₆ receptors in iMSNs shifted the dose-preference curve downward.

Rats were exposed to two additional doses of cocaine (0.375 mg/kg/infusion, and 1.5 mg/kg/infusion) for three days at each dose, starting with the low dose and followed by the high dose. We also included SA data from the last three training days at the 0.75 mg/kg/infusion dose in our analyses. All groups had decreasing preference for the highest dose of cocaine as indicated by fewer infusions taken as dose increased. Rats with increased 5-HT₆ receptors in the dMSNs did not differ from their GFP counterparts with regard to either the number of cocaine infusions (Figure 2.3a, $F_{(1,29)} = 0.77$, $p = 0.39$) or the total cocaine taken (Figure 2.3b, $F_{(1,29)} = 1.75$, $p = 0.20$). However, rats with increased 5-HT₆ receptors in iMSNs administered significantly fewer cocaine infusions (Figure 2.3c, $F_{(1,52)} = 16.85$, $p = 0.0001$) at the lower two doses as well as less cocaine across all doses (Figure 2.3d, $F_{(1,52)} = 14.9$, $p = 0.0003$).

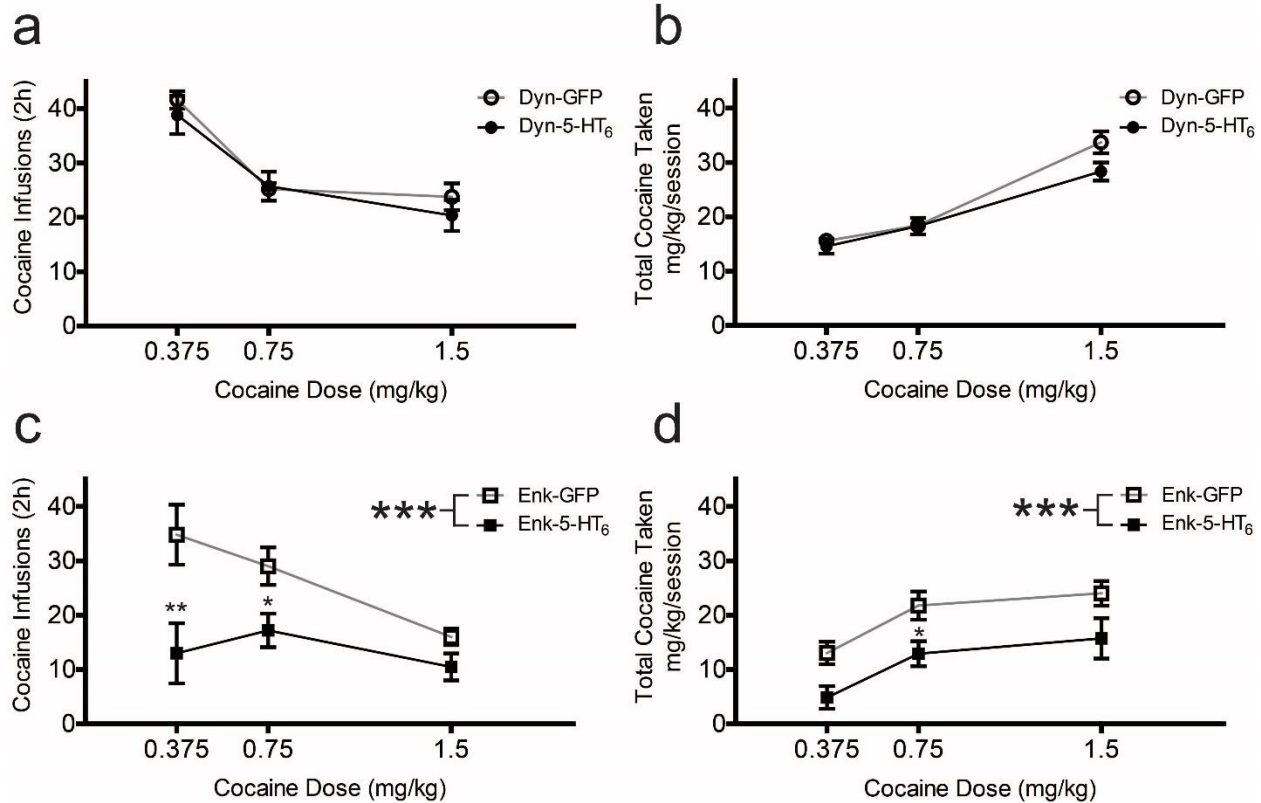


Figure 2.3 Increased expression of 5-HT₆ receptors in iMSNs shifted the dose-preference curve downward.

(a) Dose-dependent cocaine infusion (Mean \pm SEM) for rats with Dyn-GFP or Dyn-5-HT₆ (Dyn-5-HT₆, n = 4-8 depending on dose; Dyn-GFP n = 4-7 depending on dose) at three unit doses of cocaine infusion (0.375 mg/kg/infusion, 0.75 mg/kg/infusion, and 1.5 mg/kg/infusion) (2-Way ANOVA, $F_{(1,29)} = 0.77$, $p = 0.39$). (b) Total cocaine received (Mean \pm SEM) for rats with Dyn-GFP or Dyn-5-HT₆ at the three cocaine doses (0.375 mg/kg, 0.75 mg/kg, and 1.5 mg/kg) (2-Way ANOVA, $F_{(1,29)} = 1.75$, $p = 0.20$). (c) Dose dependent cocaine infusion (Mean \pm SEM) for rats with Enk-GFP or Enk-5-HT₆ (Enk-5-HT₆ n = 8-13 depending on dose; Enk-GFP n = 8-13 depending on dose) at the three doses (0.375 mg/kg, 0.75 mg/kg, and 1.5 mg/kg) (2-Way ANOVA, $F_{(1,52)} = 16.85$; $p = 0.0001$) (d) Total cocaine received (Mean \pm SEM) for rats with Enk-GFP or Enk-5-HT₆ at the three doses (0.375 mg/kg, 0.75 mg/kg, and 1.5 mg/kg) (2-Way ANOVA, $F_{(1,52)} = 14.9$, $p = 0.0003$). * = $p < 0.05$, ** = $p < 0.001$, *** = $p < 0.0001$

Increasing expression of 5-HT₆ receptors in the NAcSh iMSNs changes patterns of cocaine taking.

To assess whether increased 5-HT₆ receptors in either dMSNs or iMSNs affects the pattern of cocaine taking, we examined the time-course (15 minute intervals) of FR1 operant responding for a unit dose of 0.75 mg/kg/infusion. The number of infusions per 15 minute bin over the course of the session did not change for rats with increased 5-HT₆ receptors in dMSNs (Figure 2.4a, $F_{(1,11)} = 2.56$, $p = 0.14$). However, the average number of infusions per 15 minute bin over the course of the session was significantly decreased in rats with increased 5-HT₆ receptors in iMSNs (Figure 2.4b, $F_{(1,24)} = 9.07$, $p = 0.006$). We also examined the interval from the initiation of the session until the first response within each session. We found no change in the average time to first response for the group with increased 5-HT₆ receptors in dMSNs (Figure 2.4c, t-test, $p = 0.63$), but increased 5-HT₆ receptors in iMSNs significantly increased the interval prior to the initial lever press of each session (Figure 2.4d, t-test, $p = 0.035$).

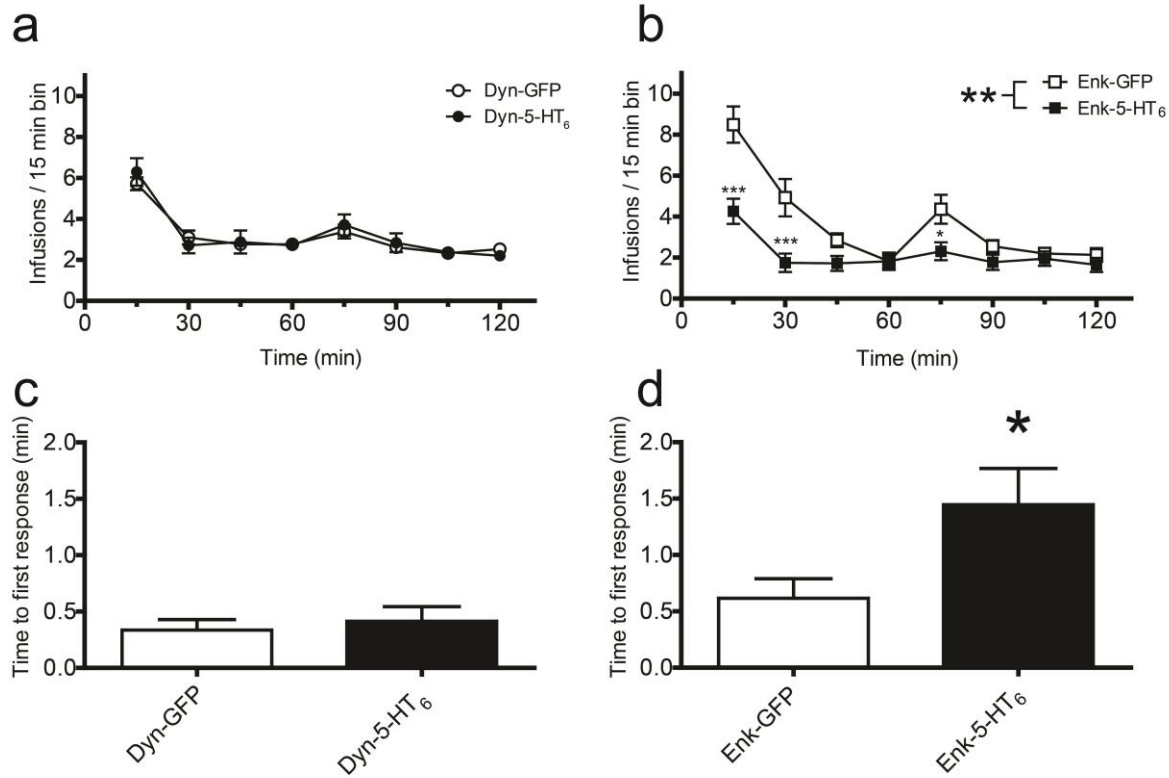


Figure 2.4 Increasing expression of 5-HT₆ receptor in the iMSNs changes pattern of cocaine taking.

(a) Average cocaine infusions (Mean ± SEM) per 15 minute bin on mid dose testing days shown for rats expressing either Dyn-GFP (n = 7) or Dyn-5-HT₆ (n = 8) (2-Way ANOVA, $F_{(1,11)} = 2.56$; $p = 0.14$). (b) Average cocaine infusions (Mean ± SEM) per 15 minute bin on mid dose testing days shown for rats expressing either Enk-GFP (n = 13) or Enk-5-HT₆ (n = 13) (Repeated measure 2-Way Anova, $F_{(1,24)} = 9.07$; $P = 0.006$). (c) Average time to initial response in minutes for Dyn-GFP (n = 7) and Dyn-5-HT₆ (n = 8) rats on all testing days (t-test $p = 0.63$). (d) Average time to initial response in minutes for Enk-GFP (n = 13) and Enk-5-HT₆ (n = 14) rats on all testing days (t-test $P = 0.035$).

Increasing expression of 5-HT₆ receptors in iMSNs reduced the preferred brain cocaine concentration.

To further probe the pattern of cocaine taking among rats we used a previously described pharmacokinetic model to estimate local brain concentration of cocaine in NAc (Pan *et al*, 1991; Zimmer *et al*, 2011). For reference, examples of how the model predicted brain cocaine concentration during the SA session are shown for Enk-GFP and Enk-5-HT₆ injected rats (Figure 2.5a and b, respectively). Estimated tissue cocaine concentrations were not different between Dyn-5-HT₆ and Dyn-GFP rats at any unit dose tested (Figure 2.5c, $F_{(1,29)} = 1.94$, $p = 0.18$). However, rats with increased 5-HT₆ receptors in iMSNs titrated around significantly lower cocaine concentrations as compared to their GFP counterparts (Figure 2.5d, $F_{(1,54)} = 7.427$, $p = 0.0086$).

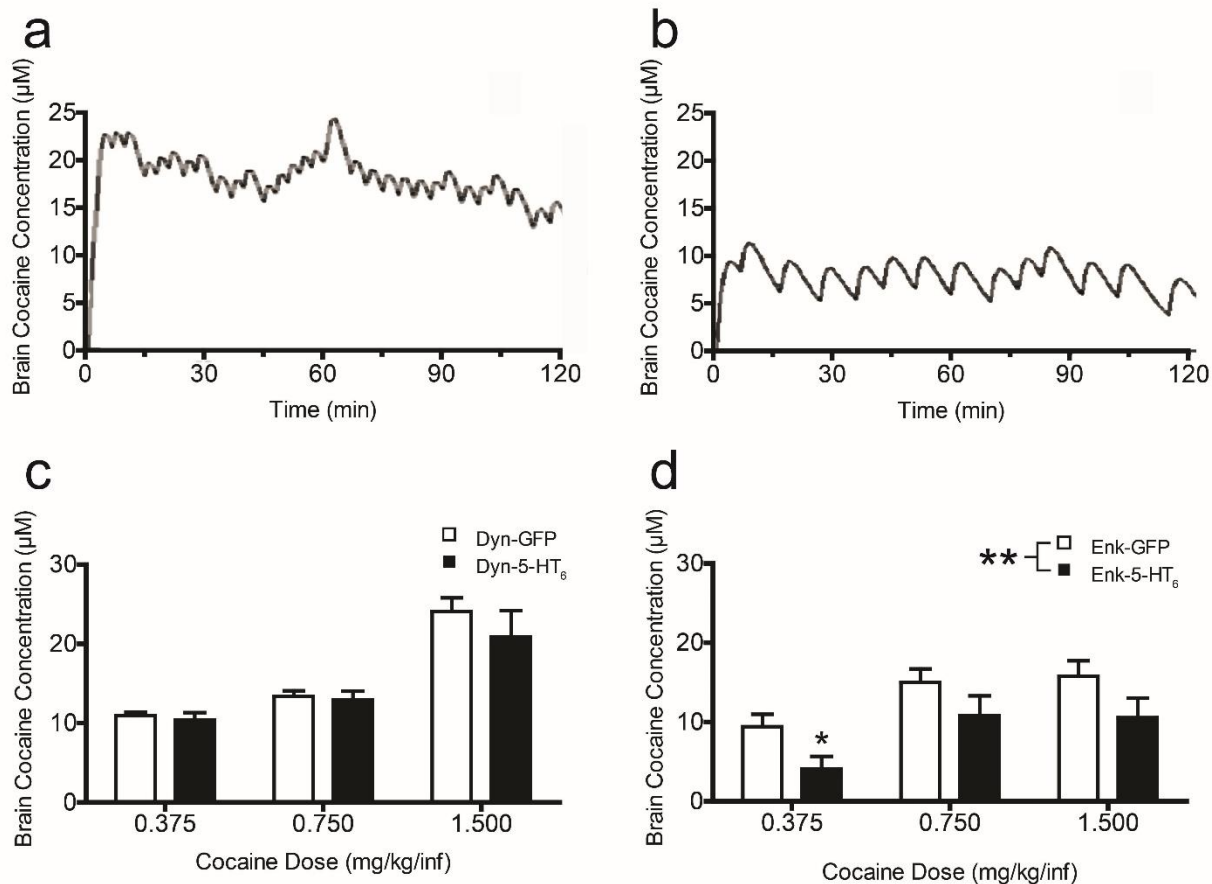


Figure 2.5 Increasing expression of 5-HT₆ receptors in iMSNs reduced the preferred brain cocaine concentration.

Example brain cocaine concentration modeling at 0.75 mg/kg/infusion are shown for reference Enk-GFP (a) and Enk-5-HT₆ (b). (c) Average estimated brain cocaine concentrations across doses (0.375 mg/kg/infusion, 0.75 mg/kg/infusion, and 1.5 mg/kg/infusion) for Dyn-5-HT₆ rats (n = 4-8 depending on dose) and Dyn-GFP rats (n = 4-7 depending on dose) (2-Way ANOVA, $F_{(1,29)} = 1.94$, $p = 0.18$). (d) Average estimated brain cocaine concentrations across doses (0.375 mg/kg/infusion, 0.75 mg/kg/infusion, and 1.5 mg/kg/infusion) for Enk-5-HT₆ rats (n = 8-14 depending on dose) and Enk-GFP rats (n = 8-14 depending on dose) (2-Way ANOVA, $F_{(1,54)} = 7.427$, $p = 0.009$). * = $p < 0.05$, ** = $p < 0.001$, *** = $p < 0.0001$.

Increasing expression of 5-HT₆ receptors in either the NAcSh iMSNs or dMSNs MSNs did not affect cocaine SA on PR reinforcement schedule

On a progressive ratio (PR) reinforcement schedule, increased 5-HT₆ receptor expression in dMSNs had no significant effect on the total number of cocaine infusions when averaged over 4 PR testing days (Figure 2.6a, t-test, $p = 0.14$) or average active-lever presses (Figure 2.6b, t-test, $p = 0.264$). Similarly, increased 5-HT₆ receptor expression in iMSNs had no significant effect on the average number of cocaine infusions over four PR testing days (Figure 2.6c, t-test, $p = 0.31$) or active-lever presses (Figure 2.6d, t-test, $p = 0.53$). Thus, we conclude that the motivation to self-administer cocaine at the dose of 0.75 mg/kg/infusion was not directly affected by increased expression of 5-HT₆ receptors in either pathway.

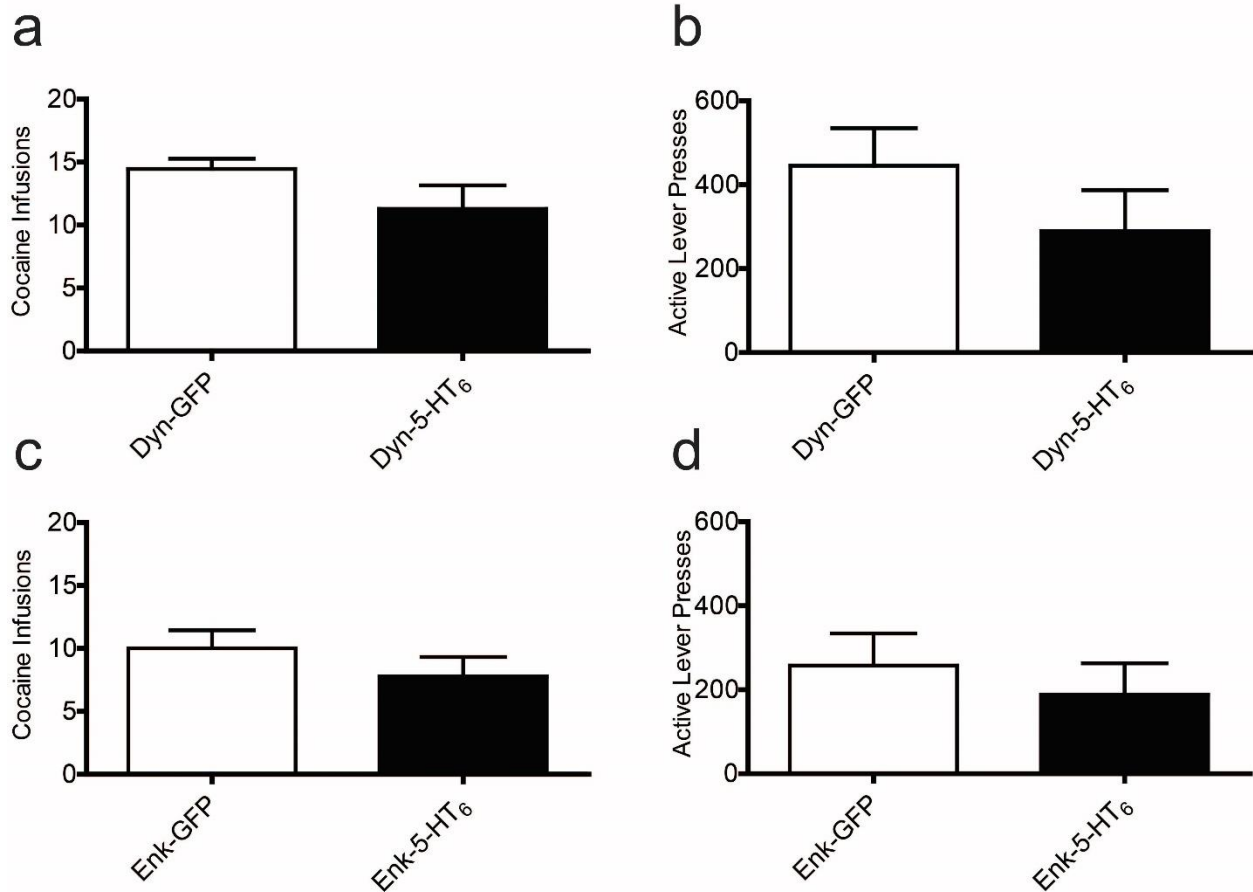


Figure 2.6 Increasing expression of 5-HT₆ receptors in either the dMSNs or iMSNs does not affect operant responding on a PR schedule. (a) Cocaine infusions (Mean \pm SEM) for the average of four PR sessions for Dyn-GFP (n = 7) and Dyn-5-HT₆ (n = 7) t-test; p=0.144). (b) Active lever presses (Mean \pm SEM) for the average of four PR sessions for Dyn-GFP (n = 7) and Dyn-5-HT₆ (n = 7) (t-test; p=0.264). (c) Cocaine infusions (Mean \pm SEM) for the average of four PR sessions for Enk-GFP (n = 12) and Enk-5-HT₆ (n = 14) (t-test; p=0.31). (d) Active lever presses (Mean \pm SEM) for the average of four PR sessions for Enk-GFP (n = 12) and Enk-5-HT₆ (n = 14) (t-test; p=0.53).

Increasing expression of 5-HT₆ receptors in NAcSh iMSNs increased preference for cocaine at a low dose.

To examine whether increased 5-HT₆ receptors in the indirect pathway altered the sensitivity to the rewarding properties of cocaine, we tested the effects of expressing 5-HT₆ receptors or GFP in iMSNs on the ability of cocaine to support conditioned place preference to a low dose of cocaine (5 mg/kg) that we previously found was too low to produce a consistent conditioned place preference in control rats (Barot *et al*, 2007; Neumaier *et al*, 2002). The rats with increased 5-HT₆ receptor expression in iMSNs spent a significantly longer period exploring the cocaine paired chamber during testing (Figure 2.7a, t-test; p=0.04).

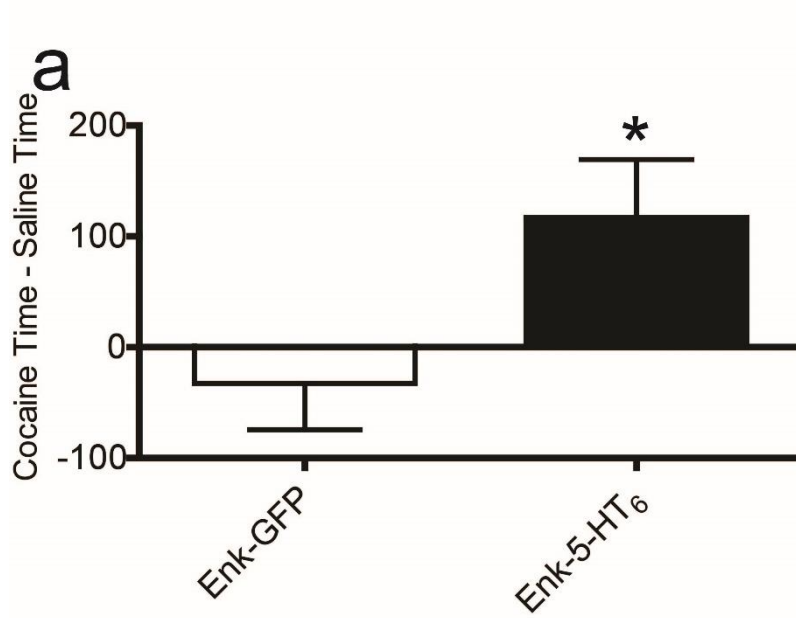
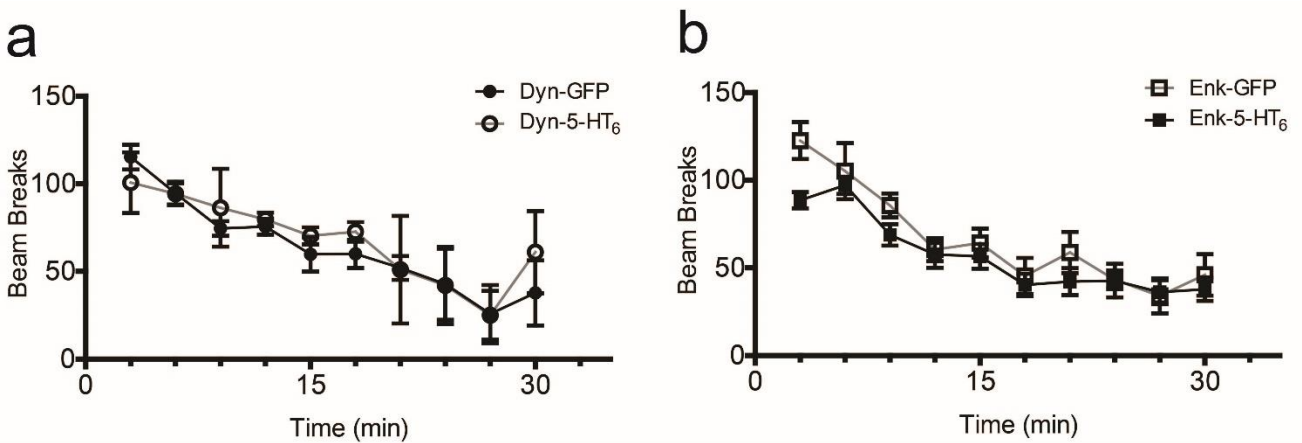


Figure 2.7 Increasing expression of 5-HT₆ receptors the iMSNs increases preference for cocaine at a low dose. (a) CPP score (cocaine time – saline time [seconds]) shown for rats with either Enk-GFP (n = 8) or Enk-5-HT₆ (n = 8) (t-test, p = 0.04). * = p < 0.05, ** = p < 0.001, *** = p < 0.0001.

Increasing expression of 5-HT₆ receptors in dMSNs or iMSNs does not influence locomotor activity.

We tested whether there were differences in locomotor activity in rats from a subset of rats from all groups by placing the individual rats into locomotor activity monitoring chambers. All of the groups demonstrated similar locomotor activity patterns with no significant between-group differences (direct pathway: Supplemental Figure 2a, $F_{(1,5)}=0.30$; $p=0.61$; indirect pathway: Supplemental Figure 2b, $F_{(1,10)}=2.42$; $p=0.151$).



Supplemental Figure 2.2 Increasing expression of 5-HT₆ receptor in either the dMSNs or iMSNs does not change locomotor activity. (a) Average beam breaks per 3 minute bin for rats with either Dyn-GFP (n = 4) or Dyn-5-HT₆ (n = 3) (2-Way ANOVA, $F_{(1,5)} = 0.30$; $p = 0.61$). (b) Average beam breaks per 3 minute bin for rats with either Enk-GFP (n = 3) or Enk-5-HT₆ (n = 6) (2-Way ANOVA, $F_{(1,10)} = 2.42$; $p = 0.15$). * = $p < 0.05$, ** = $p < 0.001$, *** = $p < 0.0001$.

		F statistics	P value
Figure 2A – Direct Pathway Fixed Ratio SA	Virus	$F_{(1,13)} = 0.87$	0.369
	Time	$F_{(10,130)} = 1.83$	0.061
	Interaction	$F_{(10,130)} = 0.92$	0.516
Figure 2C – Indirect Pathway Fixed Ratio SA	Virus	$F_{(1,24)} = 5.32$	0.03
	Time	$F_{(10,240)} = 4.50$	< 0.0001
	Interaction	$F_{(10,240)} = 0.45$	0.917
Figure 3A – Direct Pathway Dose Response SA	Virus	$F_{(1,29)} = 0.77$	0.39
	Dose	$F_{(2,29)} = 46.20$	< 0.0001
	Interaction	$F_{(2,29)} = 0.30$	0.745
Figure 3B – Direct Pathway Dose Response SA	Virus	$F_{(1,29)} = 1.75$	0.20
	Dose	$F_{(2,29)} = 64.45$	< 0.0001
	Interaction	$F_{(2,29)} = 1.7$	0.19
Figure 3C – Indirect Pathway Dose Response	Virus	$F_{(1,52)} = 16.85$	0.0001
	Dose	$F_{(2,52)} = 4.33$	0.018
	Interaction	$F_{(2,52)} = 1.95$	0.15
Figure 3D – Indirect Pathway Dose Response	Virus	$F_{(1,52)} = 14.9$	0.0003
	Dose	$F_{(2,52)} = 8.16$	0.0008
	Interaction	$F_{(2,52)} = 0.01$	0.98
Figure 4A – Direct Pathway SA Pattern	Virus	$F_{(1,11)} = 2.56$	0.14
	Time	$F_{(7,77)} = 31.13$	<0.0001
	Interaction	$F_{(7,77)} = 0.49$	0.84
Figure 4B – Indirect Pathway SA Pattern	Virus	$F_{(1,24)} = 9.07$	0.006
	Time	$F_{(7,168)} = 31.13$	< 0.0001
	Interaction	$F_{(7,168)} = 7.71$	< 0.0001
Figure 4G – Direct Pathway Estimated Brain Cocaine Concentration	Virus	$F_{(1,29)} = 1.94$	0.17
	Dose	$F_{(2,29)} = 42.71$	0.0001
	Interaction	$F_{(2,29)} = 0.53$	0.65
Figure 4H – Indirect Pathway Estimated Brain Cocaine Concentration	Virus	$F_{(1,54)} = 7.43$	0.009
	Dose	$F_{(2,54)} = 5.07$	0.01
	Interaction	$F_{(2,54)} = 0.05$	0.95

Supplemental Table 2.1 Statistical values for ANOVAs. P values less than 0.05 are shown in bold.

E. Discussion

The segregation of direct and indirect pathway MSNs in dorsal and ventral striatum is a fundamental feature of the organization of brain reward circuitry and the functional implications of the divergent roles of these pathways is currently a topic of great interest. It has been known for many years that both iMSNs and dMSNs express 5-HT₆ receptors (Ward *et al*, 1995), but past studies involving 5-HT₆ receptors and psychostimulants did not address these distinct pathways (de Bruin *et al*, 2013; Eskenazi and Neumaier, 2011a; van Gaalen *et al*, 2010; Valentini *et al*, 2012), and hence the circuitry mechanisms involved were not evaluated. Therefore, we investigated the relative contribution of 5-HT₆ receptors in each pathway on operant behaviors reinforced by cocaine.

Previously, we found that increasing 5-HT₆ receptors in both pathways of NAcSh interfered with learning of a conditioned place preference for cocaine, and that systemic treatment with a selective 5-HT₆ antagonist had the opposite effect (Ferguson *et al*, 2008). However, in that study we did not observe any direct effects on drug preference. Furthermore, we have previously observed that increasing 5-HT₆ receptors in both pathways of dorsomedial striatum interfered with acquisition of action-outcome learning when sucrose was used as the reinforcer (Eskenazi and Neumaier, 2011a; Mitchell *et al*, 2007). In dorsomedial striatum, we recently found that selective expression of 5-HT₆ receptors in indirect pathway MSNs alone was sufficient to interfere with action-outcome operant learning (Eskenazi *et al*, 2015). In contrast, we found that 5-HT₆ receptor signaling in dorsolateral striatum facilitated omission training in a contingency-specific manner, but did not affect the learning acquisition of action-outcome learning (Eskenazi and Neumaier, 2011b). Thus, the impact of 5-HT₆ receptors on striatum-

dependent behavior depends both on the pathway being manipulated and the sub region that is targeted.

The central finding of this report is that increased expression of 5-HT₆ receptors in the iMSNs reduced cocaine SA under an FR1 reinforcement schedule by about 50% when using a 0.75 mg/kg unit dose. The most obvious explanation for this result is that the animals had lower motivation to take cocaine, but increasing 5-HT₆ receptors in iMSNs had no effect on the “break point” under a PR reinforcement schedule at a dose of 0.75 mg/kg, suggesting that their motivation to take cocaine was not likely altered at this dose. In contrast, increasing expression of 5-HT₆ receptors in the dMSNs of NAcSh had no effect on cocaine SA under either FR1 or PR reinforcement schedules. While it is possible that increasing 5-HT₆ receptor expression may modulate break point at higher or lower doses of cocaine, the present evidence suggests that there were no changes in motivation using the PR “break point” method.

One possible explanation for our central observation is that the rats were more sensitive to the reinforcing properties of cocaine and therefore required less to attain a preferred subjective response to the drug. We applied a previously developed and later refined method for modeling the pharmacokinetics and tissue concentration of cocaine to estimate the brain cocaine concentration that each animal titrated to and presumably preferred for each unit dose of cocaine tested (Pan *et al*, 1991; Zimmer *et al*, 2011). This analysis suggested that the Enk-5-HT₆ rats preferred a lower tissue concentration of cocaine, further supporting the notion that they are more sensitive to the reinforcing properties of cocaine.

We further tested this idea using a different behavioral model by performing a cocaine CPP experiment using conditions that enhance the sensitivity to detecting differences in reward as compared to aversion—i.e. low dose and brief pairing of cocaine with the context (Barot *et al*,

2007; Ettenberg, 2004; Pliakas *et al*, 2001). The rats with increased 5-HT₆ receptors in iMSNs developed a stronger preference for a typically sub-threshold dose of cocaine (5mg/kg i.p.) (Barot *et al*, 2007; Neumaier *et al*, 2002), suggesting that these rats were more sensitive to the rewarding effects of cocaine at this dose.

We considered alternative interpretations as well. It is also conceivable that increasing 5-HT₆ receptors in the iMSNs altered the processing of aversive information. However, we found no differences in operant self-administration at the highest cocaine dose (1.5 mg/kg unit dose), which is most likely to generate aversive effects. We did not test place conditioning with high dose cocaine and delayed pairing, which is a sensitive method for detecting aversion (Barot *et al*, 2007; Ettenberg, 2004; Pliakas *et al*, 2001) because there were no differences in cocaine SA at the highest unit dose tested. Further, we found no differences in operant responding on a PR reinforcement schedule. Taken together, this suggests that these results are not likely due to changes in the processing of aversive information. Another explanation is that increased 5-HT₆ receptors in NAcSh altered motor activity and interfered with cocaine taking, but there were no changes in locomotion following these manipulations nor were there differences at 1.5 mg/kg cocaine, a unit dose that would be most likely to induce motor deficits. In a previous study we also found no evidence that increased 5-HT₆ receptors altered the acute locomotor response to cocaine or sensitization over several days, although in that study we expressed 5-HT₆ receptors using a pathway-nonspecific viral vector (Ferguson *et al*, 2008).

While we observed no change in PR responding at 0.75 mg/kg, which is usually interpreted to mean that there was no change in motivation to take cocaine at this dose, Enk-5-HT₆ animals displayed a consistent pattern of delaying the initial cocaine infusions during each test session, across multiple days. It is unlikely that 5-HT₆ receptors impaired the animal's

association between cocaine availability and the active lever because there was no declination in the delay to initial lever press over multiple days. However, most of the differences between Enk-GFP and Enk-5-HT₆ treated rats occurred during the beginning of each session, as the experimental animals lacked the initial burst of cocaine taking that is typical of cocaine SA (Belin *et al*, 2009; Zimmer *et al*, 2011). The higher initial rate of cocaine infusions may be taken to attain a preferred cocaine level in the brain, followed by a second phase of slower cocaine taking to maintain a preferred cocaine concentration in the brain. If this interpretation is correct, 5-HT₆ receptors in the NAcSh indirect pathway have an impact on the initial phase of cocaine SA and it is not surprising that there was no change in break point under a progressive ratio schedule, which depends on an extended cocaine SA session. In a recent study, we found that increased 5-HT₆ receptors in iMSNs in dorsomedial striatum delayed the time to initial lever press only on the first day of training for sucrose responding (Eskenazi *et al*, 2015), which is a similar result but this effect was not sustained over multiple days as it was in the present study using cocaine SA. If 5-HT₆ receptors in iMSNs of the NAcSh produce increased sensitivity to lower doses of cocaine, this might in turn reduce the likelihood of developing compulsive addiction-like behaviors which are associated with higher infusion rates of cocaine SA during early experience with drug taking (Belin *et al*, 2009, 2011). This interpretation is supported by previous work showing that activating the indirect pathway fosters resilience to compulsive cocaine use (Bock *et al*, 2013) and reducing indirect pathway activity can facilitate psychomotor sensitization to cocaine (Ferguson *et al*, 2011).

One parsimonious way to interpret all of these results together is to consider the distribution of 5-HT₆ receptors in both of these output pathways. Whereas dopamine differentially activates dMSNs via D₁ receptors and inhibits iMSNs via D₂ receptors, serotonin

will excite both pathways as both populations of MSNs express endogenous 5-HT₆ receptors (Ward *et al*, 1995). Thus, dMSNs are activated by both the endogenous G α s-coupled D₁ and 5-HT₆ receptors, leading to an accumulation of cAMP (Dobi *et al*, 2011; Surmeier *et al*, 2007). However, D₂ and 5-HT₆ receptors in iMSNs have opposite effects on adenylate cyclase activity and will tend to oppose one another. Therefore, 5-HT₆ receptors in the iMSNs are positioned to interfere with behaviors that are supported by dopamine action in NAcSh. Presumably balanced activation of endogenous 5-HT₆ receptors in both pathways simultaneously interferes with dopamine actions by reducing the differential activation of these two pathways. It is not known whether exposure to abused drugs alters the expression levels of 5-HT₆ in these pathways differentially, but this is a topic under active exploration.

The finding that increased 5-HT₆ receptor expression in the iMSNs of NAcSh leads to an increase in the sensitivity to the reinforcing properties of cocaine while maintaining normal reward motivation properties has implications toward the field of drug addiction research. While similar studies have focused on the role of 5-HT₆ receptors modulating DA in NAcSh (Valentini *et al*, 2012) during cocaine reinforcement, or the role of the indirect pathway in reducing cocaine reinforcement (Hikida *et al*, 2010), no studies have focused on the pathway specific roles for 5-HT₆ receptors. Our findings suggest that 5-HT₆ receptors are a potential target for treatment of drug addiction, in that if receptors are selectively up-regulated or stimulated in the iMSNs, the amount of drug taken by individuals might reduce, thereby impeding the progression toward compulsive, unregulated drug use.

CHAPTER III:

**Regulation of Primary Cilia Morphology in Striatal Neurons by
5-HT₆ Receptor Signaling**

* This chapter is currently in preparation as an article with the same title for publication with Adam Lesiak, Alex Croicu, Natalie Cohenca, Jane M. Sullivan^{3, 4}, and John F. Neumaier and John F. Neumaier are coauthors.

A.W.G., S.N., J.F.N. and I contributed to experimental design. A.W.G. and I collected all the experimental data with assistance from D.S. I carried out data analysis. I prepared the manuscript with A.W.G. and J.F.N.

A. Summary

The 5-HT₆ receptor has been implicated in a variety of cognitive processes including habitual behaviors, learning, and memory. It is found almost exclusively in the brain and is localized to the primary cilia of neurons, and is expressed abundantly in the striatum. The primary cilium is an antenna-like, sensory organelle found on most neurons that receives both chemical and mechanical signals from other cells and the surrounding environment; however, the effect of 5-HT₆ receptor function on cilia has not been examined. We confirmed that 5-HT₆ receptors were present and localized to primary cilia in wild-type (WT) but not 5-HT₆ knockout (5-HT₆KO) in both native mouse brain tissue and primary cultured striatal neurons then used primary neurons cultured from WT or 5-HT₆KO mice (PND 0) to study the function of these receptors. Selective 5-HT₆ antagonism reduced cilia length in neurons cultured from wild-type mice in a concentration and time-dependent manner, but had no effect on primary cilia length in neurons cultured from 5-HT₆KO mice. We examined the effect of varying the expression level of heterologously expressed 5-HT₆ receptors on the fidelity of ciliary localization in both WT and 5-HT₆KO neurons and found that increasing levels of overexpression lead to increasing amounts of 5-HT₆ localization outside of the cilia but did not alter cilia length. Introducing several discrete mutations in the third cytoplasmic loop of the 5-HT₆ receptor greatly reduced, but did not entirely eliminate, trafficking of the 5-HT₆ receptor to primary cilia but did not affect cilia length. These data suggest that blocking 5-HT₆ receptor activity reverses a tonic lengthening of cilia and that mechanisms that regulate trafficking of 5-HT₆ receptors to cilia are more complex than previously thought.

B. Introduction

Almost all mammalian cells, neurons included, have a primary cilium at some point during their life cycle (Bishop *et al*, 2007; Singla and Reiter, 2006; Wheatley *et al*, 1996). Primary cilia are microtubule-supported organelles that stem from the basal body and extend beyond the cell body into the extrasynaptic space (Arellano *et al*, 2012; Avasthi and Marshall, 2012; Louvi and Grove, 2011). Originally identified by the Swiss anatomist K.W. Zimmerman in 1898, primary cilia were neglected in biological research for many years and even misclassified as a vestigial organelle (Praetorius and Spring, 2003; Whitfield, 2004). Often referred to as “non-motile” cilia due to not being involved in generating motion, primary cilia are rich with receptors and receive both chemical and mechanical signals from the surrounding environment (Davenport and Yoder, 2005; Pazour and Witman, 2003; Praetorius and Spring, 2003). Signaling within primary cilia is transduced by a discrete set of membrane-bound receptors specifically localized to the primary cilium; while some receptors are found both in cilia and dendrites, others are exclusive to cilia and represent an interesting category of nonsynaptic receptor-mediated signals from the extracellular environment. Many studies have investigated the developmental impact of neuronal primary cilia, but their role in normal cognitive functions is not well understood and there is some evidence that ciliary signaling throughout the lifespan modulates neuronal function and cognition (Berbari *et al*, 2008a; Davenport and Yoder, 2005).

There is a rising interest in the function and significance of primary cilia, especially with regard to their role in a variety of disorders now known as “ciliopathies” (Ainsworth, 2007; Novarino *et al*, 2011). Some of the best characterized ciliopathies arise from mutations in or deletions of genes responsible for formation and function of primary cilia (Kim *et al*, 2010; Lee

and Gleeson, 2011). Ciliopathies frequently involve malformations of primary cilia such as shortened, elongated or total ablation of the primary cilia (Avasthi *et al*, 2012; Marley and von Zastrow, 2012; Ou *et al*, 2009). Although, little is known about the function of primary cilia on neurons, common symptoms of ciliopathies include brain deformation and cognitive impairments (Green *et al*, 2012; Louvi and Grove, 2011). Even less is known about more subtle alterations in the signaling events that occur within cilia and how they affect health and disease.

The 5-HT₆ receptor is the only serotonin (5-HT) receptors that localize to primary cilia in neurons (Berbari *et al*, 2007; Brailov *et al*, 2000). These G_{αs}-coupled metabotropic receptors couple to adenylyl cyclase and stimulate accumulation of cyclic adenosine monophosphate (cAMP) which, in most neurons, mediates a downstream excitatory effect (Duhr *et al*, 2014; Jacobshagen *et al*, 2014; Kohen *et al*, 2001). 5-HT₆ receptors are present during development and their localization on primary cilia may be involved in neuronal migration (Dayer *et al*, 2015; Duhr *et al*, 2014; Grimaldi *et al*, 1998). However, additional signal-transduction mechanisms separate from the canonical G-protein signaling cascades have been recently described (Dayer *et al*, 2015; Duhr *et al*, 2014; Yun and Rhim, 2011; Yun *et al*, 2007). 5-HT₆ receptors are expressed almost exclusively in the brain, especially in striatum, hippocampus, and cortex (Gerard *et al*, 1997; Monsma Jr. *et al*, 1993; Ruat *et al*, 1993). The receptor has high affinity for several psychotropic drugs, and have substantial similarities of sequence identity and pharmacological properties between rats and humans (Eskenazi and Neumaier, 2011a; Hirst *et al*, 2003; Mitchell *et al*, 2007). Due to their expression in striatal medium spiny neurons, 5-HT₆ receptors are a possible candidate for serotonin's effect on learning, memory, and reward-motivated behavior (Mitchell and Neumaier, 2005). Increased expression of 5-HT₆ receptors in neurons of the ventral striatum of rats blocks the reward-learning processes associated with cocaine (Ferguson

et al, 2008). The specific cell type that expresses these receptors is critical, as increasing 5-HT₆ expression just in indirect pathway medium spiny neurons reduced cocaine self-administration by increasing the sensitivity to cocaine reward (Brodsky, submitted). Together these studies implicate 5-HT₆ receptors as a possible therapeutic target for drug addiction and possibly other cognitive disorders (Eskenazi *et al*, 2015; Ferguson *et al*, 2011; Fukuo *et al*, 2010; Wilkinson *et al*, 2014). While a number of GPCRs in striatal circuitry have been investigated in relation to these problems, the unique role of cilia-localized signaling has not been considered. 5-HT₆ receptors, along with most other ciliary localizing GPCRs, have a five amino acid long ciliary targeting consensus sequence (CTS) within their third intracellular (i3) loop that is thought to be critical for trafficking into primary cilia (Berbari *et al*, 2008a; Nachury *et al*, 2010; Nagata *et al*, 2013). The unique subcellular localization of 5-HT₆ receptors to primary cilia in neurons creates an interesting question about the true functions of the receptor.

In the present study, we asked whether the unique functional properties of 5-HT₆ receptors are dependent upon their localization to primary cilia. In particular we evaluated whether manipulating 5-HT₆ receptor activity and expression in striatal neurons alters neuronal primary cilia morphology and signaling. In both native brain tissue and primary cultured striatal neurons, 5-HT₆ receptors were localized to cilia in wild-type (WT) but not 5-HT₆ knockout mice (5-HT₆KO). Treatment of cultured neurons with a selective 5-HT₆ receptor antagonist reduced cilia length in striatal neurons in a concentration and time-dependent manner. The degree of heterologously expression of 5-HT₆ receptors modified the fidelity with which 5-HT₆ receptors localized to primary cilia in primary cultured neurons, but had no effect on primary cilia length. Lastly, targeting of mutant 5-HT₆ receptors containing mutations within the CTS decreased but failed to eliminate targeting to the primary cilia or changes in primary cilia length. These results

suggest that the presently assumed CTS mechanism for 5-HT₆ receptor trafficking to primary cilia is not the exclusive system resulting in the unique localization. Taken together our findings suggest a role for endogenous 5-HT₆ receptor activity in the dynamic regulation of striatal primary cilia and find that regulation of cilia trafficking of 5-HT₆ receptors is more complex than previously thought.

C. Materials and Methods

Animals

All animal procedures were approved by the University of Washington's Institutional Animal Care and Use Committee and were carried out in accordance with National Institutes of Health guidelines of the "Principles of Laboratory Animal Care" (NIH publication no. 86-23, 1996). Care was taken to minimize animal discomfort. 5-HT₆KO mice on a C57BL/6 background (generously provided by Dr. Lawrence Tecott) (Bonasera *et al*, 2006) or WT C57BL/6 mice (Jackson Labs, Sacramento, CA) were housed for breeding with two females and one male per cage with access to food and water available *ad libitum*. 5-HT₆KO mice were genotyped by polymerase chain reaction performed on tail DNA utilizing 5'GCCATGCTGAACGCGCTG as the forward primer upstream of the Htr6 null mutation and either 5'GCACCCAGGATGAGCGC or 5'TGCCCCAAAGGCCTACCCGCTTCC as the reverse primer for WT or 5-HT₆KO, respectively.

Neuronal cell culture

Primary dissociated striatal cultures containing a small number of cortical neurons were generated from either postnatal day 0-1 C57BL/6 mice or postnatal day 0-1 5-HT₆ KO mice (Bonasera *et al*, 2006). Dissociation and culture method were adapted from previously described protocols (Brewer, 1997; Lesiak *et al*, 2015; Pratt *et al*, 2011). Cells were plated at a density of 7×10^4 cells per cm² on culture dishes pre-coated with poly-L-lysine (Sigma; molecular weight

300,000). Cultures were maintained in growth media consisting of Neurobasal-A (NBA) medium (Life Technologies, Carlsbad, CA) supplemented with B27 and Glutamax (Life Technologies) throughout treatment days. From the fourth day *in vitro* (DIV) until homogenization or fixation, culture medium was supplemented with 1 μ M Ara-C (Sigma), a mitotic inhibitor. This results in cultures consisting of approximately 70% neurons and 30% glia. Cultures were maintained at 37°C in 5% CO₂ from DIV0 until homogenization or fixation.

Drugs and Drug Treatments

Selective 5-HT₆ receptor agonist WAY 208466 (*N*-[2-[3-(3-fluorophenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridin-1-yl]ethyl]-*N,N*-dimethylamine) and selective 5-HT₆ receptor antagonists SB 399885 (*N*-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzenesulfonamide) HCl and SB 258585 (4-Iodo-*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]benzenesulfonamide) were obtained from Tocris Biosciences. Unless noted otherwise, drugs were diluted in warm neuronal growth medium and administered directly to primary neuronal cultures on DIV9.

Transfection/Plasmids

Cultured neurons were transfected on DIV7 using Lipofectamine 2000 (Life Technologies). Lipofectamine 2000 was added to warm NBA media (0.02 μ L/ μ L) and incubated for 5 minutes before combining with pre-mixed plasmid DNA (50 μ L/well). Total plasmid DNA for transfections consisted of 1 μ g/well (24-well plate). The percentage of total plasmid transfected for each plasmid-combination used in the experiments was as follows: 25% 5-HT₆

receptor or mutant 5-HT₆ receptors (unless specified otherwise), 50% fluorescent protein reporter (hSyn-RFP or Clover), and for each condition empty plasmid vector (pCAGGS) was added to the plasmid mix to reach 100% of total transfected plasmid. Lipofectamine 2000/NBA/DNA mix was incubated at room temperature for 20 minutes, while native culture media was collected and kept at 37°C and replaced with growth medium. Lipofectamine 2000/NBA/DNA mix was added to plates (50 µL/well), and incubated for 35–40 minutes before transfection media was aspirated and replaced with original culture media. Plasmids expressing varied receptors were all c-terminally HA tagged for identification, and expressed under the CMV promoter (Figure 3.5).

Immunohistochemistry

Cultured neurons were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) / PHEMS (Sigma-Aldrich) buffer on DIV10 for 20 minutes at room temperature. Neurons were then permeabilized with 1xPBS/0.5% Triton-x100 for 10 minutes at room temperature, and blocked in 5% Bovine Serum Albumen (BSA) in PBS for 1 hour at 4°C. Cultured cells were incubated overnight at 4°C with either of the following primary antibodies in PBS with 1% BSA: anti-adenylyl cyclase III (AC3) rabbit polyclonal antibody (sc-588; Santa Cruz Biotechnology, Santa Cruz, CA), used at 1:1000, anti- ADP-ribosylation factor-like protein 13B (Arl13b) mouse monoclonal antibody (73-287; NeuroMab, Davis, CA), used at 1:1000, anti-SR-6 (5-HT₆) (A-20) raised in goat (sc-26668; Santa Cruz Biotechnology, Santa Cruz, CA), used at 1:1000, or anti-HA (1:1000, rabbit, Cell Signaling). Next, coverslips were rinsed briefly four times in PBS and incubated in species-appropriate Alexa 488 (green) and/or Alexa 568 (red)-conjugated donkey secondary antibodies (1:400, Invitrogen) for 1-2 hours at room temperature. Coverslips were

washed 3 times in PBS, mounted on slides and using ProLong Gold Antifade media containing DAPI (Invitrogen, Carlsbad, CA).

Floating sections (40 μm) were washed in 0.5% Triton-X/PBS for 10 min, then blocked in 10% normal goat serum (NGS)-Triton-X/PBS for 1 h. Sections were then incubated in 5% NGS-Triton-X/PBS containing anti-adenylyl cyclase III (ACIII) rabbit polyclonal antibody (sc-588; Santa Cruz Biotechnology, Santa Cruz, CA), used at 1:400 and anti-SR-6 (5-HT₆) (A-20) raised in goat (sc-26668; Santa Cruz Biotechnology, Santa Cruz, CA), used at 1:400 with gentle agitation at 4°C overnight. Next, sections were rinsed 4 times in PBS and incubated with Alexa 488 or Alexa 568, conjugated goat anti-rabbit secondary antibody (1:250, Invitrogen, Carlsbad, CA) for 2 h. Sections were washed 3 times in PBS, mounted on slides and cover-slipped with ProLong Gold Antifade mounting medium.

Image Analysis/ Statistics

Immunostained primary cultured neurons or brain sections were imaged as stacks using either a Leica inverted wide field fluorescence microscope and associated ZEN software or a Leica TCS SL Confocal at the University of Washington Keck Microscopy Facility. Stacked images were compiled and images were analyzed using the NeuronJ plugin of the ImageJ software (Schneider *et al*, 2012). Unless noted otherwise, primary cilia measurements from neurons were averaged from neurons on each culture coverslip and those coverslip averages were used as each biological replicate.

Cyclic AMP analysis

Cyclic adenosine monophosphate (cAMP) accumulation was measured using IMCD-3 kidney cells (ATCC, Manassas, VA) as they possess primary cilia (Mai *et al*, 2005); they were maintained in Dulbecco's modified eagle medium: nutrient mixture F-12 (DMEM/F12) media supplemented with 10% FBS, 1.2 g/l of sodium bicarbonate, and 0.5 mM sodium pyruvate (Invitrogen). Cells were plated on 6-well plates at 100,000 cells per well and allowed to grow to overnight. On the second day IMCD3 cells were transfected using lipofectamine 2000 with plasmids containing 5-HT₆ receptors or our mutants and allowed to grow in the absence of serum for two days. The cells were then lysed with 0.1 M HCl, and then cellular cAMP levels were measured using an enzyme-linked immunosorbent assay-based detection kit (Cayman Chemicals, Ann Arbor, MI) according to the manufacturer's directions.

Data Analysis

Statistical analysis, non-linear regression modeling, and IC₅₀ calculations were performed using GraphPad Prism. Significance for multiple comparisons was assessed with one-way analyses of variance (ANOVA) followed by Tukey post hoc tests. For analysis involving only two samples we used a two-tailed t-test. Chi-squared and Fisher's exact test were used where appropriate to evaluate contingency tables of categorical data done in GraphPad Prism. For all comparisons, we used an alpha value of 0.05.

D. Results

5-HT₆ receptors localize to WT mouse neuronal primary cilia both *in vivo* and *in vitro*

Previous studies have described endogenous 5-HT₆ receptors on “cilia-like” processes in rats with electron microscopy and peroxidase staining (Brailov *et al*, 2000; Hamon *et al*, 1999). Here we confirmed that endogenous 5-HT₆ receptors co-localize with adenylyl cyclase 3 (AC3), an established marker for primary cilia, (Bishop *et al*, 2007; Wang *et al*, 2009), in sections of WT mouse striatum (Figure 3.1A). Further, 5-HT₆ receptors immunostaining was absent in 5-HT₆KO striatal sections despite continued presence AC3-positive cilia (Figure 3.1B). 5-HT₆ receptors also localized to primary cilia in WT primary cultured striatal neurons following dissociation and 10 days growth *in vitro* (Figure 3.1C); as expected, primary cultured striatal neurons from 5-HT₆ KO mice had cilia but lacked 5-HT₆ immunostaining altogether (Figure 3.1D).

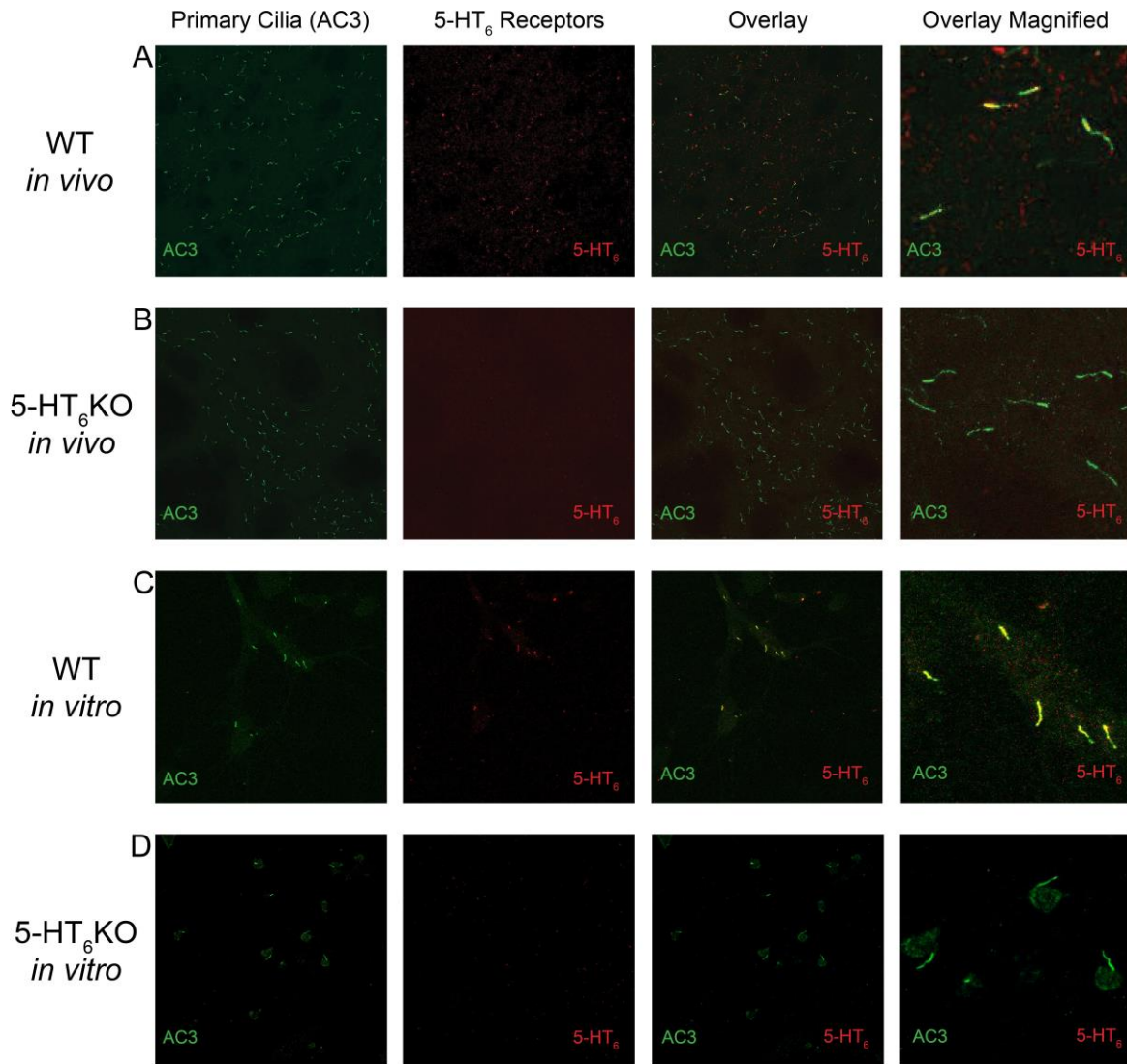


Figure 3.1 5-HT₆ receptors localize to mouse neuronal primary cilia both *in vivo* and *in vitro* A) Images of WT mouse striatum stained against cilia marker AC3, 5-HT₆ receptors, and both (also shown at higher magnification). B) Images of 5-HT₆ receptors mouse striatum stained against cilia marker AC3, 5-HT₆ receptors, and both (also shown at higher magnification). C) Images of WT primary mouse striatal neurons stained against cilia marker AC3, 5-HT₆ receptors, and both (also shown at higher magnification). D) Images of 5-HT₆ receptor KO primary mouse striatal neurons stained against cilia marker AC3, 5-HT₆ receptors, and both (also shown at higher magnification).

5-HT₆ receptor antagonists shorten primary cilia of striatal neurons

To examine the effect of endogenous 5-HT₆ receptor activity on neuronal primary cilia morphology, we used primary cultured striatal neurons to facilitate precise control of drug concentration and ready imaging and measurement of individual primary cilia. Treatment of WT cultured striatal neurons with 1 μM of either selective 5-HT₆ receptor agonist (WAY-208466) for 24 hours had no effect on primary cilia length, but treatment with the 5-HT₆-selective receptor antagonist SB-399885 for 24 hours significantly reduced the length of primary cilia (Figure 2A, $F(3,58) = 16.84$; $p < 0.0001$). Treatment with both 5-HT₆ receptor agonist and antagonist together had no effect. These drug treatments did not alter the percentage of primary neurons with primary cilia (ciliation) on the striatal neurons (Figure 2B, $F(3,33) = 0.113$; $p = 0.95$). Treatment with SB-399885 reduced the average primary cilia length in a concentration-dependent manner with an IC₅₀ value of 28 nM (Figure 2C), which is similar to the previously reported K_i for this drug (Hirst *et al.*, 2006). The effect of 1 μM SB-399885 treatments on WT cultured striatal neurons at different time intervals (0, 4, 12, and 24 hours) established that the antagonist-induced primary cilia shortening is also time dependent (Figure 2D). We observed a similar reduction in cilia length using the 5-HT₆-selective antagonist SB-258585 (1 μM, data not shown).

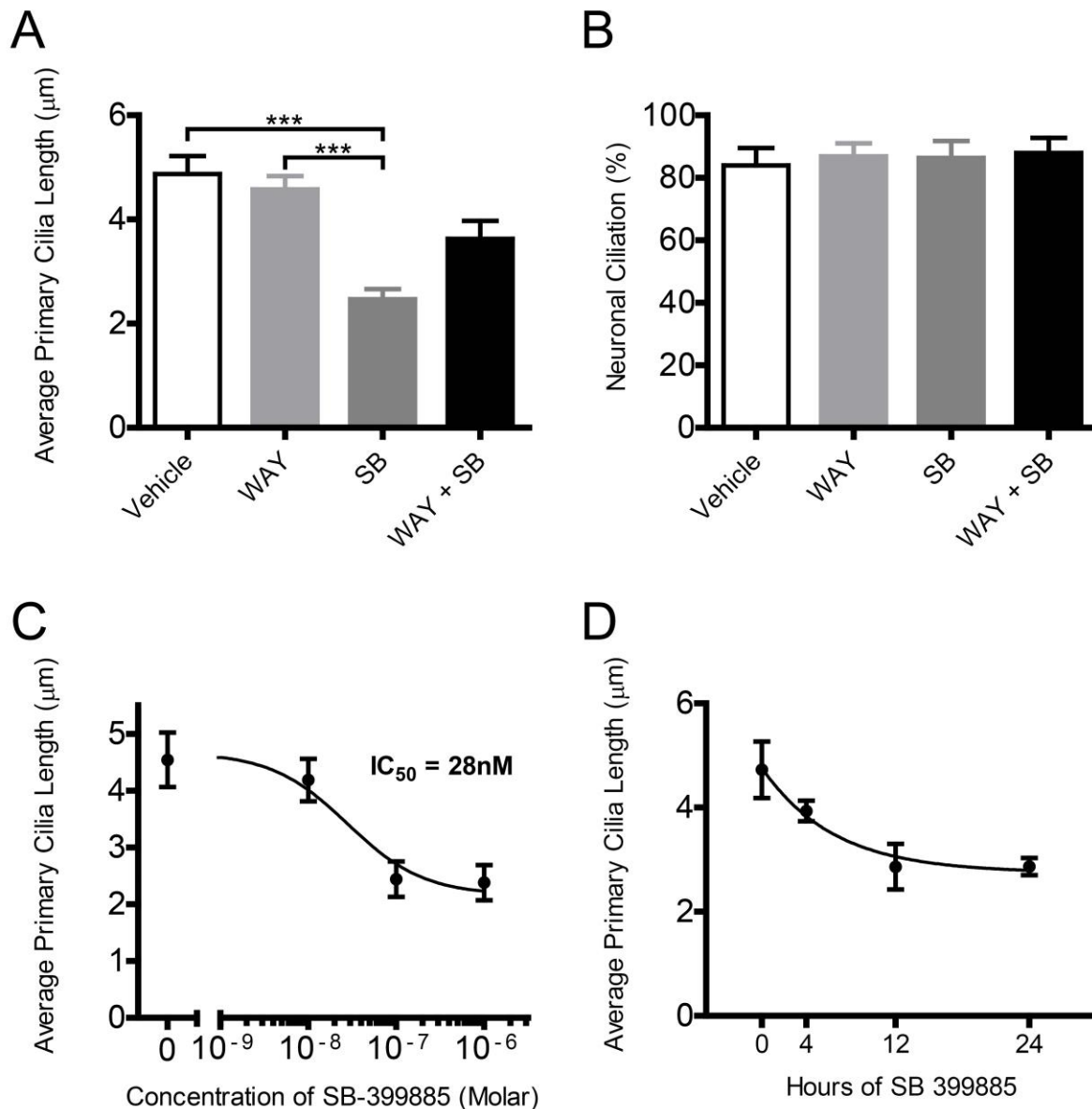


Figure 3.2 5-HT₆ receptor antagonism shortens primary cilia of striatal neurons. A) Effect of 5-HT₆ receptor selective drugs on primary cilia lengths (Mean ± SEM) of WT primary striatal neurons in culture. Vehicle (n=18 coverslips), WAY (n=17 coverslips), SB (n=18 coverslips) WAY+SB (n=8 coverslips). B) Effect of 5-HT₆ receptor selective drugs on percentage of WT striatal neurons with primary cilia (Mean ± SEM). Vehicle (n=10 coverslips), WAY (n=10 coverslips), SB (n=9 coverslips) WAY+SB (n=8 coverslips). C) Primary cilia length in response to different concentrations of SB-399885 (antagonist) (Mean ± SEM); Vehicle (n=25 cilia across 5 coverslips), 10 nM (n=36 cilia across 5 coverslips), 100 nM (n=29 cilia across 5 coverslips) and 1 µM (n=30 cilia across 5 coverslips). D) Time-dependent reduction of cilia length by SB-

399885 (Mean \pm SEM); 0 (n=6 coverslips), 4 (n=7 coverslips), 12 (n=5 coverslips), and 24 hours (n=7 coverslips). * = P < 0.05, ** = P < 0.001, *** = P < 0.0001; one-way ANOVA followed by post hoc Tukey's HSD test.

Effect SB-399885 of primary cilia of striatal neurons is 5-HT₆ receptors specific.

We confirmed that SB-399885 reduced cilia length via 5-HT₆ receptors using primary cultured striatal neurons cultured from 5-HT₆KO mice; there was no effect of 5-HT₆-selective agonist (1 μM WAY-208466), antagonist (1 μM SB-399885) or both drugs together; none of these treatments altered primary cilia length (Figure 3A, $F(3,26) = 0.46$; $p=0.71$) or ciliation (Figure 3B, $F(3,16) = 1.53$; $p=0.25$).

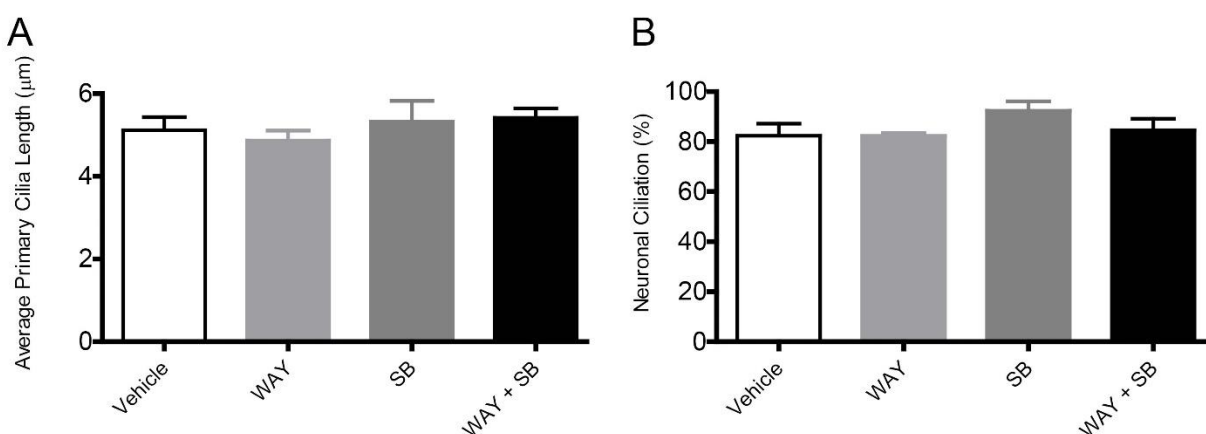


Figure 3.3 Effect of SB-399885 on primary cilia length of striatal neurons is 5-HT₆ receptors specific. A) Effect of 5-HT₆ receptor selective drugs on primary cilia lengths (Mean ± SEM) of 5-HT₆ KO primary striatal neurons in culture. Vehicle (n=5 coverslips), WAY (n=6 coverslips), SB (n=8 coverslips) WAY+SB (n=10 coverslips). B) Effect of 5-HT₆ receptor selective drugs on percentage of 5-HT₆KO striatal neurons with primary cilia (Mean ± SEM). Vehicle (n=5 coverslips), WAY (n=5 coverslips), SB (n=5 coverslips) WAY+SB (n=5 coverslips). *P < 0.05, **P < 0.01; one-way ANOVA followed by post hoc Tukey's HSD test.

High levels of heterologous expression increased nonspecific localization of 5-HT₆ receptors

Previous studies of subcellular localization of 5-HT₆ receptors to primary cilia used heterologous expression of 5-HT₆ receptors (Berbari *et al*, 2008a; Guadiana *et al*, 2013; Mahjoub and Stearns, 2012). Using Lipofection, we transfected HA-tagged 5-HT₆ receptor plasmid into primary striatal neurons using a wide range of plasmid levels. Immunostaining of the transfected neurons revealed that the heterologous 5-HT₆ receptors did not always localize to neuronal primary cilia (Figure 3.4A), and sometimes localized to the surface of the cell body (Figure 3.4B). This finding led us to vary the amount of 5-HT₆ plasmid while maintaining a constant amount of total transfected plasmid to evaluate the extent to which increased levels of overexpression affected subcellular localization of the HA-5-HT₆ receptor (Susa *et al*, 2008). In each condition, each well of WT or 5-HT₆KO neurons was transfected with a total of 1 μg of total plasmid DNA containing varying proportions of the 5-HT₆ receptor plasmid. At low levels of expression, most of the immunostaining for 5-HT₆ receptor was mostly in the primary neuronal cilia, but as the amount of 5-HT₆ receptor plasmid increased, the percentage of transfected neurons with HA-tagged receptor localized outside of primary cilia increased significantly in both WT neurons (Figure 3.4C, $\chi^2(7, N=111) = 14.13, p=0.048$) and 5-HT₆KO neurons (Figure 3.4F, $\chi^2(7, N=169) = 22.26, p=0.0023$) neurons. Proportions of receptor localization where HA-5-HT₆ receptor was found in primary cilia, out of primary cilia, or both was evaluated for both transfected WT (Figure 3.4D) and 5-HT₆KO (Figure 3.4G) neurons. Primary cilia length did not change with different amounts of transfected plasmid in either WT (Figure 3.4E, 1-way ANOVA, $p = 0.76$) or 5-HT₆KO (Figure 3.4H, 1-way ANOVA, $p = 0.12$) cultured striatal neurons.

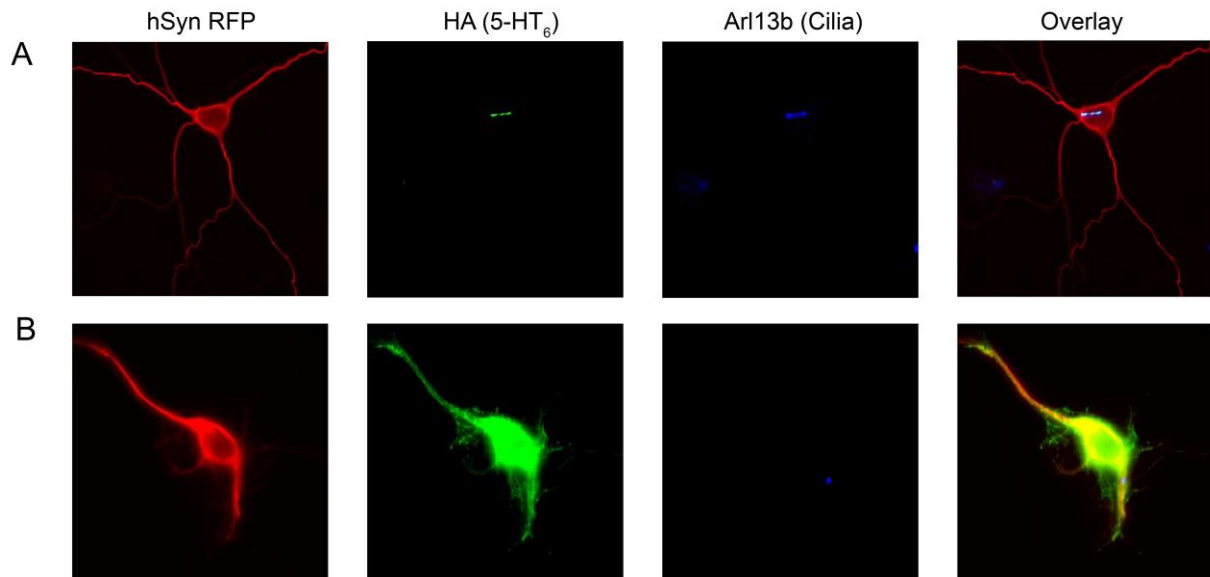


Figure 3.4. High levels of heterologous expression increases nonspecific localization of 5-HT₆ receptors. A) Representative images of transfected 5-HT₆ receptor expressing in neuronal primary cilia. Red = hSynRFP (marker for neuronal specific transfection), Green = HA (HA-tagged transfected 5-HT₆ receptors), Blue = Arl13b (marker for primary cilia). B) Representative image of transfected 5-HT₆ receptor expressing out of neuronal primary cilia and on cell surface. Red = hSynRFP (marker for neuronal specific transfection), Green = HA (HA-tagged transfected 5-HT₆ receptors), Blue = Arl13b (marker for primary cilia).

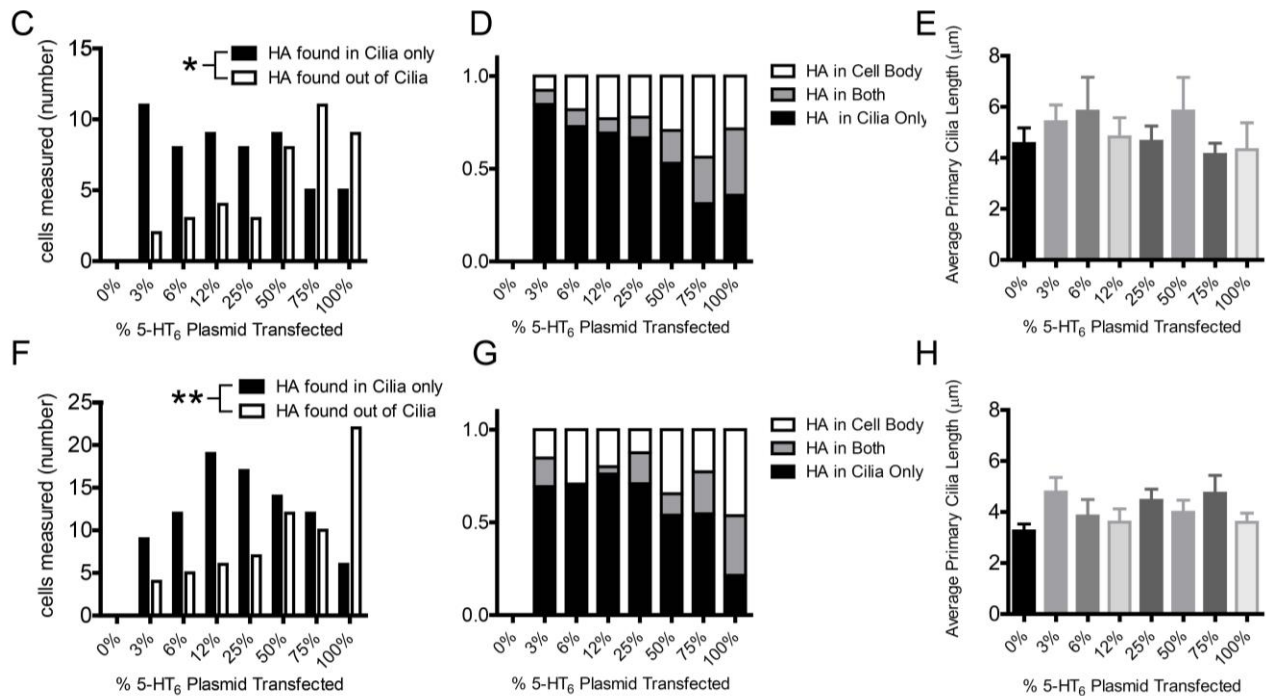


Figure 3.4 cont. High levels of heterologous expression increases nonspecific localization of 5-HT₆ receptors. C) Receptor localization from gene-dose transfection of WT 5-HT₆ receptor in WT primary culture neurons (counts). 0% (n=15 cells), 3% (n=13 cells), 6% (n=11 cells), 12% (n=13 cells), 25% (n=12 cells), 50% (n=17 cells), 75% (n=16 cells), 100% (n=14 cells). D) Proportion of total WT neurons transfected with varied amount of 5-HT₆ receptor plasmid where HA is found either in primary cilia, out of primary cilia, or both. E) Average primary cilia lengths resulting from gene-dose transfection of WT 5-HT₆ receptor in WT primary culture neurons 0% (n=29 cells), 3% (n=10 cells), 6% (n=10 cells), 12% (n=10 cells), 25% (n=17 cells), 50% (n=6 cells), 75% (n=28 cells), 100% (n=13 cells). F) Receptor localization gene-dose transfection of 5-HT₆ receptor in 5-HT₆KO primary culture neurons (count). 0% (n=15 cells), 3% (n=13 cells), 6% (n=17 cells), 12% (n=25 cells), 25% (n=24 cells), 50% (n=26 cells), 75% (n=21 cells), 100% (n=28 cells). G) Proportion of total 5-HT₆KO neurons transfected with varied amount of 5-HT₆ receptor plasmid where HA is found either in primary cilia, out of primary cilia, or both. H) Average primary cilia lengths resulting from gene-dose transfection of 5-HT₆ receptor in 5-HT₆KO primary culture neurons 0% (n=54 cells), 3% (n=19 cells), 6% (n=16 cells), 12% (n=20 cells), 25% (n=43 cells), 50% (n=17 cells), 75% (n=20 cells), 100% (n=29 cells).

Mutations to the CTS reduced cilia targeting

Previous studies of the 5-HT₆ and other GPCRs have suggested that a five amino acid long consensus sequence in the i3 loop acts as a cilia targeting sequence (CTS) (Berbari *et al*, 2008a; Domire *et al*, 2011; Nagata *et al*, 2013). We took advantage of the CTS on the rat 5-HT₆ receptor and cloned two mutant forms of receptor: one in which the five amino acid CTS was removed (Δ 5-HT₆ No CTS, Figure 5A) and one in which the entire i3 loop was replaced with that of the 5-HT₇ receptor (Δ 5-HT₆ i3-7) (Figure 5B). The 5-HT₇ receptor was chosen because it is also a G α_s -coupled receptor, but does not localize to primary cilia (Berbari *et al*, 2008a). We transfected WT 5-HT₆, Δ 5-HT₆ No CTS, and Δ 5-HT₆ i3-7 (25% of total plasmid transfected) into 5-HT₆KO cultured neurons to test localization in the absence of endogenous 5-HT₆ receptors to preclude possible WT-mutant heteromerization and thus prevent potential co-trafficking to primary cilia. These two mutants had significantly reduced trafficking to primary cilia but a modest proportion of the mutant receptors were still localized to primary cilia (Figure 5C, Fisher's Exact test; Δ 5-HT₆ No CTS, p=0.001; Δ 5-HT₆ i3-7, p=0.007). The mutant 5-HT₆ receptors did not affect the average length of primary cilia (Figure 5D, 1-way ANOVA, p = 0.6836). Since it was possible that the functional properties of these mutants affected localization or cilia morphology, we tested whether these mutants were still able to activate adenylyl cyclase using an enzyme-linked immunosorbent assay to measure cAMP accumulation in IMCD3 cells, which do not express 5-HT₆ receptors endogenously (data not shown). The WT 5-HT₆ and Δ 5-HT₆ No CTS showed both constitutive and agonist-stimulated increase in cAMP levels compared to empty vector control, but the Δ 5-HT₆ i3-7 receptor failed to activate cAMP accumulation (Figure 5E, 2-way ANOVA; WT 5-HT₆, p<0.001; WT 5-HT₆ +WAY, p<0.001; Δ 5-HT₆ No CTS, p<0.001; Δ 5-HT₆ No CTS + WAY, p<0.001; Δ 5-HT₆ i3-7, p>0.05; Δ 5-HT₆ i3-

7 + WAY, $p > 0.05$). Thus, the $\Delta 5\text{-HT}_6$ i3-7 mutant was non-functional at least in terms of the ability to increase adenylyl cyclase activity.

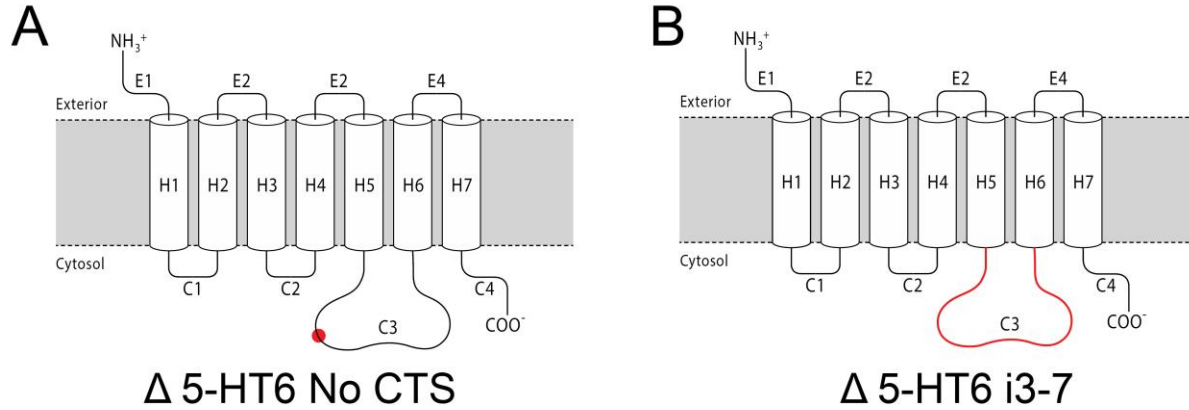


Figure 3.5. Mutations to the CTS reduces cilia targeting. A) Representative diagram of mutation on 5-HT₆ receptor to create a receptor without the primary cilia targeting sequence (Δ 5-HT₆ No CTS). B) Representative diagram of mutation on 5-HT₆ receptor to create a receptor with 5-HT₇ receptor intracellular loop 3 (IC3) (Δ 5-HT₆ i3-7).

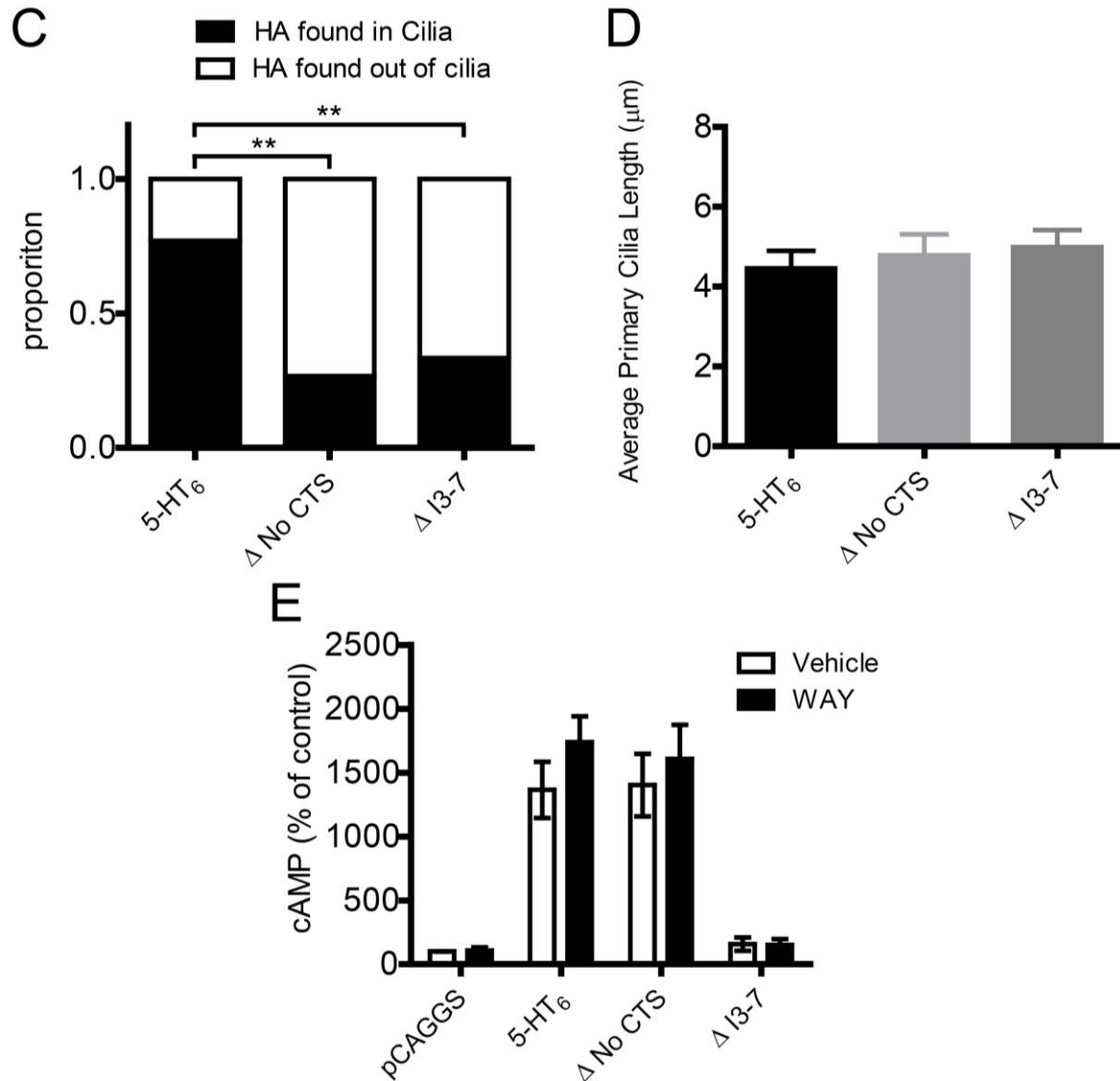


Figure 3.5. Mutations to the CTS reduces cilia targeting cont. C) Proportion primary cilia localization of 5-HT₆KO cultured neurons transfected with 25% receptor plasmid: WT 5-HT₆ (n=39 neurons), Δ5-HT₆ No CTS (n=15 neurons), or Δ5-HT₆ i3-7 (n=18 neurons). D) Average primary cilia lengths when the mutant 5-HT₆ receptors localize to cilia: WT 5-HT₆ (n=43 neurons), Δ5-HT₆ No CTS (n=28 neurons), or Δ5-HT₆ i3-7 (n=41 neurons). E) Relative levels of cAMP accumulation in IMCD3 cells transfected with empty vector (pCAGGS), WT 5-HT₆, Δ5-HT₆ No CTS, or Δ5-HT₆ i3-7; both in the presence and absence of WAY-208846 (n=3 independent biological replicates). * = P < 0.05, ** = P < 0.001, *** = P < 0.0001.

E. Discussion

The 5-HT₆ receptor has become an important target for drug development (Fone, 2008) and at least one 5-HT₆ antagonist is in later stages of development for the treatment of Alzheimer's disease (Wilkinson *et al*, 2014). However, the implications that 5-HT₆ receptors localize to primary neuronal cilia has received scant attention. Primary cilia are enriched with signaling proteins such as receptors and second messenger systems that allow them to function as neuronal sensory organelles that sample the extrasynaptic space (Koemeter-Cox *et al*, 2014; Louvi and Grove, 2011; Whitfield, 2004). This could be an important mechanism for integrating extracellular signals over a different time scale than that associated with synaptic neurotransmission. Thus, 5-HT₆ receptors on neuronal primary cilia are well positioned for detecting the extrasynaptic effects of 5-HT (Whitfield, 2004), rendering them unique targets for modulating neuronal plasticity without interfering with momentary synaptic events. Here, we demonstrate that 5-HT₆ receptors preferentially localize to primary cilia and regulate primary cilia morphology. We also find that the level of 5-HT₆ receptor expression dictates receptor localization, and that the mechanism leading to the unique neuronal primary cilia localization is more complicated than previously described.

We observed no effect of the addition of a 5-HT₆ agonist on primary cilia length but 5-HT₆ antagonists reduce striatal primary cilia length in a time and concentration dependent manner; the inclusion of an agonist and an antagonist together reversed this effect. There are at least two possible explanations for this. First, the 5-HT₆ receptor has been reported to express constitutive activity (Brouard *et al*, 2015; Duhr *et al*, 2014; Jacobshagen *et al*, 2014; Sebben *et al*, 1994) and there may be a “ceiling effect” in which additional receptor activation produces no

further elongation of primary cilia. Further, we observed no effect of increasing levels of overexpression on cilia length. Secondly, we cultured the primary neurons in the presence of FBS, which can contain a small amount of serotonin. Since 5-HT₆ receptors have moderately high affinity for 5-HT, they might be activated by residual (or extrasynaptic) serotonin much of the time. While we observed constitutive activity in our functional assay of cAMP accumulation following heterologous overexpression of the receptor, we are aware of no definitive evidence that endogenous 5-HT₆ receptors show constitutive activity under conditions where there is no trace serotonin present.

Regulation of cilia length is thought to modulate the amplitude of signaling events possible in cilia (Marley and von Zastrow, 2012), with longer cilia allowing greater signaling capacity by the receptor systems within the cilia. The effect of 5-HT₆ receptors on primary cilia morphology is particularly interesting because reduced cilia length is regulated during key stages in development and is often associated with genetic ciliopathies (Armato *et al*, 2013; Chakravarthy *et al*, 2012; Pan and Snell, 2007). Another phenotype often seen in ciliopathies is a reduction in the number of neurons possessing primary cilia (Kulaga *et al*, 2004; Lee and Gleeson, 2011; Novarino *et al*, 2011; Ross *et al*, 2005). 5-HT₆ receptor antagonism did not change the proportion of neurons bearing primary cilia.

Using 5-HT₆KO cultured neurons, no morphology changes were seen in the primary cilia of, indicating that the effects of SB-399885 were dependent upon endogenous 5-HT₆ receptors on the WT neurons. Although we were able to confirm that the antagonist effects were absent in primary striatal neurons cultured from 5-HT₆KO mice, these germ-line null mutants had normal cilia length, perhaps due to developmental compensation that has been described for 5-HT₆ effects on neuronal migration (Riccio *et al*, 2009). These data implicate a role for 5-HT₆

receptors in the dynamic regulation of neuronal primary cilia length, and provides another target, along with lithium, for studying inducible changes to primary cilia morphology (Miyoshi *et al*, 2009; Ou *et al*, 2009).

Previous findings showed that heterologous expression of 5-HT₆ receptors via *in utero* electroporation of embryonic hippocampal neurons elongated primary cilia dramatically (Guadiana *et al*, 2013). We expressed heterologous 5-HT₆ receptors in both WT and 5-HT₆KO cultured striatal neurons using lipofection and found the subcellular distribution of heterologously expressed 5-HT₆ receptors was dependent on the amount of receptor plasmid introduced to the neurons, while primary cilia length was unaffected. The previous report studied less mature, neocortical neurons and found that even mutant, nonfunctional receptors produced elongation of up to several hundred percent as well as a number of morphological and phenotypic anomalies such as branching and loss of AC3 expression (Guadiana *et al*, 2013); we did not observe these issues. Rather, we observed that transfection with increasing amounts of exogenous 5-HT₆ receptor led the receptor to localize outside of primary cilia in both WT and 5-HT₆KO cultured striatal neurons. Other investigators have used heterologous overexpression of ciliary receptors to study the effects on primary cilia, but to our knowledge the observation of gene dose-dependent mislocalization of protein has not been previously addressed. Here, we find that moderate to high levels of expression impact the fidelity of ciliary localization in primary neurons, perhaps this is due to overloading of the ciliary protein trafficking machinery. This supports the notion that the rates of protein expressed in primary cilia are likely more dependent on the machinery necessary to traffic the proteins than on the total amount of protein expressed (McGlashan *et al*, 2010). Additionally, cilia length was unaffected by reintroducing 5-HT₆ receptors into 5-HT₆KO striatal neurons or increasing 5-HT₆ expression in WT neurons. It is

possible that cilia localization depends on the maturity of the neurons, and so studying them in postnatal day 0 neurons grown in culture for another 10-14 days provides new perspective on 5-HT₆ trafficking and signaling, as we did not observe the extreme lengthening that others saw even with receptors deemed as nonfunctional.

A five amino acid consensus sequence on the i3 loop, denoted the CTS, has been found in many GPCRs that traffic into primary cilia (Berbari *et al*, 2008a; Nachury *et al*, 2010; Nagata *et al*, 2013). The 5-HT₆ receptor is the only serotonin receptor that contains this motif and no other serotonin receptors have been reported to traffic to cilia. Based on these findings, we designed two mutant 5-HT₆ receptors that disrupted the CTS in different ways and were predicted to block cilia trafficking in order to study the effect of 5-HT₆ receptors signaling inside or outside of neuronal primary cilia. By deleting the CTS or by swapping the i3 loop from a non-cilia localizing receptor into the 5-HT₆ receptor, we found that these changes significantly reduced, but did not entirely abolish, trafficking of the receptor to cilia. Additionally, cilia length was unaffected by reintroducing these mutant 5-HT₆ receptors into 5-HT₆KO striatal neurons. One possible explanation for how mutant receptors might gain access to cilia is through dimerization with endogenous 5-HT₆ receptors; however, we also expressed the mutant 5-HT₆ receptors in 5-HT₆KO neurons but the trafficking was not substantially different than in WT neurons, so we conclude that dimerization (at least with other 5-HT₆ receptors) is unlikely to explain trafficking to cilia. It still remains possible that 5-HT₆ receptors heterodimerize with some other cilia-localizing GPCR but that seems less likely since receptors heterodimerize rather freely but very few receptors gain access to cilia.

Together, our results implicate a role of 5-HT₆ receptor signaling in primary cilia function and regulation of primary cilia morphology. Our findings suggest that 5-HT₆ receptor

research should consider relative levels of receptor expression when evaluating their impact on primary cilia function. Future studies can also examine how 5-HT₆ receptor signaling alters neuronal function and interacts with signaling via other cilia-localizing receptors, and together these may provide novel avenues for treating a variety of neuropsychiatric diseases (Marley and von Zastrow, 2012).

CHAPTER IV: DISCUSSION

The results presented in this dissertation provide some insight into the importance of 5-HT₆ receptors on the structure and function of the neuronal circuitry in mammalian striatum. The implications of the experiments carried out in this study are threefold. First, I show that 5-HT₆ receptor expression in major output pathways of striatum affects the reinforcing properties of cocaine. Next, I show that the activity of 5-HT₆ receptors affects the morphology of primary cilia in striatal neurons. Finally, I show that the relative levels of 5-HT₆ receptor overexpression in striatal neurons affect the subcellular localization of the receptors. Together, my work begins to address how 5-HT₆ receptor function within striatum needs to be considered both in a sub region and subcellular-specific manner.

In chapter two, I used viral vector tools previously developed in our lab to target increased expression of 5-HT₆ receptors into either the direct or indirect pathway of the NAcSh (Eskenazi *et al*, 2015; Ferguson *et al*, 2011, 2013). By injecting viral vectors utilizing prodynorphin- or proenkephalin promoters to drive expression of 5-HT₆ receptors or GFP selectively in the dMSNs or iMSNs of the NAcSh, I showed that increased expression of 5-HT₆ receptors in the iMSNs, but not dMSNs, reduced cocaine SA under an FR1 schedule at varied doses of cocaine. Further, I saw this selective increase of 5-HT₆ receptors in NAcSh iMSNs did not lead to a decrease in the “break point” under a PR reinforcement schedule, suggesting that the rats’ motivation to take cocaine was not altered.

I postulate that the rats with increased 5-HT₆ receptors in iMSNs were more sensitive to the reinforcing properties of cocaine and therefore needed a lower dose of cocaine to reach a preferred subjective response to the drug. This hypothesis was supported when I applied a

method for modeling the pharmacokinetics and tissue concentration of cocaine to estimate the brain cocaine concentration that each animal took and presumably preferred for each dose of cocaine tested (Pan *et al*, 1991; Zimmer *et al*, 2011). Further support for my hypothesis was established by CPP, as the rats with increased 5-HT₆ receptors in iMSNs developed a preference for a typically sub-threshold dose of cocaine that did not support a conditioned place preference in GFP control rats (Barot *et al*, 2007; Neumaier *et al*, 2002). These analyses suggested that the Enk-5-HT₆ rat were more sensitive to cocaine and preferred a lower tissue concentration of cocaine, further supporting the notion that they are more sensitive to the reinforcing properties of cocaine.

As with any set of experiments, there are multiple ways to improve them or address the questions differently in future experiments. The experiments in Chapter 2 could be designed to make sure the rats go through the different cocaine SA behavioral paradigms in a way that is more consistent with previous literature. One particular concern was that the rats in Chapter 2 experienced the PR sessions before the low and high dose FR1 sessions as a possible confound to the behavioral results, because the rats would have to reacquire stable FR1 cocaine taking behavior. This point may be addressed in future experimental design, however I believe our results were not affected by our less conventional order of experiments. Since our rats changed behavior within the first few presses of each session they seemed to learn new paradigms and doses so quickly that the order of experiments once a rat has established stable cocaine taking should not matter, especially when taking averages across multiple sessions. Another way to improve similar experiments in the future would be to adapt behaviors that are more sensitive to the rats' preference and titration of cocaine dose by allowing the rats to control the speed of its

own cocaine pump and thus preferential dose (Liu *et al*, 2005; Ward *et al*, 2005; Zimmer *et al*, 2013).

As mentioned in Chapter 2, dopamine differentially activates dMSNs via D₁ receptors and inhibits iMSNs via D₂ receptors whereas 5-HT excites both populations of MSNs via endogenous 5-HT₆ receptors (Tassone *et al*, 2011; Ward *et al*, 1995). Thus, dMSNs are activated by both the endogenous Gs-coupled D₁ and 5-HT₆ receptors, leading to an accumulation of cAMP and downstream activation of many signaling pathways (Dobi *et al*, 2011; Surmeier *et al*, 2007). On the other side, D₂ and 5-HT₆ receptors in iMSNs have opposing effects on adenylate cyclase activity and work in opposition. Thus, 5-HT₆ receptors in the iMSNs are positioned to interfere with behaviors that are supported by dopamine action in NAcSh. Presumably balanced activation of endogenous 5-HT₆ receptors in both pathways simultaneously interferes with dopamine actions by reducing the differential activation of these two pathways. It is not known whether exposure to abused drugs alters the expression levels of 5-HT₆ in these pathways differentially, but this is a topic under active exploration.

There is therapeutic potential for 5-HT₆ receptors in the research and treatment of drug addiction. Since increased 5-HT₆ receptor expression in the iMSNs of NAcSh does not affect motivation while increasing the sensitivity to the reinforcing properties of cocaine, if 5-HT₆ receptors are up-regulated or stimulated selectively in the iMSNs, the amount of drug taken by individuals may decrease, thereby impeding the progression toward compulsive, unregulated drug use and reducing the risk of overdose. Another potential way to achieve a similar result would be to selectively express DREADD receptors with analogous protein coupling profiles in the indirect pathway using viral vectors and the *Penk* promoter, and stimulate using CNO.

However, these types of genetic interventions are currently unavailable and would be fairly invasive compared to the condition they potentially treat.

In Chapter 3, I focused on the subcellular localization of 5-HT₆ receptors to neuronal primary cilia. Utilizing cultured striatal neurons, I focused on whether manipulating 5-HT₆ receptor activity and expression in striatal neurons altered primary cilia morphology and signaling. There are three major findings stemming from these experiments. First, 5-HT₆ receptor antagonists reduce the average primary cilia lengths of striatal neurons in both a concentration- and time-dependent manner via direct action on 5-HT₆ receptors. Second, increasing levels of overexpression of heterologously expressed 5-HT₆ receptors led to increasing amounts of 5-HT₆ localization outside of the primary cilia in both WT and 5-HT₆KO striatal neurons. Last, mutations in the CTS of the i3 loop of the 5-HT₆ receptor substantially reduced but did not entirely eliminate trafficking of the 5-HT₆ receptor to primary cilia.

There are multiple hypotheses regarding the function of neuronal primary cilia, especially during neuronal development (Dobi *et al*, 2011; Koemeter-Cox *et al*, 2014; Ruat *et al*, 2012). Since primary cilia are enriched with sensitive signaling proteins and may function as neuronal sensory organelles, 5-HT₆ receptors likely contribute to their signaling pathways. I hypothesize that 5-HT has extrasynaptic effects via 5-HT₆ receptors on neuronal primary cilia, making 5-HT₆ receptors unique targets for modulating internal neuronal signaling without interfering with momentary synaptic events. Our finding that selective 5-HT₆ antagonists reduced cilia length in striatal cultured neurons is an example of such specific signaling. These inducible changes on the dynamic regulation of neuronal primary cilia length may be useful in the future of ciliopathy research (Avasthi and Marshall, 2012; Miyoshi *et al*, 2009; Thompson *et al*, 2015).

Our findings that the subcellular distribution of heterologously expressed 5-HT₆ receptors was dependent on the amount of receptor plasmid introduced to the neurons has repercussions for the fields of both primary cilia and 5-HT₆ receptor research. Specifically, I saw that with an increase of transfected 5-HT₆ receptor expression, the location of where the receptor was expressed changed. With more transfected receptor, there was more HA-tagged receptor expressed outside of primary cilia in both our WT and 5-HT₆KO cultured striatal neurons. Perhaps this effect is due to overloading of the ciliary protein trafficking machinery, but to our knowledge the issue of protein spilling out of or never reaching primary cilia has not been previously addressed. It is common to use heterologous overexpression of ciliary receptors in order to study the effects of those receptors on primary cilia and the amount of heterologous receptor is rarely quantified. Additionally, we observed no effect of reintroducing 5-HT₆ receptors into striatal neurons on cilia length. This result contradicts previous studies that reported dramatic lengthening and branching of primary cilia after overexpression of 5-HT₆ receptors via in-utero electroporation (Guadiana *et al*, 2013). It is possible that our use of more mature neurons led to less dramatic effects of 5-HT₆ expression on primary cilia morphology because the effect requires immature neurons.

The final finding in Chapter 3 regards the putative CTS on the i3 loop that has been proposed to be essential for selective trafficking of GPCRs into primary cilia. I designed two mutant 5-HT₆ receptors that were predicted to block cilia trafficking in order to study the effect of 5-HT₆ receptor signaling either within or outside of neuronal primary cilia. By deleting the entire CTS or by swapping the i3 loop from a non-cilia localizing receptor into the 5-HT₆ receptor, I found that these mutations reduced, but did not prevent, trafficking of the mutant receptors to cilia. In order to eliminate the possibility of our mutants being co-transported to

primary cilia with endogenous 5-HT₆ receptors as heterodimers, I transfected the mutants into 5-HT₆KO cultures as well. I still saw our mutants localizing to primary cilia. This brings previous CTS and ciliary protein trafficking research into question as it is likely not the only method by which GPCRs can localize to neuronal primary cilia.

Despite the reduction of primary cilia localization, the mutant 5-HT₆ receptors still localized to neuronal primary cilia to some extent. The mutant receptors would be an ideal tool if the mutant receptors remain functional and exclusively localize to or outside primary cilia in 5-HT₆KO cultured striatal neurons. We could use those mutant receptors to study what the morphological and cellular consequences of expressing and activating 5-HT₆ receptors exclusively in the primary cilium vs. soma of a neuron. Further, we could package the mutant receptors in viral vectors and explore whether 5-HT₆ receptor localization to primary cilia has a behavioral impact *in vivo*. Using WT and 5-HT₆KO mice, we would investigate how viral-mediated gene transfer of primary cilia or somatodendritic localizing 5-HT₆ receptors in striatal neurons alters reward-mediated learning, general locomotion, open field behavior, and a variety of cognitive tasks.

When taken together, our results implicate roles for 5-HT₆ receptors both in the reinforcing properties of cocaine, and in the function and regulation of primary cilia. The 5-HT₆ receptor proves to be of continual interest to those who study neuropsychiatric disease and to those who study the function of primary cilia on neurons. The findings presented in this thesis suggest that 5-HT₆ receptors' circuitry specific role in behavior and localization to neuronal primary cilia may be closer than previously assumed. Thus highlighting the importance of evaluating 5-HT₆ expression levels when studying their impact on primary cilia function, as well as how they function within the specific pathways in which they are expressed.

REFERENCES

- Ainsworth C (2007). Tails of the unexpected. *Nature* **448**: 638–641.
- Allers K a., Sharp T (2003). Neurochemical and anatomical identification of fast- and slow-firing neurones in the rat dorsal raphe nucleus using juxtacellular labelling methods in vivo. *Neuroscience* **122**: 193–204.
- Andrade R, Chaput Y (1991). 5-Hydroxytryptamine₄-like receptors mediate the slow excitatory response to serotonin in the rat hippocampus. *J Pharmacol Exp Ther* **257**: 930–937.
- Andrade R, Haj-Dahmane S (2013). Serotonin neuron diversity in the dorsal raphe. *ACS Chem Neurosci* **4**: 22–25.
- Andrews C, Lucki I (2001). Effects of cocaine on extracellular dopamine and serotonin levels in the nucleus accumbens. *Psychopharmacology (Berl)* **155**: 221–229.
- Arellano JI, Guadiana SM, Breunig JJ, Rakic P, Sarkisian MR (2012). Development and distribution of neuronal cilia in mouse neocortex. *J Comp Neurol* **520**: 848–73.
- Armato U, Chakravarthy B, Pacchiana R, Whitfield JF (2013). Alzheimer’s disease: An update of the roles of receptors, astrocytes and primary cilia (Review). *Int J Mol Med* **31**: 3–10.
- Avasthi P, Marley A, Lin H, Gregori-Puigjane E, Shoichet BK, Zastrow M von, *et al* (2012). A chemical screen identifies class a g-protein coupled receptors as regulators of cilia. *ACS Chem Biol* **7**: 911–9.
- Avasthi P, Marshall WF (2012). Stages of ciliogenesis and regulation of ciliary length. *Differentiation* **83**: S30–42.
- Baker LP, Nielsen MD, Impey S, Metcalf MA, Poser SW, Chan G, *et al* (1998). Stimulation of type 1 and type 8 Ca²⁺/calmodulin-sensitive adenylyl cyclases by the G_s-coupled 5-hydroxytryptamine subtype 5-HT_{7A} receptor. *J Biol Chem* **273**: 17469–17476.
- Balleine BW, Delgado MR, Hikosaka O (2007). The role of the dorsal striatum in reward and decision-making. *J Neurosci* **27**: 8161–5.
- Barnes NM, Hales TG, Lummis SCR, Peters J a (2009). The 5-HT₃ receptor--the relationship between structure and function. *Neuropharmacology* **56**: 273–84.
- Barnes NM, Neumaier JF (2011). Neuronal 5-HT Receptors and SERT. *Tocris Biosci Sci Review Ser* **34**: 1–15.
- Barnes NM, Sharp T (1999). A review of central 5-HT receptors and their function. *Neuropharmacology* **38**: 1083–1152.
- Barot SK, Ferguson SM, Neumaier JF (2007). 5-HT_{1B} receptors in nucleus accumbens efferents enhance both rewarding and aversive effects of cocaine. *Eur J Neurosci* **25**: 3125–3131.
- Belin D, Balado E, Piazza PV, Deroche-Gamonet V (2009). Pattern of Intake and Drug Craving

- Predict the Development of Cocaine Addiction-like Behavior in Rats. *Biol Psychiatry* **65**: 863–868.
- Belin D, Berson N, Balado E, Piazza PV, Deroche-Gamonet V (2011). High-novelty-preference rats are predisposed to compulsive cocaine self-administration. *Neuropsychopharmacology* **36**: 569–579.
- Bennett JP, Snyder SH (1976). Serotonin and lysergic acid diethylamide binding in rat brain membranes: relationship to postsynaptic serotonin receptors. *Mol Pharmacol* **12**: 373–389.
- Berbari NF, Bishop GA, Askwith CC, Lewis JS, Mykytyn K (2007). Hippocampal Neurons Possess Primary Cilia in Culture. **1100**: 1095–1100.
- Berbari NF, Johnson AD, Lewis JS, Askwith CC, Mykytyn K (2008a). Identification of Ciliary Localization Sequences within the Third Intracellular Loop of G Protein-coupled Receptors. **19**: 1540–1547.
- Berbari NF, Lewis JS, Bishop G a, Askwith CC, Mykytyn K (2008b). Bardet-Biedl syndrome proteins are required for the localization of G protein-coupled receptors to primary cilia. *Proc Natl Acad Sci U S A* **105**: 4242–6.
- Berger M, Gray JA, Roth BL (2009). The expanded biology of serotonin. *Annu Rev Med* **60**: 355–366.
- Bishop G a, Berbari NF, Lewis JS, Mykytyn K (2007). Type III Adenylyl Cyclase Localizes to Mouse Brain. *J Comp Neurol* **571**: 562–571.
- Boado RJ, Li JY, Nagaya M, Zhang C, Pardridge WM (1999). Selective expression of the large neutral amino acid transporter at the blood-brain barrier. *Proc Natl Acad Sci U S A* **96**: 12079–84.
- Bock R, Shin JH, Kaplan AR, Dobi A, Markey E, Kramer PF, *et al* (2013). Strengthening the accumbal indirect pathway promotes resilience to compulsive cocaine use. *Nat Neurosci* **16**: 632–8.
- Boess FG, Martin IL (1994). Review Molecular Biology of 5-HT Receptors. *Neuropharmacology* **33**: 275–317.
- Bonasera SJ, Chu H-M, Brennan TJ, Tecott LH (2006). A null mutation of the serotonin 6 receptor alters acute responses to ethanol. *Neuropsychopharmacology* **31**: 1801–13.
- Bornstein AM, Daw ND (2011). Multiplicity of control in the basal ganglia: computational roles of striatal subregions. *Curr Opin Neurobiol* **21**: 374–80.
- Brailov I, Bancila M, Brisorgueil MJ, Miquel MC, Hamon M, Vergé D (2000). Localization of 5-HT(6) receptors at the plasma membrane of neuronal cilia in the rat brain. *Brain Res* **872**: 271–5.
- Branchek T a, Blackburn TP (2000). 5-Ht6 Receptors As Emerging Targets for Drug Discovery. *Annu Rev Pharmacol Toxicol* **40**: 319–34.
- Brewer GJ (1997). Isolation and culture of adult rat hippocampal neurons. *J Neurosci Methods* **71**: 143–155.

- Brodie B, Pletscher A, Shore P (1955). Evidence That Serotonin Has a Role in Brain Function. *Science* (80-) **122**: 968.
- Brouard JT, Schweimer J V., Houlton R, Burnham KE, Qu  r  e P, Sharp T (2015). Pharmacological Evidence for 5-HT₆ Receptor Modulation of 5-HT Neuron Firing *in Vivo*. *ACS Chem Neurosci* 150410150506007doi:10.1021/acschemneuro.5b00061.
- Bruin NMWJ de, McCreary a C, Loevezijn a van, Vries TJ de, Venhorst J, Drimmelen M van, *et al* (2013). A novel highly selective 5-HT₆ receptor antagonist attenuates ethanol and nicotine seeking but does not affect inhibitory response control in Wistar rats. *Behav Brain Res* **236**: 157–65.
- Burnham KE, Baxter MG, Bainton JR, Southam E, Dawson L a, Bannerman DM, *et al* (2010). Activation of 5-HT(6) receptors facilitates attentional set shifting. *Psychopharmacology (Berl)* **208**: 13–21.
- Carr G V, Lucki I (2012). The role of serotonin receptor subtypes in treating depression: a review of animal studies. *Psychopharmacol* **213**: 265–287.
- Carr G V, Schechter LE, Lucki I (2011). Antidepressant and anxiolytic effects of selective 5-HT₆ receptor agonists in rats. *Psychopharmacology (Berl)* **213**: 499–507.
- Chakravarthy B, Gaudet C, M  nard M, Brown L, Atkinson T, LaFerla FM, *et al* (2012). Reduction of the immunostainable length of the hippocampal dentate granule cells' primary cilia in 3xAD-transgenic mice producing human A β 1-42 and tau. *Biochem Biophys Res Commun* **427**: 218–222.
- Chamba A, Holder MJ, Barnes NM, Gordon J (2008). Characterisation of the endogenous human peripheral serotonin transporter SLC6A4 reveals surface expression without N-glycosylation. *J Neuroimmunol* **204**: 75–84.
- Chandra R, Francis TC, Konkalmatt P, Amgalan a., Gancarz a. M, Dietz DM, *et al* (2015). Opposing Role for Egr3 in Nucleus Accumbens Cell Subtypes in Cocaine Action. *J Neurosci* **35**: 7927–7937.
- Corbit LH, Nie H, Janak PH (2012). Habitual alcohol seeking: time course and the contribution of subregions of the dorsal striatum. *Biol Psychiatry* **72**: 389–95.
- C  t   F, Th  venot E, Fligny C, Fromes Y, Darmon M, Ripoch   M-A, *et al* (2003). Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. *Proc Natl Acad Sci U S A* **100**: 13525–13530.
- Cowen P, Lucki I (2011). Serotonin Revisited. *Psychopharmacology (Berl)* **213**: 167–169.
- Cunningham K a, Fox RG, Anastasio NC, Bubar MJ, Stutz SJ, Moeller FG, *et al* (2011). Selective serotonin 5-HT(2C) receptor activation suppresses the reinforcing efficacy of cocaine and sucrose but differentially affects the incentive-saliency value of cocaine- vs. sucrose-associated cues. *Neuropharmacology* **61**: 513–23.
- Davenport JR, Yoder BK (2005). An incredible decade for the primary cilium: a look at a once-forgotten organelle. *Am J Physiol Renal Physiol* **289**: F1159–69.

- Dayer AG, Jacobshagen M, Chaumont-Dubel S, Marin P (2015). 5-HT₆ Receptor: A New Player Controlling the Development of Neural Circuits. *ACS Chem Neurosci* doi:10.1021/cn500326z.
- Dobi A, Seabold GK, Christensen CH, Bock R, Alvarez V a (2011). Cocaine-induced plasticity in the nucleus accumbens is cell specific and develops without prolonged withdrawal. *J Neurosci* **31**: 1895–904.
- Dölen G, Darvishzadeh A, Huang KW, Malenka RC (2013). Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. *Nature* **501**: 179–84.
- Domire JS, Green J a, Lee KG, Johnson AD, Askwith CC, Mykytyn K (2011). Dopamine receptor 1 localizes to neuronal cilia in a dynamic process that requires the Bardet-Biedl syndrome proteins. *Cell Mol Life Sci* **68**: 2951–60.
- Domire JS, Mykytyn K (2009). Markers for neuronal cilia. *Methods Cell Biol* **91**: 111–21.
- Duhr F, Délérís P, Raynaud F, Séveno M, Morisset-Lopez S, Mannoury la Cour C, *et al* (2014). Cdk5 induces constitutive activation of 5-HT₆ receptors to promote neurite growth. *Nat Chem Biol* **10**: 590–7.
- Eskenazi D, Brodsky M, Neumaier JF (2015). Deconstructing 5-HT₆ receptor effects on striatal circuit function. *Neuroscience* **299**: 97–106.
- Eskenazi D, Neumaier JF (2011a). Increased expression of the 5-HT₆ receptor by viral mediated gene transfer into posterior but not anterior dorsomedial striatum interferes with acquisition of a discrete action-outcome task. *J Psychopharmacol* **25**: 944–51.
- Eskenazi D, Neumaier JF (2011b). Increased expression of 5-HT₆ receptors in dorsolateral striatum decreases habitual lever pressing, but does not affect learning acquisition of simple operant tasks in rats. *Eur J Neurosci* **34**: 343–51.
- Ettenberg A (2004). Opponent process properties of self-administered cocaine. *Neurosci Biobehav Rev* **27**: 721–728.
- Everitt BJ, Parkinson JA, Olmstead MC (1999). Associative Processes in Addiction and Reward The Role of Amygdala-Ventral Striatal Subsystems. *Ann N Y Acad Sci* 412–438.
- Ferguson SM, Eskenazi D, Ishikawa M, Wanat MJ, Phillips PEM, Dong Y, *et al* (2011). Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization. *Nat Neurosci* **14**: 22–4.
- Ferguson SM, Mitchell ES, Neumaier JF (2008). Increased expression of 5-HT₆ receptors in the nucleus accumbens blocks the rewarding but not psychomotor activating properties of cocaine. *Biol Psychiatry* **63**: 207–13.
- Ferguson SM, Phillips PEM, Roth BL, Wess J, Neumaier JF (2013). Direct-pathway striatal neurons regulate the retention of decision-making strategies. *J Neurosci* **33**: 11668–76.
- Fienberg a a, Hiroi N, Mermelstein PG, Song W, Snyder GL, Nishi a, *et al* (1998). DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. *Science* **281**: 838–842.
- Fijal K, Pachuta A, McCreary AC, Nowak E, Papp M, Bieńkowski P, *et al* (2010). Effects of

- serotonin (5-HT) 6 receptor ligands on responding for cocaine reward and seeking in rats. *Pharmacol Reports* **181187**: 1005–1014.
- Foley AG, Murphy KJ, Hirst WD, Gallagher HC, Hagan JJ, Upton N, *et al* (2004). The 5-HT(6) receptor antagonist SB-271046 reverses scopolamine-disrupted consolidation of a passive avoidance task and ameliorates spatial task deficits in aged rats. *Neuropsychopharmacology* **29**: 93–100.
- Fone KCF (2008). An update on the role of the 5-hydroxytryptamine6 receptor in cognitive function. *Neuropharmacology* **55**: 1015–1022.
- Frantz KJ, Hansson KJ, Stouffer DG, Parsons LH (2002). 5-HT 6 receptor antagonism potentiates the behavioral and neurochemical effects of amphetamine but not cocaine. *Neuropharmacology* **42**: 170–180.
- Fukuo Y, Kishi T, Yoshimura R, Kitajima T, Okochi T, Yamanouchi Y, *et al* (2010). Serotonin 6 receptor gene and mood disorders: case-control study and meta-analysis. *Neurosci Res* **67**: 250–5.
- Fuller RW, Wong DT (1990). Serotonin uptake and serotonin uptake inhibition. *Ann N Y Acad Sci* **600**: 68–78; discussion 79–80.
- Gaalen MM van, Schetters D, Schoffemeer ANM, Vries TJ De (2010). 5-HT6 antagonism attenuates cue-induced relapse to cocaine seeking without affecting cocaine reinforcement. *Int J Neuropsychopharmacol* **13**: 961–5.
- Gaddum JH, Picarelli ZP (1957). Two kinds of tryptamine receptor. *Br J Pharmacol Chemother* **12**: 323–8.
- Gerard C, Martres M, Lefevre K, Verge D, Lanfumey L, Doucet E, *et al* (1997). Immunolocalization of serotonin 5-HT 6 receptor-like material in the rat central nervous system. 207–219.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, *et al* (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* **250**: 1429–1432.
- Gerfen CR, McGinty JF, Young WS (1991). Dopamine differentially regulates dynorphin, substance P, and enkephalin expression in striatal neurons: in situ hybridization histochemical analysis. *J Neurosci* **11**: 1016–1031.
- Göthert M (2013). Serotonin discovery and stepwise disclosure of 5-HT receptor complexity over four decades. Part I. General background and discovery of serotonin as a basis for 5-HT receptor identification. *Pharmacol Reports* **65**: 771–786.
- Graybiel A, Ragsdale CW (1978). Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. *Proc Natl Acad Sci U S A* **75**: 5723–5726.
- Graybiel AM (2005). The basal ganglia: learning new tricks and loving it. *Curr Opin Neurobiol* **15**: 638–44.

- Green J a, Gu C, Mykytyn K (2012). Heteromerization of ciliary G protein-coupled receptors in the mouse brain. *PLoS One* **7**: e46304.
- Grimaldi B, Bonnin A, Fillion MP, Ruat M, Traiffort E, Fillion G (1998). Characterization of 5-HT6 receptor and expression of 5-HT6 mRNA in the rat brain during ontogenetic development. *Naunyn Schmiedebergs Arch Pharmacol* **357**: 393–400.
- Gruber AJ, McDonald RJ (2012). Context, emotion, and the strategic pursuit of goals: interactions among multiple brain systems controlling motivated behavior. *Front Behav Neurosci* **6**: 50.
- Guadiana SM, Semple-Rowland S, Daroszewski D, Madorsky I, Breunig JJ, Mykytyn K, *et al* (2013). Arborization of dendrites by developing neocortical neurons is dependent on primary cilia and type 3 adenylyl cyclase. *J Neurosci* **33**: 2626–38.
- Hamon M, Ph D, Doucet E, Ph D, Lefèvre K, Ph D, *et al* (1999). Antibodies and Antisense Oligonucleotide for Probing the Distribution and Putative Functions of Central 5-HT 6 Receptors. *Neuropsychopharmacology* **21**: 68–76.
- Hannon J, Hoyer D (2008). Molecular biology of 5-HT receptors. *Behav Brain Res* **195**: 198–213.
- Heath MJ, Hen R (1995). Serotonin receptors. Genetic insights into serotonin function. *Curr Biol* **5**: 997–999.
- Helboe L, Egebjerg J, Jong IEM de (2015). Distribution of serotonin receptor 5-HT6 mRNA in rat neuronal subpopulations: a double in situ hybridization study. *Neuroscience* **310**: 442–454.
- Hikida T, Kimura K, Wada N, Funabiki K, Nakanishi S (2010). Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. *Neuron* **66**: 896–907.
- Hirst WD, Abrahamsen B, Blaney FE, Calver AR, Aloj L, Price GW, *et al* (2003). Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. *Mol Pharmacol* **64**: 1295–308.
- Hirst WD, Stean TO, Rogers DC, Sunter D, Pugh P, Moss SF, *et al* (2006). SB-399885 is a potent, selective 5-HT6 receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. *Eur J Pharmacol* **553**: 109–19.
- Holt DJ, Graybiel a MNNM, Saper CB (1997). Neurochemical architecture of the human striatum. *J Comp Neurol* **384**: 1–25.
- Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, *et al* (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev* **46**: 157–203.
- Hoyer D, Hannon JP, Martin GR (2002). Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* **71**: 533–554.

- Hoyer D, Martin GR (1996). Classification and nomenclature of 5-HT receptors: a comment on current issues. *Behav Brain Res* **73**: 263–268.
- Hyman SE, Malenka RC, Nestler EJ (2006). Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu Rev Neurosci* **29**: 565–98.
- Jacobs BL, Azmitia EC (1992). Structure and function of the brain serotonin system. *Physiol Rev* **72**: 165–229.
- Jacobshagen M, Niquille M, Chaumont-Dubel S, Marin P, Dayer A (2014). The serotonin 6 receptor controls neuronal migration during corticogenesis via a ligand-independent Cdk5-dependent mechanism. *Development* **141**: 3370–3377.
- Jin H, White SR, Shida T, Schulz S, Aguiar M, Gygi SP, *et al* (2010). The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. *Cell* **141**: 1208–19.
- Kandel E (McGraw-Hill Health Professions Division: New York, 2000). *Principles of neural science*. at <<http://uwashington.worldcat.org/title/principles-of-neural-science/oclc/42073108>>.
- Kendall I, Sloten H a., Codony X, Burgueño J, Pauwels PJ, Vela JM, *et al* (2011). E-6801, a 5-HT₆ receptor agonist, improves recognition memory by combined modulation of cholinergic and glutamatergic neurotransmission in the rat. *Psychopharmacology (Berl)* **213**: 413–430.
- Kim J, Lee JE, Heynen-Genel S, Suyama E, Ono K, Lee K, *et al* (2010). Functional genomic screen for modulators of ciliogenesis and cilium length. *Nature* **464**: 1048–51.
- Kimchi EY, Laubach M (2009). The dorsomedial striatum reflects response bias during learning. *J Neurosci* **29**: 14891–902.
- King M V, Sleight a J, Woolley ML, Topham I a, Marsden C a, Fone KCF (2004). 5-HT₆ receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation--an effect sensitive to NMDA receptor antagonism. *Neuropharmacology* **47**: 195–204.
- Kirby LG, Zeeb FD, Winstanley C a (2011). Contributions of serotonin in addiction vulnerability. *Neuropharmacology* **61**: 421–32.
- Koemeter-Cox AI, Sherwood TW, Green J a, Steiner R a, Berbari NF, Yoder BK, *et al* (2014). Primary cilia enhance kisspeptin receptor signaling on gonadotropin-releasing hormone neurons. *Proc Natl Acad Sci U S A* **111**: 10335–40.
- Kohen R, Fashingbauer LA, Heidmann DEA, Guthrie CR, Hamblin MW (2001). Cloning of the mouse 5-HT₆ serotonin receptor and mutagenesis studies of the third cytoplasmic loop. *Mol Brain Res* **90**: 110–117.
- Kohen R, Metcalf M a, Khan N, Druck T, Huebner K, Lachowicz JE, *et al* (1996). Cloning, characterization, and chromosomal localization of a human 5-HT₆ serotonin receptor. *J Neurochem* **66**: 47–56.

- Kulaga HM, Leitch CC, Eichers ER, Badano JL, Lesemann A, Hoskins BE, *et al* (2004). Loss of BBS proteins causes anosmia in humans and defects in olfactory cilia structure and function in the mouse. *Nat Genet* **36**: 994–998.
- Lee JE, Gleeson JG (2011). A systems-biology approach to understanding the ciliopathy disorders. *Genome Med* **3**: 1–9.
- Lee SH, Lee KJ, Lee HJ, Ham BJ, Ryu SH, Lee MS (2005). Association between the 5-HT₆ receptor C267T polymorphism and response to antidepressant treatment in major depressive disorder. *Psychiatry Clin Neurosci* **59**: 140–145.
- Lesiak AJ, Brodsky M, Neumaier JF (2015). RiboTag is a flexible tool for measuring the translational state of targeted cells in heterogeneous cell cultures. *Biotechniques* **58**: 308–317.
- Liu Y, Roberts DCS, Morgan D (2005). Sensitization of the reinforcing effects of self-administered cocaine in rats: effects of dose and intravenous injection speed. *Eur J Neurosci* **22**: 195–200.
- Louvi A, Grove E a (2011). Cilia in the CNS: the quiet organelle claims center stage. *Neuron* **69**: 1046–60.
- Lucki I (1998). The spectrum of behaviors influenced by serotonin. *Biol Psychiatry* **44**: 151–162.
- Macpherson T, Morita M, Hikida T (2014). Striatal direct and indirect pathways control decision-making behavior. *Front Psychol* **5**: 1–7.
- Mahjoub MR, Stearns T (2012). Supernumerary Centrosomes Nucleate Extra Cilia and Compromise Primary Cilium Signaling. *Curr Biol* **22**: 1628–1634.
- Mai W, Chen D, Ding T, Kim I, Park S, Cho S, *et al* (2005). Inhibition of Pkhd1 Impairs Tubulomorphogenesis of Cultured IMCD Cells. *Mol Biol Cell* **16**: 4398–4409.
- Marazziti D, Baroni S, Borsini F, Picchetti M, Vatteroni E, Falaschi V, *et al* (2013). Serotonin receptors of type 6 (5-HT₆): from neuroscience to clinical pharmacology. *Curr Med Chem* **20**: 371–7.
- Marley A, Zastrow M von (2012). A simple cell-based assay reveals that diverse neuropsychiatric risk genes converge on primary cilia. *PLoS One* **7**: e46647.
- Martinowich K, Lu B (2008). Interaction between BDNF and serotonin: role in mood disorders. *Neuropsychopharmacology* **33**: 73–83.
- Masellis M, Basile VS, Meltzer HY, Lieberman JA, Sevy S, Goldman DA, *et al* (2001). Lack of association between the T→C 267 serotonin 5-HT₆ receptor gene (HTR6) polymorphism and prediction of response to clozapine in schizophrenia. *Schizophr Res* **47**: 49–58.
- Massinen S, Hokkanen M-E, Matsson H, Tammimies K, Tapia-Páez I, Dahlström-Heuser V, *et al* (2011). Increased expression of the dyslexia candidate gene DCDC2 affects length and signaling of primary cilia in neurons. *PLoS One* **6**: e20580.
- Matthes H, Boschert U, Amlaiky N, Grailhe R, Plassat JL, Muscatelli F, *et al* (1993). Mouse 5-hydroxytryptamine_{5A} and 5-hydroxytryptamine_{5B} receptors define a new family of

- serotonin receptors: cloning, functional expression, and chromosomal localization. *Mol Pharmacol* **43**: 313–9.
- McDevitt R a, Tiran-Cappello A, Shen H, Balderas I, Britt JP, Marino R a M, *et al* (2014). Serotonergic versus Nonserotonergic Dorsal Raphe Projection Neurons: Differential Participation in Reward Circuitry. *Cell Rep* **8**: 1857–1869.
- McGlashan SR, Knight MM, Chowdhury TT, Joshi P, Jensen CG, Kennedy S, *et al* (2010). Mechanical loading modulates chondrocyte primary cilia incidence and length. *Cell Biol Int* **34**: 441–446.
- Meffre J, Chaumont-Dubel S, Mannoury la Cour C, Loiseau F, Watson DJG, Dekeyne A, *et al* (2012). 5-HT₆ receptor recruitment of mTOR as a mechanism for perturbed cognition in schizophrenia. *EMBO Mol Med* **4**: 1043–1056.
- Meneses A (1999). 5-HT system and cognition. *Neurosci Biobehav Rev* **23**: 1111–1125.
- Meneses A (2001). Effects of the 5-HT₆ receptor antagonist Ro 04-6790 on learning consolidation. *Behav Brain Res* **118**: 107–110.
- Michaelides M, Anderson SAR, Ananth M, Smirnov D, Thanos PK, Neumaier JF, *et al* (2013). Technical advance Whole-brain circuit dissection in free-moving animals reveals cell-specific mesocorticolimbic networks. *J Clin Invest* **123**: .
- Mitchell ES, Hoplight BJ, Lear SP, Neumaier JF (2006). BGC20-761, a novel tryptamine analog, enhances memory consolidation and reverses scopolamine-induced memory deficit in social and visuospatial memory tasks through a 5-HT₆ receptor-mediated mechanism. *Neuropharmacology* **50**: 412–20.
- Mitchell ES, Neumaier JF (2005). 5-HT₆ receptors: a novel target for cognitive enhancement. *Pharmacol Ther* **108**: 320–33.
- Mitchell ES, Neumaier JF (2008). 5-HT₆ receptor antagonist reversal of emotional learning and prepulse inhibition deficits induced by apomorphine or scopolamine. *Pharmacol Biochem Behav* **88**: 291–8.
- Mitchell ES, Sexton T, Neumaier JF (2007). Increased expression of 5-HT₆ receptors in the rat dorsomedial striatum impairs instrumental learning. *Neuropsychopharmacology* **32**: 1520–30.
- Miyoshi K, Kasahara K, Miyazaki I, Asanuma M (2009). Lithium treatment elongates primary cilia in the mouse brain and in cultured cells. *Biochem Biophys Res Commun* **388**: 757–62.
- Monsma Jr. FJ, Shen Y, Ward RP, Hamblin MW, Sibley DR (1993). Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol Pharmacol* **43**: 320–327.
- Müller CP, Homberg JR (2015). The role of serotonin in drug use and addiction. *Behav Brain Res* **277**: 146–192.
- Nachury M V, Seeley ES, Jin H (2010). Trafficking to the ciliary membrane: how to get across the periciliary diffusion barrier? *Annu Rev Cell Dev Biol* **26**: 59–87.

- Nagata A, Hamamoto A, Horikawa M, Yoshimura K, Takeda S, Saito Y (2013). Characterization of ciliary targeting sequence of rat melanin-concentrating hormone receptor 1. *Gen Comp Endocrinol* **188**: 159–165.
- Nair SG, Furay AR, Liu Y, Neumaier JF (2013). Differential effect of viral overexpression of nucleus accumbens shell 5-HT1B receptors on stress- and cocaine priming-induced reinstatement of cocaine seeking. *Pharmacol Biochem Behav* **112**: 89–95.
- Neumaier JF, Vincow ES, Arvanitogiannis A, Wise R a, Carlezon W a (2002). Elevated Expression of 5-HT 1B Receptors in Nucleus Accumbens Efferents Sensitizes Animals to Cocaine. *J Neurosci* **22**: 10856–10863.
- Noda M, Higashida H, Aoki S, Wada K (2004). Multiple signal transduction pathways mediated by 5-HT receptors. *Mol Neurobiol* **29**: 31–39.
- Novarino G, Akizu N, Gleeson JG (2011). Modeling human disease in humans: the ciliopathies. *Cell* **147**: 70–9.
- O'Connor JM (1912). Über den Adrenalinegehalt des Blutes. *Arch für Exp Pathol und Pharmakologie* **67**: 195–232.
- Olsen MA, Nawoschik SP, Schurman BR, Schmitt HL, Burno M, Smith DL, *et al* (1999). Identification of a human 5-HT6 receptor variant produced by alternative splicing. *Mol Brain Res* **64**: 255–263.
- Ou Y, Ruan Y, Cheng M, Moser JJ, Rattner JB, Hoorn F a van der (2009). Adenylate cyclase regulates elongation of mammalian primary cilia. *Exp Cell Res* **315**: 2802–17.
- Packard MG, Knowlton BJ (2002). Learning and memory functions of the Basal Ganglia. *Annu Rev Neurosci* **25**: 563–93.
- Pan H-T, Menacherry S, Justice JB (1991). Differences in the Pharmacokinetics of Cocaine in Naive and Cocaine-Experienced Rats. *J Neurochem* **56**: 1299–1306.
- Pan J, Snell W (2007). The primary cilium: keeper of the key to cell division. *Cell* **129**: 1255–7.
- Parsons LH, Justice JB (1993a). Perfusate serotonin increases extracellular dopamine in the nucleus accumbens as measured by in vivo microdialysis. *Brain Res* **606**: 195–199.
- Parsons LH, Justice JB (1993b). Serotonin and Dopamine Sensitization in the Nucleus Accumbens, Ventral Tegmental Area, and Dorsal Raphe Nucleus Following Repeated Cocaine Administration. *J Neurochem* **61**: 1611–1619.
- Pazour GJ, Witman GB (2003). The vertebrate primary cilium is a sensory organelle. *Curr Opin Cell Biol* **15**: 105–110.
- Peroutka SJ, Lebovitz RM, Snyder SH (1981). Two distinct central serotonin receptors with different physiological functions. *Science* **212**: 827–9.
- Pliakas AM, Carlson RR, Neve RL, Konradi C, Nestler EJ, Carlezon WA (2001). Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens. *J Neurosci* **21**: 7397–7403.

- Praetorius HA, Spring KR (2003). The renal cell primary cilium functions as a flow sensor. *Curr Opin Nephrol Hypertens* **12**: 517–20.
- Pratt KG, Zimmerman EC, Cook DG, Sullivan JM (2011). Presenilin 1 regulates homeostatic synaptic scaling through Akt signaling. *Nat Neurosci* **14**: 1112–4.
- Prosser R a, Stowie a, Amicarelli M, Nackenoff a G, Blakely RD, Glass JD (2014). Cocaine modulates mammalian circadian clock timing by decreasing serotonin transport in the SCN. *Neuroscience* **275**: 184–93.
- Rapport MM (1949). Serum Vasoconstrictor (Serotonin) The presence of creatinine in the complex; a proposed structure of the vasoconstrictor principle. *J Biol Chem* 961–970.
- Rapport MM, Green AA, Page IH, Rapport MM, Green AA, Page IH (1948). Crystalline Serotonin. *Science (80-)* **108**: 329–330.
- Rehm J, Mathers C, Popova S, Thavorncharoensap M, Teerawattananon Y, Patra J (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *Lancet* **373**: 2223–33.
- Riccio O, Potter G, Walzer C, Vallet P, Szabo G, Vutskits L, *et al* (2009). Excess of serotonin affects embryonic interneuron migration through activation of the serotonin receptor 6. *Mol Psychiatry* **14**: 280–290.
- Riccioni T, Bordi F, Minetti P, Spadoni G, Yun HM, Im BH, *et al* (2011). ST1936 stimulates cAMP, Ca²⁺, ERK1/2 and Fyn kinase through a full activation of cloned human 5-HT₆ receptors. *Eur J Pharmacol* **661**: 8–14.
- Richardson NR, Roberts DCS (1996). Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *J Neurosci Methods* **66**: 1–11.
- Ross AJ, May-Simera H, Eichers ER, Kai M, Hill J, Jagger DJ, *et al* (2005). Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat Genet* **37**: 1135–40.
- Rover M de, Lodder JC, Kits KS, Schoffelmeer AN, Brussaard AB (2002). Cholinergic modulation of nucleus accumbens medium spiny neurons. *Eur J Neurosci* **16**: 2279–2290.
- Ruat M, Roudaut H, Ferent J, Traiffort E (2012). Hedgehog trafficking, cilia and brain functions. *Differentiation* **83**: S97–104.
- Ruat M, Traiffort E, Arrang JM, Tardivel-Lacombe J, Diaz J, Leurs R, *et al* (1993). A novel rat serotonin (5-HT₆) receptor: molecular cloning, localization and stimulation of cAMP accumulation. *Biochem Biophys Res Commun* **193**: 268–76.
- Schechter LE, Lin Q, Smith DL, Zhang G, Shan Q, Platt B, *et al* (2008). Neuropharmacological profile of novel and selective 5-HT₆ receptor agonists: WAY-181187 and WAY-208466. *Neuropsychopharmacology* **33**: 1323–35.
- Schneider CA, Rasband WS, Eliceiri KW (2012). HISTORICAL commentary NIH Image to ImageJ : 25 years of image analysis. *Nat Methods* **9**: 671–675.
- Schweimer J V., Mallet N, Sharp T, Ungless M a. (2011). Spike-timing relationship of

- neurochemically-identified dorsal raphe neurons during cortical slow oscillations. *Neuroscience* **196**: 115–123.
- Sebben M, Ansanay H, Bockaert J, Dumuis A (1994). 5-HT₆ receptors positively coupled to adenylyl cyclase in striatal neurones in culture. *Neuroreport* **5**: 2553–2557.
- Shore PA, Silver SL, Brodie BB, Foundation EBR (1955). Interaction of Reserpine, Serotonin, and Lysergic Acid Diethylamide in Brain. *Science* (80-) **122**: 284–285.
- Singla V, Reiter JF (2006). The primary cilium as the cell's antenna: signaling at a sensory organelle. *Science* **313**: 629–33.
- Sleight AJ, Boess FG, Bös M, Bourson A (1998). The putative 5-HT₆ receptor: localization and function. *Ann NY Acad Sci* **861**: 91–6.
- Sora I, Hall FS, Andrews a M, Itokawa M, Li XF, Wei HB, *et al* (2001). Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. *Proc Natl Acad Sci U S A* **98**: 5300–5.
- Stuber GD, Roitman MF, Phillips PEM, Carelli RM, Wightman RM (2005). Rapid dopamine signaling in the nucleus accumbens during contingent and noncontingent cocaine administration. *Neuropsychopharmacology* **30**: 853–863.
- Stuber GD, Sparta DR, Stamatakis AM, Leeuwen W a van, Hardjoprajitno JE, Cho S, *et al* (2011). Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature* **475**: 377–80.
- Sugita S, Shen KZ, North RA (1992). 5-hydroxytryptamine is a fast excitatory transmitter at 5-HT₃ receptors in rat amygdala. *Neuron* **8**: 199–203.
- Surmeier DJ, Ding J, Day M, Wang Z, Shen W (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci* **30**: 228–35.
- Susa T, Kato T, Kato Y (2008). Reproducible transfection in the presence of carrier DNA using FuGENE6 and Lipofectamine 2000. *Mol Biol Rep* **35**: 313–319.
- Svenningsson P, Tzavara ET, Qi H, Carruthers R, Witkin JM, Nomikos GG, *et al* (2007). Biochemical and behavioral evidence for antidepressant-like effects of 5-HT₆ receptor stimulation. *J Neurosci* **27**: 4201–9.
- Tamir H, Kuhar MJ (1975). Association of serotonin-binding protein with projections of the midbrain raphe nuclei. **83**: 169–172.
- Tassone A, Madeo G, Schirinzi T, Vita D, Puglisi F, Ponterio G, *et al* (2011). Activation of 5-HT₆ receptors inhibits corticostriatal glutamatergic transmission. *Neuropharmacology* **61**: 632–7.
- Thompson CL, Wiles A, Poole CA, Knight MM (2015). Lithium chloride modulates chondrocyte primary cilia and inhibits Hedgehog signaling. *FASEB J* 1–11doi:10.1096/fj.15-274944.
- Twarog BM, Page IH (1953). Serotonin content of some mammalian tissues and urine and a

- method for its determination. *Am J Physiol* **175**: 157–161.
- Tzschentke TM (1998). Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol* **56**: 613–672.
- U.S. Department of Justice National Drug Intelligence Center (2011). National Drug Threat Assessment 2011. 1–72doi:10.1037/e618352012-001.
- Uchimura N, North R (1990). Actions of cocaine on rat nucleus accumbens neurones in vitro. *Br J Pharmacol* **740**: 736–740.
- United States Department of Health and Human Services (2014). The Health Consequences of Smoking—50 Years of Progress A Report of the Surgeon General. *A Rep Surg Gen* 1081.
- Valentini V, Piras G, Luca M a De, Perra V, Bordi F, Borsini F, *et al* (2012). Evidence for a role of a dopamine/5-HT₆ receptor interaction in cocaine reinforcement. *Neuropharmacology* **65C**: 58–64.
- Waeber C, Grailhe R, Yu X-J, Hen R, 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.
- Walther DJ, Peter J-U, Bashammakh S, Hörtnagl H, Voits M, Fink H, *et al* (2003). Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* **299**: 76.
- Wang RY, Aghajanian GK (1982). Correlative firing patterns of serotonergic neurons in rat dorsal raphe nucleus. *J Neurosci* **2**: 11.
- Wang Z, Li V, Chan GCK, Phan T, Nudelman AS, Xia Z, *et al* (2009). Adult type 3 adenylyl cyclase-deficient mice are obese. *PLoS One* **4**: 1–11.
- Ward R, Hamblin M, Lachowicz J, Hoffman BJ, Sibley DR, Dorsa DM (1995). Localization of serotonin subtype 6 receptor messenger RNA in the rat brain by in situ hybridization histochemistry. *Neuroscience* **64**: 1105–1111.
- Ward SJ, Morgan D, Roberts DCS (2005). Comparison of the reinforcing effects of cocaine and cocaine/heroin combinations under progressive ratio and choice schedules in rats. *Neuropsychopharmacology* **30**: 286–95.
- Wheatley DN (2005). Landmarks in the first hundred years of primary (9+0) cilium research. *Cell Biol Int* **29**: 333–9.
- Wheatley DN, Wang a M, Strugnell GE (1996). Expression of primary cilia in mammalian cells. *Cell Biol Int* **20**: 73–81.
- Whitaker-Azmitia PM (1999). The discovery of serotonin and its role in neuroscience. *Neuropsychopharmacology* **21**: 2S–8S.
- Whitfield JF (2004). The neuronal primary cilium--an extrasynaptic signaling device. *Cell Signal* **16**: 763–7.
- Wilkinson D, Windfeld K, Colding-Jorgensen E (2014). Safety and efficacy of idalopirdine, a 5-

- HT6 receptor antagonist, in patients with moderate Alzheimer's disease (LADDER): a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol* **13**: 1092–1099.
- Wise RA, Newton P, Leeb K, Burnette B, Pocock D, Justice JB (1995). Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. *Psychopharmacology (Berl)* **120**: 10–20.
- Witten IB, Lin S-C, Brodsky M, Prakash R, Diester I, Anikeeva P, *et al* (2010). Cholinergic interneurons control local circuit activity and cocaine conditioning. *Science* **330**: 1677–81.
- Woods S, Clarke NN, Layfield R, Fone KCF (2012). 5-HT(6) receptor agonists and antagonists enhance learning and memory in a conditioned emotion response paradigm by modulation of cholinergic and glutamatergic mechanisms. *Br J Pharmacol* **167**: 436–49.
- Yager LM, Garcia AF, Wunsch AM, Ferguson SM (2015). The ins and outs of the striatum: Role in drug addiction. *Neuroscience* **301**: 529–541.
- Yun H-M, Kim S, Kim H-J, Kostenis E, Kim J Il, Seong JY, *et al* (2007). The novel cellular mechanism of human 5-HT6 receptor through an interaction with Fyn. *J Biol Chem* **282**: 5496–505.
- Yun H-M, Rhim H (2011). The serotonin-6 receptor as a novel therapeutic target. *Exp Neurobiol* **20**: 159–68.
- Zimmer BA, Dobrin C V, Roberts DCS (2011). Brain-Cocaine Concentrations Determine the Dose Self-Administered by Rats on a Novel Behaviorally Dependent Dosing Schedule. *Neuropsychopharmacology* **36**: 2741–2749.
- Zimmer BA, Dobrin C V., Roberts DCS (2013). Examination of behavioral strategies regulating cocaine intake in rats. *Psychopharmacology (Berl)* **225**: 935–944.

ABBREVIATIONS

5-HT	5-hydroxytryptamine (serotonin)
DA	Dopamine
5-HT ₆	5-hydroxytryptamine ₆
NAc	Nucleus accumbens
NAcSh	Nucleus accumbens shell
GFP	Enhanced green fluorescent protein
Dyn	Dynorphin (marker of striatonigral)
Enk	Enkephalin (marker of striatopallidal)
D ₁	Dopamine receptor type 1
D ₂	Dopamine receptor type 2
HSV	Herpes-simplex virus
MSN	Medium spiny neuron
dMSN	Direct pathway medium spiny neuron
iMSN	Indirect pathway medium spiny neuron
SA	Self-administration
FR1	Fixed-interval reinforcement schedule
PR	Progressive Ratio
AC3	Adenylyl Cyclase 3 (marker of primary cilia)
cAMP	Cyclic adenosine Monophosphate
hSyn	Human Synapsin-1 promoter
RFP	Red fluorescence protein
WT	Wild-type
5-HT ₆ KO	5-HT ₆ knockout mice
CMV	Cytomegalovirus promoter
pcDNA	Plasmid cytomegalovirus promoter deoxyribonucleic acid
CTS	Ciliary targeting sequence

i3 loop	Third intracellular loop
DIV	Days <i>in vitro</i>
NBA	Neurobasal-A medium
PBS	Phosphate buffered saline
BSA	Bovine serum albumin
Arl13b	ADP-ribosylation factor-like protein 13B (marker of primary cilia)
HA	Hemagglutinin tag
Δ 5-HT ₆ No CTS	5-HT ₆ receptor mutated to remove CTS
Δ 5-HT ₆ i3-7	5-HT ₆ receptors mutated to swap i3 loop with that of 5-HT ₇ receptor
IMCD3	Inner medullary collecting duct
FBS	Fetal bovine serum

CURRICULUM VITA

MATTHEW BRODSKY

Phone: (510) 253-8226

Mbrod47@uw.edu

4319 NE 65th St.

Seattle, WA 98115

Education

Ph.D. (Candidate)

University of Washington – Seattle, Washington

Fall 2010 - Present

Neuroscience (In Progress)

Dissertation advisor: John F. Neumaier

BS University of California, Santa Cruz – Santa Cruz, California

Fall 2005 -2009

Neuroscience and Behavior

Publications

Journal Publications

M. Brodsky, A. W. Gibson, D. Smirnov, S. Nair and J. F. Neumaier "Striatal 5-HT₆ receptors regulate cocaine reinforcement in a pathway-selective manner." **Neuropsychopharmacology**, in revision.

M. Brodsky, A.J. Lesiak, J.M. Sullivan, and J. F. Neumaier "Regulation of primary cilia morphology in striatum by 5-HT₆ receptor signaling," in preparation

D. Eskenazi, **M. Brodsky**, and J. F. Neumaier “Deconstructing 5-HT₆ receptor effects on striatal circuit function” *Neuroscience*, Vol 299, pp. 97–106, July 2015.

A. Lesiak, **M. Brodsky**, and J. F. Neumaier “RiboTag: a novel and flexible tool for measuring gene expression within discrete populations of primary neuronal cultures” *BioTechniques*, Vol. 58, No. 6, pp. 308–317 June 2015.

C. Rippey, T. Walsh, S. Gulsuner, **M. Brodsky**, A. S. Nord, M. Gasperini, S. Pierce, C. Spurrell, B. P. Coe, N. Krumm, M. K. Lee, J. Sebat, J. M. McClellan, and M.-C. King, “Formation of chimeric genes by copy-number variation as a mutational mechanism in schizophrenia” *Am. J. Hum. Genet.*, vol. 93, no. 4, pp. 697–710, Oct. 2013.

I. B. Witten, E. E. Steinberg, S. Y. Lee, T. J. Davidson, K. Zalocusky, **M. Brodsky**, O. Yizhar, S. L. Cho, S. Gong, C. Ramakrishnan, G. D. Stuber, K. M. Tye, P. H. Janak, and K. Deisseroth, “Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement” *Neuron*, vol. 72, no. 5, pp. 721–33, Dec. 2011.

I. Goshen, **M. Brodsky**, R. Prakash, J. Wallace, V. Gradinaru, C. Ramakrishnan, and K. Deisseroth, “Dynamics of retrieval strategies for remote memories” *Cell*, vol. 147, no. 3, pp. 678–89, Oct. 2011.

I. B. Witten*, S.-C. Lin*, **M. Brodsky***, R. Prakash*, I. Diester, P. Anikeeva, V. Gradinaru, C. Ramakrishnan, and K. Deisseroth, “Cholinergic interneurons control local circuit activity and cocaine conditioning” *Science*, vol. 330, no. 6011, pp. 1677–81, Dec. 2010.

Posters and Presentations

M. Brodsky, A. W. Gibson, D. Smirnov, S. Nair and J. F. Neumaier "Striatal 5-HT₆ receptors regulate cocaine reinforcement in a pathway-selective manner." (Poster) Society for Neuroscience, Oct. 16-21, 2015 Chicago, IL

M. Brodsky, A.J. Lesiak, K.Garcia-Nakata, J.M. Sullivan, and J. F. Neumaier “Regulation of primary cilia morphology in striatum by 5-HT₆ receptor signaling,” (Poster) Society for Neuroscience, Nov. 15-19, 2014 Washington, DC

M. Brodsky, and J. F. Neumaier “Regulation of Neuronal Primary Cilia Morphology in Striatal Neurons by 5-HT₆ Receptors” (Presentation and Poster) International Society for Serotonin Research, July 9-12, 2014 Arabella, South Africa

M. Brodsky, J.M. Sullivan, A.L. Kostrinsky-Thomas, and J. F. Neumaier “Regulation of primary cilia morphology in striatum by 5-HT₆ receptor signaling,” (Poster) Society for Neuroscience, Nov. 9-13, 2013 San Diego, CA

M. Brodsky “Dissecting the Relationship between 5-HT₆ Receptors and Neuronal Primary Cilia” (Presentation) Annual NIDA T32 Trainees Retreat, March 28th 2013, Seattle, WA

M. Brodsky, J.M. Sullivan, and J. F. Neumaier “5-HT₆ receptor signaling effects neuronal primary cilia morphology” (Poster) Society for Neuroscience, Oct. 13-17, 2012 New Orleans, LA

M. Brodsky, J.M. Sullivan, and J. F. Neumaier “5-HT₆ receptor antagonism modulates neuronal primary cilia morphology” (Poster) Center for Integrated Neuroscience Annual Symposium, May 14 2013, Seattle, WA

M. Brodsky “5-HT₆ Receptors and Drug Addiction” (Presentation) Annual NIDA T32 Trainees Retreat, May 31st 2012, Seattle, WA

I. B. Witten*, S.-C. Lin*, **M. Brodsky***, P. Anikeeva, I. Diester, C. Ramakrishnan, and K. Deisseroth, “Cholinergic interneurons of the nucleus accumbens control local circuit activity and reward behavior.” (Poster) Society for Neuroscience, Nov. 13-17, 2010 San Diego, CA

I. Goshen, **M. Brodsky**, V. Gradinaru, C. Ramakrishnan, and K. Deisseroth, “Optogenetic inhibition of dorsal CA1 enables reversible interruption of memory acquisition and recall.” (Poster) Society for Neuroscience, Nov. 13-17, 2010 San Diego, CA

Research Experience

Neumaier Lab, University of Washington – Seattle, Washington 2011- Present

Ph. D. Candidate

Advisor: John F. Neumaier

- Examining the function of 5-HT₆ receptor localization on neuronal primary cilia in mammalian striatum.

Deisseroth Lab, Stanford University - Stanford, CA 2009 to 2010

Research Assistant,

- Identifying neural correlates of reaching in the rodent motor cortex, with a focus on elucidating the key differences between healthy and Parkinsonian animals.
- Optically dissecting the neural circuits underlying addiction

Zuo Lab, University of California, Santa Cruz – Santa Cruz, California 2008 to 2009

Undergraduate Researcher

- Visualizing the synaptic plasticity of learning, with regards to dendritic spine density. In normal and stroke model rodents.

Lab Rotations

Chavkin Lab, University of Washington – Seattle, Washington Fall 2010

- Modulating serotonergic projections in stress related behaviors using a novel TPH2 floxed transgenic mouse line

Phillips Lab, University of Washington – Seattle, Washington Winter 2011

- The role of phasic dopamine in the nucleus accumbens core in computing the utility of reward transactions.

Neumaier Lab, University of Washington – Seattle, Washington Spring 2011

- Developing a mi/shRNA mediated knock-down of the 5-HT₆ receptor

Teaching Experience

University of Washington – Seattle, Washington

Jan 2012 to April 2012

Teaching Instructor, Neurobiology

- Taught NeuroBio 301 lab course. Taught electrophysiological techniques, provided technical lectures, managed laboratory equipment, graded lab reports and exams.

Service

Pacific Science Center, Portal to the Public – Seattle, WA

Science Communication Fellow

University of Washington, Neuroscience Graduate Admissions Committee – Seattle, WA

Committee Member 2012 - Present

University of Washington, Neurobiology Community Outreach – Seattle, WA

Member September 2010 - Present

Dominican Hospital Emergency Dept. – Santa Cruz, CA

Emergency Department Volunteer Jan 2008- Nov 2008

Honors and Awards

Neuroscience Scholarship for full tuition to attend Pacific Science Center's Science Communication Fellowship short course (Summer 2015)

National Institutes of Drug Abuse (NIDA) Travel Award to attend the 2014 meeting of the International Society for Serotonin Research (ISSR) in South Africa

NIDA T32 Training Grant Awardee (2011-2014)