ROLE OF DOPAMINE D2 RECEPTORS IN THE CONSOLIDATION OF AMPHETAMINE-CUE MEMORY IN CONDITIONED ACTIVITY IN RATS

by

Rebekah Helena Wickens

A thesis submitted to the Department of Psychology

In conformity with the requirements for

the degree of Master of Science

Queen's University

Kingston, Ontario, Canada

(June, 2016)

Abstract

The neurotransmitter dopamine (DA) plays an essential role in reward-related incentive learning, whereby neutral stimuli gain the ability to elicit approach and other responses. In an incentive learning paradigm called conditioned activity, animals receive a stimulant drug in a specific environment over the course of several days. When then placed in that environment drug-free, they generally display a conditioned hyperactive response. Modulating DA transmission at different time points during the paradigm has been shown to disrupt or enhance conditioning effects. For instance, blocking DA D2 receptors before sessions generally impedes the acquisition of conditioned activity. To date, no studies have examined the role of D2 receptors in the consolidation phase of conditioned activity; this phase occurs immediately after acquisition and involves the stabilization of memories for long-term storage. To investigate this possible role, I trained Wistar rats (N = 108) in the conditioned activity paradigm produced by amphetamine (2.0 mg/kg, intraperitoneally) to examine the effects of the D2 antagonist haloperidol (doses 0.10, 0.25, 0.50, 0.75, 1.0, & 2.0 mg/kg, intraperitoneally) administered 5 min after conditioning sessions. Two positive control groups received haloperidol 1 h before conditioning sessions (doses 1.0 mg/kg and 2.0 mg/kg). The results revealed that post-session haloperidol at all doses tested did not disrupt the consolidation of conditioned activity, while pre-session haloperidol at 2.0 mg/kg prevented acquisition, with the 1.0 mg/kg group trending toward a block. Additionally, post-session haloperidol did not diminish activity during conditioning days, unlike pre-session haloperidol. One possible reason for these findings is that the consolidation phase may have begun earlier than when haloperidol was administered, since the conditioned activity paradigm uses longer learning sessions than those generally used in consolidation studies. Future studies may test if conditioned activity can be achieved with shorter sessions; if so, haloperidol would then be re-tested at an earlier time point. D2 receptor second messenger systems may also be investigated in consolidation. Since drug-related incentive stimuli can evoke cravings in those with drug addiction, a better understanding of the mechanisms of incentive learning may lead to the development of solutions for these individuals.

Acknowledgements

First and foremost, to my supervisor and mentor, Dr. Beninger: Our conversations about dopamine and the mind were the highlight of my time at Queen's. Your passion for the topic is contagious and helped instill in me a lifelong curiosity about the brain. To Drs. Booij, Olmstead, and Andrew, who examined my thesis: Thank you for challenging me and giving me invaluable input. To the 4th-floor facility members and alumni—Jeff, Chloe, Farhana, Claudia, Alex, Rachel, Kathleen, Suzi, Charelle, Cynthia, and many more: Your wit and humour gave me extra *incentive* to go to Humphrey. Finally, to my wonderful parents: Thank you for your savvy advice and your unflinching devotion to my education and well-being.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AMPH	Amphetamine
ANOVA	Analysis of variance
cAMP	Cyclic adenosine monophosphate
CPP	Conditioned place preference
CREB	cAMP-response element-binding protein
DA	Dopamine
GSK3β	Glycogen synthase kinase-3β
Hal	Haloperidol
LSD	Least significant difference
LTP	Long-term potentiation
LTD	Long-term depression
NAc	Nucleus accumbens
NMDA	*N*-methyl-D-aspartate
PKA	Protein kinase A
SEM	Standard error of the mean
SN	Substantia nigra
VTA	Ventral tegmental area

Chapter 1

Introduction and Literature Review

This thesis focuses on the role of dopamine D2 receptors in the consolidation of conditioned activity produced by amphetamine. I will first describe reward-related incentive learning and the role that dopamine and its receptor subtypes play in such learning. I will then view incentive learning through a memory-based lens characterized by time-dependent memory stages. Noting the paucity of studies on the D2 receptor in the consolidation phase of incentive learning, I will attempt to synthesize what is known about its role in other phases of incentive learning as well as its role in the consolidation of other types of learning in order to put forth a hypothesis. I will finish by proposing a study using the conditioned activity paradigm to elucidate this role.

Dopamine and Reward

Reward-related incentive learning occurs when contextual cues gain the ability to elicit approach and other responses (Bindra, 1968; Beninger, 1983). During this learning, cues that predict biologically important stimuli, such as food and shelter, become attractive, increasing the organism's likelihood of attaining goals needed for survival. However, cues that predict the expected rewarding properties of psychoactive drugs share the same ability to attract an organism. In both cases, the incentive learning is largely mediated by the catecholamine neurotransmitter dopamine (DA) in the brain (Beninger, 1983).

Some of the initial discoveries on reward-related learning pertained to homeostatic and reproductive needs governed by the hypothalamus (Kelley, 2004). Olds & Milner (1956) were the first to show that animals electrically self-stimulate the medial forebrain bundle of the hypothalamus. It was then found that DA antagonists eliminated this effect, which implicates DA in reward (Wise & Rompré, 1989). This was bolstered by findings that natural rewards and nearly all drugs of abuse increase brain DA

concentrations (Scheel-Krüger, 1971). The role of DA in reward-related learning is now widely accepted (Bromberg-Martin, Matsumoto, & Hikosaka, 2010).

Despite the fact that DA is often associated with reward, DA release is not always triggered by the experience of rewards per se, and the neurotransmitter may be more aptly considered a communicator of salience (Bromberg-Martin et al., 2010). For example, in studies on monkeys, the unexpected presentation of rewards cause a burst in DA cell firing, whereas the omission of expected rewards cause dips in this firing (Schultz, 1998). Expected rewards generally do not change DA cell firing. The response of DA cells to aversive stimuli appears to differ by neuronal population: in certain populations they increase DA cell firing, and in others, they diminish it; the reason may be that some populations encode salience and others, value (Bromberg-Martin et al., 2010). The above suggests that DA helps attract animal to salient stimuli and helps tune out non-salient stimuli, which then adjusts the organism's motivation and improves its ability to predict future oucomes (Kelley, 2004). This learning effect might explain why, over time, there is a shift from *natural* rewards causing a spike in DAergic neuron firing rates to *reward-predicting* stimuli causing such a spike (Schultz, Apicella, & Ljungberg, 1993).

Dopaminergic Pathways and Receptor Subtypes

DA is synthesized in two adjacent areas of the midbrain, the substantia nigra (SN) and the ventral tegmental area (VTA), which give rise to three major DAergic pathways. Neurons of the SN that synapse onto the dorsal striatum create the nigrostriatal pathway, which controls goal-directed movement. Degraded neurons in the SN are the main pathology found in Parkinson's disease (Dawson & Dawson, 2003). DAergic cells of the VTA form the mesolimbic pathway and innervate the nucleus accumbens (NAc; part of the ventral striatum), the hippocampus, the amygdala, and the olfactory tubercle (Adinoff, 2004). This system controls many aspects of cognition, motivation, and reward learning, and nearly all psychoactive drugs of abuse increase DA transmission in this pathway (Di Chiara & Imperato, 1988). It should be noted that the nigrostriatal and mesolimbic pathways overlap in their functions. For example,

stimulating neurons of the SN produces a rewarding effect, and the dorsal striatum plays a role in reward learning (Wise & Rompré, 1989; Wise, 2009). Lastly, the mesocortical pathway is comprised of neurons from the VTA that innervate the prefrontal cortex, a region involved in attention and executive control. This system is implicated in attention deficit hyperactivity disorder and in certain cognitive symptoms of schizophrenia (Wise, 1996). The SN, VTA, and striatum are part of a larger system referred to as the basal ganglia, a group of structures involved in the coordination of movement (Packard & Knowlton, 2002).

When DA is released into the synaptic cleft, it can bind to any one of five DA receptor subtypes, termed D1-D5 receptors. These receptors are metabotropic and G-protein-coupled (Beaulieu & Gainetdinov, 2011). They are divided into two main categories of DA receptor classes: D1-like receptors, which include D1 and D5 receptors, and D2-like receptors, which include D2, D3, and D4 receptors. D1-like receptors activate adenylyl cyclase, which then triggers a second messenger cascade of the intracellular signaling molecule cyclic adenosine monophosphate (cAMP), which phosphorylates protein kinase A (PKA; Kebabian and Calne, 1979). D2-like receptors, however, inhibit adenylyl cyclase and suppress cAMP-PKA activity.

In general, D1-like and D2-like receptors are expressed on different medium spiny neurons in the striatum, and each type corresponds to one of two opposing pathways of movement control: a direct ('Go') pathway that facilitates the execution of a planned action and an indirect ('NoGo') pathway that inhibits action. The difference is driven by the excitation versus the inhibition, respectively, of the thalamus, one of the output regions of the basal ganglia (Frank, 2005). D1 receptor binding stimulates the direct pathway and inhibits the indirect pathway, whereas D2 receptor binding does the opposite. For normal movement learning, the stimulation of one pathway generally needs to be accompanied by the reduction in the other pathway (Frank, 2005).

Dopamine, Motor Activity, and the Conditioned Activity Paradigm

Balanced DA signaling in the basal ganglia is crucial for proper motor function. In general, DA depletion leads to reduced motor activity levels and difficulties in initiating movement, as seen in Parkinson's disease, whereas nonselective DA agonists such as amphetamine or cocaine generally cause hyperactivity and stereotypy (repetitive movements; Beninger, 1983).

When an animal receives a stimulant drug for several days in the same environment, the contextual cues gain the ability to elicit approach responses as well as certain behaviours produced by the drug itself (Beninger, 1993; Eikelboom & Stewart, 1982). This phenomenon is seen in locomotor sensitization: repeated daily injections of stimulant drugs heighten activity to levels that surpass the initial response, an effect that is environment-dependent (Stewart & Vezina, 1987). A similar phenomenon exists in a paradigm called conditioned activity. Animals experience daily conditioning sessions in an activity-measuring chamber: one group receives a stimulant drug right before the sessions ('drug-paired'), while the other group receives the same drug 1 h after the sessions ('drug-unpaired'). Control saline injections are given at reciprocal times to counterbalance injection time. On a drug-free test day, when placed into the chamber, the drug-paired group shows more locomotor activity than the drug-unpaired group, therefore displaying conditioned activity (Adams, Carreri, Efferen, & Rotrosen, 2001). The phenomenon demonstrates that the pairing, and not the drug history, drives the difference.

DA transmission has been shown to mediate the establishment of conditioned activity. The paradigm can be induced by nonselective DA agonists, such as apomorphine, which activates DA receptors (Dias, Carey, & Carrera, 2006), cocaine, which blocks the DA transporter therefore preventing DA reuptake (Post et al., 1981; Beninger & Herz, 1986) and amphetamine, which reverses the DA transporter therefore preventing DA reuptake and increasing DA release into the synaptic cleft (Pickens & Crowder, 1967; Beninger & Hahn, 1983). Conditioned activity can also be induced by agonists selective

for D1 receptors (e.g., SKF38393; White, Packard, & Hiroi, 1991) or D2 receptors (e.g., quinprole; White et al., 1991; Mazurski & Beninger, 1991).

In conditioned activity, the neutral stimulus (i.e., context) that precedes the unconditioned stimulus (i.e., drug) becomes capable of eliciting the unconditioned response (i.e., hyperactivity; Martin-Iverson & Fawcett, 1996). Although it has been viewed as a Pavlovian stimulus-response habit, conditioned activity appears to also involve a motivational component (Beninger, 1983; Ahmed, Stinus, & Cador, 1998). For example, the structure of activity has been shown to be different when it is unconditioned versus conditioned, indicating that the conditioned response is not an exact replication of the initial response (Martin-Iverson & Fawcett, 1996).

Mechanism Underlying Incentive Learning

One theory of the mechanism behind incentive learning maps the stimulus-response interface onto the striatum. This model joins together the excitatory neurotransmitter glutamate—specifically its receptor *N*-methyl-D-aspartate (NMDA)—and DA. It is thought that cortical pyramidal neurons that carry sensory information about the context synapse onto medium spiny neurons, where they release glutamate at the terminals and cause Ca^{2+} influx into the cells (Beninger, 1983). Concurrently, rewarding stimuli such as drugs cause VTA neurons to release DA onto the medium spiny neurons. This series of events at the D1 receptor triggers a second messenger cascade and protein synthesis that then strengthens the synapse, increasing the possibility for motor responses to be elicited by these contextual stimuli in the future (Beninger, 1983; Kelley, 2004). In effect, this stamps together the stimulus and response system (i.e., the cortical inputs and striatal motor outputs). This theory is supported by evidence coming from studies showing that NMDA receptor antagonists inhibit the acquisition of incentive learning tasks (Kelley, Smith-Roe, & Holahan, 1997) and that coincident NMDA and D1 receptor activation is needed for the acquisition of lever-pressing tasks (Smith-Roe & Kelley, 2000). Although the above theory discusses sensory inputs from the cortex, it should be noted that limbic structures like the hippocampus

and amygdala, which are involved in motivation and affective processing, also synapse onto medium spiny neurons in the striatum (Nestler et al., 2009).

Learning can be thought as the experience-dependent modification of synapses (Cajal, 1894; Hebb, 1949). Generally, if there is a repeating tendency for cell A to fire immediately before the nearby activation of cell B, growth at the synapse will occur to enhance the efficacy of cell B's firing in response to cell A (Hebb, 1949; Kandel, 2012). The consistent patterns of activity across cells forms a neuronal ensemble, which is thought to encode memories (Takeuchi, Duszkiewicz, & Morris, 2014; Josselyn, Köhler, & Frankland, 2015). There is evidence that reward-related memories, such as the preference for a cocaine-paired context, are linked to consistent neuronal ensembles (Hsiang et al., 2014). There is also evidence to suggest that incentive learning recruits some neural mechanisms involved in general memory formation, such as synaptic plasticity mechanisms (Takeuchi et al., 2014). One such agent is long-term potentiation (LTP), whereby high-frequency stimulation of a presynaptic cell leads to a strengthened postsynaptic response (Bliss & Lomo, 1973). In certain cases, LTP requires the convergence of presynaptic glutamate release and heightened post-synaptic cellular Ca^{2+} concentrations (via NMDA receptors)—a pattern that also occurs in the incentive learning model described above (Beninger, 1983; Nestler, Hyman & Malenka, 2009). In rats, prolonged self-administration of cocaine leads to increases in LTP in the VTA, and so does exposure to cocaine-predicting cues (Lüscher & Malenka, 2011). In a rodent study of intra-cranial self-stimulation (of DAergic neurons), the degree of synaptic potentiation in the striatum correlated with the subsequent rate of acquisition of the task (Reynolds, Hyland, & Wickens, 2001). It has been shown that D1-like receptors generally mediate LTP formation in the striatum, whereas D2-like receptors mediate long-term depression (LTD; the opposite process of LTP; Wise, 2009). Broadly speaking, this pattern could selectively strengthen the direct 'Go' pathway via D1-dependent LTP and a dampening of the 'NoGo' pathway via D2-dependent LTD to bring about a particular motor response

(Wickens, 2009; Frank, 2005). Therefore, incentive learning recruits neural plasticity mechanisms differentially according to the DA receptor subtype.

Memory Phases in Incentive Learning

Incentive learning requires the formation of memories between stimulus and response systems (White & Carr, 1985; Wu et al., 2011). Memory has been shown to undergo several time-dependent stages: in chronological order, they include acquisition, consolidation, expression (i.e., retrieval), and reconsolidation (Abel & Lattal, 2001; Dudai, 2004). These phases have been shown to be somewhat dissociable in terms of the neural mechanisms that mediate them (Beninger & Hahn, 1983; Banasikowski et al., 2010).

Studies on incentive learning have mostly examined the acquisition and expression phases of memory. In studies on DA and the conditioned activity paradigm, D1 or D2 receptor antagonists given before conditioning sessions prevented the acquisition of conditioned activity (Beninger & Hahn, 1983; Beninger & Herz, 1986). However, D1 or D2 receptor antagonists do not block conditioned activity when given right before expression, unless given at very high doses (Beninger & Hahn, 1983; Beninger & Herz, 1986; Dias, Carey, & Carrera, 2006). A similar pattern implicating D1 or D2 receptors more strongly in acquisition than in expression has been found for other incentive learning paradigms, such as the conditioned place preference paradigm (CPP), which tests an animal's preference for spending time in a drug-paired versus neutral-paired context (Hiroi & White, 1991; Cervo & Samanin, 1995; Banasikowski, McLeod, & Beninger, 2012).

Few studies have examined the neural mechanisms behind consolidation of incentive learning (Hernandez, Sadeghian, & Kelley, 2002). The concept of consolidation arose when it was discovered that electroconvulsive shocks or protein synthesis inhibitors given immediately after learning produced amnesia (Duncan, 1949; McGaugh, 1966; Inda, Delgado-Garcia, & Carrion, 2005). During consolidation, the memory trace (neural representation of the memory) engages in second messenger signaling, gene

transcription, and translation (protein synthesis). This protein synthesis leads to the growth of synaptic processes that stabilize the memory for long-term storage (Kandel, 2012; Igaz, Vianna, Medina & Izquierdo, 2002). There are two types of consolidation discussed in the literature: synaptic consolidation, which begins immediately after learning and is described above, and systems consolidation, a more gradual process (e.g., over weeks) that involves the transfer of the memory trace from one brain region to another. For instance, lesion studies have shown that declarative memory is hippocampus-dependent during encoding, but over time becomes neocortex-dependent, which indicates that the brain circuitry underlying the memory undergoes reorganization (Squire & Alvarez, 1995). There is some research suggesting that systems consolidation is in fact brought forth by a particular pattern of synaptic consolidation over time (Dudai, 2004). Although the two types of consolidation appear to be linked, this thesis focuses on more immediate synaptic consolidation, which will be referred to simply as 'consolidation'.

Interestingly, consolidation may involve more than one stage; these stages can occur many hours after the learning. In a set of studies that tracked gene expression and protein synthesis across time, results pointed to two time windows of contextual fear learning: one immediately after learning and the other 3-6 hours after learning (Igaz et al., 2002; Igaz, Bekinschtein, Vianna, Izguierdo, & Medina, 2004). Multiple 'waves' of consolidation have been found for other types of learning as well (Inda et al., 2005). It has been proposed that sleep is involved in consolidating memories formed during the day (Nishida, Pearsall, Buckner, & Walker, 2009). In a study of decision-making using the Iowa Gambling Task, heatlhy individuals were trained on the task and were then retested on it 12 h later after an intervening period of either sleep or wakefulness. Those who had slept demonstrated better performance than those who had not. This therefore indicates that memories underlying decision-making undergo sleep-dependent consolidation some time (perhaps hours) after learning (Seeley, Beninger & Smith, 2014).

The final stage of memory processing is called reconsolidation. Retrieval of a memory reactivates the memory trace and causes it to become unstable, and protein synthesis is required to consolidate the memory once again (Nader, Schafe, & LeDoux, 2000); exposure to associated cues alone is often sufficient to return memories to an unstable state (Mactutus, Riccio, & Ferek, 1979). One study examined the role of DA in the reconsolidation of apomorphine-produced conditioned activity. The context-paired animals received 2.0 mg/kg of apormophine, s.c., during conditioning days and developed conditioned activity (Carrera, Carey, Dias, & de Matos, 2012). After the test, they had three daily drug-free sessions in the conditioning chamber, each followed by 2.0 mg/kg of apomorphine or 0.05 mg/kg of apomorphine, a low dose that primarily stimulates presynaptic D2 auto-receptors that then *inhibits* DA release (Benoit-Marand, Borelli, & Gonon, 2001). The next day, the apomorphine 2.0 mg/kg group demonstrated more conditioned activity, while the 0.05 mg/kg group showed an attenuation of activity. Post-trial treatments delayed by 2 hrs did not show the above effects, which points to the role of DA in the early reconsolidation of conditioned activity. A very similar study by the same research group that examined apomorphine-produced locomotor sensitization found similar results: after a drug-free test, a single injection of 0.05 mg/kg or 2.0 mg/kg of apomorphine eliminated or strengthened, respectively, the sensitization (Carrera, Carey, Dias, & de Matos, 2011). Two reconsolidation studies of mice in the cocaine-produced CPP paradigm found that exposure to the cocaine-paired context while in a drug-free state, combined with an inhibitor of the signaling molecule glycogen synthase kinase-3β, downstream of D2 receptor activity, abolished CPP in a future test (Wu et al., 2011; Shi et al., 2014). Receiving the inhibitor alone without the retrieval did not produce this effect (Wu et al., 2011). These findings implicate DA, and to some degree, D2 receptors, in the early reconsolidation of incentive learning. These findings may also implicate D2 receptor signaling in the *consolidation* of learning.

Post-Training Injection Studies

Post-training injection studies involve the injection of various compounds after training sessions. The protein synthesis inhibitor anisomycin can be given after learning to detect the existence of a consolidation phase, as consolidation requires protein synthesis (Abel & Lattal, 2001; Nader et al., 2000). Anisomycin studies have implicated a consolidation phase in incentive learning: anisomycin infused into the NAc after lever-pressing sessions lowered the rate of lever-pressing on subsequent days compared to controls (Hernandez et al., 2002).

Although post-training studies can track protein synthesis in general (i.e., with anisomycin), they can also investigate upstream receptor systems that may affect this synthesis (Packard & Knowlton, 2002). Post-training studies have implicated the neuromodulators norepinephrine, acetylcholine, glutamate, and DA in the consolidation of various types of learning. Post-training low to moderate doses of epinephrine, a nonendogenous adrenergic agonist, improves performance on aversively and appetitively motivated tasks, whereas high doses impede performance (McGaugh, 1989). A biphasic trend also exists for acetylcholine: low and high post-training doses, i.p., of an anti-cholinergic agent facilitate visual discrimination, whereas intermediate doses have no effect (McGaugh & Krivanek, 1970). Post-training i.p. injections of glutamic acid increase memory retention in the foot-shock task (Flood, Baker, & Davis, 1990). Importantly, each of the above neuromodulators interacts with the DA system in the striatum, which suggests that DA may be involved as well (Joyce & Marshall, 1987; Theodororou, Reavill, Jenner, & Marsden, 1981).

DA agonists have generally been shown to enhance the consolidation of various types of learning (e.g., discrimination and spatial), which is consistent with DA's characterization as a reinforcer (Wise, 2009; Grogan, Bogacz, Tsivos, Whone & Coulthard, 2015). Several studies have examined DA's role in passive avoidance tasks in mice or rats. In this single-trial paradigm, stepping onto one side of the apparatus or onto a platform produces a foot-shock. During testing the following day, the rodents' latency

to step onto the 'shock' side or platform is measured. Performance has been enhanced by the following given post-training: D1-like receptor agonists and cAMP agonists administered to the hippocampus 3-6 h after training sessions (Bernabeu et al., 1997); and D1 or D2 agonists given i.p. immediately but not 2 h after training (Castellano, Cestari, Cabib, & Puglisi-Allegra, 1991). D1 or D2 agonists have been shown to increase performance in the win-stay (non-spatial) version of the radial arm maze task when infused into part of the striatum immediately after the last day of training (Packard & White, 1991). However, when injected i.p., D2 agonists but not D1 agonists enhance performance (Packard & White, 1989). Additionally, D1 or D2 antagonists (SCH23390 and sulpiride, i.p., respectively) have been shown to block consolidation of novel object recognition training in guinea pigs (Lee & Chirwa, 2015). In the above studies that also tested antagonists (in addition to agonists), the agonists increased consolidation and the antagonists impaired consolidation. However, one study produced results that differ from the above: it was a Morris water maze study in which the D2 antagonist sulpiride given after a final trial *decreased* the time rats needed to find the platform in the spatial (hippocampal) and cued (striatal) versions of the task (Setlow & McGaugh, 2000). Therefore, although D1- and D2-like receptors play a role in consolidation, the role of the D2 receptor might require further characterization. This may be especially true since cAMP and PKA, which have been proposed as more downstream mediators of consolidation, are regulated by D1 and D2 receptors differently (Kebabian & Calne, 1979; Bernabeu et al., 1997). Therefore, more research is needed regarding the role of D2 receptors in the consolidation of incentive learning tasks.

Introduction to My Research

Given that D1 and D2 receptors have been strongly implicated in the *acquisition* of incentive learning and that D2 receptors have been implicated in the *consolidation* of various types of learning, my goal in the present study was to investigate the possible role of D2 receptors in the consolidation of conditioned activity, which has not yet been tested. Conditioned activity requires daily 60-min pairing

sessions, unlike many post-training injection studies mentioned above that involved either single-trial tasks or tasks with shorter training lengths. However, previous consolidation studies have employed multi-trial tasks (e.g., radial arm maze; Morris water maze; appetitive lever-pressing), either over the course of one day or multiple days (Packard & White, 1989, 1991; Setlow & McGaugh, 2000; Hernandez et al., 2002). Additionally, several studies have used long delays between training and treatment. For instance, Bernabeu et al. (1997) found that D1 receptor agonists and antagonists given 3-6 h after foot-shock learning affected the consolidation. In a human study of the Iowa Gambling Task, learning was shown to be dependent on sleep-based consolidation (Seeley et al., 2014). Finally, one study examined a two-day place aversion task (60 min of conditioning per day) induced by lithium chloride and found that a D1 agonist (intra-hippocampal) given 12 h after each conditioning session strengthened the memory persistence (Kramar, Chefer, Wise, Medina & Barbano, 2014). The same study tested a six-day CPP paradigm induced by cocaine (30 min of conditioning per day) and found that a D1 receptor agonist given 12 h after each session impaired the memory persistence. Therefore, several experiments have shown that sleep or DAergic modifications can affect learning, despite long learning sessions (30 or 60 min) across multiple days, as well as long delays (e.g., 12 h) between learning and treatment (Kramar et al., 2014; Seeley et al., 2014). Despite that the conditioned activity paradigm has never been tested before with post-training injections, the present study has commonalities with other studies that have found significant results.

I obtained conditioned activity with the DA transporter reverser amphetamine (Sulzer et al., 1995) and used haloperidol, a D2 receptor-preferring competitive antagonist, to possibly block the consolidation of conditioned activity. Haloperidol is a typical antipsychotic drug that produces bradykinesia at high doses (Ahlenius & Hillegaart, 1986) and demonstrates high selectivity for the D2 receptor over the D1 receptor (55 nM vs. 1.2 nM; Martelle & Nader, 2008). I administered haloperidol 5

min after conditioning sessions, given studies suggesting that D2 is involved the immediate rather than delayed consolidation of learning (Packard & White, 1991; Castellano et al., 1991; Lee & Chirwa, 2015).

I hypothesized that post-session haloperidol will dose-dependently block the consolidation of incentive learning in the conditioned activity procedure. Additionally, I hypothesized that pre-session haloperidol will block the acquisition of conditioned activity: this positive control will check if haloperidol blocks conditioning effects, as previously shown with another D2 receptor antagonist, pimozide (Beninger & Hahn, 1983). Finally, I anticipated that rats receiving post-session haloperidol will be less active during conditioning days, due to blocked consolidation of the chamber's ability to elicit hyperactivity.

Chapter 2

Method

Subjects

Experimentally naive male Wistar rats (*N* = 108; Charles River, Quebec, Canada) weighing 225-250 g upon arrival were pair-housed in clear Plexiglas cages with sterilized wood chip bedding (BetaChips, NEPCO, Warresnburg) on a 12-hour reverse dark-light cycle (lights on at 19:00 h) and in a climatically controlled (21 ± 2° C) colony room. Rodent chow (Lab Diet #5001, PMI Nutrition Intl., Brentwood, MO, USA) and tap water were available *ad libitum*. After arrival to the colony, rats were handled for approximately 3-5 min daily for 5 days. Treatment of rats was in accordance with the guidelines of the Animals for Research Act and the Canadian Council on Animal Care, and experiments were approved by Queen's University Animal Care Committee.

Drugs

d-Amphetamine sulfate (AMPH; Sigma-Aldrich, Oakville ON) was dissolved in saline (0.9% NaCl) at a concentration of 2.0 mg/kg. This dose has been shown to produce conditioned activity in previous studies (Mazurski & Beninger, 1991).The D2-receptor preferring antagonist haloperidol (4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one; Sigma, St. Louis, MO, USA) was dissolved at concentrations of 0.10, 0.25, 0.50, 0.75, 1.0, and 2.0 mg/ml in a solution of 0.3% distilled water solution of tartaric acid. All injections were administered at a volume of 1.0 ml/kg, i.p.

Apparatus

Locomotor activity was assessed using six chambers made of Plexiglas (50.0 cm long x 41.0 cm wide x 37.0 cm high) and housed in wooden, Styrofoam-insulated boxes. Each chamber contained seven emitters and detectors located 5.0 cm above the metal rod floor, spaced 10 cm apart from one another

(three pairs along the 41 cm long sides, and four pairs along the 50.0-cm long sides). Chambers were lit with a 2.5 W incandescent bulb and ventilated by a small fan that also produces background noise. Each horizontal beam break was recorded on a circuit board connected to a 1-GHz IBM computer (White Plains, NY).

Procedure

Experiment 1: Establishing Conditioned Activity

The total procedure lasted 7 days. On days 1-3 (habituation), each animal spent 60 min in the activity chamber, drug-free. On days 4-7 (conditioning days), one group of animals received AMPH (2.0 mg/kg, i.p.) immediately prior to entry into the chambers, where they remained for an hour; the other group received saline (1.0 ml/mg) prior to conditioning. After removal from the chambers, all rats were given home-cage injections one hour later of saline (1.0 ml/mg) for the AMPH-paired rats, or AMPH (2.0 mg/kg, i.p.) for the AMPH-unpaired rats. On the test day, all rats entered the activity chambers without any drug or saline injections.

One control group followed the same protocol as the unpaired group and also received 2.0 mg/kg, i.p., of haloperidol 5 min after conditioning sessions, which is the same timeframe for post-session haloperidol groups (see below). This group was used to detect possible changes in activity due to physical tolerance 24 hours after the last administration of haloperidol. Rebound effects in motor activity have noted in previous studies (Herz & Beninger, 1987; see Discussion).

Experiment 2: The Effects of Post-Session Haloperidol on Conditioned Activity

Groups underwent the same procedure as the AMPH-paired group (above), except that on conditioning days, they also received injections of vehicle or haloperidol at 0.10, 0.25, 0.50, 0.75, 1.0, and 2.0 mg/kg, i.p. These injections were given 5 min after removal from the chambers and placement into home cages.

Experiment 3: The Effects of Pre-Session Haloperidol on Amphetamine-Induced Locomotion and Conditioned Activity

Two additional groups underwent the same protocol as the AMPH-paired group (above) but also received either 1.0 or 2.0 mg/kg, i.p., of haloperidol in their home cages 1 h prior to conditioning sessions. Doses lower than 1.0 mg/kg, i.p. have been shown to prevent the acquisition of conditioned activity when administered 1 h before conditioning (Banasikowski et al, 2010).

Groups

There was a total of 12 groups (*n*s = 9). The AMPH-paired and AMPH-unpaired groups are termed the 'paired' and 'unpaired' groups, respectively, and the unpaired group receiving 2.0 mg/kg pre-session haloperidol was referred to as the 'unpaired + hal' group. For Experiment 2, groups receiving post-session injections are called the 'vehicle group' and the hal 0.10, hal 0.25, hal 0.50, hal 0.75, hal 1.0, and hal 2.0 mg/kg groups. For Experiment 3, the two pre-session haloperidol groups are termed the hal 1.0 mg/kg before and hal 2.0 mg/kg before groups. Protocols for all groups are described in the procedure (above).

Data Analysis

Activity levels were measured as the total horizontal beam breaks within the 60-min session. All statistical tests were conducted using IBM SPSS v. 21 (SPSS Inc., Chicago, IL). For experiment 1, a one-way analysis of variance (ANOVA) compared the unpaired, paired, and unpaired + hal groups on their activity on test day. To follow up the ANOVA, Dunnett's post hoc comparisons were performed using the unpaired group as the reference group. A higher beam break count for the paired group versus the unpaired group determined the presence of conditioned activity; differences in beam break counts between the unpaired + hal group versus the unpaired group determined a withdrawal effect of haloperidol. The comparison between the unpaired + hal and paired group was of little importance and was therefore eliminiated with the use of Dunnett's comparisons. For experiment 2, a one-way ANOVA

analyzed the unpaired, paired, vehicle and post-session haloperidol groups (doses 0.10, 0.25, 0.50, 0.75, 1.0 and 2.0 mg/kg) on their test day activity. The follow-up for the ANOVA were Fisher's least significant difference (LSD) post hoc comparisons between groups. For experiment 3, a one-way ANOVA assessed the test day activity levels of the unpaired, paired, and haloperidol 1.0 mg/kg before and 2.0 mg/kg before groups. To follow up for significance, the LSD test was used.

The final analysis was not based on a procedure but was instead an analysis of spontaneous locomotor activity during conditioning days. A one-way ANOVA compared all 12 groups on the activity levels averaged over the three conditioning days; LSD comparisons were used as the follow-up for significance. Additionally, a mixed-model ANOVA (group [10] x day [3]) assessed groups on activity scores over the three conditioning days. Technical malfunctions caused data loss for groups hal 0.25 mg/kg and 0.75 mg/kg on conditioning day 3 such that these groups could not be included in the mixed-model ANOVA; malfunctions on day 3 also eliminated all but two subjects from the paired group in this ANOVA. Simple main effects following a significant interaction consisted of one-way, within-subjects ANOVA run for each group comparing the conditioning days. In order to include the groups that were eliminated from the mixed-model ANOVA and to increase the number of subjects in the paired group, planned one-way within-subjects ANOVA were run on these groups across days 1 and 2 only (*n*s = 6 for each group). Follow-up tests for significant one-way ANOVA consisted of LSD comparisons of day.

Chapter 3

Results

Because beam breaks varied for groups on the last day of habituation (F[11, 96] = -2.12, p = .026), which occurred before the start of any experimental manipulations, beam breaks on conditioning or test days were instead expressed as a percentage of levels on the last (third) day of habituation (e.g.: test day beam breaks ÷ habituation day beam breaks x 100). This therefore eliminated any variability produced by pre-existing group differences. (For original data, see Appendix.)

Experiment 1: Establishing Conditioned Activity

The one-way ANOVA comparing the unpaired, paired, and unpaired + hal groups on test day activity had a significant effect of group, $F(2, 24) = 11.13$, $p < .001$, $\eta_p^2 = .481$ (see Figure 1). The paired group displayed signficantly higher activity than the unpaired group on test day ($p < .001$) by Dunnett's post hoc test. Therefore, the conditioned activity paradigm was established: despite the similar drug histories, the animals experiencing the drug-environment pairing were significantly more active when tested drug-free. The activity of the unpaired + hal group did not significantly differ from the unpaired group ($p = .811$) by Dunnett's test. Therefore, post-session haloperidol alone did not alter test day responding in unpaired animals.

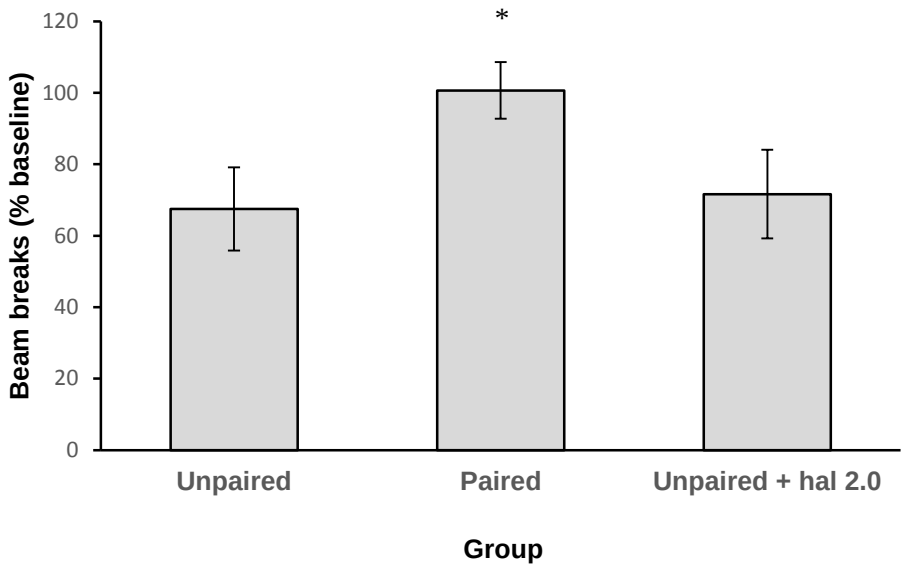


Figure 1. Effects of amphetamine-environment pairing on conditioned activity. Mean beam breaks (±2 *SEM*) during the 60 min test session for the unpaired, paired, and unpaired + hal groups. Values are expressed as a percentage of baseline (last day of habituation). The unpaired + hal 2.0 group received 2.0 mg/kg of haloperidol 5 min after conditioning sessions. * significantly ($p < .05$) different from the unpaired group by Dunnett's post hoc comparisons. Abbreviations: hal = haloperidol. All *ns* = 9.

Experiment 2: The Effects of Post-Training Haloperidol on Conditioned Activity

A one-way ANOVA compared the test day activity levels of the unpaired, paired, vehicle group, and post-session haloperidol groups: hal 0.10, 0.25, 0.50, 0.75, 1.0, and 2.0 mg/kg (see Figure 2). There was a significant effect of group, $F(8, 72) = 4.04$, $p = .001$, $\eta_p^2 = .310$. The LSD comparisons revealed that the paired and vehicle groups did not differ significantly ($p = .949$); none of the post-session haloperidol groups significantly differed from the paired or vehicle groups (*ps* > .183); and all groups had higher activity than the unpaired group (*ps* < .027). Amongst the post-session haloperidol groups, hal 0.1 mg/kg and hal 1.0 mg/kg were significantly less active on test day than the hal 0.25 mg/kg and hal 2.0 mg/kg groups.

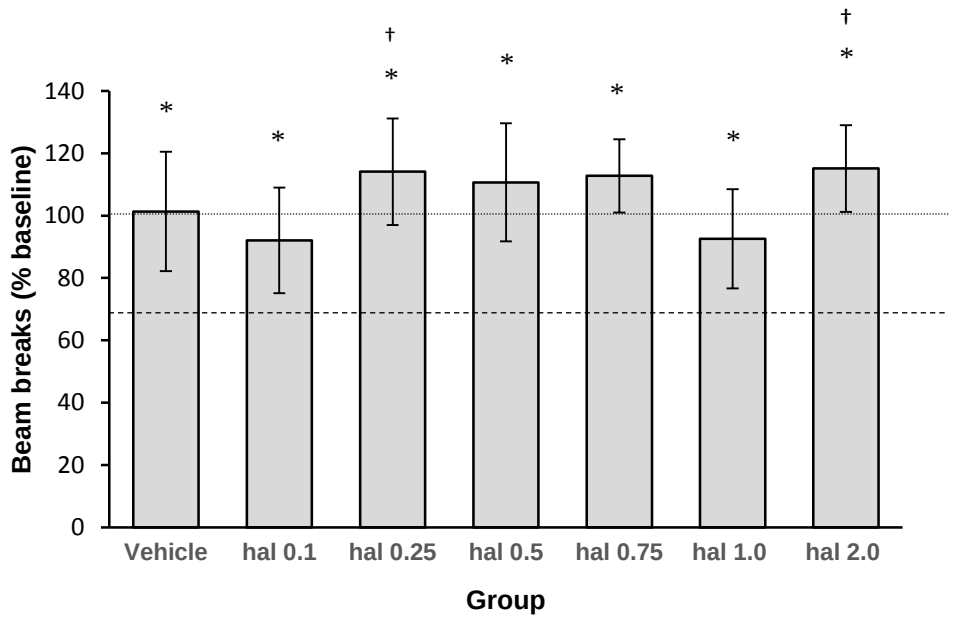


Figure 2. Effects of post-session haloperidol on the consolidation of conditioned activity. Mean beam breaks (±2 *SEM*) during the 60 min test session for the vehicle and post-session haloperidol groups. Values are expressed as a percentage of baseline (last day of habituation). The dotted line (top) denotes the paired value and the dashed line (bottom) denotes the unpaired value. Numbers beside "hal" represent doses of haloperidol in mg/kg given 5 min after conditioning sessions; the vehicle group received vehicle 5 min after conditioning. * significantly ($p < .05$) greater than unpaired by LSD post hoc comparisons. † significantly ($p < .05$) greater than groups hal 0.1 and hal 1.0 mg/kg by LSD test. All *n*s = 9. Abbreviations: hal = haloperidol.

Experiment 3: The Effects of Pre-Training Haloperidol on Conditioned Activity

A one-way ANOVA compared the unpaired, paired and pre-session haloperidol groups (hal 1.0 mg/kg before and hal 2.0 mg/kg before) on test day activity (see Figure 3). There was a significant difference in groups, $F(3,32) = 6.49$, $p = .001$, $\eta_p^2 = .378$. In the LSD follow-up test, the hal 1.0 mg/kg before group was more active than the unpaired group ($p = .023$), whereas the hal 2.0 mg/kg before group was not ($p = .078$). There was no significant difference between the hal 1.0 mg/kg before and hal 2.0

mg/kg before groups (p = .569). The hal 2.0 mg/kg before group was less active than the paired group (p = .016); the hal 1.0 mg/kg before group was less active than the paired group by a value that approached significance (p = .056, Cohen's d = 0.66). Therefore, using the paired group as the benchmark, halopeirdol at the dose 2.0 mg/kg blocked the acquisition of conditioned activity and the dose of 1.0 mg/kg came close to blocking the acquisition.

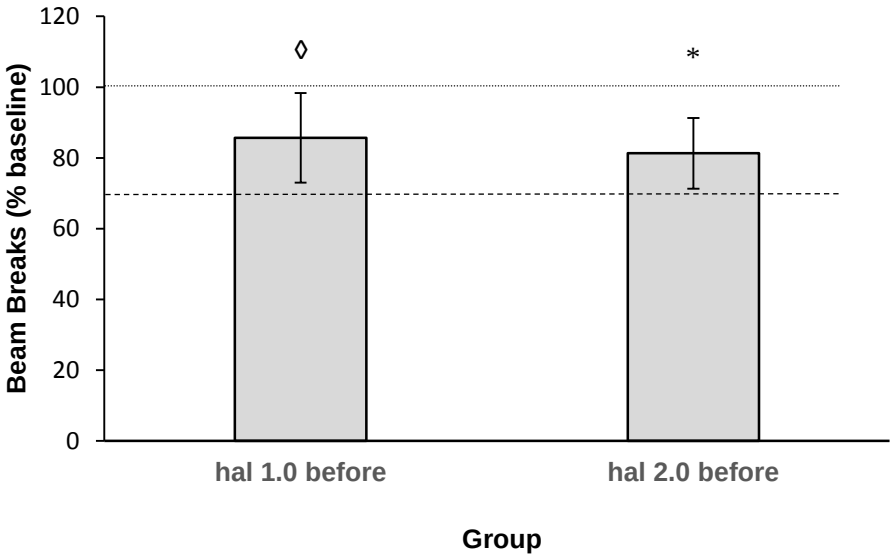


Figure 3. Effects of pre-session haloperidol on the acquisition of conditioned activity. Mean beam breaks (±2 *SEM*) during the 60 min test sessions for the groups that received 1.0 mg/kg or 2.0 mg/kg of haloperidol 1 h before amphetamine conditioning sessions. Values are expressed as a percentage of baseline (last day of habituation). The dotted line (top) denotes the paired value and the dashed line (bottom) denotes the unpaired value. Numbers represent doses of haloperidol in mg/kg that were given 1 h before conditioning sessions. * significantly (p < .05) different from paired group by LSD post hoc comparisons. ◊ different from paired with approaching significance (p = .056) by LSD test. All ns = 9.

The Effects of Pre- and Post-Session Haloperidol on Locomotor Activity during Conditioning

A one-way ANOVA compared all groups on their locomotor activity, averaged over the three conditioning sessions (see Table). It should be noted that only pre-session haloperidol groups had

haloperidol on board during conditioning sessions; the rest of the groups that were not unpaired had amphetamine on board during conditioning. The ANOVA revealed a significant difference, $F(11, 96) = 21.58$, $p < .001$, $\eta_p^2 = .735$. In the LSD comparisons, the paired, vehicle, and post-session haloperidol groups each had higher activity than the pre-session haloperidol groups, the unpaired group, and the unpaired + hal group (*ps* < .001). There were no differences between the unpaired, unpaired + hal, and both pre-session haloperidol groups (*p*s > .154). The paired group did not differ signifcantly from the vehicle group ($p = .986$), and neither groups differed from the post-session haloperidol groups (*p*s > .150). Amongst the post-session haloperidol groups, hal 0.1 mg/kg was less active than hal 2.0 mg/kg ($p = .043$).

Table: Locomotor activity averaged over conditioning days.

Group	***n***	**Mean Beam Breaks (% baseline)**	***SEM***
Unpaired	9	83.33	5.60
Unpaired + hal 2.0 mg/kg	9	77.57	11.67
Paired	9	379.47*	49.95
Vehicle	9	378.46*	35.36
hal 0.10 mg/kg	9	344.50*	42.05
hal 0.25 mg/kg	9	450.73*	35.99
hal 0.50 mg/kg	9	413.35*	76.10
hal 0.75 mg/kg	9	441.85*	26.04
hal 1.0 mg/kg	9	425.74*	48.41
hal 2.0 mg/kg	9	459.53*	54.61
hal 1.0 mg/kg before	9	17.58	2.39
hal 2.0 mg/kg before	9	6.93	0.44

Note. Values are expressed as a percentage of baseline (last day of habituation). All groups had AMPH (2.0 mg/kg) on board during conditioning except unpaired groups. The hal 1.0 mg/kg and 2.0 mg/kg before groups received haloperidol 1 h before conditioning sessions; all other haloperidol groups received it 5 min after. * significantly ($p < .05$) different than unpaired, unpaired + hal, hal 1.0 mg/kg before, and hal 2.0 mg/kg groups by LSD post hoc test. Abbreviations: hal = haloperidol.

A mixed-model ANOVA analyzed the group (10) x conditioning day (3) interaction on activity levels. Due to missing data (see Method), the hal 0.25 mg/kg and hal 0.75 mg/kg groups were not included in this ANOVA. There was a significant interaction effect (F[13.94, 136] = 3.22, p = .001, η_p^2 = .299), as well as a significant main effect of day (F[1.55, 136] = 8.87, p < .001, η_p^2 = .115) and a significant main effect of group (F[9, 136] = 17.86, p < .001, η_p^2 = .703). Therefore, slopes of activity across days differed by group (see Figure 4). A simple main effects analysis probed day effects for individual groups using within-subjects ANOVA. In order to re-include the hal 0.25 mg/kg and hal 0.75 groups that had been excluded, and to include more subjects for the paired group (see Method), planned one-way ANOVA compared days 1 and 2 only for those three groups.

Activity for the unpaired group decreased over time, F(2, 16) = 6.87, p = .007, with activity being higher on day 1 than on days 2 and 3 (p =.012; p = .005). Paired group activity did not significantly change between days 1 and 2, F(1, 5) = 0.14, p = .723. Activity did not change for the unpaired + hal group (F[2, 10] = 0.313, p =.738) or the vehicle group (F[2, 16] = 1.52, p = .249). Amongst the post-session haloperidol groups, all groups except the hal 0.5 mg/kg group (F[2, 16] = 2.12, p = .153) showed increases over time, which indicates sensitization. The hal 0.1 mg/kg group experienced an increase over days, F(2, 16) = 11.21, p = .001, with activity being higher on days 2 and 3 than on day 1 (ps < .008) but not differing between days 2 and 3 (p = .164). For the hal 0.25 mg/kg and hal 0.75 mg/kg groups, day 2 activity was significantly higher than day 1 activity (F[1, 5] =7.50, p = .014; F[1, 5] = 27.53, p = .003). For the hal 1.0 mg/kg group, activity changed across days, F(2, 16) = 14.74, p < .001; both days 2 and 3 were more active than day 1 (ps < .004), but days 2 and 3 did not significantly differ (p = .310). Activity also heightened across days for the hal 2.0 mg/kg group (F[2, 16] = 10.00, p = .002), with each day being more active than the previous (ps < .023). Neither of the pre-session haloperidol groups (1.0 mg/kg and 2.0 mg/kg) showed changes over days (F[2, 12] = 0.45, p = .651; F[2, 16] = 0.48, p = .629).

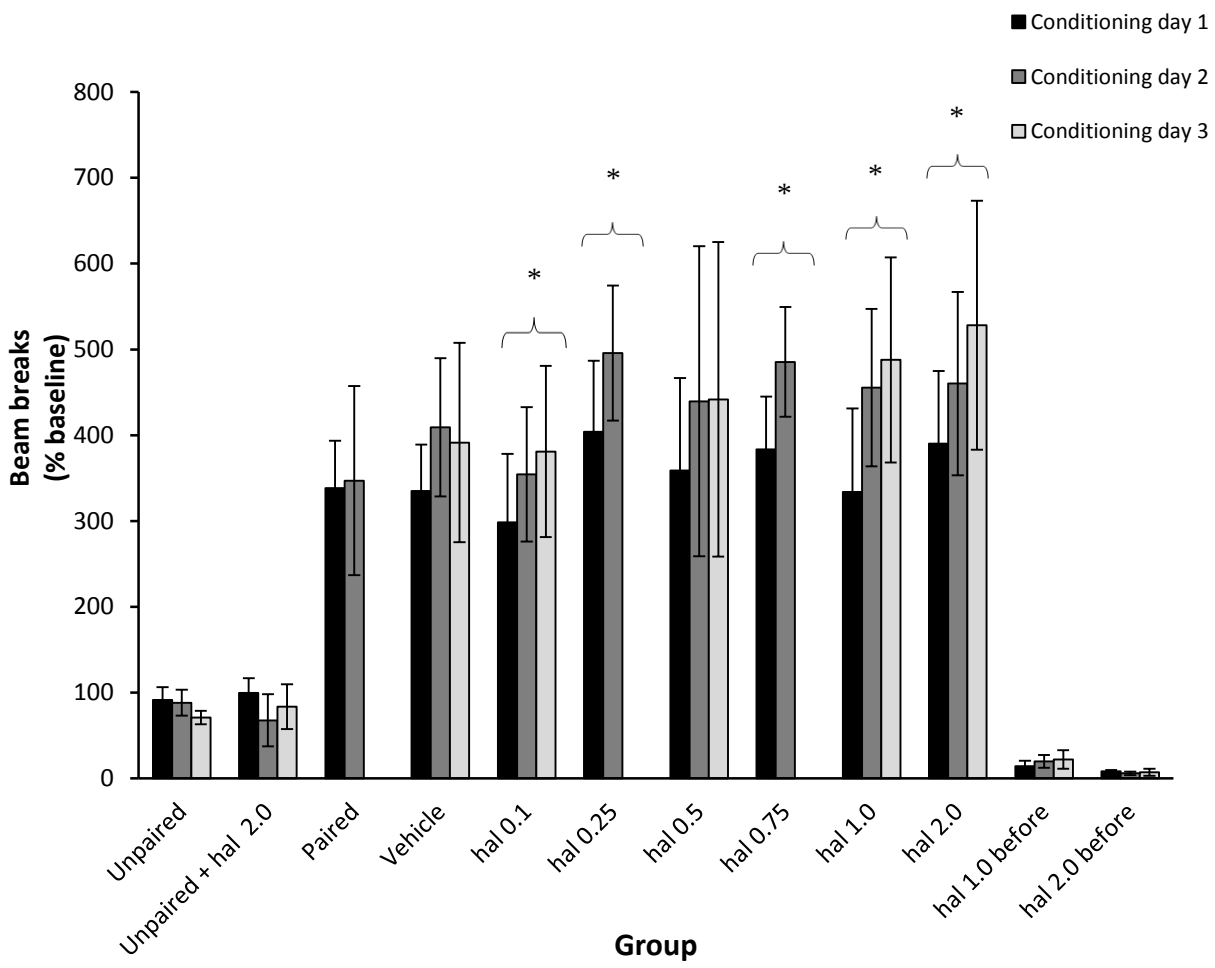


Figure 4. Locomotor activity across each conditioning day. Mean beam breaks (± 2 *SEM*) during days 1, 2, and 3 of conditioning for all groups. Means displayed are those used in follow-up or planned one-way ANOVA of each group. Values are expressed as a percentage of baseline (last day of habituation). Numbers beside 'hal' represent haloperidol dose in mg/kg. The hal 1.0 mg/kg and 2.0 mg/kg before groups received haloperidol 1 h before conditioning sessions; all other haloperidol groups received it 5 min after. * significant ($p < .05$) increase over days by within-subjects ANOVA. For the paired, hal 0.25 mg/kg and hal 0.75 mg/kg groups, ANOVA were conducted across days 1 and 2 only. *Ns* = 6 for the paired, hal 0.25 mg/kg, hal 0.75 mg/kg, and unpaired + hal groups; *n* = 7 for the hal 1.0 mg/kg group; *ns* = 9 for all other groups.

Chapter 4

Discussion

This was the first study to examine the role of D2 receptors in the consolidation of incentive learning measured by the conditioned activity paradigm. The main results can be summarized as follows: (1) animals that had received the amphetamine-context pairing showed higher test day activity than those that had not, confirming conditioned activity; (2) haloperidol at a range of doses (0.1-2.0 mg/kg) 5 min after amphetamine conditioning sessions did not block the consolidation of conditioned activity, whereas I had hypothesized that it would; (3) haloperidol at 2.0 mg/kg (but not at 1.0 mg/kg) 1 h before conditioning sessions blocked the acquisition of conditioned activity, supporting my prediction; and (4) post-session haloperidol did not produce any differences in activity over conditioning days, whereas I had hypothesized that it would mildly suppress activity. These findings will be expanded upon below.

The groups showed pre-existing differences in activity levels before any conditioning had started. One possible reason is that they were tested across the summer and fall, and the facility is not fully climate controlled. Our laboratory has found that rats' locomotor activity levels tend to decline as heat and humidity increase (Rocca, 2014). Although I tested several groups at a time to counteract this effect, it could only help to a limited extent because of the number of groups in the experiment (k = 12). As such, activity on the last day of habituation was used as the baseline, and pre-existing variability was eliminated.

It should be noted that no saline injection was given before the test, even though this is often done in the conditioned activity paradigm (e.g., Mazurski & Beninger, 1988). An injection on the test day might serve as an additional incentive cue because it simulates the conditioning day more closely, which could elicit a larger conditioned response. However, all groups forewent a saline injection, so it is unlikely that the patterns of results were affected. In fact, the absence of the injection resulted in the

habituation and test day injection schedules being identical, which then made it possible for the test day scores to be expressed as a percentage of habituation without any extra variability produced a possible response to the injection.

Experiment 1 demonstrated that animals receiving amphetamine in the test context displayed higher activity than those that had received it in home cages, establishing the paradigm. Therefore, stimuli in the testing environment became incentive stimuli as expected. Experiment 1 also served to check if haloperidol produced a withdrawal effect, manifested as increased activity in the unpaired + hal group; it did not. This check was done because a previous study in our lab on conditioned activity found that rats displayed a hypoactive response 23 h after caffeine injections (Herz & Beninger, 1987). The authors had first speculated that the rats were anticipating caffeine injections and were showing a compensatory effect. However, they ruled out this possibility because rats unaccustomed to receiving caffeine injections after sessions also showed this hypoactive response. They therefore deduced that the hypoactivity was a physiological withdrawal response to caffeine (Herz & Beninger, 1987). In the present study, I examined if haloperidol produces an analogous effect—a *heightening* of activity 23 h later—because haloperidol generally lowers activity (Ahlenius & Hillegaart, 1986). The effect would be seen on the test day, which is 23 h after the last post-session injection of haloperidol (2.0 mg/kg). The results indicated that the unpaired + haloperidol group did not differ significantly from the unpaired group on the test day, eliminating this concern. The minimal difference between the two groups also indicates that haloperidol did not produce lingering hypoactivity 23 h afterward, either.

In experiment 2, post-session haloperidol at all doses tested (0.1-2.0 mg/kg, i.p.) neither disrupted nor enhanced the consolidation of amphetamine-produced conditioned activity. There were differences among post-session haloperidol groups, but there was no discernible pattern between dose and effect. These differences might have resulted from chance or sampling error, since rats supplied by the breeder may have originated from various colonies, which may have resulted in genetic differences that affected

behavioural responses (Harris, 1965). I had predicted that haloperidol would block consolidation for several reasons: (1) DA is implicated in the reconsolidation of conditioned activity (Carrera et al., 2011); (2) D2 antagonists impede the acquisition of conditioned activity (Beninger & Hahn, 1983); and (3) D2 antagonists disrupt the early consolidation of various types of learning, such as striatal-based radial arm maze learning (Packard & White, 1991). Although the results of the present study suggest that D2 receptors are not implicated in the consolidation of incentive learning, certain novel factors of the design may have affected the outcome. Possible reasons are discussed below.

Experiment 3 investigated whether haloperidol would disrupt the acquisition of conditioned activity. Haloperidol at 2.0 mg/kg blocked the acquisition of conditioned activity, but 1.0 mg/kg of haloperidol did not, although it trended toward a block. Haloperidol at 1.0 mg/kg, i.p., is a high dose (Mattingly, Rowlett, Ellison, & Rase, 1996), and although its may appear surprising that it did not block acquisition, amphetamine at 2.0 mg/kg, i.p., is also a relatively high dose. Haloperidol at 1.0 mg/kg might not have been able to fully block acquisiton produced by this higher dose. Nonetheless, experiments 2 and 3 together suggest that haloperidol blocked the acquisition of conditioned activity at a dose that did not block the consolidation of amphetamine-produced conditioned activity.

The ANOVA in experiments 2 and 3 were not followed up with Dunnett's post hoc tests as was done in experiment 1, since in experiments 2 and 3 it was of interest to examine how haloperidol groups compared against one another, as well as to see where they stood relative to the unpaired group (lower reference) and paired group (upper reference). It should be noted that the Fisher's LSD tests that were instead used are more liberal than other post hoc tests. More conservative tests were not used because group sizes were relatively low (*ns* = 9) and because the design was between-subjects and not within-subjects; these factors generally reduce statistical power. Nonetheless, the fact that liberal post hoc testing was used should be noted as a possible limitation in this study.

The final analysis examined activity during the conditioning phase. Groups were generally separated into two levels of activity: low ($M \approx 46\%$) for the unpaired and pre-session haloperidol groups, and high ($M \approx 412\%$) for all other groups (paired, vehicle, and post-session haloperidol). Therefore, haloperidol inhibited the stimulant effect of amphetamine when given 1 h before, but injections 5 min after did not appear to influence the stimulant effect on subsequent days. There was a pattern that the haloperidol 0.1 mg/kg group was less active than the haloperidol 2.0 mg/kg during the conditioning and test phases. Although this might appear to indicate a parallel between acute activity (influenced by the drugs) and conditioned activity, other group relationships found on the test day were not found on the conditioning days. For instance, the pre-session haloperidol 1.0 mg/kg group was slightly less active than the unpaired group during conditioning, yet it was significantly more active than unpaired on the test day. This observation therefore suggests that the *acute* motor effects influenced by the drug do not fully correspond to the *conditioned* activity revealed on the test day.

When the activity curve is examined across days, it appears that all the post-session groups, with the exception of the haloperidol 0.5 mg/kg group, experienced a general escalation. This trend might reflect sensitization, namely, that the chamber gained the ability to elicit activity that surpassed the initial unconditioned response to the drug (de Wit & Stewart, 1981; Badiani & Stewart, 1993). Therefore, haloperidol likely did not impede the consolidation of sensitization. Paired group activity increased slightly from day 1 to day 2, and day 3 data are missing. Vehicle group activity increased non-significantly from day 1 to day 2. Thus, although not significant, activity of these groups was tending to show sensitization like that seen in haloperidol groups. Activity for the unpaired rats declined over days, demonstrating habituation, and activity for the rats receiving pre-session haloperidol remained flat. Therefore, haloperidol during conditioning sessions possibly blocked the acquisition of the sensitization effect. In general, the activity curves did not predict the presence of conditioned activity, an observation previously reported (Mazurski & Beninger, 1991). Activity during the drug-free test may be a better index

of incentive learning than that during conditioning because it eliminates acute drug effects and compares groups on an 'equal' footing.

One of the benefits of post-training injections is that they do not directly influence motivational or sensory processes during the animal's active learning stage. Despite this, there are some possible sources of interference. One is that the anticipation of an injection could cause anxiety, which may influence locomotion. However, this may not have occurred in this study because the paired group and the vehicle group (differing only by a post-session vehicle injection) had nearly identical activity during the conditioning and test phases. Secondly, memory consolidation researchers Carey (1987) and McGaugh (1989) have questioned whether post-training injections could change an animal's perception of the session's emotional valence. For instance, if the animal receives epinephrine after a passive avoidance session, it might misattribute the high arousal as having resulted from the shocks that occurred during the training (Carey, 1987). This argument has been proposed for fear-motivated tasks, but it should be noted that incentive learning is also an associative task that corresponds to emotional states (Baik, 2013). Perhaps the 'incentive valence' is influenced. However, there is no evidence in this study that either of these possibilities occurred, given that the unpaired + haloperidol group did not show any differences in incentive learning compared to the unpaired group. Additionally, these conjectures are not necessarily based on neural mechanisms but instead on subjective states.

One somewhat novel aspect of this study's design was that it used 60-min training sessions. The majority of post-training injection studies mentioned above generally used sessions ranging from 5 to 15 min, although several experiments used tasks lasting 30 or 60 min (Kramar et al., 2014). One can ask at what point during the 60-min sessions animals acquired conditioned activity. This question might prove challenging because conditioned activity is a continuous measure and does not have a discrete endpoint, such as reaching a platform. To obtain an answer, one can test whether shorter sessions (e.g., lasting 45 or 30 min, or even shorter) can reproduce the paradigm, as shown by a significant difference between the

unpaired and paired groups. If a significant difference occurred with 30-min sessions, for instance, one could argue that 30 min is the acquisition phase and that the time afterward spills over into the consolidation phase. If so, in this study, haloperidol would have been administered 35 min after consolidation began (i.e., the remaining 30 min of the session plus the 5 min after removal from the chamber), and the delivery of haloperidol via injection would have taken additional time to block D2 receptors. This timeframe may be too long to measure early consolidation and too late to test the possible second 'wave' of consolidation, since treatments that are not immediate or delayed by 3-6 h generally do not affect the consolidation phase (Igaz et al., 2002; Abel & Lattal, 2001). Moreover, in this scenario, the presence of amphetamine during the second half of the session would have further consolidated the incentive memory trace (Wise, 2009). Either or both of these reasons could have contributed to the null findings in this study, assuming that acquisition takes less than 60 min. In the future, testing a D2 receptor antagonist with a shorter onset of action than haloperidol might also help target the early consolidation. Once the consolidation period of conditioned activity is established, post-session haloperidol, or a D2 antagonist with a shorter onset of action than haloperidol, could then be used to re-examine the role of DA in the consolidation phase of conditioned activity.

This study was also somewhat atypical in that it involved multiple training sessions that were each followed by an injection. Only two post-training studies mentioned in this thesis had this design; both involved incentive learning tasks. In one study, rats lever-pressed for 15 min each day for 5 days, each followed by anisomycin injections; this method attenuated lever response rates (Hernandez et al., 2002). The other study involved a six-day cocaine-CPP experiment and found that intra-hippocampal D1 agonism 12 after each trial weakened the persistence the memories (Kramar et al., 2014). Despite these findings, certain researchers have criticized multi-trial consolidation studies, stating that they muddle the stages of learning, and instead recommend single-trial procedures, such as passive avoidance training (Izquierdo et al., 2006; Abel & Lattal, 2001). If a certain type of learning takes a minimum of two

sessions to acquire, one could argue that it is unclear whether the injection after the first day blocked *consolidation* or the broader *acquisition* of the memory trace. Similarly, it might be challenging to distinguish consolidation from reconsolidation in multi-trial paradigms. A previous study in our laboratory demonstrated that conditioned activity could be achieved with *two* 60-min sessions using 2.0 mg/kg of amphetamine (Mazurski & Beninger, 1987). Therefore, this could indicate that the third conditioning day in the present study reconsolidated, rather than consolidated, conditioned activity, given that on the third day, exposure to cues might have reactivated the putatively already-established (and -consolidated) memory trace and returned it to a labile state (Nader et al., 2000). Despite these possibilities, D2 receptors have been implicated in the acquisition of conditioned activity (Beninger & Hahn, 1983) and in the reconsolidation of drug cue memories (Wu et al., 2011), so it may not be the case that overlaps in memory phases produced the negative results in this study.

It might be inevitable that certain types of learning require multiple spaced trials for the memory trace to enter into a long-term state (Kandel, 2012). This could be especially true of habit-based and striatal forms of memory (Pinsker, Hening, Carew, & Kandel, 1973). In one post-training injection study that involved a *two*-day passive avoidance task with mice, the authors used central injections to deduce that the hippocampus consolidated the first trial whereas the striatum consolidated the second trial; the same inhibitors (e.g., of PKA and NMDA receptors) blocked consolidation on both days (Cammarota, Bevilaqua, Köhler, Medina, & Izquierdo, 2005). The authors stated that the memory trace likely 'shifted' from the hippocampus to the striatum, a type of systems consolidation that possibly demonstrates a transition from declarative to habit memory (Izquierdo et al., 2006). A single-trial paradigm would have overlooked both the synaptic and systems consolidation that occurred on the second day. Incentive learning relies greatly on habit formation, so multi-trial paradigms might be necessary for its full characterization (Beninger, 1983). In general, the dynamic nature of memory, as well as the lack of consensus in memory phase definitions, proves it difficult to clearly delineate memory stages (Abel &

Lattal, 2001; Kandel, 2012). This said, certain steps can be taken to clarify the stages of paradigms such as conditioned activity. One would be to test the minimum number of sessions needed to acquire the paradigm. With this information, it might be possible to avoid instances of reconsolidation in later sessions. Additionally, one could give a post-training injection after the critical point to ensure that acquisition remains unchanged. For instance, Packard and White (1991) gave intra-striatal infusions in rats only after the fifth day of radial arm maze training and obtained a significant block. Finally, other techniques, such as functional neuroimaging or neuronal tagging, could provide high temporal and spatial resolution in tracking memory traces throughout their lifespan (Josselyn et al., 2015). These findings could clarify at what point during training that memory traces become stable.

Attempting this study with certain changes, as outlined above, might reveal a role of the D2 receptor in the consolidation of incentive learning. An alternative interpretation of this study is that D2-like receptors are simply not as involved in the early consolidation of conditioned activity as they are in its acquisition. The widely-established involvement of the D1/cAMP/PKA pathway in long-term memory formation and consolidation provides another mechanism (Kandel et al., 2012; Bernabeu et al., 1997). One key player appears to be the cAMP-responsive element binding protein (CREB), a transcription factor phosphorylated by PKA that influences gene expression and produces synaptic modifications (Josselyn et al., 2015). A recent study in mice employed genetic techniques to track the 'cocaine memory' trace in rats trained in the cocaine-CPP paradigm. It was found that neurons made to overexpress CREB before conditioning were preferentially allocated to the memory trace (Hsiang et al., 2014). Because CREB is activated primarily by D1/cAMP/PKA signaling, findings like these appear to place further importance on D1-like receptor activity. Perhaps D2 receptors are needed during acquisition to induce long-term depression of the 'NoGo' pathway in the basal ganglia in order to amplify the 'Go' signal of D1/cAMP/PKA/CREB signaling, which carries forth the synaptic growth, and therefore, the consolidation (in other words, the increased 'Go' signal would bring forth the hyperactive conditioned

response; Wickens, 2009). Another possibility is that signaling molecules downstream of D2 receptor activity act in a delayed manner to consolidate incentive memories. The signaling molecule glycogen synthase kinase-3β (GSK3β), activated by D2 receptor binding, has been implicated in acquisition and reconsolidation of amphetamine and cocaine-based incentive learning paradigms, respectively (Rocca, 2014; Shi et al., 2014). The same dose of amphetamine used in this study, 2.0 mg/kg, has been shown to cause a peak in GSK3β activity in the striatum 90 min after injection (Beaulieu et al., 2004). Therefore, perhaps D2 receptor stimulation during the acquisition of learning leads to the delayed activation of GSK3β that then affects consolidation. If this were the case, it might explain why D2 receptor blockade during consolidation did not produce deficits despite that D2 receptors could play an indirect role. More studies are needed to elucidate the dynamic relationship between D1- and D2-like receptors and their related second messengers during the acquisition and consolidation of incentive learning.

Finally, a better understanding of incentive learning mechanisms may lead to the development of solutions for those suffering with addiction. For the chronic methamphetamine user, the sight of a syringe can trigger many reactions within the body, such as increased heart rate and feelings of craving (Kalivas & Volkow, 2005) and can precipitate a relapse, even after a period of abstinence (O'Brien, Childress, Ehrman, & Robbins, 1998). Cue exposure without reinforcement is unfortunately not sufficient to extinguish the cues permanently (de Wit & Stewart, 1981). Memory reconsolidation interference techniques, however, may hold some promise in effectively extinguishing cues. One such therapy in humans could work as follows: first, the patients would be exposed to cues highly associated with the drug (e.g., a needle) in order to reactivate the specific incentive memories, rendering the memory trace labile (Nader et al., 2000). Immediately afterward, the patients would receive a treatment (e.g., pharmacological) shown to block the reconsolidation of incentive memories. This would then impede the permanent stability of the incentive memory, possibly dampening the incentive value of drug cues. Given animal studies of reconsolidation, these effects might last several weeks and reduce cue-elicited drug-

seeking, all the while leaving contextual learning intact (Lee, Milton & Everitt, 2006; Shi et al., 2014). However, more research is needed to ensure the safety of this treatment. Better insight into reward-related memory mechanisms may lead to innovative methods of memory manipulation that were previously inconceivable. Ultimately, these therapies may hold promise in bringing relief to those with addiction.

Conclusion

In summary, this study was the first to investigate the role of DA D2 receptors in the consolidation of reward-related memories, which was achieved by administering haloperidol shortly after conditioned activity sessions in rats. It was found that haloperidol blocked the acquisition but not consolidation of amphetamine-cue memoires. Although this may indicate that D2 receptors are not involved in the consolidation phase, further tests are needed to delineate the acquisition and consolidation phases in this paradigm in order to accurately interpret these data. A clearer understanding of incentive learning mechanisms may lead to the development of memory manipulation techniques for those suffering with drug addiction, a disorder of incentive learning.

References

Abel, T., & Lattal, K. M. (2001). Molecular mechanisms of memory acquisition, consolidation and retrieval. *Current Opinion in Neurobiology*, *11*(2), 180–187.

Adams, J. U., Careri, J. M., Efferen, T. R., & Rotrosen, J. (2001). Differential effects of dopamine antagonists on locomotor activity, conditioned activity and conditioned place preference induced by cocaine in rats. *Behavioural Pharmacology*, *12*(8), 603–11.

Adinoff, B. (2004). Neurobiologic processes in drug reward and addiction. *Harvard Review of Psychiatry*, *12*(6), 305–320.

Ahlenius, S., & Hillegaart, V. (1986). Involvement of extrapyramidal motor mechanisms in the suppression of locomotor activity by antipsychotic drugs: A comparison between the effects produced by pre- and post-synaptic inhibition of dopaminergic neurotransmission. *Pharmacology Biochemistry & Behavior*, *24* (5), 1409-1415.

Ahmed, S. H., Stinus, L., & Cador, M. (1998). Amphetamine-induced conditioned activity is insensitive to perturbations known to affect Pavlovian conditioned responses in rats. *Behavioral Neuroscience*, *112*(5), 1167-1176.

Badiani, A., & Stewart, J. Enhancement of the prophagic but not of the antidipsogenic effect of U-50,488H after chronic amphetamine pretreatment. *Pharmacology Biochemistry & Behavior*, 1993, 44, 77-86.

Baik, J.-H. (2013). Dopamine signaling in reward-related behaviors. *Frontiers in Neural Circuits*, *7*(10), 152.

Banasikowski, T. J., Bespalov, K., Drescher, B., Behl, L., Unger, A. Haupt, H.,… & Beninger, R. J. (2010). Double dissociation of the effects of haloperidol and the dopamine D3 receptor antagonist

ABT-127 on acquisition vs. expression of cocaine-conditioned activity in rats. *Pharmacology and Experimental Therapeutics*, *335*(2), 506-515.

Banasikowski, T. J., McLeod, L. S., & Beninger, R. J. (2012). Comparison of nafadotride, CNQX, and haloperidol on acquisition versus expression of amphetamine-conditioned place preference in rats. *Behavioural Pharmacology*, *23*(1), 89-97.

Beaulieu, J.-M., & Gainetdinov, R.R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological Reviews*, *63*, 1182-1217

Beaulieu, J.-M., Sotnikova, T. D., Yao, W.-D. Kockeritz, L., Woodgett, J. R., Gainetdinov, R.R, & Caron, M.G. (2004). Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(14), 5099-104

Beninger, R. J. (1983). The role of dopamine in locomotor activity and learning. *Brain Research Reviews*, *6*, 173-196.

Beninger, R. J. (1993). Role of D1 and D2 receptors in learning. In J. L. Waddington (Ed.), *D1:D2 dopamine receptor interactions: Neuroscience and neuropharmacology* (115-157). London: Academic Press.

Beninger, R. J., & Hahn, B. L. (1983). Pimozide blocks establishment but not expression of amphetamine-produced environment-specific conditioning. *Science*, *220*(4603), 1304–1306.

Beninger, R.J., & Herz, R.S. (1986). Pimozide blocks establishment but not expression of cocaineproduced environment-specific conditioning. *Life Sciences*, *38*, 1425-1431.

Benoit-Marand, M., Borelli, E., & Gonon, F. (2001). Inhibition of dopamine release via presynaptic d2 receptors: Time course and functional characteristics *in vivo. The Journal of Neuroscience*, *21*(23), 9134-9141.

Bernabeu, R., Bevilaqua, L., Ardenghi, P., Bromberg, E., Schmitz, P., Bianchin, M., … Medina, J. H. (1997). Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(13), 7041–7046.

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*, *28*(3), 309–369.

Bindra, D. (1968). Neuropsychological interpretation of the effects of drive and incentive-motivation on general and instrumental behavior. *Psychological Review, 75*, 1-22.

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *The Journal of Physiology*, *232*(2), 331 – 356.

Bromberg-Martin, E. S., Matsumoto, M., & Hikosata, O. (2010). Dopamine in motivational control: rewarding, aversive, and alerting. *Neuron*, *68*(5), 815-834.

Cajal, S. R. Y. (1894) The Croonian Lecture: La Fine Structure des Centres Nerveux. *Proceedings of the Royal Society of London*, *55*, 444-468.

Cammarota, M., Bevilaqua, L. R. M., Köhler, C., Medina, J. H., & Izquierdo, I. (2005). Learning twice is different from learning once and from learning more. Neuroscience, *132*(2), 273–279.

Carey, R. J. (1987). Posttrial hormonal treatment effects - memory modulation or perceptual-distortion. *The Journal of Neuroscience Methods*, *22*(1), 27–30.

Carrera, M. P., Carey, R. J., Dias, F. R., & De Matos, L. W. (2011). Reversal of apomorphine locomotor sensitization by a single post-conditioning trial treatment with a low autoreceptor dose of apomorphine: A memory re-consolidation approach. *Pharmacology Biochemistry and Behavior*, *99*(1), 29–34.

Carrera, M. P., Carey, R. J., Dias, F. R., & De Matos, L. W. (2012). Memory re-consolidation and drug conditioning: An apomorphine conditioned locomotor stimulant response can be enhanced or reversed by a single high versus low apomorphine post-trial treatment. *Psychopharmacology*, *220*(2), 281–291.

Castellano, C., Cestari, V., Cabib, S., & Puglisi-Allegra, S. (1991). Post-training receptor agonists and antagonists affect memory storage in mice irrespective of their selectivity to D1 and D2 receptors. *Behavioral and Neural Biology*, *56*(3), 283-291.

Cervo L., & Samanin R. (1995). Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. *Brain Research, 673*, 242-250.

Dawson, T. M., & Dawson, V. L. (2003). Molecular pathways of neurodegeneration in Parkinson's disease. *Science*, *302*(5646), 819-822.

de Wit, H., & Stewart, J. (1981). Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology*, *75*(2), 134-143.

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(14), 5274-5278.

Dias, F. R., Carey, R. J., & Carrera, M. P. (2006). Conditioned locomotion induced by unilateral intrastriatal administration of apomorphine: D(2) receptor activation is critical but not the expression of the unconditioned response. Brain Res, *1083*(1), 85-95.

Dudai, Y. (2004). The neurobiology of consolidations, or, how stable is the engram? *Annual Review of Psychology*, *55*, 51–86.

Duncan, C. P. (1949). The retroactive effect of electroshock on learning. *Journal of Comparative and Physiological Psychology*, *42*(1), 32-44.

Eikelboom, R., & Stewart, J. (1982). Conditioning of drug-induced physiological responses. *Psychological Review*, *89*(5), 507-528.

Flood, J. F., Baker, M. L., & Davis, J. L. (1990). Modulation of memory processing by glutamic acid receptor agonists and antagonists. *Brain Research*, *521*(1-2), 197–202.

Flood, J. F., Rosenzweig, M. R., Bennett, E. L., & Orme, A. E. (1973). The influence of duration of protein synthesis inhibition on memory. *Physiology and Behavior*, *10*(3), 555–562.

Frank, M. J. (2005). Dynamic Dopamine Modulation in the Basal Ganglia: A Neurocomputational Account of Cognitive Deficits in Medicated and Nonmedicated Parkinsonism. *Journal of Cognitive Neuroscience*, *17*(1), 51–72.

Grogan J., Bogacz, R., Tsivos, D., Whone, A. & Coulthard, E. (2015) Dopamine and consolidation of episodic memory: Timing is everything. *Journal of Cognitive Neuroscience*, *10*, 2035-2050

Harris (1965). Differences in responses between rat strains and colonies. *Food and Cosmetics Toxicology*, *3*, 199-202.

Hebb, D. O. (1949). *Organization of behavior: A neuropsychological theory*. New York, NY: John Wiley & Sons.

Hernandez, P. J., Sadeghian, K., & Kelley, A. E. (2002). Early consolidation of instrumental learning requires protein synthesis in the nucleus accumbens. *Nature Neuroscience*, *5*(12), 1327–1331.

Herz, R. S., & Beninger, R. J. (1987). Comparison of the ability of (+)-amphetamine and caffeine to produce environment-specific conditioning. *Psychopharmacology*, *92*(3), 365–370.

Hiroi, N., & White, N. M. (1991). The amphetamine conditioned place preference: Differential involvement of dopamine receptor subtypes and two dopaminergic terminal areas. *Brain Research*, *552*(1), 141-152.

Hsiang, H.-L. L., Epp, J. R., van den Oever, M. C., Yan, C., Rashid, A. J., Insel, N., … Josselyn, S. A. (2014). Manipulating a "cocaine engram" in mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *34*(42), 14115–14127.

Igaz, L. M., Bekinschtein, P., Vianna, M. M. R., Izquierdo, I., & Medina, J. H. (2004). Gene expression during memory formation. *Neurotoxicity Research*, *6*(3), 189–203.

Igaz, L. M., Vianna, M. R., Medina, J. H., & Izquierdo, I. (2002). Two time periods of hippocampal mRNA synthesis are required for memory consolidation of fear-motivated learning. *The Journal of Neuroscience*, *22*(15), 6781–6789.

Inda, M. C., Delgado-Garcia, J. M., Carrion, Á. M. (2005). Acquisition, consolidation, reconsolidation, and extinction of eyelid conditioning responses require de novo protein synthesis. *The Journal of Neuroscience*, *25*(8), 2070-2080.

Izquierdo, I., & Medina, J. H. (1997). Memory formation: The sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiology of Learning and Memory*, *68*(3), 285–316.

Izquierdo, I., Bevilaqua, L. R. M., Rossato, J. I., Bonini, J. S., Da Silva, W. C., Medina, J. H., & Cammarota, M. (2006). The connection between the hippocampal and the striatal memory systems of the brain: A review of recent findings. *Neurotoxicity Research*, *10*(2), 113–121.

Josselyn, S.A., Köhler, S., & Frankland, P. W. (2015). Finding the engram. *Nature Reviews Neuroscience*. *16*(9), 521-534.

Joyce, J. N., & Marshall, J. F. (1987). Quantitative autoradiography of dopamine D2 sites in rat caudate-putamen: Localization to intrinsic neurons and not to neocortical afferents. *Neuroscience*, *20*, 773–795.

Kalivas, P. W., & Volkow, N. D. (2005). The neural basis of addiction: A pathology of motivation and choice. *The American Journal of Psychiatry*, *162*(8), 1403-1413

Kandel, E. R. (2012). The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB, *Molecular Brain, 5*(1), 14.

Kebabian, J. W., & Calne, D. B. (1979). Multiple receptors for dopamine. *Nature, 277*, 93-96.

Kelley, A. E. (2004). Memory and addiction shared neural circuitry and molecular mechanisms. *Neuron, 44*(1), 161–179.

Kelley, A. E., Smith-Roe, S. L., & Holahan, M. R. (1997). Response-reinforcement learning is dependent on *N*-methyl-D-aspartate receptor activation in the nucleus accumbens core. *Proceedings of the National Academy of Sciences of the United States of America, 94*(22), 12174–9.

Kramar, C. P., Chefer, V. I., Wise, R. A., Medina, J. H., & Barbano, M. F. (2014). Dopamine in the dorsal hippocampus impairs the late consolidation of cocaine-associated memory. *Neuropsychopharmacology, 39*(4), 1645-1653.

Lee, J. L. C., Milton, A. L., & Everitt, B. J. (2006). Cue-Induced Cocaine Seeking and Relapse Are Reduced by Disruption of Drug Memory Reconsolidation. *The Journal of Neuroscience, 26*(22), 5881–5887.

Lee, K. N., & Chirwa, S. (2015). Blocking dopaminergic signaling soon after learning impairs memory consolidation in Guinea Pigs. *Public Library of Science ONE, 10*(8), 1–15.

Lüscher & Malenka (2011). Drug-evoked synaptic plasticity in addiction: From molecular changes to circuit remodeling. *Neuron, 69*(4), 650-663.

Mactutus, F. C., Riccio, D. C., & Ferek, J. M. (1979). Retrograde amnesia for old (reactivated) memory: some anomalous characteristics. *Science, 204*(4399), 1319-1320.

Martelle, J. L., & Nader, M. A. (2008). A review of the discovery, pharmacological characterization, and behavioral effects of the dopamine D2-like receptor antagonist eticlopride. *CNS Neuroscience & Therapeutics, 14*(3), 248-262.

Martin-Iverson, M. T., & Fawcett, S. L. (1996). Pavlovian conditioning of psychomotor stimulant-induced behaviours: Has convenience let us astray? *Behavioural pharmacology, 7*(1), 24-41

Mattingly, B. A., Rowlett, J. K., Ellison, T., & Rase, K. (1996). Cocaine-induced behavioral sensitization: Effects of haloperidol and SCH 23390 treatments. *Pharmacology Biochemistry & Behavior, 53*(3), 481-186.

Mazurski, E. J., & Beninger, R. J. (1987). Environment-specific conditioning and sensitization with (+)-amphetamine. Pharmacology, Biochemistry and Behavior, *27*(1), 61–65.

Mazurski, E. J., & Beninger, R. J. (1988). The dopamine D-2 agonist quinpirole produces environment-specific conditioned activity. *Pharmacology, Biochemistry and Behavior, 30*(2), 525–527.

Mazurski, E. J., & Beninger, R. J. (1991). Effects of selective drugs for dopaminergic D1 and D2 receptors on conditioned locomotion in rats. *Psychopharmacology*, *105*, 107-112.

McGaugh, J. L. (1966). Time-dependent processes in memory storage. *Science, 153*(2742), 1351-1358.

McGaugh, J. L. (1989). Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. *Annual Review of Neuroscience, 12*(1), 255–287.

McGaugh, J. L., & Krivanek, J. A. (1970). Strychnine effects on discrimination learning in mice: Effects of dose and time on administration. *Physiology & Behavior, 5*(12), 1437-1442.

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature, 406*, 722-726.

Nestler, E. J., Hyman, S., & Malenka, R. (2009). *Molecular neuropharmacology: A foundation for clinical neuroscience* (2nd ed.). New York, NY: McGraw-Hill Medical.

Nishida, M., Pearsall, J., Buckner, R. L., & Walker, M. P. (2009). REM sleep, prefrontal theta, and the consolidation of human emotional memory. *Cerebral Cortex, 19*(5), 1158-1166.

O'Brien, C. P., Childress, A. R., Ehrman, R., & Robbins, S. J. (1998). Conditioning factors in drug abuse: Can they explain compulsion? *Journal of Psychopharmacology, 12*(1), 15–22.

Olds, J. (1956). A preliminary mapping of electrical reinforcing effects in the rat brain. *Journal of Comparative and Physiological Psychology*, *49*(3), 281-285.

Packard, M. G., & Knowlton, B. J. (2002). Learning and memory functions of the Basal Ganglia. *Annual Review of Neuroscience, 25*, 563-593.

Packard, M. G., & White, N. M. (1989). Memory facilitation produced by dopamine agonists: Role of receptor subtype and mnemonic requirements. *Pharmacology Biochemistry & Behaviour*, *33*(3), 511-518.

Packard, M. G., & White, N. M. (1991). Dissociation of hippocampus and caudate nucleus memory systems by posttraining intracerebral injection of dopamine agonists. *Behavioral Neuroscience*, *105*(2), 295-306.

Pickens, R. W., & Crowder, W. F. (1967). Effects of CS-US interval on conditioning of drug response, with assessment of speed of conditioning. *Psycopharmacologia*, *11*, 88-94.

Pinsker, H. M., Hening, W. A., Carew, T. J. & Kandel, E. R. (1973). Long-term sensitization of a defensive withdrawal reflex in Aplysia. *Science, 182*(4116), 1039-42.

Post, R. M., Lockfeld, A., Squillace, K. M., & Contel, N. R. Drug-environment interaction: Context dependency of cocaine-induced behavioral sensitization. *Life Science*, *28*, 755-760.

Quartarone, S. E. (2014). *Effect of glycogen synthase kinase-3β on the acquisition & expression of intra-accumbal amphetamine-induced conditioned place preference in rats*. (Master's thesis, Queen's University, Kingston, Ontario). Retrieved from http://hdl.handle.net/1974/8544

Reynolds, J. N., Hyland, B. I., & Wickens, J. R. (2001). A cellular mechanism of reward-related learning. *Nature, 413*(6851), 67-70.

Rocca, J. F. (2014). *Effect of glycogen synthase kinase-3 inhibition on the acquisition and expression of cocaine-cue conditioned activity and spontaneous locomotion in rodents*. Master's thesis, Queen's University, Kingston, Ontario). Retrieved from http://hdl.handle.net/1974/12576

Scheel-Krüger, J. (1971). Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines in the brain. *European Journal of Pharmacology, 14*(1), 47-59.

Schultz, W. (1998). Predictive reward signal of dopamine neurons. *Journal of Neurophysiology, 80,* 1-27.

Schultz, W., Apicella, P. & Ljungberg, T. (1993). Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *The Journal of Neuroscience, 13*(3), 900-913.

Seeley, C. J., Beninger, R. J., & Smith, C. T. (2014). Post learning sleep improves cognitive emotional decision-making: Evidence for a 'Deck B Sleep Effect' in the Iowa Gambling Task. *Public Library of Science ONE, 9*(11); e112056.

Setlow, B., & McGaugh, J. L. (2000). D2 Dopamine receptor blockade immediately post-training enhances retention in hidden and visible platform versions of the water maze. *Learning and Memory, 7,* 187-191.

Shi, X., Miller, J. S., Harper, L. J., Poole, R. L., Gould, T. J., & Unterwald, E. M. (2014). Reactivation of cocaine reward memory engages the Akt/GSK3/mTOR signaling pathway and can be disrupted by GSK3 inhibition. *Psychopharmacology, 231*(16), 3109–3118.

Smith-Roe, S. L., & Kelley, A. E. (2000). Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. *The Journal of Neuroscience, 20*(20):7737-7742.

Squire, L. R., & Alvarez, P. (1995). Retrograde amnesia and memory consolidation: A neurobiological perspective. *Current Opinions in Neurobiology, 5*(2), 169-177.

Stewart, J., & Badiani, A. (1993). Tolerance and sensitization to the behavioral effects of drugs. *Behavioural Pharmacology, 4*(4), 289-312.

Stewart, J., de Wit, H., & Eikelboom, R. (1984). Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychological Review, 91*(2), 251–268.

Stewart, J., & Vezina, P. (1987). Environment-specific enhancement of the hyperactivity induced by systemic or intraVTA morphine injections in rats preexposed to amphetamine. *Psychobiology,15*(2), 144-153.

Sulzer, D., Chen, T. K., Lau, Y. Y. Kristensen, H., Rayport, S., & Ewing, A. (1995) Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *The Journal of Neuroscience, 15*, 4102-4108.

Takeuchi, T., Duszkiewicz, A. J., & Morris, R. G. M. (2014). The synaptic plasticity and memory hypothesis: encoding, storage and persistence. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1633), 20130288.

Theodorou, A., Reavill, C., Jenner, P. & Marsden, C. D. (1981). Kainic acid lesions of striatum and decortication reduce specific [3H]sulpiride binding in rats, so D-2 receptors exist post-synaptically on corticostriate afferents and striatal neurons. *Journal of Pharmacy and Pharmacology*, *33*, 439–444.

White, N. M., & Carr, G. D. (1985). The conditioned place preference is affected by two independent reinforcement processes. *Pharmacology, Biochemistry and Behavior*, *23*(1), 37–42.

White, N.M., Packard, M. G., & Hiroi, N. (1991). Place conditioning with dopamine D1 and D2 agonists injected peripherally or into nucleus accumbens. *Psychopharmacology, 103*(2), 271-276.

Wickens (2009). Synaptic plasticity in the basal ganglia. *Behavioural Brain Research, 199*(1), 119-128.

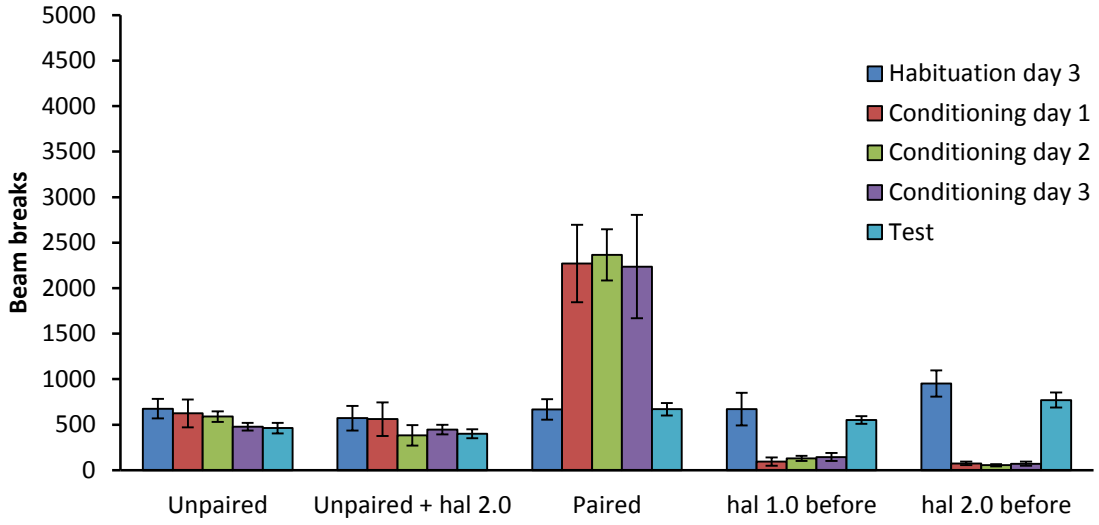
Wise, R. A. (2009). Roles for nigrosriatal—not just mesocorticolimbic—dopamine in reward and addiction. *Trends in Neurosciences*, *32*(10), 517–524.

Wise, R. A., & Rompré, P. P. (1989). Brain Dopamine and Reward. *Annual Review of Psychology, 40*, 191-225.

Wu, P., Xue, Y.X., Ding, Z.B., Xue, L.F., Xu, C.M., Lu, L. (2011). Glycogen synthase kinase 3β in the basolateral amygdala is critical for the reconsolidation of cocaine reward memory. *Journal of Neurochemistry, 118*, 113-125.

Appendix

(A)



(B)

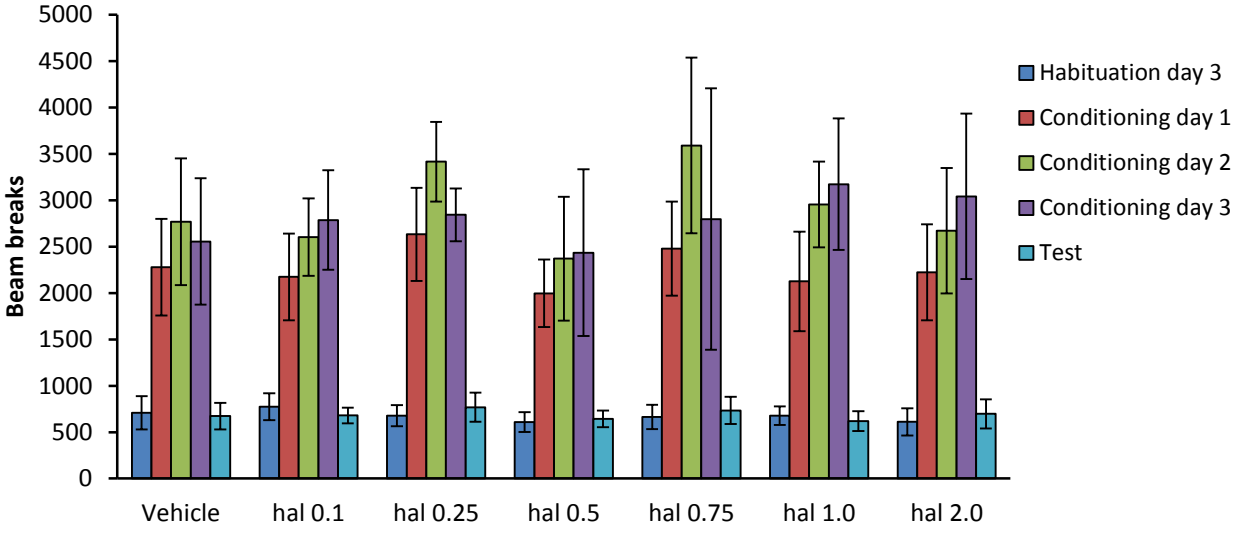


Figure 5. Original (non-adjusted) beam breaks on habituation day 3, conditioning days 1-3, and test day. Mean (± 2 *SEM*) counts during the 60 min sessions displayed for the (A) unpaired, unpaired + hal, and paired groups, and (B) the vehicle and all post-session haloperidol groups. Numbers beside 'hal' represent haloperidol dose in mg/kg.