ABSTRACT

D2 and D3 receptors are subtypes of D2-like dopamine receptors, and both have been shown to be involved in drug addiction with different roles. Their mRNA are expressed moderately highly in striatum, where dopamine plays a crucial role in drug reward pathways, and are expressed at low level in hippocampus, where dopamine has recently been implicated in the long-term, memory components of addiction. In our studies, we did functional and gene expression assays on dorsal and ventral fractions of striatum and hippocampus from eight cocaine-treated and control rats. We found that the potency of D2/D3 receptor agonist was highest in dorsal striatum. The potency of receptor activity was more correlated with D2 receptor gene expression than D3 receptor expression. Cocaine did not show any convincing effect on D2 and D3 receptor function and gene expression.

INDEX WORDS: D2-like dopamine receptors, D2 dopamine receptor, D3 dopamine receptor, Cocaine, Addiction, Dorsal striatum, Ventral striatum, Dorsal hippocampus, Ventral hippocampus
EFFECTS OF COCAINE ON D2 AND D3 DOPAMINE RECEPTOR EXPRESSION AND FUNCTION IN THE RAT BRAIN

by

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CHAPTER I
INTRODUCTION AND LITERATURE REVIEW

Dopamine Receptors

Dopamine and Dopamine Pathways

Dopamine (DA) is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation. It also plays multiple roles in the periphery as a modulator of cardiovascular function, hormone secretion, vascular tone, renal function, and gastrointestinal motility. Several neuropsychiatric disorders such as schizophrenia and Tourette’s syndrome and neurological disorders such as Parkinson’s disease have been linked to a dysregulation of dopaminergic transmission (1-3). Dopamine is also extensively implicated in the mechanism of drug addiction as the substrate of psychostimulant reward and as a substrate of drug-related learning and neuroadaptation (4).

Dopaminergic neurons in the substantia nigra pars compacta, the ventral tegmental area (VTA) and the hypothalamus give origin to three main pathways in the central nervous system (CNS), respectively: 1) the nigrostriatal pathway, mainly serving the dorsal striatum (primarily composed of putamen and the caudate nucleus), which contains 70% of the dopamine in the whole brain, 2) the mesolimbocortical pathway, mainly innervating the prefrontal cortex and the ventral striatum (primarily composed of nucleus accumbens (NAc)), and 3) the tuberoinfundibular pathway, playing an important
role in modulation of neuroendocrine system (1, 5). The mesolimbocortical pathway is further divided into mesocortical and mesolimbic pathways. The mesocortical pathway connects the VTA to the cerebral cortex, particularly the mesioprefontal lobes, and is essential to the normal cognitive function of the dorsolateral prefrontal cortex and involved in motivation and emotional response. The mesolimbic pathway links the VTA to the ventral striatum/NAc in the limbic system, and is involved in producing pleasurable feeling and is associated with feelings of reward and desire (6, 7) (Figure 1.1).

**Dopamine Receptors Classification**

Dopamine acts via dopamine receptors (DR), which are G protein-coupled receptors (GPCR), in a neuromodulatory characterized fashion. Dopamine release sites are located immediately outside the synaptic cleft. The released dopamine diffuses in the extracellular fluid, and reaches effective concentrations onto its receptors after a relatively longer delay than a transmitter released inside the cleft (4).

Molecular genetic studies have defined two types of dopamine receptors, the D1-like receptors composed of D1 and D5 receptor subtypes, and D2-like receptors composed of D2, D3, and D4 receptor subtypes based upon their structural and pharmacological similarities. Dopamine receptors are G protein-coupled receptors. They share most of their structural characteristics, displaying considerable conservation within transmembrane. Their signaling is mediated by coupling and activation of heterotrimeric G proteins, which is controlled by the nucleotide binding cycle (Figure 1.2). D1-like receptors have about seven times longer COOH terminus than D2-like receptors and a short third intracellular loop (IL3). D1 receptors are positively linked to
the activation of adenylyl cyclase via coupling to the $G_s/G_{olf}$ class of G proteins. D2-like receptors have a long IL3, and stimulation of the D2-like receptors results in coupling with the $G_s/G_o$ class of G proteins, leading to the inhibition of adenylyl cyclase activity (1, 3, 8).

**D2 and D3 Dopamine Receptor Subtypes**

*Structures and Distribution*

The D2 and D3 dopamine receptors have approximately 50% overall amino acid homology. However, the transmembrane spanning (TMS) regions of the D2 and D3 receptors, which are thought to construct the ligand binding site, share 79% homology (9, 10). The difference in the length of the sequences of the D2-like receptor subtypes largely depends on the length of the huge IL3. Two splice variants of the D2 receptor and two of the D3 receptor show variations in the IL3, differing in the insertion of a stretch of 29 and 21 amino acids, respectively (1, 10). Studies with chimeric receptors with interchanged regions of the D2 and D3 receptors generally indicated that the IL3 is critical for the ability of agonist binding and G protein coupling (2, 3, 11).

Despite the similarities in the structure of the D2 and D3 receptors, the D2 and D3 receptors differ in their neuroanatomical localization, levels of receptor expression, efficacy in response to agonist stimulation, and regulation and desensitization (9). The mRNA of both D2 and D3 receptors are expressed in the striatum and the olfactory tubercle at moderately high levels. While D2 receptor is expressed throughout the dorsal striatum and the ventral striatum/NAc, D3 receptor is expressed primarily in the ventral striatum/NAc but poorly in the dorsal striatum. D3 receptor has a specific distribution to limbic areas such as the ventromedial shell of the NAc, the olfactory...
tubercle and the islands of Calleja. D2 and D3 receptors are also found in the hippocampus, substantia nigra and the VTA at low expression levels (1, 2, 10), although the relative expression levels in these regions have not been defined.

**Functions**

Activation of D2 and D3 receptors in ventral striatum has been shown to have effects on the degree of forward locomotion. Activation of D2 autoreceptors results in decreased DA release and decreases locomotor activity, whereas activation of postsynaptic D2 receptors slightly increases locomotion. The D3 receptor, mainly postsynaptically located in the ventral striatum/NAc, seems to inhibit locomotor activity (1).

Mesolimbocortical DA is implicated in reward and reinforcement mechanisms. Activation of D2-like receptors has been shown to mediate stimulant drug reinforcement (1, 2). Postmortem and preclinical studies point to the possibility that chronic abuse of cocaine, nicotine and opioids may be associated with an adaptive change in D3 receptors (12). Mesolimbocortical DA also plays a role in learning and memory. Activation of D2 receptors in hippocampus improves acquisition and retention of different working memory tasks in the rat. D3 receptor is expressed in the hippocampus and septal area, suggesting a role in cognition and a possible contribution to the behavioral effects of D2 agonists. It has been shown that the effects of dopamine on memory consolidation are the result of a balance between D2 receptor-mediated facilitation and D3 receptor-mediated inhibition (13).

The D2-like dopamine receptors are currently of great therapeutic interest. The D2 receptor has been targeted successfully by a growing number of agonists and antagonists that reduce symptoms of movement disorders and psychiatric disorders,
respectively. Although the D2 receptor is the predominate CNS D2-like receptor subtype in that it is expressed at the highest levels in the CNS and the majority of clinically used antipsychotics are antagonists at D2 receptors, it is recently becoming clear that the D3 receptor subtype may also play a role in the development of pharmacotherapeutic strategies for the treatment of neuropsychiatric and neurological disorders, as well as for the rehabilitation of individuals who abuse psychostimulants, because of its selective anatomical distribution in limbic brain areas which suggests potential involvement in those pathological conditions (7, 9, 14).

**Drug Addiction and D2 and D3 Dopamine Receptors**

**Definitions and Theories of Drug Addiction**

Rewards can broadly be defined as desirable outcomes that serve to influence behavior. The three basic functions of rewards are inducing subjective feelings of pleasure (hedonia) and positive emotional states, serving as goals of voluntary behavior, and increasing the frequency and intensity of behaviors that lead to rewards (positive reinforcing effects) (15, 16). Reward pathways play a key role in drug use and addiction, but addiction is a complex disease that extends beyond reward. Drug addiction is characterized as a compulsive pattern of drug-seeking and drug-taking behavior that continues despite adverse consequences (17, 18). The key features that distinguish drug *addiction* from drug *use* are 1) recurrent failure to control the use of one or more drugs, and 2) continuation of drug use despite significant harmful consequences (19).

The mechanisms underlying compulsive drug use and relapse (the resumption of drug-seeking and drug-taking behaviors following a prolonged period of abstinence) are
currently major areas of emphasis in addiction neuroscience. Theories proposed to explain the compulsive and relapse elements of drug addiction include positive and negative reinforcement, incentive-sensitization and learning-based theories. However, none of them can fully account for all aspects of addiction (17, 18).

**Common Neurobiology of Drug Addiction**

Although drugs of abuse often produce differential behavioral effects and have diverse pharmacological profiles, one common feature they share is an enhancement in mesolimbocortical dopamine activity, although their interaction with this system occurs at different levels. The mesolimbocortical dopamine system consists of dopamine projections from cell bodies in the VTA of the midbrain to limbic structures such as hippocampus, amygdala and in particular the ventral striatum/NAc (mesolimbic pathway), and to cortical areas (mesocortical pathway) (17, 18, 20, 21) (Figure 1.3). This neural circuitry in the brain normally controls responses to natural rewards such as food, water, sex and social interactions. However, drugs of abuse not only ‘hijack’ this system, but also produce persistent rewarding and reinforcing effects, consolidate the reward response to drug-associated stimuli, and further promote the repeated use of the addictive substance (18). These neural circuits operate in parallel, but may have somewhat different roles in addiction. It is suggested that the mesolimbic pathway is involved in the acute reinforcing effects of drugs and various conditional responses related to craving and relapse, whereas the mesocortical pathway mediates the conscious drug experience, drug craving and a loss of behavioral inhibition related to compulsive drug-seeking and drug-taking behaviors (18).
Role of Striatum and Hippocampus in Drug Addiction

**Striatum**

The striatum is the main input unit of the basal ganglia, which receives synaptic input from cortical and subcortical afferents, such as motor cortical input and dopaminergic projections from substantia nigra and the VTA. As mentioned above, the dorsal striatum, primarily composed of the caudate nucleus and putamen, receives dopaminergic input from the substantia nigra, while the ventral striatum, primarily composed of the NAc, receives dopaminergic input from VTA.

VTA-NAc pathway seems to be a site where virtually all drugs of abuse converge to produce their acute reward signals; it is a key detector of a rewarding stimulus and all drugs of abuse increase dopamine-mediated transmission in the ventral striatum/NAc (17, 21). However, it is argued that NAc dopamine does not mediate the primary motivational functions but should be considered a modulator of several functions related to motivated behavior, including decision making, responsive to conditioned stimuli, learning and cognition (22). Moreover, dopaminergic innervations of NAc, in particular the NAc shell, have been shown to play an important role in relapse to drug-seeking and drug-taking behaviors (18).

The dorsal striatum has also been showed to play a role in drug addiction. For example, addictive drugs increase extracellular dopamine in the dorsal striatum (caudate-putamen) although less preferentially as compared to the ventral striatum/NAc (4). Significant increases in DA release in dorsal striatum have been observed during cocaine self-administration in rats (15). It has been shown that increases in craving for cocaine are correlated with increases in dopamine in the dorsal striatum (18). There are
studies suggesting that the dorsal striatum, specifically the caudate nucleus, responds to the reinforcement of an action, rather than the reward per se, and thus it is involved in reward processing, specifically learning and updating actions that lead to reward, rather than representing and identifying rewards (15).

**Hippocampus**

Hippocampus is a brain region known to participate in declarative memory and spatial memory (23, 24). The current appreciation of the ability of dopamine to act via multiple signaling pathways in the hippocampus has evolved from the previous presumption that dopamine had no significant role in this area (25). Data obtained from rats utilizing drug self-administration models (25), along with the fact that the hippocampus has direct excitatory efferent to the ventral striatum/NAc and can activate dopaminergic neurons of the VTA (23), have suggested that the hippocampus may be involved in development and/or maintenance of drug addiction and drug relapse.

It has been demonstrated that the hippocampus is a heterogeneous entity comprising two distinct parts, the dorsal and the ventral hippocampus. The dorsal part of hippocampus receives a dopaminergic pathway from substantia nigra and plays a role in exploratory behavior and memory consolidation, whereas the ventral part mainly receives dopaminergic signaling from the VTA (26). It is now believed that hippocampus is crucial for conditioned learning engaged in the process of addiction (17, 18). The ventral hippocampus involves discrete stimulus-reward associations, while the dorsal hippocampus mediates stimulus-stimulus associations that may be particularly important for contextual learning (18). Recent studies have demonstrated a significant role for dorsal hippocampus in contextual reinstatement of drug-seeking behavior (18).


Cocaine Addiction

Cocaine is a potent CNS stimulant and a powerful reinforcer. The main characteristics of cocaine addiction are compulsive drug use despite adverse consequences and high rates of relapse during a period of abstinence. The mesolimbocortical dopamine system underlies cocaine reward and contributes to relapse to cocaine (27).

The initial site of action leading to cocaine reinforcement is the dopamine transporter (DAT) (28). DAT is a Na⁺/Cl⁻ -dependent symport transporter which mediates the reuptake of dopamine from extracellular spaces and recycles it back into dopamine neurons thereby tightly regulating the extracellular lifetime of dopamine (29-31). Reuptake of dopamine via DAT is the primary mechanism for terminating dopamine transmission; the rapid and efficient clearance of dopamine prevents overstimulation of dopamine receptors, reduces the metabolic demands on the presynaptic neuron for new synthesis and vesicular storage of dopamine, and controls the homeostasis of both presynaptic and postsynaptic dopamine (29-30).

Cocaine binds to the DAT and blocks dopamine uptake, which results in a higher synaptic concentration of dopamine and a potentiation of dopaminergic neurotransmission in the limbic pathways. This ultimately leads to reinforcement of the behavior that is associated with the cocaine binding and uptake inhibition (28-31) (Figure 1.4). Cocaine exposure has been shown to increase DAT cell surface expression, possibly via transporter trafficking from intracellular compartments to the plasma membrane, and this DAT expression increase is possibly due to the increased extracellular dopamine levels after uptake inhibition (30, 31). It is hypothesized that following the removal of cocaine, the elevated DAT cell surface expression could then
cause the extracellular concentration of dopamine to fall below that of basal which may contribute to the triggering of the relapse of cocaine abuse following withdrawal (30).

A current influential hypothesis is that cocaine addiction is due to drug-induced neuroadaptations in reward-related learning and memory processes in the mesolimbocortical dopamine system and glutamatergic corticolimbic circuitry in which the dopamine projections are embedded. These neuroadaptations have been hypothesized to cause hypersensitivity to cocaine-associated cues, impulsive decision making and abnormal habit-like learned behaviors that are insensitive to adverse consequences (27). As an indirect agonist at dopamine receptor by inhibiting DAT, cocaine has effects on various signaling cascades downstream of dopamine receptor activation including cAMP signaling pathway whose upregulation has been extensively characterized as an adaptation to chronic exposure to drugs of abuse (17).

**D2 and D3 Dopamine Receptors in Drug Addiction**

As mentioned above, D2 and D3 dopamine receptors play a role in drug reinforcement. It has been suggested that D2 receptors positively regulate DAT function to enhance dopamine clearance capacity by redistributing DAT from inside the cell to the membrane surface through inhibitory G protein (G\(_{\text{i/o}}\)) signaling and regulating the turnover and degradation rates for DAT. The impairment of this modulation by long-term exposure to psychostimulant drugs acting on the DAT may contribute to drug addiction (31). Imaging studies and preclinical studies have shown that high D2 receptor levels in striatum could protect against drug self administration (32, 33). D3 receptor is receiving more interest recently because of its specific distribution in the ventral striatum/NAc which is one of the main regions involved in drug addiction. Recent studies have
reported that D3 receptor selective antagonists such as SB-277011-A and NGB 2904, and putative partial D3 receptor agonist BP 897 attenuate drug conditioned cue-induced reinstatement such as relapse to drug-seeking behavior in the rats (34-40), but minimally affect drug primary reinforcing properties (36, 37, 40), which suggests a more important role for D3 receptor in the expression rather than in the acquisition of incentive learning (40).

D2 receptor antagonists have also been reported to prevent drug-seeking behavior induced by the reintroduction of cocaine-associated cues, with no effect on the primary reinforcing properties (37). Considering the different distribution of D2 and D3 receptor in the brain, D2 and D3 receptor ligands might both affect relapse but through different neural sites or mechanisms of action. However, some recent research has pointed to a different role for D2 and D3 receptors in conditioned incentive learning. D2 receptor appears to be important for the establishment (acquisition) of incentive learning while D3 receptor is important for the expression of incentive learning (40). Although there are some controversies on the role of D2 and D3 receptors, it is clear that D2 and D3 receptors possess different regulatory processes, which implies that they might regulate the same neural processes through different signaling mechanism. For example, the extracellular signal-regulated kinase (ERK) pathway, which is implicated in drug-induced neuroadaptations in reward-related learning and memory processes (27), could be regulated flexibly through alternative usage of either the D2 or D3 receptor pathway depending on the cellular situation (41).

Dopamine receptors in different brain areas might play a different role and be involved to a different extent in drug addiction. It has been reported that D3 receptor
mRNA and protein (after a 16 h delay) was increased in ventral striatum/NAc after a single injection of cocaine in rats, and in dorsal striatum following chronic alcohol intake (40). A study performed on rats with a history of cocaine self-administration also revealed increased D3 receptor in the ventral region of the caudate (in the dorsal striatum) and NAc core (in the ventral striatum) following a cocaine challenge after 30-31 days of cocaine withdrawal (40, 42). Another conditioned activity experiment showed a significant increase in D3 but not D2 receptor mRNA and receptor in ventral striatum/NAc in conditioned mice but not unconditioned mice, indicating a role for D3 receptor in incentive learning (40).

In hippocampus, cocaine exposure has been shown to enhance long-term potentiation (LTP) in the CA1 region which may generally indicate neural adaptations as a result of cocaine administration (23), and this action could be prevented by a D2-like receptor antagonist (43). Cocaine has also been shown to inhibit the monosynaptic inhibitory postsynaptic currents (IPSCs) in the CA1 region of the hippocampus, and this action could be prevented effectively by a selective D3 receptor antagonist, which can lead to a net excitation of pyramidal neurons in this region of the hippocampus (25).

**Objective of This Research**

D2 and D3 dopamine receptors, as well as the brain regions where they distributed, have been shown to be involved in drug addiction. However, the exact role for them, the distinct function of D2-like receptor subtypes in different brain regions and subdivision of striatum and the hippocampus have not yet been fully defined.
In this study, brain tissues of eight male Sprague-Dawley rats, four cocaine-treated and four control, were used to examine the function and gene expression of D2 and D3 receptor subtype distinction, as well as the effects of cocaine exposure on the D2 and D3 receptors, in four different brain areas, dorsal striatum, ventral striatum, dorsal hippocampus and ventral hippocampus.

Agonist stimulated \( ^{35}\text{S} \) GTP\(\gamma\)S binding assays were used in our study to examine the function of D2 and D3 receptors as well as the effect of cocaine exposure on these functions. Recently, more attention has been focused on the events following ligand binding to dopamine receptors, particularly on the first step of the intracellular signaling cascade, the interaction of dopamine receptors and G proteins (44). Agonist stimulated \( ^{35}\text{S} \) GTP\(\gamma\)S binding assay provides direct information about the activation of G proteins following agonist binding to the receptor. The biochemical characteristics of the heterotrimeric G proteins are used to investigate the functional activation of G proteins coupled with dopamine receptors (44, 45). The agonist stimulated \( ^{35}\text{S} \) GTP\(\gamma\)S binding assay is a radiolabeling assay, in which GTP is substituted by \( ^{35}\text{S} \) GTP\(\gamma\)S, a non-hydrolysable, radiolabeled analog of GTP. When GDP is released from G-proteins upon agonist stimulation, \( ^{35}\text{S} \) GTP\(\gamma\)S binds to the active conformation of G-proteins with similar kinetics and affinity as GTP, but cannot be hydrolyzed by G\(\alpha\) subunits; the \( ^{35}\text{S} \) GTP\(\gamma\)S-bound G\(\alpha\) subunits cannot be stimulated again, and therefore, the \( ^{35}\text{S} \) GTP\(\gamma\)S binding measures a single exchange event (Figure 1.5). This technique was first applied to examine dopamine receptor activation in cell lines transfected with recombinant human dopamine receptors. Now, agonist stimulated \( ^{35}\text{S} \) GTP\(\gamma\)S binding has been modified so that it can also be applied to tissue sections, and it has been used
to study the functional coupling of dopamine receptors and G proteins in rat striatal membranes (44).

Gene expression assays were performed to detect the expression of D2 and D3 receptor transcripts in different brain regions, and to determine whether cocaine administration changed the receptor expression. As mentioned above, cocaine challenge has been shown to increase the D3 receptor mRNA in striatum particularly in NAc. Since the hippocampus appears to play an important role in conditioned learning engaged in the process of addiction, and D2-like receptor seems to be involved in this event, we also investigated the expression of D2 and D3 receptors in the hippocampus, subdivided into dorsal and ventral regions, in our study.
**Figure 1.1. Four dopamine pathways in the brain.** There are four major dopamine pathways in the brain, nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular pathways. Mesolimbic and mesocortical pathways are also called as mesolimbocortical pathway since they both originate from ventral tegmental area (VTA).
Figure 1.2. Model of G protein signaling. Upon agonist binding, $\text{G}\alpha$ exchanges GDP for GTP. The binding of GTP releases the heterotrimer from the receptor and $\text{G}\alpha$ from the $\text{G}\beta\gamma$ dimer, which are then able to regulate the activity of second messenger-producing effector molecules. The G protein signal is “turned off” when $\text{G}\alpha$ hydrolyzes GTP to GDP. Regulators of G protein Signaling (RGS) proteins enhance the rate of GTP hydrolysis, thereby controlling the amplitude and kinetics of the GPCR signal.
Figure 1.3. **Dopaminergic circuits involved in addiction.** The mesolimbocortical dopamine pathway, particularly the mesolimbic dopamine system, is thought to be a critical substrate for drug reward. This system originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc) and other limbic structures, including ventral domains of the striatum/caudate-putamen (C-P) and prefrontal cortex (PFC). Dotted lines indicate limbic afferents to the NAc, including a potential role for the hippocampus. Arrows represent efferents from the NAc thought to be involved in drug reward. AMG: amygdala; DMT: dorsomedial thalamus.
Figure 1.4. Dopamine transporter (DAT) inhibition by cocaine at the dopaminergic terminal. Dopamine release sites located immediately outside the synaptic cleft. The released dopamine diffuses in the extracellular fluid, and reaches effective concentrations onto its receptors after a relatively longer delay than a transmitter released inside the cleft. Dopamine is slowly cleared as a result of reuptake mainly by DAT. Cocaine binds to the DAT and blocks the reuptake of dopamine in the mesolimbocortical pathway. This potentiates dopaminergic neurotransmission, and initiates the sequence of events that ultimately cause the rewarding and addictive effects of the drug.
Figure 1.5. Rationale of the agonist stimulated $[^{35}S]$ GTPγS binding assay. In the agonist stimulated $[^{35}S]$ GTPγS binding assay, GTP is substituted by $[^{35}S]$ GTPγS, a non-hydrolysable, radiolabeled analog of GTP. When GDP is released from G-proteins upon agonist stimulation, $[^{35}S]$ GTPγS binds to the active conformation of G-proteins with similar kinetics and affinity as GTP, but cannot be hydrolyzed by Gα subunits; the $[^{35}S]$ GTPγS-bound Gα subunits cannot be stimulated again, and therefore, the $[^{35}S]$ GTPγS binding measures a single exchange event.
CHAPTER II
MATERIALS AND METHODS

Materials

Rat Brain Tissues
The brain tissues of four cocaine-treated, two saline-treated and two untreated male Sprague-Dawley rats were obtained from Dr. John J Wagner's lab in Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia.

Chemicals
Guanosine 5′-(y-thio) triphosphate, \[^{35}\text{S} \] was purchased from Perkin Elmer Inc. 2-Bromo-\(\alpha\)-ergocryptine methanesulfonate salt (bromocriptine) was purchased from Sigma-aldrich Co. TRIZOL Reagent was purchased from Invitrogen Co. High capacity cDNA reverse transcription kit, TaqMan universal PCR Master Mix and TaqMan gene expression assays for Gapdh (Rn01775763_g1), Drd2 (Rn00561126_m1, Dopamine receptor 2) and Drd3 (Rn00691132_m1, Dopamine receptor 3) from Rattus norvegicus were purchased from Applied Biosystems.

Apparatus
A 24-sample Harvester operated in conjunction with a Deposit/Dispenser System (Brandel, Inc) was used for the \[^{35}\text{S} \] GTP\(\gamma\)S binding assays. A LS 6500 Multi-Purpose Scintillation Counter (Beckman Coulter) was used for the counting of \[^{35}\text{S} \]. A PTC-150 MiniCycler (MJ Research Inc, now Bio-Rad Laboratories) was used for cDNA preparation. An iCycler Real-Time PCR Detection System with thermal cycler and
optical module (Bio-Rad Laboratories) was used for the TaqMan expression assays. A Biophotometer (Eppendorf) was used for the detection of RNA concentrations. A SPECTRAmax M2 Multi-detection Microplate Reader (Molecular Devices, Co) was used for the detection of the concentration of tissue membrane proteins.

Methods

Rat Treatment and Dissection (performed in Dr. Wagner’s lab)

Six male Sprague-Dawley rats were given an i.p. injection of 10 mg/kg cocaine (n=4) or saline (n=2) for one day, and then were subjected to a conditioning session of four days with i.p. injection of 20 mg/kg cocaine or saline every day. After seven drug-free days, the rats were challenged with an i.p. 10 mg/kg cocaine or saline (46).

The brains of the six treated rats and two naïve/untreated rats were collected, snap frozen, and stored at -70 °C. One brain from the cocaine-treated rats was collected one week after the last cocaine exposure, and others were collected one day after the last cocaine exposure. For dissection, the frozen brains were thawed in ice-cold buffer, and then the tissue fractions of dorsal and ventral striatum, and dorsal and ventral hippocampus were separated for functional and gene expression assays.

Rat Brain Tissue Membrane Preparation

The rat brain tissues were added to 2.5 ml of ice-cold Buffer A (20 mM Hepes, pH 7.4, 5 mM MgCl₂, 1 mM EDTA, 1 mM DTT, plus the protease inhibitors pepstatin, aprotinin, leupeptin, and PMSF) containing 10% sucrose per 100 mg of tissue. The tissues were disrupted briefly using a glass dounce homogenizer, and 100 µl or 25% of the briefly-homogenized tissues were added into 1 ml TRIZOL Reagent and saved for RNA
isolation. The remaining tissues were further homogenized, and subjected to 400 × g centrifugation for 10 minutes at 4 °C. The pellets were added to the same volume of ice-cold Buffer A containing sucrose, disrupted and centrifuged at 400 × g again. The two supernatants were combined and this cleared lysate was diluted with Buffer A containing no sucrose to yield a final sucrose concentration of 3.3% and subjected to centrifugation at 100,000 × g for 40 minutes at 4 °C. The final membrane pellets were re-suspended in minimum volume of Buffer A with 10% sucrose, aliquoted, snap frozen in liquid nitrogen or dry ice/ethanol slurry, and stored at -70 °C.

**Agonist Stimulated [35S] GTPγS Binding Assay**

Rat brain tissue membranes were thawed on ice, diluted in membrane buffer (20 mM Hepes, 2 mM MgCl₂, 1 mM EDTA, 100 mM NaCl) to a concentration of 250 µg/ml. In parallel, a reaction buffer (50 mM Tris-HCl (pH8), 5 mM MgCl₂, 100 mM NaCl, 20 µM GDP, 0.5 mM EDTA, 0.5 mM DTT, 50000 cpm/µl [35S]GTPγS) was prepared, and different amount of drugs were added to the reaction buffer to make binding mix at desired concentration of drugs. Before reaction, 90 µl of binding mixture containing different concentration of drugs were added to reaction tubes on ice. The effect of each drug concentration was measured in triplicate. The reaction was started with the addition of 10 µl 250 µg/ml diluted tissue membrane (total 2.5 µg membrane protein per reaction) to the binding mix and transferred to a 30 °C water bath. After incubation at 30 °C for 30 min, the reaction was stopped by addition of 5 ml of ice cold stop buffer (20 mM Tris, 25 mM MgCl₂, 120 mM NaCl). Then the reaction solutions were filtered over Whatman GF/B glass fiber filter paper, washed twice with 3 ml wash buffer (stop buffer) and added to scintillation fluid using a 24-sample Harvester with Deposit/Dispenser.
system (Brandel, Inc). The filters were then soaked in the fluid for at least 3 hours, and subjected to counting in a LS6500 Multi-purpose Scintillation Counter (Beckman Coulter).

**RNA Isolation from Rat Brain Tissue**

Homogenized rat brain tissues in 1 ml TRIZOL Reagent were incubated at room temperature for 5 min. The tissues were then combined with 0.2 ml of chloroform per 1 ml TRIZOL Reagent, shaken vigorously by hand for 15 sec, incubated at room temperature for 2 - 3 min, and subjected to centrifugation at 3270 × g for 20 min at 4 °C. The colorless upper aqueous phase containing RNA was transferred to fresh tubes, added by 0.5 ml of isopropyl alcohol, incubated at room temperature for 10 min, and centrifuged at 3270 × g for 30 min at 4 °C. The supernatant were removed. The pellets were washed by adding 1 ml 75% ethanol in DEPC-H$_2$O, mixing and centrifuging at 3270 × g for 10 min at 4 °C. The pellets were dried briefly (not completely), dissolved in 30 µl RNAase-free water, incubated at 55 – 60 °C for 10 min, and stored at -70 °C.

**cDNA Preparation**

A high capacity cDNA reverse transcription kit (Applied Biosystems) was used to prepare cDNA from RNA. Briefly, a 10 µl 2× RT master mix was prepared on ice by mixing 2.0 µl 10× RT Buffer, 2.0 µl 10× RT random primers, 0.8 µl 25× dNTP mix, 0.2 µl RNase inhibitor, 1 µl MultiScribe reverse transcriptase and 4 µl nuclease-free H$_2$O. In parallel, RNA samples were diluted in DEPC-H$_2$O to a concentration of 0.2 µg/µl. Then, 10 µl of diluted RNA samples were mixed with the 10 µl master mix, and run on thermal cycler with the following conditions: 1) 25 °C, 10 min, 2) 37 °C, 120 min, 3) 85 °C, 5 sec and 4) 4 °C, ∞.
TaqMan Expression Assay (Q-RT-PCR)

A 23 µl reaction master mix was prepared by mixing 1.25 µl 20× TaqMan gene expression assay, 12.5 µl TaqMan universal PCR master mix and 9.25 µl nuclease-free H₂O, and then loaded into iCycler iQ optical-quality 96-well PCR plate reaction tubes. 2 µl of cDNA samples were added into reaction tubes. The expression was measured in duplicates for each cDNA sample and each TaqMan gene expression assay. The optical plate was sealed with iCycler iQ optical-quality sealing tape and run on iCycler Real-Time PCR Detection System (Bio-Rad Laboratories) with the following conditions: 1) 95 °C, 10 min, 2) 95 °C, 15 sec and 60 °C, 1 min, 40 repeats.

Statistical Analysis

All data were compiled and analyzed using GraphPad Prism version 3.00 (GraphPad Software Inc). Unpaired two-tailed t-tests were used for all comparisons between treatment groups. Correlation between function activity and gene expression of D2 and D3 receptors were analyzed by two-tailed Pearson Correlation. Differences were considered statistically significant at p<0.05.
CHAPTER III

RESULTS AND DISCUSSION

Results

In this study, eight rats were treated (four were treated with cocaine and four were controls), and total membrane fractions and RNA fractions of four different brain regions were prepared as described in Methods. GTP\(\gamma\)S binding assays were performed on the membrane fractions to compare functional D2-D3 receptor G-protein coupling, and semi-quantitative RT-PCR was performed on the RNA fraction to compare expression of transcripts encoding D2 and D3 receptor subtypes.

Activities of D2-like Dopamine Receptors in Various Brain Regions

Bromocriptine, a non-selective agonist at D2 and D3 dopamine receptors, was used in agonist stimulated \(^{35}\)S GTP\(\gamma\)S binding assays. Bromocriptine stimulated G protein coupling activities of D2-like (D2 and D3) receptors in ventral and dorsal hippocampus, and ventral and dorsal striatum were assayed, and the maximal efficacies and the EC\(_{50}\)s were calculated according to the concentration-response curves (Figure 3.1, Table 3.1). The efficacy of bromocriptine in different brain regions ranged from 10.8% to 64.1% above basal, and the potency ranged from 0.033 \(\mu\)M to 1.020 \(\mu\)M.

The properties of bromocriptine stimulated \(^{35}\)S GTP\(\gamma\)S binding in the same brain regions from different rats varied significantly (Table 3.1), especially the potency of bromocriptine (Figure 3.2). Further, data appeared to segregate into two groups according to when assays were performed. The data for group 1 (rat 1-4, two cocaine-
treated and two controls) and group 2 (rat 5-8, two cocaine-treated and two controls) were generated at different times, including brain dissection, tissue membrane preparation and all assays. The functional data of group 1 were very different from those of group 2 (Table 3.1, Figure 3.2, 3.3A and 3.4B and C). The efficacies of bromocriptine in the ventral and dorsal hippocampus and the ventral striatum from group 1 were much lower than those from group 2 (Figure 3.3A), and the EC$_{50}$s of bromocriptine in ventral and dorsal striatum from group 1 were much lower than those from group 2 (Figure 3.2 and 3.4B and C).

Nonetheless, clear distinctions in the properties of bromocriptine dose-response curves in different brain regions were evident. In both groups, efficacy was significantly higher in ventral and dorsal hippocampus than in ventral and dorsal striatum (Figure 3.3); potency was highest in dorsal striatum and lowest in dorsal hippocampus (Figure 3.4). There was no significant difference in maximal efficacies between ventral (%E$_{\text{max}}$=44.77%±4.63%) and dorsal hippocampus (%E$_{\text{max}}$=43.64%±3.49%), and between ventral (%E$_{\text{max}}$=18.24%±2.21%) and dorsal striatum (%E$_{\text{max}}$=16.18%±1.22%).

EC$_{50}$s of bromocriptine in ventral and dorsal striatum from group 2 shifted a lot compared to group 1 and became closer to those in ventral hippocampus (Figure 3.4C). However, combining the results of this study with a previous study (data not shown), bromocriptine is, in general, most potent in dorsal striatum (EC$_{50}$=0.1016 µM, Log EC$_{50}$=-0.9930 ±0.1431), followed by ventral striatum (EC$_{50}$=0.1983 µM, Log EC$_{50}$=-0.7026 ±0.1321), ventral hippocampus (EC$_{50}$=0.3159 µM, Log EC$_{50}$=-0.5004 ±0.0508) and dorsal hippocampus (EC$_{50}$=0.5504 µM, Log EC$_{50}$=-0.2593 ±0.0687) (Figure 3.1 and 3.4).
Dopamine Receptor activity in Cocaine-treated versus Control Rats

We next compared the potency of bromocriptine in cocaine-treated and control rats (Figure 3.5). We observed a general trend that bromocriptine was more potent in cocaine-treated animals (Figure 3.5A). This effect was most evident in group 2 animals (rat 5-8) when analyzed separately. The potency was higher in all four brain regions from cocaine-treated rats although only statistically significant in dorsal hippocampus (p<0.05) (Figure 3.5C).

Since the range of EC$_{50}$s of group 1 and 2 was different, we normalized values for cocaine treated animals to control animals within each group, and then combined the data to analyze the effects of cocaine treatment on functional receptor activity across all eight animals. Specifically, we calculated the Δ Log EC$_{50}$s between Log EC$_{50}$s from cocaine-treated and control rats for each group, and performed t-test on the Δ Log EC$_{50}$ means compared to zero (no change) (Figure 3.5D). We found that Δ Log EC$_{50}$ means in dorsal hippocampus was significantly different from zero (p<0.05), which meant that the EC$_{50}$ of bromocriptine in dorsal hippocampus from cocaine-treated rats was lower than in dorsal hippocampus from control rats. We also observed a trend that Δ Log EC$_{50}$ in dorsal striatum was different from zero suggesting an increase in D2/D3 receptor activity in this brain region following cocaine treatment; however, this difference was not statistically significant due to higher variability in the data (p=0.11).

Gene Expression of D2 and D3 Dopamine Receptors in Cocaine-treated and control Rat Brain Tissues

To detect the gene expression of D2 and D3 receptors in different brain regions from differently treated animals, total RNAs were isolated from brain tissues, and cDNAs
were prepared from the RNAs and subjected to quantitative RT-PCR for quantification of the transcripts. The TaqMan reagents we used to perform gene expression assays have been optimized to have same annealing temperature and efficiency for all cDNAs, so that the results of the assays are comparable. The gene expression data were more consistent through all eight rats than the functional data (Figure 3.6A). The gene expression of D2 and D3 receptors was presented by ΔCT values which were calculated by subtracting the CT values of GAPDH from the CT values of D2 and D3 receptors. In quantitative RT-PCR, gene transcripts are doubled in every cycle, and the CT value represents the number of cycles needed for the amount of the amplified transcripts to cross the threshold. Therefore, the lower the ΔCT values, the higher the expression of the receptor transcripts.

The gene expression is different between D2 and D3 receptors in the same brain region, as well as in D2 and D3 receptors themselves among different brain regions (Figure 3.6B, Table 3.2). D2 receptor was found to be expressed significantly much more abundantly than D3 receptor in all four brain regions we tested (p<0.05). The difference of D2 and D3 expression by ratio was largest in dorsal striatum, followed by ventral striatum, ventral hippocampus and dorsal hippocampus (Table 3.2A). D2 and D3 receptor themselves expressed significantly differently among the four brain regions (p<0.05) except D3 receptor expression between ventral and dorsal hippocampus which was significantly different at 90% level (p=0.0824) (Table 3.2B). Both D2 and D3 receptor transcripts were expressed significantly more abundantly in striatum than in hippocampus, but the expression ratio of D2 receptor in striatum vs. hippocampus was much higher than that of D3 receptor. In striatum, D2 receptor was more expressed in
dorsal than in ventral regions, whereas D3 receptor was more expressed in ventral than in dorsal regions.

In general, cocaine exposure had little effect on the gene expression of D2 and D3 receptors in brain tissues, except for D3 receptor expression in ventral hippocampus (Figure 3.7). The ∆CT value of D3 receptor in ventral hippocampus of cocaine-treated rats was a little lower than that of control rats (p=0.0884 two-tailed, p=0.0442 one-tailed, Figure 3.7B), which meant that D3 receptor expression in ventral hippocampus of cocaine-treated rats was a little higher than that of control rats. The D3 receptor transcript expression was increased approximately 60% in cocaine treated rats, based on the formula: \( \frac{\text{transcript}_{\text{cocaine}}}{\text{transcript}_{\text{control}}} = 2^{(\Delta CT_{\text{cocaine}} - \Delta CT_{\text{control}})} \). This difference fails to meet statistical criteria for significance at the 95% level (p=0.069) using a two-tailed t-test, but is significant (p=0.039) using the less stringent one-tailed t-test (see Discussion, Figure 3.7C).

**Correlation between Functional Activity and Gene Expression of D2 and D3 Receptors**

We found that the potency of bromocriptine stimulated G protein coupling activity was more correlated with D2 receptor (R=0.9210, p=0.0790) than D3 receptor (R=0.7237, p=0.2763) (Figure 3.8A). The functional activity and gene expression were more correlated for group 1 (R=0.9626, p=0.0001 for D2; R=0.7825, p=0.0217 for D3, Figure 3.8B) than for group 2 (R=0.5864, p=0.1266 for D2; R=0.4025, p=0.3228 for D3, Figure 3.8C). Cocaine did not show effects on the correlation (data not shown).
**Discussion**

In the present study on the function and gene expression of D2 and D3 dopamine receptors in cocaine-treated and control rat brain tissues, we found that the potency of the D2-like receptor agonist to stimulate G protein coupling was highest in dorsal striatum, followed by ventral striatum, ventral hippocampus and dorsal hippocampus; the efficacy of the agonist in the hippocampus was significantly higher than in the striatum. D2 receptor transcript expression was significantly higher than D3 receptor in all four brain regions. D2 receptor gene was expressed highest in dorsal striatum followed by ventral striatum, ventral hippocampus and dorsal hippocampus, whereas D3 receptor gene was highest in ventral striatum followed by dorsal striatum, ventral hippocampus and dorsal hippocampus. Although cocaine exposure seemed to increase the potency of the agonist in the rat brain particularly in the dorsal hippocampus, and might slightly increase the D3 receptor transcript expression in ventral hippocampus, no convincing effect of cocaine on D2 and D3 receptor function and gene expression were observe in our study.

Agonist stimulated $[^{35}\text{S}]$ GTPγS binding assay has been used to examine D2 and D3 receptors in the human brain, and higher efficacy of dopamine and quinpirole, two D2-like receptor agonists, in human dorsal striatum than in the hippocampus was reported (44). However, in our study, the non-selective D2/D3 receptor agonist, bromocriptine, had higher efficacy in rat hippocampus than in the striatum.

It was consistent with the previous findings that D3 receptor gene was expressed more in ventral than in dorsal striatum. It was interesting to find that in contrast with D3 receptor, D2 receptor expressed more in dorsal than in ventral striatum, which may
indicate a different role for D2 and D3 receptors in drug addiction since dorsal and ventral striatum have been suggested to have different functions in drug reward (15). A slight increase of D3 receptor gene expression in cocaine-treated rats compared with control rats was found, which is not significant at the 95% level but is significant at the 90% level \( (p=0.069) \). We also performed a one-tailed t-test on this data, and found that the increase was significant at the 95% level \( (p=0.039) \). The one-tailed test may be appropriate because based on previous reports that cocaine exposure increased the D3 receptor mRNA in the ventral and dorsal striatum, such that we anticipated a positive effect of cocaine treatment in D3 receptor expression, but is nonetheless not the most stringent test of our data. Overall, these data suggest that cocaine had little effect on the gene expression of D2 and D3 receptors in our study and further studies using more animals and diverse methods are needed to confirm a possible increase in D3 receptor expression in the ventral hippocampus in response to cocaine exposure.

The rats used in our study, have also been analyzed for conditioned place preference (CPP) and behavioral sensitization before they were killed. CPP is commonly used to measure drug reward, involving repeatedly pairing a compartment with a specific stimulus while pairing a separate distinct compartment with a neutral stimulus. Behavior sensitization refers to a progressively enhanced behavioral response following the repeated administration of drugs, which might be involved in the development and maintenance of drug addiction through enhanced incentive salience (46). During a one-day activity session, a four-day drug-conditioning session, a seven-day drug-free session and finally a cocaine challenge session, a significance shift in preference for the drug-paired compartment and a sensitized locomotor response were
observed in cocaine-conditioned rats (46). An increase in activity was displayed during
the challenge session compared to the activity session following i.p. injection, which
might indicate the learned associations with the drug experience. The slightly increased
D3 receptor gene expression in the ventral hippocampus from cocaine-treated rats we
found in our study may be associated this increased activity. Cocaine exposure also
appeared to increase the agonist potency in stimulating activity in dorsal hippocampus
in our study, which could also be related to the sensitized behavior. Notably, the length
of treatment with cocaine in these studies is not the standard for acute cocaine
treatment as is used in most of the studies cited herein that measured the effects of
cocaine treatment on D2 and D3 receptor expression.

Although there might be a little trend that cocaine exposure increases the
potency of bromocriptine in brain regions of some rats, the data were varied from rat to
rat. Because the assays on rat 1-4 and rat 5-8 were performed at different times, we
divided them into two groups and found significant differences between them.
Furthermore, even within the same group, there were individual variances. Since
individual differences among rats were so great, we could not reach such a simple
conclusion; further studies with more carefully performed experiments to reduce the
individual variance caused by experimental error are needed to investigate this
possibility.

The D2-like receptor (D2/D3) agonist stimulated activity was found to be more
correlated with D2 than D3 receptor transcript expression. This might be because that
D2 receptor transcript is expressed much more abundantly in all the brain regions than
D3 receptor, and therefore the activity of D3 receptor is covered by the D2 receptor
activity. GTPγS binding dose-response curves were also determined for PD128907, a selective D3 receptor agonist and U99194A and GR218237, two semi-selective antagonists of D3 receptors to determine the distinction of the D2 and D3 receptor activities. However, we did not get consistent results (data not shown), which may be due to their insufficient selectivity. The agonist stimulated $[^{35}S]$ GTPγS binding assay allows obtaining functional information of D2-like dopamine receptors in the native anatomical environment; however, highly selective and stable ligands are required to enable the most effective utility of this technique, such as the functional distinction between D2 and D3 receptors. The gene expression assay is more reliable, but it cannot provide information on non-gene level respects of drugs of abuse, and thus need to be combined with other functional assays for a thorough study of drug actions and addiction mechanism.

In summary, functional and gene expression assays were performed on brain tissue membranes of cocaine-treated and control rats to investigate the involvement of D2 and D3 dopamine receptor in cocaine addiction. The efficacy and potency of D2-like receptor agonist to stimulate G-protein coupling, as well as the distribution of D2 and D3 receptors RNA, varied significantly among different brain regions. Cocaine exposure did not show convincing effect on the function and gene expression of D2 and D3 receptors in spite of the possible slight increase in the D3 receptor expression in ventral hippocampus and in the potency of agonist in dorsal hippocampus. Further research with more animals is required to confirm these findings.
Figure 3.1. Properties of bromocriptine stimulated $[^{35}\text{S}]$ GTP$\gamma$S binding in rat brain tissues. Representative concentration-response curves of bromocriptine stimulated $[^{35}\text{S}]$ GTP$\gamma$S binding in membranes of ventral and dorsal hippocampus, and ventral and dorsal striatum from one of the eight rats.
Table 3.1. Properties of bromocriptine stimulated $[^{35}\text{S}]$ GTPγS binding in rat brain tissue membranes. (Data are presented as mean ± SEM. n, number of dose-response curves generated.) Odd numbered rats (shaded rows): Cocaine treated.

<table>
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<tr>
<th>Rat</th>
<th>n</th>
<th>Log EC$_{50}$ (µM)</th>
<th>EC$_{50}$ (µM)</th>
<th>%E$_{\text{max}}$</th>
<th>n</th>
<th>Log EC$_{50}$ (µM)</th>
<th>EC$_{50}$ (µM)</th>
<th>%E$_{\text{max}}$</th>
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<table>
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Figure 3.2. Potency of bromocriptine to stimulate $[^{35}\text{S}]$ GTPγS binding in different brain regions from each rat. VH, ventral hippocampus; DH, dorsal hippocampus; VS, ventral striatum; DS, dorsal striatum. Legends are presented as rat number (treatment).
Figure 3.3. **Efficacy of bromocriptine in different brain regions.** The efficacy of bromocriptine in different brain regions are presented by $\% E_{\text{max}}$ means calculated for (A) group 1 (rat 1-4) and group 2 (rat 5-8) separately (a, b, c, d and e are significantly different from one another, $p<0.05$), and (B) eight rats altogether. (a is significantly higher than b, $p<0.05$) VH, ventral hippocampus; DH, dorsal hippocampus; VS, ventral striatum; DS, dorsal striatum.
**Figure 3.4. Potency of bromocriptine in different brain regions.** The potency of bromocriptine in different brain regions from (A) all eight rats, (B) group 1 (rat 1-4), and (C) group 2 (rat 5-8) are presented by Log EC\textsubscript{50} means. (In each figure, a, b and c are significantly different from one another, p<0.05.) VH, ventral hippocampus; DH, dorsal hippocampus; VS, ventral striatum; DS, dorsal striatum.
Figure 3.5. Potency of bromocriptine in brain tissues from cocaine-treated and control rats. The potency of bromocriptine in different brain regions for cocaine-treated and control rats from (A) all eight rats, (B) group 1 (rat 1-4), and (C) group 2 (rat 5-8) are presented by Log EC$_{50}$ means (*: significantly different from control rats, p<0.05). (D) The differences between Log EC$_{50}$ means of cocaine-treated and those of control rats from group 1 and 2 are presented by Δ Log EC$_{50}$ (#: significantly different from zero, p<0.05). VH, ventral hippocampus; DH, dorsal hippocampus; VS, ventral striatum; DS, dorsal striatum.
Figure 3.6. Gene expression of D2 and D3 dopamine receptors in rat brain tissues.

(A) Expression of D2 and D3 receptors in rat brain tissues from each rat are presented by ΔCT values means (Legends are presented as rat number (treatment)). (B) Expression of D2 and D3 receptors in different brain regions are presented by the means of ΔCT values for all eight rats (a through g are significantly different from one another, p<0.05). VH, ventral hippocampus; DH, dorsal hippocampus; VS, ventral striatum; DS, dorsal striatum.
Table 3.2. Comparison of gene expression of D2 and D3 dopamine receptors in different brain regions. (*: significantly different from one. VH, ventral hippocampus; DH, dorsal hippocampus; VS, ventral striatum; DS, dorsal striatum.)

<table>
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<tr>
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<th>B. ΔCT value ratio of different brain regions in D2 and D3 receptors</th>
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<td>D3</td>
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Figure 3.7. The effects of cocaine exposure on gene expression of D2 and D3 dopamine receptors in different brain regions. (A) The effect of cocaine exposure on D2 receptor transcripts expression. (B) The effect of cocaine exposure on D3 receptor transcripts expression. (C) Receptor transcript expression ratio between cocaine-treated and control rats are calculated via $2^{-(\text{cocaine } \Delta \text{CT} - \text{control } \Delta \text{CT})}$. VH, ventral hippocampus; DH, dorsal hippocampus; VS, ventral striatum; DS, dorsal striatum.
Figure 3.8. Correlation between functional activity and gene expression of D2 and D3 receptors. Correlation is presented by Log EC$_{50}$ means with ∆CT value means in the four brain regions for (A) all eight rats, (B) group 1 (rat 1-4), and (C) group 2 (rat 5-8).
REFERENCES


