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Comparison of the role of dopamine in egocentric and allocentric learning, two subtypes of navigation

A dissertation submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Ph.D.)

in the Graduate Program in Neuroscience of the College of Medicine 2014 by Amanda A. Braun B.A. College of Wooster 2007

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Successful navigation requires interactions among multiple but overlapping neural pathways mediating distinct capabilities, including egocentric (self-oriented, route-based) and allocentric (external, map-based) learning. Multiple neurotransmitters are involved in both navigation types, including dopamine. These studies sought to elucidate the region-specific role of dopamine in egocentric and allocentric learning.

The dopaminergic-rich dorsal striatum (dStr) is involved in both egocentric and allocentric navigation. We first tested whether dStr dopamine loss using bilateral 6-hydroxydopamine (6-OHDA) injection impaired one or both types of navigation. Direct dStr 6-OHDA injection resulted in 80% dStr dopamine depletion pre- and post-testing. Two weeks after 6-OHDA injections, rats began testing in the Cincinnati water maze (CWM) followed by Morris water maze (MWM), tests of egocentric and allocentric navigation, respectively. dStr 6-OHDA treatment significantly impaired CWM and MWM learning, but not MWM cued performance. These data support that dStr dopamine modulates both navigation types.

The dStr is divided into two heterogeneous sub-regions, the dorsolateral (DLS) and dorsomedial (DMS) striatum. Both regions have been implicated in egocentric learning, with the DMS also involved in allocentric learning. We next tested how selective DMS or DLS dopamine loss via 6-OHDA injection would impact one or both types of navigation. Both DMS and DLS lesioned animals were significantly impaired in CWM, but not allocentric or cued MWM performance. Dopamine loss in the DMS (62%) and DMS (75%) were region specific indicating independent roles for DMS and DLS dopamine in egocentric, but not allocentric learning. While the DMS is involved in allocentric learning, these processes do not appear to depend on DMS dopamine.
The nucleus accumbens (Nacc), another dopaminergic-rich striatal region, is involved in learning. Nacc dopamine depleted rats (60%) were tested in either the CWM or MWM. Nacc dopamine is implicated in reversal learning, thus this study tested allocentric reversal learning and egocentric reverse path CWM learning. Nacc dopamine depletion significantly impaired CWM, but not CWM reverse path performance. Lesioned animals were impaired in MWM acquisition, reversal, and cued trials. Off-target dopamine depletion in the dStr (20%) was at a sub-threshold level to influence egocentric or allocentric learning indicating a Nacc specific dopaminergic modulatory role in both navigation types.

Egocentric learning is dependent on the medial prefrontal cortex (mPFC), however the role of mPFC dopamine in egocentric learning has not been tested. 6-OHDA lesioned animals were tested in the CWM followed by the MWM. mPFC dopamine depletion (88%) did not alter CWM or MWM learning. These findings suggest that while the mPFC is necessary for egocentric learning, it is not dependent on or modulated by mPFC dopamine.

These data suggest independent roles for striatal sub-region (Nacc, dStr, DMS and DLS) dopamine involvement in egocentric learning. Allocentric learning is independently modulated by Nacc and dStr dopamine. Egocentric and allocentric learning do not appear to depend on mPFC dopamine levels.
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CHAPTER 1: Introduction

Navigation

Successful navigation requires highly complex interactions among multiple distinct but parallel cognitive processes including visual perception, spatial orientation, and learning and memory (Van Asselen et al., 2006). Spatial orientation can be subdivided into egocentric (route-based, self-oriented) and allocentric (map-based) frames of reference which can both represent the same spatial area (Byrne, 1982). In the allocentric Euclidean learning process, the navigator’s spatial orientation to objects in the environment is fluid and represented in a common coordinate system external to the navigator (Byrne, 1982). Euclidean navigation demands proper angle and distance representations between landmarks, and allows for flexibility in navigating between different target points (Garber, 2000). Route knowledge is different as it is grounded within an egocentric coordinate frame and encodes information as a sequential record of turning points leading from the starting point to the destination (Aguirre and D'Esposito, 1999). A central nervous system (CNS) representation of the path is maintained through sets of decision points without a need to encode specific distances or angles between points or requiring a constant updating of position within space (Di Fiore and Suarez, 2007). While the use of spatial information is necessary for route learning in determining what becomes a decision point, this is still an egocentric navigational technique as no spatial information is given from these landmarks or “nodes” as they merely serve as signposts to evoke the next egocentric course of action. Route-based navigation differs from other navigational techniques such as Euclidean navigation and path integration due to the presumed inflexibility of knowledge (Aguirre and D'Esposito, 1999; Byrne, 1982). This inflexibility arises from the representation of nodes as a linear set of
instructions because change or removal of a node can render the learned path useless (Di Fiore
and Suarez, 2007).

The navigational technique of path integration, a different type of egocentric navigation,
is the least reliant on environmental representations. For path integration, the navigator can
return to a starting point through vector ‘integration’, i.e., by computation of the incremental
addition of the route taken via self-movement cues such as direction, speed, and distance and
determine a direct path home without having to retrace steps (Etienne et al., 1996). For the
purpose of this project, egocentric learning will be referring to route-based navigation and not
path integration.

**Navigational Impairments**

A variety of human conditions can result in navigational impairments, or topological
disorientation (TD), with deficits occurring in egocentric and/or allocentric navigation without
necessarily altering the other. Egocentric TD is characterized by a deficit in representing the
relative location of objects with respect to self, whereas allocentric TD is characterized by
difficulty in recalling or forming a link between directional information and landmark identity
(Aguirre and D'Esposito, 1999). This section will examine only a few examples of the diversity
of insults that can result in TD.

Some insults or conditions result in both egocentric and allocentric TD. William’s
syndrome, a genetic neurodevelopmental disorder, creates spatial deficits with both egocentric
and allocentric frames of reference (Bernardino et al., 2013). The aging process, with or without
dementia can cause both egocentric and allocentric TD. People 60+ years old took longer and
were less accurate when learning a route in a hospital, navigating a university using landmarks,
and navigating a virtual reality maze (all egocentric tasks) compared with younger controls
(Wilkniss et al., 1997, Barrash et al., 2000, Moffat and Resnick, 2002). Aging also impaired allocentric learning tasks such as navigating in an unfamiliar neighborhood, route retracing, as well as making and using cognitive maps (Iaria et al., 2009; Lipman, 1991; Wiener et al., 2012). All of the aged groups tested did not have dementia or mild cognitive impairment with or without amnesia, each of which cause allocentric or egocentric impairment. This implicates the aging process as the reason behind the deficits observed. Other studies looking at mild cognitive impairment with amnesia show increased egocentric and allocentric TD compared with aged matched controls in landmark recognition and map placement tasks, as well as learning in both virtual mazes and parks (delpolyi et al., 2007; Sanders et al., 2008; Weniger et al., 2011). Mild cognitive impairment with amnesia is a high risk factor for developing Alzheimer’s disease. Consistent with spatial (both allocentric and egocentric) navigation deficits in mild cognitive impairment with amnesia, spatial navigation deficits are often observed early in Alzheimer’s disease, and reports of getting lost in familiar places can often help lead to the diagnosis of Alzheimer’s disease (Gazova et al., 2012). These observed congruent allocentric and egocentric impairments indicate that there is a degree of convergent neural pathways for both allocentric and egocentric navigational learning and memory.

Other disease states will affect only egocentric or allocentric impairments, giving evidence of divergent neural pathways for each spatial learning type. Huntington’s disease, which affects the striatum, can impair egocentric navigation. The brain regions involved and that are affected in Huntington’s disease correspond with functional neuroimaging studies of healthy controls that show increased activation of the basal ganglia during egocentric-dependent maze tasks (Cook and Kesner, 1988; Packard and Knowlton, 2002). Huntington’s disease patients consistently demonstrate difficulty correctly completing a route-finding task when moving in the
reverse direction and in compensating for a self-produced movement task; both tasks of egocentric localization (Brouwers et al., 1984; Potegal, 1971).

While Huntington’s disease affects the striatum primarily, schizophrenia is generally associated with explicit memory deficits that are linked with the medial temporal lobe, while implicit memory processes that are dependent on striatal connections are normally left intact (Clare et al., 1993; Danion et al., 2001; Perry et al., 2000). Schizophrenics are impaired in allocentric navigation when tested in virtual reality maze tests that assess allocentric navigation but show no impairments in virtual egocentric navigation (Folley et al., 2010; Hanlon et al., 2006; Weniger and Irle, 2006). The schizophrenic subjects reported using egocentric strategies more often than controls during both types of tests (Weniger and Irle, 2006). This dissociation of impairment suggests that the neural network underlying egocentric learning is less affected by schizophrenia than regions important for allocentric navigation. Therefore, egocentric networks may offer compensatory navigational strategies in the face of allocentric impairment in schizophrenic patients.

Injuries to the brain via traumatic brain injury or stroke also differentially impair spatial navigational impairments, depending on the injured region. Traumatic brain injury is caused by a blow to the head, and often results in TD both to familiar and unfamiliar locations. In a virtual Morris water maze (MWM) task moderate to severe traumatic brain injury patients showed a large allocentric deficit, but no egocentric deficit compared with controls (Livingstone and Skelton, 2007). This was not due to difficulty in understanding or remembering task instructions, as patients were equally able as controls to locate a visible target platform in the virtual MWM. The temporal region, especially the hippocampus, is highly vulnerable to injury following traumatic brain injury, making these deficits consistent with observed allocentric
deficits following hippocampal lesions in rodent studies (Livingstone and Skelton, 2007; Morris, 1981).

Patients with frontal lobe damage exhibit deficits on an egocentric right-left discrimination task, without impairment in a task that evaluated allocentric representation of space (Butters et al., 1972). Patients were also unable to perform a task requiring knowledge of one’s body’s orientation in space, indicative of egocentric disorientation (Semmes et al., 1963). Unilateral parietal cortex lesions caused impaired learning in an egocentric virtual maze with no landmarks available, but had no effect when patients were required to navigate in an allocentric-based virtual park setting (Weniger et al., 2009). The hemisphere of the brain the lesion was located did not make a difference in terms of learning impairment, indicating that both parietal cortices are actively involved in egocentric navigational learning, but not allocentric learning. Unilateral neglect following stroke will also frequently present itself with egocentric TD, preferentially on the identical side as the neglect (Aguirre and D'Esposito, 1999; Palermo et al., 2012). This is also seen in virtual reality maze testing for subjects presenting with right brain damage and hemineglect (Palermo et al., 2012).

The human data available shows that William’s syndrome, the natural aging process, mild cognitive impairment with amnesia, and Alzheimer’s disease result in both egocentric and allocentric TD. General schizophrenia and traumatic brain injury cause allocentric, but not egocentric, TD. Schizophrenia and traumatic brain injury primarily affect the hippocampus compared to the striatum. The hippocampus in humans, non-human primates and rodents is a necessary brain region for allocentric learning (Penner and Mizumori, 2012). Huntington’s disease, frontal lobe damage, and parietal lobe damage cause egocentric, but not allocentric TD. Huntington’s disease preferentially causes damage to the striatum, which along with the frontal
and parietal lobes are considered necessary for egocentric learning (Penner and Mizumori, 2012). These data available from human conditions is helpful in determining the regions of interest for both allocentric and egocentric learning. They also indicate both convergent and divergent neural pathways involved in both spatial learning types.

All of the aforementioned disorders are characterized by other cognitive impairments as well; however navigational learning tasks are easily tested in humans, non-human primates, and rodents thereby offering a reliable means of cross-species comparison. Human studies on navigation rely on patients with variable brain injuries, or neuroimaging techniques, which limits mechanistic study. Utilizing navigational learning tasks in rodents can provide valuable information not possible in human studies.

**Common animal behavioral tests for spatial learning**

The majority of information known about allocentric and egocentric spatial learning and memory comes from testing rodents. Since successful navigation through known and unknown environments is a complex process that utilizes both egocentric and allocentric reference frames, it is very difficult to tease out different learning mechanisms in a real world setting. People have different preferences for navigational strategy use, and these preferences can switch with practice (Etchamendy and Bohbot, 2007; Iaria et al., 2009). This has led experimenters to design both human virtual reality mazes and rodent mazes that control for the strategy being used to successfully solve the maze. In the following section, 3 different rodent mazes are described. The first two manipulate the environmental surroundings to allow for only an allocentric or an egocentric strategy to navigate while the last maze can be learned via either strategy and then a probe test is employed to determine which type was used.
Morris water maze

Originally designed by Richard Morris (Morris, 1981), the Morris water maze (MWM) has been adapted to test many aspects of cognitive functions (Figure 1). The classic protocol tests allocentric navigation, requiring that the animal learn to swim from pseudo random start positions to a submerged escape platform (Morris, 1981). Navigation to this fixed but hidden goal is guided by memory of its spatial relationship to visible extramaze cues, distal to the platform. Acquisition of the place navigation task is disrupted by hippocampal lesions regardless of the lesioning technique used (Morris et al., 1982, Sutherland et al., 1983). Many neurotransmitter systems have been shown to be involved, including dopamine (DA), norepinephrine (NE) and serotonin (5-HT) (Myhrer, 2003). Glutamate (Glu) appears to be the largest modulator of MWM learning. Systemic or hippocampal exposure to N-methyl-D-aspartate (NMDA) or \(\alpha\)-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists in rodents impairs MWM learning while systemic AMPA receptor agonist exposure improves learning (Myhrer, 2003). Using the MWM in animal studies is also beneficial when creating animal models of human conditions. The use of virtual reality tests of the MWM in humans allows for a direct comparison between human and rodent allocentric learning.

Cincinnati water maze

The Cincinnati water maze (CWM) is a 9-unit multiple-T swimming maze of egocentric route-based navigation (Vorhees, 1987) (Figure 2). By running the maze in complete darkness, all extramaze cues are eliminated and animals must rely on egocentric navigation to learn the maze and find the hidden platform. The CWM is sensitive to pharmacological, developmental and genetic manipulations. For example, genetic knockdown of phosphodiesterase-4D improves CWM performance (Schaefer et al., 2012), while prenatal exposure to maternal immune
response (Vorhees et al., 2012), and developmental exposure to (+)-methamphetamine, (±)-3,4-
methylenedioxyamphetamine (MDMA) or citalopram (Vorhees et al., 2008, Skelton et al.,
2009, Schaefer et al., 2013) all impair CWM performance in adulthood. Adult exposure to a
neurotoxic dose of methamphetamine impairs CWM learning, but neurotoxic doses of MDMA
or fenfluramine do not (Herring et al., 2008; Vorhees et al., 2010). These data implicate post-
developmental DA, but not 5-HT, in route-based navigation (Herring et al., 2008; Vorhees et al.,
2010). Both the MWM and CWM give the experimenter control over the spatial learning
strategy used by the tested rodent and are good companion tests. The CWM is very similar to
virtual reality tests for route-based egocentric learning in humans as well, giving a test for
rodents that can be directly compared to human findings.

T-maze

The T-maze can be learned utilizing either an allocentric place strategy or an egocentric
response strategy and a probe test following training is employed to determine which strategy the
rodent used (Figure 3). During training trials, food is placed on the same arm of the maze with
the rodent starting from a location that is 90° from the reward. The start and food positions are
kept stationary. As training continues, the animal learns which arm the reward is located by
extramaze cues as guidance (place learning) or by body positioning as a right or left turn
(response learning). Following training, the T-maze is rotated for the probe trial and the
animal’s start position is 180° from the training position. If a place strategy was used, the animal
will go to the arm where the reward was previously, and if a response strategy was used the
animal will make an identical right or left turn similar to training.
These 3 mazes have been used extensively to study different neurotransmitter systems and neural pathways in regards to spatial learning. The purpose of the studies herein is to study the regional role of DA in both egocentric and allocentric learning using the CWM and MWM.

**The dopaminergic system**

Dopamine is a catecholamine neurotransmitter important in many cognitive and emotional processes including learning and memory, attention, reward, and cognitive flexibility (Dalley et al., 2004). Dysfunction in DA systems is implicated in multiple neurological and neurodegenerative disease states including Tourette’s syndrome, Parkinson’s disease, Huntington’s disease, Alzheimer’s disease, schizophrenia, and attention deficit hyperactivity disorder (El-Ghundi et al., 2007). Due to the involvement of DA in CNS conditions that manifest as both egocentric and allocentric TD, it is probable that DA is a modulator in these navigational learning types.

DA shares a biosynthetic pathway with NE and is synthesized from the essential amino acid tyrosine (Goridis and Rohrer, 2002). The rate-limiting enzyme tyrosine hydroxylase (TH) oxidizes tyrosine into L-dihydroxyphenylalanine (L-DOPA), which is then decarboxylated by L-aromatic amino acid decarboxylase into DA and CO₂ (Smeets and Gonzalez, 2000). DA is packaged into synaptic vesicles by a vesicular monoamine transporter and released into the synapse in a CA²⁺-dependent manner (Smeets and Gonzalez, 2000). Regulation of synaptic DA levels is maintained through the Na⁺/Cl⁻ dependent transporters NE and DA transporters (NET & DAT, respectively) that are located on the plasma membrane (Smeets and Gonzalez, 2000). DA metabolism is mediated by both monoamine oxidase and catechol-O-methyltransferase (Smeets and Gonzalez, 2000).
DA neurons originate in cell groups A8-A17, with about 85% of them coming from A8-A10 (the retrorubral area, substantia nigra (SN), and ventral tegmental area (VTA), respectively) (Goridis and Rohrer, 2002). These neurons travel through the medial forebrain bundle to innervate almost every cortical and limbic brain structure with three distinct projection systems. The nigrostriatal tract innervates the striatum from the SN and is involved in cognition, motor control, and emotion (Bjorklund and Dunnett, 2007). The mesolimbic pathway, which is implicated in motivated behaviors, reward, and attention, begins in the VTA and projects to the nucleus accumbens (Nacc), amygdala, hypothalamus, prefrontal cortex (PFC), and other limbic structures (Bjorklund and Dunnett, 2007). Also originating in the VTA, the mesocortical tract projects to cortical areas including the medial prefrontal (mPFC), cingulate, and entorhinal cortices and is involved in motivation, devising abstract concepts, prioritizing the significance of stimuli, and monitoring the temporal sequence of stimuli and behavior (Bjorklund and Dunnett, 2007). These 3 pathways make up the dopaminergic pathways and systems that are addressed in later sections and chapters herein. Several brain regions, including the striatum and mPFC, that receive DA projections from the VTA and SN have been implicated in egocentric, allocentric, or both learning types. DA cells are also found in the periaqueductal gray (A11) cell groups, hypothalamic cell groups (A12, A14, A15) and in the ventral thalamus (A13) (Smits et al., 2006). To a lesser extent, DA cell bodies are also found in A16 and A17 (the olfactory bulb and retina, respectively) (Goridis and Rohrer, 2002). The high percentage of DA neurons in the VTA and SN, along with the multiple regions associated with allocentric and egocentric learning they project to, have the highest probability of involvement in these learning types compared to other DA cell groups.
There are 5 DA receptors, all of which are G-protein coupled receptors and are differentially expressed throughout the brain (Missale et al., 1998). D₁-like receptors (D₁ & D₅) are characterized by increasing adenylate cyclase activity resulting in increased cyclic adenosine monophosphate (cAMP) levels, whereas D₂-like receptors (D₂, D₃, D₄) inhibit adenylate cyclase activity and decrease cAMP levels (Missale et al., 1998). All DA receptors can be found postsynaptically, but D₂ and D₃ are also found presynaptically functioning as autoreceptors (El-Ghundi et al., 2007). D₂ receptors have 2 isoforms, the D₂-long form that is mainly found at postsynaptic sites and the D₂-short form that is the autoreceptor (El-Ghundi et al., 2007). These autoreceptors decrease DA release, acting as an inhibitory factor on DA neurotransmission. The differential roles of D₁-like receptors and D₂-like receptors in neuronal activation allow DA signaling to affect neuronal activation in a complex fashion.

These opposing roles of DA receptor signaling and the diversity of dopaminergic projections to many areas of the brain associated with cognition gives insight into the multiple ways DA signaling could influence egocentric and/or learning.

**Striatum**

The striatum is the major target for DA projections from the SN and VTA. The striatum refers to the part of the basal ganglia containing the caudate nucleus, putamen, Nacc and olfactory tubercle. Dorsal striatum (dStr) or neostriatum are used to refer to the caudate and putamen, especially in rodents. While in other mammals, such as humans and nonhuman primates, the caudate and putamen are separated by the internal capsule into distinct regions, in rodents there is no such topographical divide. The dStr sub-regions dorsomedial striatum (DMS) and dorsolateral striatum (DLS) are considered the rat homologues for the caudate and putamen, respectively (Figure 4).
Inhibitory γ-aminobutyric acid (GABA) medium spiny neurons (MSNs) make up 90-95% of the striatal neuronal population and are the major efferent target site from cortical glutamatergic neurons (Ribak et al., 1979, Wilson and Groves, 1980). Two types of interneurons make up the remaining 5-10% of striatal neurons, large cholinergic neurons and smaller GABAergic cells (DiFiglia et al., 1976). Both types of interneurons act in an inhibitory fashion on striatal MSNs and are responsible for most of the tonic activity in the striatum (Parent and Hazrati, 1995). As the only striatal output projection neuron, MSNs are relatively evenly divided into two subpopulations (striatonigral and striatopallidal) based on their projection targets that are evenly distributed between striatal sub-regions. The striatonigral pathway has direct inhibitory axon collaterals to GABAergic neurons in the entopenduncular nucleus and SN pars reticulata, decreasing signaling from these regions (Gerfen et al., 1990, Parent and Hazrati, 1995, Pan et al., 2010, Surmeier et al., 2011). Both the entopenduncular nucleus and SN pars reticulate operate in an inhibitory manner as the output regions from the basal ganglia to the rest of the brain (Parent and Hazrati, 1995, Pan et al., 2010). The striatopallidal pathway has indirect projections to the internal basal ganglia nuclei which in turn disinhibits the entopenduncular nucleus and SN pars reticulata (Tritsch and Sabatini, 2012). Normal striatal output is dependent on the dynamic balance and coordination between these opposing neuronal populations (Parent and Hazrati, 1995).

Dopamine is an integral neurotransmitter in proper striatonigral and striatopallidal neuronal function. Both striatonigral and striatopallidal neurons can be characterized by their DA receptor profile with excitatory D₁ receptors on striatonigral neurons and inhibitory D₂ receptors on striatopallidal neurons (Parent and Hazrati, 1995). Insults to striatal DA systems can differentially affect D₁ and D₂ receptor activation leading to altered inhibitory and excitatory...
influences on GABAergic MSNs thus disrupting basal ganglia signaling and whole brain activity (Gerfen et al., 1990, Bertran-Gonzalez et al., 2010). For example, dStr DA depletion from dStr 6-hydroxydopamine (6-OHDA) injection decrease D1 expression and increase D2 expression (Gerfen et al., 1990). This imbalance decreases inhibitory striatonigral neuronal signaling and increases excitatory striatopallidal signaling, thus increasing overall dStr excitation (Gerfen et al., 1990). This excitatory shift decreases dStr inhibitory signals to the entopeduncular nucleus and SN pars reticulate, thereby increasing inhibitory basal ganglia output (Gerfen et al., 1990). This and other disruptions from altered striatal DA signaling have far reaching consequences on cognition, motor skills and more.

The striatum receives neuronal projections from all areas of cortex and each subregion is involved in a specific and non-overlapping loop starting with the neocortex to striatum, to the globus pallidus, and then thalamus before going back to the neocortex (Parent and Hazrati, 1995, Penner and Mizumori, 2012). The DLS ‘sensorimotor’ loop links the DLS with the somatosensory and motor cortices, the DMS ‘associative’ loop connects with the mPFC, visual and auditory cortices, and the Nacc ‘limbic’ loop connects with ventromedial PFC (Parent and Hazrati, 1995, Penner and Mizumori, 2012). The Nacc network also extends through the parahippocampal area, the hippocampus, and the amygdala (Mizumori et al., 2009). Each of these loops connects with regions of the brain that have also been shown to be necessary for egocentric and/or allocentric learning and could indicate independent functionality for each striatal subregion in both types of spatial learning. If either type of spatial learning is disrupted after specific DA insults in any striatal subregion it would support a theory for divergent striatal neuronal pathways for egocentric and/or allocentric learning. If allocentric or egocentric
learning is only disrupted following wide spread striatal or dStr DA loss, it would support a convergent theory for striatal neuronal pathways in that spatial learning type.

**Striatum and Navigational Learning**

**Dorsal Striatum**

The dStr has previously been shown to be an integral region for egocentric learning. Both the DMS and DLS have neurons that only fire to specific egocentric response movements such as turns, forward movement, or head direction (Lavoie and Mizumori, 1994; Ragozzino et al., 2001; Wiener, 1993). Electrolytic lesions of the dStr impair egocentric learning in the radial arm maze (RAM). For example, dStr lesioned animals are impaired in learning the position of a food reward that is always in a constant direction relative to the starting point of the animals (Potegal, 1969). dStr lesions also impair a RAM egocentric right-left discrimination task with no effect on allocentric RAM learning (Cook and Kesner, 1988). The right-left discrimination RAM test isolates egocentric learning and tests rodent ability to determine which of two available doors was the right or left one from their current position, only one of which contained the food reward across testing. The allocentric RAM task has baited arms in a constant position relative to the distal cues around the room, allowing the animal to learn the placement of the reward through use of distal cues. dStr Glu is implicated in egocentric learning; dStr injection of the NMDA receptor antagonist (2R)-amino-5-phosphonopentanoate (AP-5) results in egocentric frame of reference, but not allocentric frame of reference, deficits in a modified spatial object recognition test (De Leonibus et al., 2005). In the traditional spatial object recognition test, reactivity to spatial and non-spatial changes is measured in a circular open field containing 5 objects. Animals are introduced to the open field in a consistent place throughout testing. Observing reactivity in rodents to object displacement tests spatial novelty detection, and
reactivity to object substitution tests non-spatial novelty detection (Coccurello et al., 2000). Animals placed at the same starting point use stationary distal cues to determine spatial object change via an allocentric frame of reference. The modified test uses a procedure where some animals are placed in the open field at random points throughout testing, thus diminishing their use of distal cues to recognize spatial displacement of the objects, encouraging an egocentric frame of reference (De Leonibus et al., 2005). These studies implicate the dStr as an important region for egocentric learning, with dStr Glu as an important modulatory factor in this learning type.

Ablation of the dStr does not normally result in allocentric learning deficits (McDonald and White, 1993, 1994, Oliveira et al., 1997); however DA in this area has been shown to be involved. Direct dStr 6-OHDA administration causing more than 60% dStr DA depletion results in allocentric deficits as seen in the MWM and the spatial object recognition test (De Leonibus et al., 2007; Lindner et al., 1999; Whishaw and Dunnett, 1985). 6-OHDA injections into the medium forebrain bundle result in allocentric MWM deficits (Mura and Feldon, 2003). 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injections into either the SN or dStr with less than 60% DA depletion do not result in allocentric MWM deficits indicating the threshold for DA loss to result in allocentric deficits is above 60% (Miyoshi et al., 2002, Da Cunha et al., 2003).

While the dStr itself does not appear to be involved in allocentric learning, DA projections to this region have been shown to be a necessary modulator for this learning type. It is currently unknown why dStr DA-specific lesions, but not ablation of the dStr, result in allocentric deficits. It has been hypothesized that dStr DA may be essential for choosing the correct sensorimotor subsystems necessary for allocentric learning to occur, and is not actively
involved in retaining the location of objects or plotting the correct allocentric path (Whishaw and Dunnett, 1985). Evidence suggests that dStr DA could also have a modulatory role in egocentric learning as well, however this has yet to be fully tested. Drugs that target DA systems and produce decreases in dStr DA levels (i.e., (+)-methamphetamine and (+)-amphetamine) affect CWM performance whereas drugs that preferentially act on 5-HT (i.e. (+)-fenfluramine or MDMA) do not alter CWM performance (Herring et al., 2008; Vorhees et al., 2010). However, drugs such as methamphetamine have widespread effects making it unclear which mechanism contributes most to the effect of the drug on route-based navigation. Given the modulatory role of dStr DA in allocentric learning, and the importance of the dStr in egocentric learning it can be hypothesized that dStr DA also modulates egocentric learning.

The subregions of the dStr show different and independent roles for allocentric and egocentric learning. Similarly to the dStr, allocentric learning deficits have not been observed following DLS lesions, regardless of lesion type (Devan and White, 1999; Mizumori et al., 2009; Packard and McGaugh, 1996; Packard, 2009; Penner and Mizumori, 2012; Yin and Knowlton, 2004; Yin and Knowlton, 2006). Both electrolytic and excitotoxic DMS lesions do result in allocentric learning deficits in the hidden platform MWM as well as create a preference for a cued response during a place-cued competition test in the MWM (Whishaw et al., 1987, Devan et al., 1999, Devan and White, 1999). Allocentric RAM performance is impaired after DMS electrolytic lesions, even when rats are pre-trained in the maze prior to surgery (Colombo et al., 1989). NMDA receptor blockade with propyl-1-phosphonic acid in the DMS impairs RAM learning and long-term allocentric retention in the MWM at similar impairment levels to hippocampal NMDA receptor blockade (Smith-Roe et al., 1999, Holahan et al., 2005). NMDA lesions in the posterior DMS impair place learning in a T-maze (Yin and Knowlton, 2004).
DMS DA depletion decreases place learning in a T-maze (Lex et al., 2011), but MWM learning following DMS DA loss has yet to be tested. This functional heterogeneity between the DLS and DMS in allocentric learning has been attributed to the indirect connections the DMS shares with the hippocampus (Whishaw et al., 1987, Devan et al., 1999, Devan and White, 1999).

While the DLS and DMS exhibit functional heterogeneity concerning allocentric learning, both regions have been shown to be involved in egocentric learning. Excitotoxic lesions of either the DMS or DLS impair egocentric procedural learning in a 14-unit T-maze (Pistell et al., 2009). DLS Glu has a modulatory role in egocentric response learning; AP-5 injections into the DLS disrupt response learning, but not allocentric place learning, in the T-maze (Palencia and Ragozzino, 2005). Post-training silencing of the DLS with lidocaine also inhibits an egocentric response in the T-maze (Packard and McGaugh, 1996, Palencia and Ragozzino, 2005). Electrolytic DLS lesions produce a preference for utilizing a place strategy in a MWM competition test (Devan and White, 1999). In rhesus macaques, pharmacological inhibition of the DMS reduces route-based (direction-based) learning, but not allocentric or cued learning (Etienne et al., 2012).

Both regions are involved in egocentric learning, although the role of DA in the DMS or DLS on route-based navigation has yet to be examined. Given the independent roles of the DMS and DLS in egocentric learning, it can be hypothesized that DMS and DLS DA also modulate egocentric learning. The role of DLS DA has not yet been explored in allocentric learning. It is unlikely that DLS DA is involved in allocentric learning, however it cannot be completely discounted, as the dStr does not normally influence allocentric learning but dStr DA depletion impairs it. DMS DA loss decreases place learning in the T-maze, which could extend to a modulatory role in MWM allocentric learning.
Nucleus Accumbens

The Nacc is a major target for DA innervation from the VTA, and has frequently been found to be involved in navigation, making it an area of interest for determining the modulatory roles of striatal DA in allocentric and egocentric learning. In addition to DA projections from the VTA, the Nacc receives converging afferents from the PFC and hippocampus. It has been hypothesized the Nacc maintains associations between locations, actions, and goals to implement navigational strategies (Mogenson et al., 1980, Redish and Touretzky, 1997). Both behavioral and electrophysiological data suggest a role for the Nacc in allocentric navigation. Some Nacc cells fire relative to allocentric cues related to different reward sites and Nacc neuronal activity correlates to spatial conditions in the RAM, indicative of Nacc cellular position sensitivity (Lavoie and Mizumori, 1994; Shibata et al., 2001). These Nacc cellular firing patterns have similar features to hippocampal place cell firing, and are thought to occur via direct interaction with the hippocampus regarding allocentric orientation in space (Lavoie and Mizumori, 1994; Shibata et al., 2001).

Behavioral data support a role for the Nacc in allocentric learning. Electrolytic and excitotoxic Nacc lesions also impair MWM hidden platform learning and RAM performance but not MWM cued platform learning (Annett et al., 1989, Sutherland and Rodriguez, 1989). Intra-Nacc injections of lidocane disrupt allocentric but not cued RAM learning (Seamans and Phillips, 1994). A differential role for the glutamatergic NMDA and AMPA receptors has been shown in the Nacc. Focal AP-5 injection impair allocentric learning in the MWM, but the AMPA antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) has no effect (Sargolini et al., 2003). AP-5 injections in the Nacc core impair RAM allocentric learning (Smith-Roe et al., 1999). Nacc protein kinase C function is necessary for allocentric learning, indicating that Nacc
neuronal plasticity is required for acquisition of allocentric learning, not unlike the hippocampus (Ferretti et al., 2010). Electrophysiological and behavioral data all clearly support a necessary role for the Nacc in allocentric learning. The modulatory effect of Nacc DA on allocentric learning is less clear.

There are conflicting data regarding the role of Nacc DA in allocentric learning. 6-OHDA injections in the Nacc resulting in at least 70% DA depletion has not affected MWM acquisition or reversal, but allocentric spatial discrimination in a T-maze is impaired after Nacc DA loss (Grigoryan et al., 1996; Hagan et al., 1983; Whishaw and Dunnett, 1985). Pharmacological manipulation of DA receptors does implicate a role for DA in allocentric learning. D2 antagonist haloperidol Nacc injections impair MWM learning at high doses, but not low doses (Ploeger et al., 1994). Nacc injection of the D2 agonist quinpirole enhance both MWM and RAM learning, while the D2 antagonist sulpiride impair MWM learning and RAM learning (Packard and White, 1991, Cools et al., 1993, Setlow and McGaugh, 1998). Since Nacc DA is also involved in reward processes, Coccurello et al. (2002) used the spatial object recognition test as a non-associative allocentric test with no explicit reward following intra-Nacc injections of either sulpiride or the D1 receptor antagonist SCH 23390 (Coccurello et al., 2000). SCH 23390 had a very specific effect by decreasing the reactivity to spatial changes of objects in an open field, but had no locomotor effect and only a small effect on the novel objects present. Sulpiride had a wider range of effects on all measured variables, indicating D1 receptors are more selectively involved in allocentric learning than D2 receptors, although to some extent both are involved (Coccurello et al., 2000). These data implicate D1 and D2 receptors, and suggest a role for Nacc DA, in allocentric learning that is unrelated to reward processes. The conflicting Nacc 6-OHDA data makes understanding this role difficult, however.
Discerning the role of the Nacc in egocentric learning has received considerably less research than allocentric learning. In one study, animals preferred an egocentric learning strategy to an allocentric one in the RAM following intra-Nacc injections of the glutamatergic antagonists AP-5 or DNQX (Klein et al., 2004). However, both egocentric and allocentric reference frames were impaired following intra-Nacc injections of AP-5 in a spatial object recognition task (De Leonibus et al., 2005). The role of Nacc DA on egocentric learning has yet to be explored.

The dStr, DMS, DLS and Nacc are all implicated in egocentric learning, DA in these regions could also be involved as well. It is unknown if DA integrity in Nacc, DMS, and DLS is individually required for egocentric learning, or if DA damage in the dStr or whole striatum is required. The Nacc and dStr DA modulate allocentric learning; the roles of DMS and DLS DA in MWM allocentric learning have yet to be determined.

**The Medial Prefrontal Cortex and Navigational Learning**

The PFC in rodents is an area of cortex most commonly defined by the reciprocal connections from the mediodorsal thalamus (Rose and Woolsey, 1948, Uylings et al., 2003). It can further be divided into several distinct subregions defined by heterogeneous connections and function, including the mPFC. The mPFC consists of the medial precentral and anterior cingulate cortices in the dorsum and the prelimbic, infralimbic, and medial orbital cortices on the ventrum (Dalley et al., 2004). mPFC networks extend throughout numerous brain regions, including the hippocampus, DMS, Nacc, and VTA (Ongur and Price, 2000).

There are conflicting reports of mPFC involvement in allocentric learning. Early studies using a modified MWM protocol found deficits following aspiration of the mPFC (Kolb et al., 1982, Sutherland et al., 1982, Kolb and Whishaw, 1983), while later studies using a different
MWM protocol found no allocentric impairments following electrolytic or excitotoxic mPFC lesions (de Bruin et al., 1994, Maaswinkel et al., 1996, de Bruin et al., 2001, Ethier et al., 2001, Lacroix et al., 2002). These differences are most likely the result of the method of lesion induction since some are selective for specific neurotransmitters and some simply ablate all cells in a target region. Procedural differences may also be important (de Bruin et al., 1994, de Bruin et al., 2001, Lacroix et al., 2002). In some studies that found differences, lesioned animals that did not find the platform were not placed on it during the ITI thereby limiting access to distal cue information whereas in studies that found no differences, animals were placed on or guided to the platform if they did not locate it within the time limit (de Bruin et al., 1994). Other studies using different allocentric learning tasks have also failed to show an allocentric deficit following mPFC electrolytic lesioning or mPFC DA depletion, further supporting the idea that an intact mPFC is not necessary for allocentric learning (Poucet, 1989; Poucet, 1990; Kesner et al., 1989; King and Corwin, 1992; Rawson et al., 2010; Bubser, 1994; Bubser and Schmidt, 1990).

The role of the PFC in egocentric learning has been studied extensively in rodents, humans and non-human primates. Both egocentric learning and working memory are impaired following disruption of the mPFC in rodents. The mPFC receives afferents from somatosensory and motor cortices, making it a prime region to maintain the proper proprioceptive information necessary for egocentric movement (McGeorge and Faull, 1989). Following aspiration, electrolytic, and pharmacological lesions of the mPFC, rodents are impaired in the adjacent arm task in the RAM and an egocentric response task in the MWM (de Bruin et al., 1997; de Bruin et al., 2001; Ethier et al., 2001; Kesner et al., 1989; Kolb et al., 1994). The egocentric response task in the MWM differs from the traditional allocentric one in that both the start and hidden platform
positions are changed prior to the beginning of each trial. The spatial relationship between the start position and the hidden platform is kept constant throughout testing.

The dorsolateral prefrontal cortex (dlPFC) in humans and nonhuman primates is considered analogous to the mPFC in rodents. Human subjects with frontal cortex damage are impaired in egocentric-based tasks with no allocentric impairment (Butters et al., 1972; Semmes et al., 1963). Aspiration lesions of the dlPFC in nonhuman primates impair an egocentric right-left discrimination task with no allocentric discrimination reversal impairment (Pohl, 1973). Furthermore, when nonhuman primates with aspiration lesions of the dlPFC were tested in two spatial working memory tasks that each could only be solved using either an allocentric or egocentric strategy, only egocentric working memory was impaired (Ma et al., 2003). Neuronal activity in the dlPFC does not appear to correlate with hippocampal place cells during task learning, suggesting that the dlPFC does not maintain allocentric information during that learning type (Ma et al., 2004). These data have shown consistently across species that integrity of the mPFC and corresponding dlPFC is necessary for egocentric learning.

The direct role of mPFC DA on egocentric learning has yet to be explored. DA afferents from the VTA act as an inhibitory signal on mPFC Glu neurons, decreases spontaneous activity of these neurons, and acts as a “gate” on both interreference signaling and overall activity levels (Tzschentke, 2001). These DA processes in the mPFC may extend to egocentric learning. Previous data indicate 6-OHDA injection into the mPFC would not affect MWM learning.

**Hippocampal Roles in Spatial Navigation**

The hippocampus is another vital region for spatial learning. Electrolytic and excitotoxic lesions of the hippocampus severely impair allocentric learning in the MWM (Morris et al., 1982). Spatial information arrives to the hippocampus mainly from the medial entorhinal cortex,
which projects to all hippocampal subregions (Aggleton et al., 2000). The hippocampus is characterized, in part, through the presence of place cells, which fire when rats enter specific areas of the environment (Moser et al., 2008).

The role of hippocampal DA in allocentric learning has also been well documented. Roughly 10% of VTA projections to the hippocampus are dopaminergic (Bjorklund and Dunnett, 2007). 6-OHDA injections in the hippocampus result in MWM learning deficits with no effect on other performance-based factors (Gasbarri et al., 1996). Hippocampal D₁ and D₅ receptors have also proved to be necessary for allocentric learning, pre-treatment hippocampal infusion with SCH 23390 interferes with long-term potentiation (LTP), which is an established cellular correlate of allocentric learning (Li et al., 2003, Lemon and Manahan-Vaughan, 2006, O'Carroll et al., 2006).

Dorsal hippocampal place cells also fire to temporal and/or internal sensory cues in the absence of visual external stimuli supporting a role in egocentric information processes (Bjorklund and Dunnett, 2007). However, hippocampal lesions do not result in egocentric deficits, indicating that the striatum is sufficient to support egocentric learning following hippocampal ablation (Kesner, 1990, McDonald and White, 1994, Devan et al., 1996). The role of hippocampal DA in egocentric learning has not yet been tested. However since complete ablation of the hippocampus does not affect egocentric learning ability, and the percentage of dopaminergic neurons received are significantly less than that of the striatum and mPFC, it can be hypothesized that loss of hippocampal DA would either have minimal or no effect on egocentric learning.

For such reasons as the lack of whole hippocampal involvement in egocentric learning and the well-known involvement of hippocampal DA in allocentric learning, hippocampal DA
loss will not tested in the studies herein. However it cannot be completely discounted that there is the potential for a hippocampal DA role in egocentric learning in conjunction with its role in allocentric learning.

**Dissertation Synopsis**

The focus of this dissertation is to elucidate the regionally specific role of DA in the striatum and mPFC in allocentric MWM and egocentric CWM learning. In Chapter 2, the role of dStr DA was investigated using direct bilateral injections of 6-OHDA to eliminate the majority of DA terminals within this region while sparing SN DA projections to other brain regions. Following completion of DA neuronal death, animals were tested in both the CWM and MWM. To ensure that motivational and motor processes were not involved in any observed impairment, straight channel performance was tested prior to the start of maze testing. Motor ability was also confirmed by analysis of swim speed during MWM testing. This showed that swimming ability did not degrade over the course of testing. Procedural learning ability was tested as another control following MWM place learning by using the MWM cued protocol. Pre-surgical weight was maintained through nutritional supplementation when necessary.

As both subregions of the dStr have been shown to be functionally heterogeneous, Chapter 3 examined the role of DA in the DMS or DLS for egocentric and allocentric learning using the same surgical and behavioral protocols as in Chapter 2. Chapter 4 expanded on the striatal DA role by looking at the specific role of Nacc DA in both learning types. Similarly, stereotaxic surgery was used, however because the known role of the Nacc in reversal learning and strategy switching, animals were run through only the CWM or the MWM (Penner and Mizumori, 2012). Allocentric reversal learning and CWM reverse path performance were also assessed. Chapter 5 examined the role of VTA projections to the mPFC in egocentric and
allocentric learning. Similar surgical, behavioral, and histological protocols were used as in Chapters 2 and 3.

Following completion of behavioral testing, monoamines and their metabolites were analyzed using high performance liquid chromatography (HPLC). Qualification for statistical inclusion in lesion groups was set at a minimum of 50% DA loss. For each lesion, a separate group of animals were given unilateral 6-OHDA injections corresponding to the proper lesion coordinates. This group was used for immunohistochemistry histology by staining for TH immunoreactivity and observing the regional extent of DA loss.


Whishaw IQ, Dunnett SB (1985) Dopamine depletion, stimulation or blockade in the rat disrupts spatial navigation and locomotion dependent upon beacon or distal cues. BehavBrain Res 18:11-29.


Figure 1. **Morris water maze.** A test of allocentric spatial learning. Animals are placed within the circular pool and learn the location of the hidden platform through use of the external visual cues.
Figure 2. Cincinnati water maze. A test of egocentric spatial learning. Animals are started at point “S” and without the use of spatial cues learn to find the escape platform at point “G”.
Figure 3. The T-maze. This maze can be solved using either an allocentric place strategy or an egocentric response strategy. Training occurs with a stationary start and reward position with a probe test given at the end of testing. The start position is reversed in regards to the external spatial cues but the reward position stays stationary. If the animal correctly goes to the reward, it used the extramaze cues to learn (allocentric). If the animal goes to the other arm, it learned to make a right or left turn to find the reward (egocentric).
Figure 4. Major domains of the dorsal striatum; dorsolateral striatum (DLS) and dorsomedial striatum (DMS). Atlas from Paxinos and Watson brain atlas (Paxinos et al., 1985).
CHAPTER 2:  
Dorsal striatal dopamine depletion impairs both allocentric and egocentric navigation in rats  
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Abstract

Successful navigation requires interactions among multiple but overlapping neural pathways mediating distinct capabilities, including egocentric (self-oriented, route-based) and allocentric (spatial, map-based) learning. Route-based navigation has been shown to be impaired following acute exposure to the dopaminergic (DA) drugs (+)-methamphetamine and (+)-amphetamine, but not the serotoninergic (5-HT) drugs (±)-3,4-methylenedioxyamphetamine or (±)-fenfluramine. The dopaminergic-rich neostriatum is involved in both allocentric and egocentric navigation. This experiment tested whether dorsal striatal DA loss using bilateral 6-hydroxydopamine (6-OHDA) injections impaired one or both types of navigation. Two weeks following 6-OHDA injections, rats began testing in the Cincinnati water maze (CWM) followed by the Morris water maze (MWM) for route-based and spatial navigation, respectively. 6-OHDA treatment significantly increased latency and errors in the CWM and path length, latency, and cumulative distance in the MWM with no difference on cued MWM trials. Neostriatal DA levels were reduced by 80% at 2 and 7 weeks post-treatment. In addition, 6-OHDA increased DA turnover and decreased norepinephrine (NE) levels. 6-OHDA injections did not alter monoamine levels in the prefrontal cortex. The data support that neostriatal DA modulates both types of navigation.
Introduction

Impairments in navigational ability are present in numerous human conditions including Huntington’s disease, Alzheimer’s disease, schizophrenia, Parkinson’s disease, stroke, traumatic brain injury, as well as during normal aging (Aguirre and D’Esposito, 1999; Iaria et al., 2009; Laczo et al., 2009; Livingstone and Skelton, 2007; Sanders et al., 2008; Weniger and Irle, 2006). Successful navigation requires complex interactions among multiple distinct, but parallel cognitive processes that can be subdivided into egocentric (self-oriented) and allocentric (spatial, map-based) wayfinding. In the allocentric process, the navigator’s spatial orientation to distal cues in the environment is fluid and represented in a common coordinate map system external to the navigator (Byrne, 1982; Garber, 2000). Spatial learning is frequently studied in rodents using the Morris water maze (MWM), and acquisition of the place navigation task is dependent on the hippocampus (Kesner, 1990; Morris, 1981; Morris et al., 1982; Sutherland et al., 1983), although other regions also influence the process. For example, MWM learning is sensitive to damage to cortical regions including frontal, cingulate, and parietal areas (Galani et al., 2002; Kesner et al., 1989; Whishaw et al., 2001), as well as the neostriatum (Devan and White, 1999; Devan et al., 1999).

Egocentric wayfinding is subdivided into path integration and route-based navigation. For path integration, the navigator can return to a starting point through vector addition of the route segments taken on an outbound journey using cues of direction, speed, and distance to determine a direct path home without having to retrace steps (Etienne et al., 1996). Route-based navigation is a self-oriented representation of space that is connected by “nodes” or choice points representing successive navigational decision points (Aguirre and D’Esposito, 1999; Byrne, 1982). The neostriatum has been implicated in egocentric learning pathways (Cook and Kesner,
Striatal head direction cells are thought to signal context-dependent directional information as opposed to orientating relative to a visual cue (Mizumori et al., 2009; Ragozzino et al., 2001; Taube, 1998). Dorsolateral striatal lesions in rodents impair egocentric adjacent-arm radial arm maze (RAM) performance and right-left discrimination tasks, with no effect on allocentric 8-arm RAM performance, motivation, or motor ability (Cook and Kesner, 1988).

The Cincinnati water maze (CWM) is a 9-unit multiple-T swimming maze that when run under infrared conditions eliminates spatial cues, thus leaving only self-movement cues to make it a route-based learning task (Vorhees, 1987; Vorhees et al., 1991). CWM deficits are observed under infrared conditions following exposure to drugs that reduce the levels of neostriatal dopamine (DA) (i.e., (+)-methamphetamine and (+)-amphetamine), but not to drugs that primarily reduce the levels of forebrain serotonin (5-HT) (i.e., (±)-3,4-methylenedioxymethamphetamine (MDMA) or (±)-fenfluramine) (Herring et al., 2008; Herring et al., 2010; Vorhees et al., 2010a). These data suggest that route-based navigation may be predominately mediated by dopaminergic neurons in the neostriatum. However, route-based navigation may also be affected by pathways outside the neostriatum, as the effects of the aforementioned drugs are not regionally-specific.

While striatal DA reductions have previously been shown to impair spatial learning (De Leonibus et al., 2007a; Whishaw and Dunnett, 1985a; Lindner et al., 1999; Mura and Feldon, 2003), this appears to depend on the magnitude of DA loss (Da Cunha C. et al., 2003; Miyoshi et al., 2002; Hagan et al., 1983). To test the role of neostriatal DA reduction on route-based and spatial learning, rats were injected with 6-hydroxydopamine (6-OHDA) into the dorsal striatum and tested in both the CWM and MWM tasks.
Methods

Animals

Adult male Sprague-Dawley CD IGS rats (300-325 g) were purchased from Charles River Laboratories, Raleigh, NC. Animals were pair-housed in polycarbonate cages (46 x 24 x 20 cm) containing woodchip bedding for at least a 2-week acclimation period prior to surgery. Animals had free access to food and water and were housed in an environmentally controlled vivarium (21 ± 1°C), and were on a 14 h light-dark cycle (lights on at 600 h). Body weights were taken prior to surgery and weekly thereafter. Some animals were provided wet food if they failed to restart eating spontaneously pelleted rat chow. All procedures were in compliance with the Institutional Animal Care and Use Committee and the vivarium is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Surgery (day 0)

Rats were anesthetized with isoflurane (IsoThesia; Butler Animal Health Supply, Dublin OH), with continuous administration via a nose cone throughout surgery. Animals were then placed in a motorized, computer-controlled stereotaxic apparatus (StereoDrive, Stoelting Co., Wood Dale, IL). Animals for behavioral testing were given bilateral injections of 6-hydroxydopamine hydrobromide (6-OHDA; Sigma, St. Louis, MO) in the dorsal striatum. A volume of 2 µl of a 1.25 µg /µl in 0.2% ascorbic acid saline solution (each animal received 10 µg 6-OHDA total, 5 µg on each side) was injected at each site automatically (Quintessential Stereotaxic Injector, Stoelting Co., Wood Dale, IL) at a rate of 0.2 µl/min using a 26 gauge 10 µl Hamilton Gastight syringe (Reno, NV). The syringe was left in place for 5 min following completion of each injection to maximize absorption. Coordinates were based on the Paxinos and Watson brain atlas (Paxinos et al., 1985) (from bregma AP: +1.6 mm; ML: ± 2.4 mm; DV: -
4.2 mm; AP: +0.2 mm; ML: ± 2.6 mm; DV: -7.0 mm). Control animals (SHAM) received the same amount of vehicle using the same procedure. Following surgery, animals were given 0.1 ml buprenorphine hydrochloride to minimize pain, and placed in a new cage singly. Animals were allowed to recover for 2 weeks before the beginning of testing.

To determine striatal monoamine alterations 2 weeks after surgery, a separate group of animals was given unilateral 6-OHDA lesions (2 µl/injection site, total 5 µg of 6-OHDA given), along with contralateral vehicle injections which were used for comparison. All other surgery conditions were identical to those described above.

Behavioral Testing

Straight Channel (day 13)

One day prior to CWM testing, animals were tested for swimming in a 244 cm long x 15 cm wide x 51 cm high water filled (38 cm deep) straight channel for 4 consecutive trials with a maximum time limit of 2 min/trial (Herring et al., 2008; Vorhees et al., 2008). Straight channel swimming served three functions: (a) swimming acclimation, (b) to teach that escape was possible by climbing on the submerged platform at the opposite end of the channel, and (c) to determine if all animals had comparable swimming ability.

Cincinnati water maze (days 14-28)

The CWM is a nine-unit multiple T maze placed in water (21 ± 1 °C) as described previously (Vorhees, 1987; Vorhees et al., 2008; Vorhees et al., 1991). Animals had to locate a submerged escape platform; the room was dark in order to eliminate visual cues with infrared lighting for the camera. Two trials/day (5 min limit/trial) were given. If an animal failed to find the escape within 5 min on trial-1 of each day, they were given not less than 5 min of rest before trial-2. If they found the escape on trial-1 in less than 5 min, trial-2 was given immediately.
Animals reaching the time limit were removed and not guided to the goal. Latency to escape and number of errors (defined as head and shoulder entry in a stem or arm of a T that was not on the path to the goal) were recorded. To correct for animals that stopped searching for the escape, animals failing to find the platform within 5 min were given an error score equal to the highest number of errors made by the animal that did find the escape and had the most errors in under 5 min + 1. Data for the CWM were analyzed in 2-day (4 trials) blocks similar to the 4-trial blocks used to analyze MWM data.

Morris water maze hidden platform (days 29-35)

MWM hidden platform testing began the day following CWM completion. Animals were placed in a 244 cm diameter tank of water (21 ± 1 °C) and were required to find a submerged platform (10 cm diameter) in a stationary position with pseudo-randomized, balanced cardinal and ordinal start positions. For 6 days, rats were given 4 trials/day with a 2 min trial limit and an ITI of 15 s (on the platform). If a rat failed to find the platform within the time limit, it was placed on the platform. On the 7th day, a 30 s probe trial was from a novel start position with the platform removed. Data were collected using video tracking software (AnyMaze, Stoelting Co., Wood Dale, IL).

Morris water maze cued (days 36-37)

Cued MWM testing began the day following the hidden platform phase for 2 days. Curtains were closed around the tank to minimize distal cues, and a yellow plastic ball was attached to the top of a brass rod mounted in the center of the submerged platform (10 cm diameter) to mark its location. On each day, animals were given 4 trials with the locations of the platform and starting positions randomized (2 min trial limit with ITI of 15 s on the platform + 15-20 s to reposition the platform). Latency was manually recorded.
Tissue Collection

Tissue collection took place following the completion of behavioral testing for animals that received bilateral 6-OHDA lesions, or 14 days following unilateral 6-OHDA lesions for those not tested. Animals were brought to an adjacent suite and decapitated. Brains were removed and dissected and the neostriatum, hippocampi, and prefrontal cortex (PFC) were frozen for later monoamine assay as previously described (Williams et al., 2007).

Monoamine assays

Monoamines were assayed via high performance liquid chromatography with electrochemical detection (HPLC-ECD). Frozen tissues were weighed, thawed, and sonicated in appropriate volumes of 0.1 N perchloric acid (Fisher Scientific, Pittsburgh, PA). Samples were centrifuged for 14 min at 13,000 RCF at 4°C. The supernatant sample was transferred to a new vial for injection onto a Supelco Supelcosil™ LC-18 column (150 × 4.6 mm, 3 µm; Sigma-Aldrich Co., St. Louis, MO). The HPLC system consisted of a Waters 717plus autosampler (Waters Corp., Milford, MA), ESA 584 pump, and Coulochem III electrochemical detector. The potential settings were -150 mV for E1 and +250 mV for E2, with a guard cell potential set at +350 mV. MD-TM mobile phase (ESA Inc.) was used and consisted of 75 mM sodium dihydrogen phosphate (monohydrate), 1.7 mM 1-octanesulfonic acid sodium salt, 100 µl/l triethylamine, 25 µM EDTA, and 10% acetonitrile, with a final pH of 3.0. The pump flow rate was set at 0.7 ml/min, and the samples were run at 28°C. Standards for DA, 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine (NE), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) (all obtained from Sigma-Aldrich Co., St. Louis, MO) were prepared in 0.1 N perchloric acid. All neurotransmitters were run on a single chromatogram.
**Statistical Analysis**

Data were analyzed using mixed linear ANOVA models (SAS Proc Mixed, SAS Institute 9.2, Cary, NC). The covariance matrix for each dataset was checked using best fit statistics. In most cases, the best fit was to the autoregressive-1 covariance structure. Kenward-Rodger adjusted degrees of freedom were used. Measures taken repetitively on the same animal, such as week, day, or block, were within-subject factors. For the MWM, an analysis of covariance (ANCOVA) using swim speed as a covariate was also performed to account for lesion-induce motor differences. Significant interactions were analyzed using simple-effect slice ANOVAs at each level of the repeated measure factor. Biochemical data were analyzed using two-tailed t-tests. Significance was set at $p \leq 0.05$ and trends at $p \leq 0.10$. Data are presented as least squared (LS) mean ± LS SEM.

**Results**

**Body Weights**

No differences between body weights were observed on the day of surgery (Fig. 1, week 0). At 5 and 6 weeks post-surgery, lesioned animals weighed significantly less than their SHAM counterparts [lesion x week interaction: 5 weeks: $F(1,30.5) = 4.43$, $p < 0.05$; 6 weeks: $F(1,34.7) = 5.41$, $p < 0.05$]. However, the main effect of lesion was not significant.

**Straight Channel**

No difference in time to swim the straight channel was observed across trials between 6-OHDA-lesioned and SHAM control animals (LS mean ± LSSEM across trials: 6-OHDA: 15.67 ± 1.85 s; SHAM: 13.87 ± 2.00 s).

**Cincinnati water maze**
6-OHDA-treated animals had significantly increased latencies to find the platform compared with SHAM animals (F(1,26.5) = 6.09, p < 0.01; Fig 2A) with significantly longer latencies observed from block-5 through block-9 (treatment x block, F(8,146) = 2.77, p < 0.01). 6-OHDA-treated animals committed significantly more errors overall compared with SHAM controls (F(1, 24.1) = 5.01, p < 0.05; Fig 2B) with significantly more errors observed during blocks 5-7 and block 9 (treatment x block effect: F(8,145) = 2.68, p < 0.01).

Because of the difficulty of finding the escape under infrared lighting, 100% of animals had one or more trials in which they reached the 5-min time limit. Most 5-min trials occurred on early test days (mostly days 1-3) then declined rapidly thereafter. For SHAM, 40.5% of trials reached the time limit whereas for 6-OHDA animals, 57.3% reached the time limit (p < 0.05), providing further evidence that lesioned rats had greater difficulty learning the task than SHAM.

*Morris water maze*

All animals learned to find the hidden platform during testing, however latency to find the platform (F(1,23.3) = 13.03, p < 0.001; Fig 3A), path length (F(1,23.3) = 5.55, p < 0.05; Fig 3B), and cumulative distance (F(1,23.4) = 14.67, p < 0.001; not shown) were significantly increased in 6-OHDA-treated animals compared with SHAM controls. There was no treatment interaction with day. 6-OHDA-treated animals had reduced speed compared with SHAM animals (F(1,23) = 8.71, p < 0.01; 6-OHDA: 0.23 ± 0.01 m/s, SHAM: 0.24 ± 0.01 m/s). However, ANCOVA with swim speed as the covariate showed that speed did not account for the increase in latency (F(1,22.2) = 5.79, p < 0.05), path length (F(1,22.2) = 7.53, p < 0.01), or cumulative distance (F(1,22.3) = 6.33, p < 0.01) of 6-OHDA-treated animals compared with SHAM animals.
During the probe trial, 6-OHDA-treated animals showed a decreased percentage of time in the target quadrant compared with SHAM animals ($t(23) = 1.84, p < 0.05$; 6-OHDA: 31.79% ± 4.4%, SHAM: 43.63% ± 4.6%) and had greater average distance from the platform site compared with SHAM animals ($t(23) = 1.89, p < 0.05$; 6-OHDA: 0.76 ± 0.05 m, SHAM: 0.61 ± 0.06 m). ANCOVA with swim speed as the covariate did not alter these results (percent time in target quadrant: $t(22) = 1.79, p < 0.05$; average distance: $t(22) = 1.83, p < 0.05$, respectively).

For cued platform trials, there were no significant differences between 6-OHDA-treated and SHAM-treated animals for latency to find the platform (data not shown) further supporting the notion that performance factors cannot account for the spatial learning and retention deficits observed in the 6-OHDA lesioned animals.

**Monoamine Assessment**

In the neostriatum at 2 weeks, DA concentrations on the 6-OHDA-injected side were decreased by 80% compared with the vehicle-injected side ($t(10) = 9.55, p < 0.001$) and were decreased bilaterally in 6-OHDA-lesioned-behaviorally tested animals at 7 weeks compared with SHAM-treated animals ($t(23) = 8.85, p < 0.001$; **Fig 4A**). DOPAC levels were also decreased at both time points (2 weeks: $t(10) = 3.65, p < 0.01$; 7 weeks: $t(23) = 6.16, p < 0.001$; **Table 1**). 6-OHDA also decreased striatal HVA levels (2 weeks: $t(10) = 4.58, p < 0.001$; 7 weeks: $t(23) = 7.48, p < 0.001$; **Table 1**) and increased DOPAC/DA ratios (**Table 1**; 2 weeks $t(10) = -4.25, p < 0.01$; 7 weeks: $t(23) = -5.36, p < 0.001$) compared with the SHAM-treated animals. Similar patterns were found for both intracellular (DOPAC/DA ratio) and extracellular (HVA/DA ratio) DA ratios. A trend was observed in 5-HT reductions at both time points (2 weeks: $t(10) = 1.92, p = 0.08$; 7 weeks: $t(23) = 1.84, p = 0.08$; **Fig 4B**) in 6-OHDA-injected striata compared with the appropriate controls. No differences were observed between treatments for 5-HIAA levels at
either time point. Levels of NE were decreased in 6-OHDA-injected animals 7 weeks post-surgery (t(23) = 2.26, p < 0.05; Fig 4C), but not in 6-OHDA-injected striata 2 weeks post-surgery compared with vehicle-injected striata.

**Hippocampus**

Monoamine levels in the hippocampus and PFC were only collected at the 7 week time point. NE levels were decreased in the 6-OHDA-treated animals compared with SHAM-treated animals (t(23) = 10.02, p < 0.001; 6-OHDA: 125.9 ± 17.3 pg/mg, SHAM: 349.8 ± 13.8 pg/mg). No differences were observed between treatments for 5-HT, 5-HIAA, or the 5-HT utilization ratio (5-HIAA/5-HT).

**Prefrontal Cortex**

Monoamine levels for the PFC at the 7-week time point were not different between treatment groups for NE, 5-HT, 5-HIAA levels, or the 5-HIAA/5-HT ratio.

**Discussion**

Both spatial learning in the MWM and route-based learning in the CWM were impaired following 80% reductions of neostriatal DA via bilateral injections of 6-OHDA. These deficits were present independently of motivational factors (no differences in straight channel or visible platform MWM escape times), or motor deficits (slightly slower swim speeds in the MWM that did not significantly affect efficient platform finding parameters). The observed reductions in DA metabolites (DOPAC and HVA) are consistent with previous reports following bilateral 6-OHDA neostriatal injection (Aguiar et al., 2008; Chen et al., 2007; Henze et al., 2005; Tadaiesky et al., 2008). Monoamines in the PFC were unaffected by 6-OHDA striatal treatment, and only NE levels were altered in the hippocampus. As hippocampal NE levels do not play a significant role in spatial navigation (Hagan et al., 1983; Thomas and Palmiter, 1997) and route-based
navigation is thought to be independent of hippocampal function (Devan et al., 1999; Devan and White, 1999; McDonald and White, 1993; McDonald and White, 1994), the deficits seen herein are most likely a result of the neostriatal DA reductions.

6-OHDA treatment resulted in an impairment of route-based navigation. This finding is consistent with data that drugs that target DA systems and produce decreases in DA levels affect CWM performance, while drugs that preferentially act on 5-HT do not (Herring et al., 2008; Herring et al., 2010; Vorhees et al., 2010b). However, drugs such as methamphetamine affect DA, 5-HT, and glutamate making it unclear which mechanism contributes most to the effect of the drug on route-based navigation. However, 6-OHDA is specific; therefore, this is the first study to show that deficits in the CWM may be attributed to DA depletion in the dorsal striatum.

Striatal DA has been shown to be involved in procedural learning of another kind: cued MWM deficits after intrastriatal 6-OHDA (Tadaiesky et al., 2008) or substantia nigra pars compacta 6-OHDA or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injections (Ferro et al., 2005; Miyoshi et al., 2002; Whishaw and Dunnett, 1985b). We did not observe such a deficit in this study, and this may be the result of testing differences. Here, the cued task followed spatial acquisition and conducting spatial learning prior to procedural learning has been shown to eliminate deficits in cued learning following intranigral MPTP injections (Da Cunha et al., 2007); therefore, test order may account for this apparent inconsistency. Cued vs. hidden platform MWM testing was not counterbalanced here in order to keep the methods between this study and our previous study consistent. Furthermore, while test order shows practice effects, order does not affect spatial learning per se. There are examples where drugs that induce sensorimotor interference impair MWM performance and these effects can be attenuated by
giving practice trials (cued or nonspatial) prior to spatial trials (Saucier et al., 1996), but there is no evidence for such effects in the present context with 6-OHDA lesions.

In addition, only large nigrostriatal DA reductions result in spatial navigation deficits following 6-OHDA or MPTP- injections (Whishaw and Dunnett, 1985b). We and others have observed MWM spatial learning acquisition deficits when DA levels are depleted by 60% or more (De Leonibus et al., 2007b; Lindner et al., 1999; Mura and Feldon, 2003; Whishaw and Dunnett, 1985b), but smaller reductions do not produce this effect (Da Cunha C. et al., 2003; Miyoshi et al., 2002).

Multiple lines of evidence indicate that motoric effects are unlikely to account for the learning and memory impairments observed in the 6-OHDA lesioned rats. First, the 6-OHDA group showed no change in straight channel swimming times, a task in which there is essentially no learning required. Accordingly, this task assesses a relatively direct measure of swim speed that reflects motor ability and motivation to escape from the water. The results show that 6-OHDA rats swim a straight corridor as fast as sham controls. Second, the 6-OHDA group showed increased CMW errors, a measure not influenced by swim speed or motor coordination. Third, the 6-OHDA group showed no deficits in the MWM on measures immune from performance factors, included path length and cumulative distance. Fourth, the 6-OHDA group, while they swam slower in the MWM on hidden platform acquisition trials, this did not affect learning indices based on ANCOVA results using swim speed as a covariate for each dependent measure (latency, path length, and cumulative distance) and showed no change in the finding of impaired spatial learning in the lesioned group. Fifth, a similar covariate analysis with swim speed during the probe trial confirmed that this did not alter the finding that the 6-OHDA group was impaired during the transfer trial requiring the rats to relocate the spot where the platform
had formerly been. Sixth, the cued trials showed no reduction in ability to reach the platform
compared with sham control group, providing no evidence that 6-OHDA impaired these animals’
ability to see and swim directly the platform even though it was moved unpredictably on every
trial. Overall, the small swim speed reduction found during the MWM hidden platform trials but
not on no other indices of swimming provides convergent evidence that the allocentric and
egocentric navigation deficits seen in the 6-OHDA group are upon learning and memory
processes.

In summary, we show here that both route-based and spatial navigation are substantially
determined by dorsal striatal DA. This provides further evidence for the role of neostriatal DA
on these forms of navigation, and is the first study to explore this in route-based egocentric
navigation in the CWM. Future research may benefit from a dose-response investigation of DA
reduction on performance to elucidate the threshold of DA for both types of navigational
processes and determine if there is a differential sensitivity for the effect of DA on these two
mazes. In addition, selective subregional lesions within the neostriatum have differential effects
on spatial and non-spatial learning as it has been shown that dorsolateral striatum is implicated in
egocentric tasks, whereas the dorsomedial striatum is implicated more heavily in spatial
acquisition (Devan and White, 1999; Devan et al., 1999; Divac et al., 1967).

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**Figure 1. Body Weights.** No initial weight difference was observed between groups prior to surgery. While there was no significant overall effect of 6-OHDA on body weight, animals that received striatal 6-OHDA injections weighed less at 5 and 6 weeks post-surgery compared with Sham controls. Arrow denotes start of behavioral testing. N = 13/6-OHDA; 12/Sham. * p < 0.05.
Figure 2. Cincinnati water maze. Throughout testing, striatal 6-OHDA injections increased latency to find the submerged platform (A), as well as number of errors made during the trial (B), compared with Shams. N = 13/6-OHDA; 12/Sham. * p < 0.05, ** p < 0.01, *** p < 0.001.
Figure 3. **Morris water maze.** Throughout testing, striatal 6-OHDA injections increased latency to find the submerged platform (A), as well as path length (B), compared with Shams. N = 13/6-OHDA; 12/Sham. * p < 0.05, *** p < 0.001.
Figure 4. Striatal monoamine levels. Striatal 6-OHDA injections decreased striatal DA (A), 5-HT (B), and NE (C) levels at both 2 and 7 weeks post-surgery. 2 weeks: N = 6/6-OHDA, 6/Sham. 7 weeks: N = 13/6-OHDA, 12/Sham. * p < 0.05, *** p < 0.001.
CHAPTER 3:
Dopamine depletion in either the dorsomedial or dorsolateral striatum impairs egocentric Cincinnati water maze performance while sparing allocentric Morris water maze learning
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Abstract

Both egocentric route-based learning and spatial learning, as assessed by the Cincinnati water maze (CWM) and Morris water maze (MWM), respectively, are impaired following an 80% dopamine (DA) loss in the neostriatum after 6-hydroxydopamine (6-OHDA) injection in rats. The dorsolateral striatum (DLS) and the dorsomedial striatum (DMS) have been implicated in different navigational learning types, namely the DLS is implicated in egocentric learning while the DMS is implicated in spatial learning. This experiment tested whether selective DA loss through 6-OHDA injection in the DMS or DLS would impair one or both types of navigation. Both DLS and DMS DA loss significantly impaired route-based CWM learning, without affecting spatial or cued MWM performance. DLS 6-OHDA caused 75% DA loss in this region, with no changes in other monoamine levels in the DLS or DMS. DMS 6-OHDA injection caused a 62% DA loss in this region, without affecting other monoamine levels in the DMS or DLS. The results indicate a role for DA in DLS and DMS regions in route-based egocentric but not spatial learning and memory. Spatial learning deficits may require more pervasive monoamine reductions before exhibiting deficits. This is the first study to implicate DLS and DMS DA in route-based navigation.
Introduction

Impairments in navigational ability are present in numerous human disorders where they impair the quality of life and increase dependency (Aguirre and D'Esposito, 1999, Weniger and Irle, 2006, Livingstone and Skelton, 2007, Sanders et al., 2008, Iaria et al., 2009). Successful navigation requires complex interactions among multiple distinct, but parallel cognitive processes that can be subdivided into egocentric (self-oriented path integration and route-based) and allocentric (map-based) wayfinding. Route-based navigation involves a representation of space connected by “nodes” or choice points representing successive decision points in a virtual grid or pathway (Byrne, 1982, Aguirre and D'Esposito, 1999). In the allocentric process, the navigator’s spatial orientation to distal cues in the environment is fluid and represented in a common coordinate map system external to the navigator (Byrne, 1982, Garber, 2000).

Considerable behavioral, anatomical, and electrophysiological evidence suggests that the neostriatum is an important modulator in both egocentric and allocentric learning (Potegal, 1969, 1972, Whishaw and Dunnett, 1985, Whishaw et al., 1987, Cook and Kesner, 1988, McGeorge and Faull, 1989, Packard et al., 1989, McDonald and White, 1994, Taube, 1998, Devan et al., 1999, Devan and White, 1999, Jog et al., 1999, Ragozzino et al., 2001, Mizumori et al., 2004, Mizumori et al., 2009, Packard, 2009, Braun et al., 2012, Penner and Mizumori, 2012). The neostriatum is a heterogeneous structure with anatomical subregions for different functions. The dorsomedial striatum (DMS) receives primary inputs from multiple sensory and association areas such as the hippocampus and medial prefrontal cortex, and while lesions in this area have widespread effects, they often produce impairments in allocentric learning (Whishaw et al., 1987, Colombo et al., 1989, McGeorge and Faull, 1989, Devan et al., 1999, Devan and White, 1999). For example, DMS lesions or DMS dopamine (DA) depletion result in allocentric
learning and place strategy deficits in the Morris water maze (MWM) and T-maze, respectively (Devan et al., 1999, Devan and White, 1999, Lex et al., 2011). Sensory and motor cortices have major projections to the dorsolateral striatum (DLS), which has been associated with egocentric or response learning and stimulus-response habit formation (McGeorge and Faull, 1989, Reading et al., 1991, Packard and McGaugh, 1996, White, 1997, Devan and White, 1999, Yin and Knowlton, 2004, Yin et al., 2004, Palencia and Ragozzino, 2005, Yin and Knowlton, 2006, Yin et al., 2006). However, this heterogeneity of function within the neostriatum may not be fully preserved in regards to egocentric learning. Excitotoxic lesions of the DMS and DLS each result in a severe learning impairment in a 14-unit T-maze procedural learning task, implicating both regions in egocentric learning (Pistell et al., 2009).

The focus of the present experiments was to elucidate the regionally-specific role of neostriatal DA in egocentric and allocentric navigation. DA in the neostriatum influences both glutamatergic afferents and striatal medium spiny neuronal efferents that modulate striatal output (Penner and Mizumori, 2012). Previously, we showed that widespread neostriatal 6-hydroxydopamine (6-OHDA)-induced DA reduction impaired learning in both the allocentric MWM and route-based Cincinnati water maze (CWM) (Braun et al., 2012). While DMS DA has been implicated in allocentric T-maze learning strategy (Lex et al., 2011), it has not been tested for involvement in either route-based or allocentric navigation. Moreover, the role of DA in DLS-mediated route-based or allocentric navigation has yet to be tested. Accordingly, we tested groups of animals given selective 6-OHDA injections in either the DMS or DLS and evaluated them in the CWM and MWM, respectively (test order was examined previously (Broening et al., 2001, Skelton et al., 2009) compared with sham-operated controls. Motivation and swimming ability were assessed to control for potential performance changes not associated with learning.
Methods

Animals

Adult male Sprague-Dawley rats (225-250 g) were purchased from Charles River Laboratories, Raleigh, NC. Animals were pair-housed in polypropionate cages (46 x 24 x 20 cm) containing woodchip bedding for at least a 1-week acclimation period prior to surgery. Animals had free access to food and water, were housed in an environmentally controlled vivarium (21 ± 1°C), and were on a 14 h light-dark cycle (lights on at 600 h). All procedures were in compliance with the Institutional Animal Care and Use Committee and the vivarium is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Surgery

Rats were anesthetized with 2-4% isoflurane (IsoThesia; Butler Animal Health Supply, Dublin, OH) with continuous administration via a nose cone throughout surgery. Animals were placed in a motorized, computer-controlled stereotaxic apparatus (StereoDrive, Stoelting Co., Wood Dale, IL), and were given bilateral injections of 6-OHDA (Sigma, St. Louis, MO) using a 26 gauge 10 µl Hamilton Gastight syringe (Reno, NV). Coordinates were based on the Paxinos and Watson brain atlas (Paxinos et al., 1985). For the DLS lesions, a volume of 3 µl [4 µg /µl 6-OHDA in 0.2% ascorbic acid saline solution] was injected over 9 min (from bregma: AP: +0.2 mm; ML: ± 3.5 mm; from skull: DV: -4.8 mm), with the needle left in place for 1 min following injection. For the DMS lesions, a volume of 0.4 µl [30 µg /µl] was injected in each site over 4 min (from bregma: AP: +1.0 mm; ML: ± 1.7 mm; DV: -5.0 mm; and AP: -0.4 mm; ML: ± 2.6 mm; DV: -4.5 mm), with the needle left in place for 5 min following completion of injection. Control animals (SHAM) received an identical amount of saline in 0.2% ascorbic acid vehicle
(VEH) using the same procedure for its particular group. Following surgery, animals were given 0.1 ml buprenorphine hydrochloride to minimize pain. Animals were allowed to recover for 2 weeks before the beginning of testing.

Behavioral Testing

Straight Channel

One day prior to CWM testing, animals were tested for swimming ability in a 244 cm long x 15 cm wide x 51 cm high water filled (38 cm deep) straight channel for 4 consecutive trials with a maximum time limit of 2 min/trial (Herring et al., 2008, Vorhees et al., 2008). Straight channel swimming served three functions: (a) to acclimate animals to swimming, (b) to teach that escape was possible by climbing on the submerged platform at the opposite end of the channel, and (c) to determine if all animals had comparable swimming ability.

Cincinnati water maze

The CWM is a nine-unit multiple T water maze (21 ± 1°C) as described previously (Vorhees, 1987, Vorhees et al., 1991, Vorhees et al., 2008). Animals had to locate a submerged escape platform; the room was illuminated with infrared lighting in order to eliminate visual cues; a video camera was mounted above the maze sensitive to light in the near infrared range and fed to a monitor in another room. Two trials/day (5 min limit/trial) were given. If an animal failed to find the escape within 5 min on trial-1 of each day, there was at least a 5 min intertrial interval (ITI) before trial-2. If they found the escape on trial-1 in less than 5 min, trial-2 was given immediately. Animals reaching the time limit were removed from the maze from wherever they were when the time limit was reached. Latency to escape and number of errors (defined as head and shoulder entry in a stem or arm of a T or reentry into the start channel) were recorded. To correct for animals that stopped searching, they were given an error score equal to
the number of errors + 1 made by the animal that found the escape and made the most errors in < 5 min. Animals that never found the platform were removed from analysis. Data for the CWM were analyzed in 2-day (4 trials) blocks similar to the 4-trial blocks used to analyze MWM data.

*Morris water maze hidden platform*

To test spatial navigational learning, MWM hidden platform testing began the day following CWM completion (Morris, 1981). Animals were placed in a 244 cm diameter tank of water (21 ± 1 °C) and were required to find a submerged platform (10 cm diameter) in a stationary position with pseudo-randomized, balanced cardinal and ordinal start positions. For 6 days, rats were given 4 trials/day with a 2 min trial limit and an ITI of 15 s (on the platform). If a rat failed to find the platform within the time limit, it was placed on the platform. On the 7th day, a 30 s probe trial was given from a novel start position with the platform removed. Data were collected using video tracking software (AnyMaze, Stoelting Co., Wood Dale, IL).

*Morris water maze cued*

Cued MWM testing began the day following the hidden platform testing and was conducted over two days. A yellow plastic ball was attached to the top of a brass rod mounted in the center of the submerged platform (10 cm diameter) to mark its location. On each day, rats were given 4 trials with the locations of the platform and starting positions randomized (2 min trial limit with an ITI of 15 s on the platform + 15-20 s to reposition the platform). Latency was recorded (AnyMaze could not track rats under these lighting conditions).

*Tissue Collection*

Tissue collection took place following the completion of testing. Animals were brought to an adjacent suite and decapitated. Brains were removed and the neostriatum dissected and
further segmented into the DMS and DLS. Brain regions were rapidly frozen for later monoamine assay as described (Williams et al., 2007).

**Monoamine assays**

Monoamines were assayed via high performance liquid chromatography with electrochemical detection (HPLC-ECD). Frozen tissues were weighed, thawed, and sonicated in appropriate volumes of 0.1 N perchloric acid (Fisher Scientific, Pittsburgh, PA). Samples were centrifuged for 14 min at 13,000 RCF at 4°C. The supernatant sample was transferred to a new vial for injection on a Supelco Supelcosil™ LC-18 column (150 × 4.6 mm, 3 µm; Sigma-Aldrich Co., St. Louis, MO). The HPLC system consisted of a Waters 717plus autosampler (Waters Corp., Milford, MA), ESA 584 pump, and Coulochem III electrochemical detector. The potential settings were -150 mV for E1 and +250 mV for E2, with a guard cell potential set at +350 mV. MD-TM mobile phase (ESA, Inc.) was used and consisted of 75 mM sodium dihydrogen phosphate (monohydrate), 1.7 mM 1-octanesulfonic acid sodium salt, 100 µl/l triethylamine, 25 µM EDTA, and 10% acetonitrile, with a final pH of 3.0. The pump flow rate was set at 0.7 ml/min, and the samples were run at 28°C. Standards for DA, 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine (NE), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) (all obtained from Sigma-Aldrich Co., St. Louis, MO) were prepared in 0.1 N perchloric acid. All neurotransmitters were run on a single chromatogram.

**Immunohistochemistry**

Using the same surgical procedures as in behavioral testing, animals were given a unilateral injection of 6-OHDA in the DLS, and a VEH injection on the contralateral side (N = 3). This was also done in other animals using the DMS coordinates (N = 3). Two weeks after
surgery the animals were brought into an adjacent suite, perfused transcardially with 4% paraformaldehyde, and the brains dissected, postfixed, and sunk in sucrose overnight. Brains were sectioned (at 30-μm thickness) on a microtome, and the free-floating sections processed for tyrosine hydroxylase (TH) immunohistochemistry as previously described (Hemmerle et al., 2014), using mouse monoclonal anti-TH primary antibody (MAB318, diluted 1:8000; EMD Millipore, Telecuma, CA), biotinylated horse anti-mouse IgG secondary antibody (BA-2000, diluted 1:200; Vector Laboratories, Burlingame, CA), and ABC Elite Kit reagents (Vector Laboratories) with diaminobenzidine as chromagen. Striatal immunostaining for TH was analyzed for the regional specificity of 6-OHDA injections as indicated by TH depletion in the DMS or DLS. Sections were viewed and scanned at 20X on the Aperio AT2 slide scanner, and uploaded to Aperio eSlide Manager (Leica Biosystems, Buffalo Grove, IL).

Statistical Analysis

DLS and DMS groups were tested separately therefore data from each experiment were analyzed independently. Data were analyzed using mixed linear ANOVA models (SAS Proc Mixed, SAS Institute 9.2, Cary, NC). The covariance matrix for each dataset was checked using best fit statistics. In most cases, the best fit was to the autoregressive-1 covariance structure. Kenward-Rodger adjusted degrees of freedom were used. Measures taken repetitively on the same animal, such as day or block, were within-subject factors. Significant interactions were analyzed using simple-effect slice ANOVAs at each level of the repeated measure factor. Biochemical data were analyzed using two-tailed t-tests. Significance was set at p ≤ 0.05. Data are presented as least square (LS) mean ± LS SEM.

Results

Immunohistochemistry
A representative of sections from one animal that received a DLS or DMS lesion is showed herein. To ensure that TH neuronal loss due to DLS 6-OHDA injections was specific for the DLS, TH immunolabeling in the striatum after unilateral 6-OHDA injection into the DLS was examined. Unilateral 6-OHDA injection into the DLS resulted in DLS-specific loss of TH immunostaining (Fig 1B). No reduction of striatal TH immunoreactivity was observed in the contralateral DLS in the same animal that was injected with VEH (Fig 1A).

The striatal regional specificity of the DMS injections of 6-OHDA was also confirmed by analyzing TH immunostaining. Thus, unilateral 6-OHDA injections into the DMS resulted in DMS-specific loss of TH immunolabeling (Fig 1D). The contralateral DMS injected with VEH in the same animal demonstrated no such reduction in TH immunostaining (Fig 1C).

**Dorsolateral Striatal 6-OHDA Lesions**

**Straight Channel**

No difference in time to swim the straight channel was observed across trials between DLS 6-OHDA-treated and SHAM animals (LS mean ± LS SEM across trials: 6-OHDA: 13.8 ± 1.1 s; SHAM: 12.3 ± 1.2 s).

**Cincinnati water maze**

6-OHDA-treated animals had significantly increased latencies to find the platform compared with SHAM animals (F (1,37.7) = 5.75, p ≤ 0.05; Fig 2A) with significantly longer latencies observed from block-3 through block-8 (treatment x block: F(8, 177) = 2.18, p ≤ 0.05; Fig 2B). DLS 6-OHDA-treated animals committed significantly more errors compared with SHAM animals (F(1,39) = 6.56, p ≤ 0.05; Fig 2A) with significantly more errors observed during block-3 through block-6 and block-8 (treatment x block: F(8,177) = 2.24, p ≤ 0.05; Fig 2C).
Morris water maze

6-OHDA-treated animals showed no difference in MWM performance compared with SHAM animals. No significant difference was found in latency to find the platform (Fig 3), path length, or cumulative distance to the platform. Swim speed did not differ between groups (6-OHDA: 0.41 ± 0.07 m/s; SHAM: 0.47 ± 0.09 m/s). Initial heading error and average heading error were not significantly different between 6-OHDA-treated animals and SHAM controls. During the probe trial, 6-OHDA-treated animals were not affected on the number of platform crossovers (6-OHDA: 0.66 ± 0.28; SHAM: 0.5 ± 0.26) or average distance from the platform site compared with SHAM animals (6-OHDA: 0.83 ± 0.06 m; SHAM: 0.85 ± 0.06 m). For cued platform trials, there was no significant latency difference between 6-OHDA-treated animals and SHAM controls (averaged across days and trials: 6-OHDA: 28.76 ± 3.31 s; SHAM: 23.48 ± 3.54 s).

Monoamine Assessment

6-OHDA injection caused a 75% decrease in DLS DA compared with SHAM animals (t(22) = 11.2, p ≤ 0.001; Fig 4A) with significant decreases in DA metabolites (DOPAC: t(22) = 6.36, p ≤ 0.001; HVA = t(20) = 6.06, p ≤ 0.001) and utilization ratios (DOPAC/DA = t(22) = 5.38, p ≤ 0.001; overall turnover ratio: t(20) = 3.10, p ≤ 0.001) (Table 1). NE and 5-HT levels in the DLS were not altered in 6-OHDA-treated animals compared with SHAM controls (Fig 4B and 4C, respectively). To determine if DLS 6-OHDA injections affected the DMS this region was also analyzed. DA concentrations (Fig 4D), metabolites and turnover in the DMS after 6-OHDA DLS injection were not significantly different compared with SHAM animals. NE (Fig 4E) and 5-HT (Fig 4F) levels were also not changed.

Dorsomedial Striatal 6-OHDA Lesions
**Straight Channel**

No difference in time to swim the straight channel was observed between 6-OHDA-treated animals and SHAM controls (LS mean ± LS SEM across trials: 6-OHDA: 18.53 ± 2.52 s; SHAM: 17.33 ± 2.22 s).

**Cincinnati water maze**

6-OHDA-treated rats had increased latency to find the platform compared with SHAM animals (F(1,19.4) = 4.36, p ≤ 0.05; Fig 5A), but the treatment x block interaction was not significant (F(8, 109) = 1.80, p ≤ 0.10; Fig 5B). A trend towards significantly more errors overall in 6-OHDA-treated animals was also seen (F(1, 19.8) = 4.04, p ≤ 0.10; Fig 5A), however on blocks 4-6 and block 9 lesioned animals made significantly more errors (treatment x block: F(8, 109) = 2.59, p ≤ 0.05; Fig 5C) compared with SHAM control animals.

**Morris water maze**

6-OHDA-treated animals showed no difference in MWM performance compared with SHAM animals. No significant differences were found in latency to reach the platform (Fig 6), path length, or cumulative distance to the platform. Swim speed was not significantly different between the groups (6-OHDA: 0.29 ± 0.01 m/s; SHAM: 0.28 ± 0.01 m/s). Initial and average heading errors were not significantly altered. During the probe trial, 6-OHDA-treated rats showed no significant effect on the number of platform crossings (6-OHDA: 1.00 ± 0.58; SHAM: 1.00 ± 0.38), but exhibited a trend towards significantly longer average distance from the platform site (6-OHDA: 0.93 ± 0.06 m; SHAM: 0.73 ± 0.07 m; t(13) = 2.07, p ≤ 0.10). For cued platform trials, there was no significant latency difference between 6-OHDA-treated animals and SHAM animals (averaged across days and trials: 6-OHDA: 27.66 ± 9.30 s; SHAM: 36.27 ± 8.21 s).
Monoamine Assessment

Following 6-OHDA injection in the DMS, DA concentrations were decreased 62% compared with SHAM controls (t(14) = 8.87, p ≤ 0.001; Fig 7A). 6-OHDA injection in the DMS also decreased DA metabolites (DOPAC: t(14) = 3.86, p ≤ 0.01; HVA: t(14) = 2.74, p ≤ 0.05) and increased turnover (DOPAC/DA: t(14) = 2.42, p ≤0.01; HVA/DA: t(14) = 5.29, p ≤ 0.001; overall turnover: t(14) = 3.47, p ≤ 0.01) compared with SHAM controls (Table 2). DMS NE (Fig 7B) and 5-HT (Fig 7C) were not altered following DMS 6-OHDA injection compared with SHAM injections.

DA concentrations following 6-OHDA DMS injection were not significantly different in the DLS compared with SHAM controls (Fig 7D). DA metabolites and turnover and NE (Fig 7E) and 5-HT (Fig 7F) were not significantly altered in the DLS following 6-OHDA DMS injection compared with SHAM controls.

Discussion

6-OHDA injections in the DLS reduced DA levels by 75% and resulted in CWM route-based navigation deficits, but had no effect on hidden platform allocentric learning in the MWM. DMS DA depletion of 62% also resulted in route-based CWM navigational deficits, without altering MWM-based allocentric learning. These deficits were independent of motivational or motoric impairments (no differences in straight channel, visible platform MWM, or swim speed in the MWM). The DMS has been implicated in other behaviors, such as modulation of expected reward value, initiation behavior, and goal-directed behavior (White, 1997, Calaminus and Hauber, 2009, Mizumori et al., 2009, Penner and Mizumori, 2012, Fouquet et al., 2013, Kim et al., 2013). These other processes are likely not contributing to the egocentric impairment seen in the present study as the other tested behaviors were unaltered. For each of the striatal
subregions, the 6-OHDA injection damage was limited to the targeted area, precluding potential
effects from the other region to explain the route-based navigational deficits. NE and 5-HT were
not altered, regardless of which striatal subregion was lesioned, leaving DA loss to account for
the observed learning impairments. It is unlikely that brain regions outside of the neostriatum
were involved, as whole neostriatal DA loss has not been shown to affect monoamine levels in
other brain regions associated with learning (Braun et al., 2012). The observed changes in DA
metabolites and turnover are consistent with what others have found for these regions (Henze et
al., 2005, Chen et al., 2007, Aguiar et al., 2008, Tadaiesky et al., 2008, Braun et al., 2012).

The neostriatum has long been associated with egocentric learning (Potegal, 1969, 1972,
McDonald, 2002, Mizumori et al., 2004, Yin and Knowlton, 2004, Yin et al., 2004, Mizumori et
al., 2005, Palencia and Ragozzino, 2005, Yin and Knowlton, 2006, Yin et al., 2006, Packard,
2009, Braun et al., 2012, Penner and Mizumori, 2012). While a separation of function between
the DMS and DLS in regards to allocentric learning tasks has been observed, both regions have
independently been implicated in egocentric learning. Glutamate in the DLS has a modulatory
role in egocentric response learning in a T-maze and post-training silencing of the DLS inhibits
egocentric response (Packard and McGaugh, 1996, Palencia and Ragozzino, 2005). Recently,
Etienne et al. showed that pharmacological inhibition of the DMS reduced route-based
(direction-based) learning, but not allocentric or cued learning in rhesus macaques (Etienne et al.,
2012). Excitotoxic lesions of either the DMS or DLS impaired procedural learning in a 14-unit
T-maze (Pistell et al., 2009). While both Pistell et al. and this study implicate the DMS and DLS
in complex egocentric learning, the 14-unit T maze and the CWM have several differences that
distinguish the CWM as a test of route-based egocentric navigation rather than a task of procedural memory as the 14 unit T maze. For example, in the CWM there are no spatial cues available, it uses water as the motivator, and animals are not forced into making a left-right response choice during navigation. The 14 unit T maze has spatial cues available, uses shock for the motivator, guillotine doors close off previously visited arms, and there is a forced left-right response to navigate correctly. Testing in the CWM lasts for 18 days (2 trials/day) allowing animals to demonstrate long-term learning and memory ability, whereas testing in the 14-unit T-maze lasts for 1 day with 15 trials.

How DA signaling in the DMS and DLS modulates egocentric learning is currently unknown. The DMS and DLS have been shown to possess both allocentric place cells and neurons that fire only to specific egocentric response movements such as turns, forward movement, and head direction (Wiener, 1993, Lavoie and Mizumori, 1994, Ragozzino et al., 2001). The egocentric response cells in the DLS and/or DMS could be influenced by DA projections and compromised following DA loss. Striatal DA could also be influencing egocentric learning through its direct regulation of glutamatergic input to medium spiny neurons, inputs that are necessary for initial egocentric learning in the DLS (Sesack et al., 2003, Palencia and Ragozzino, 2005).

The finding that allocentric learning was spared following DLS DA loss is consistent with the literature that shows this area is not necessary for this type of learning, regardless of lesion type (Devan et al., 1999, Yin and Knowlton, 2004, Yin et al., 2006, Mizumori et al., 2009, Packard, 2009). Electrolytic and excitotoxic lesions of the DMS cause deficits in hidden platform MWM learning (Devan et al., 1999, Devan and White, 1999). DA in the posterior DMS has been implicated in place learning, however not specifically in MWM-based allocentric
When given a choice between solving a T-maze using an egocentric response strategy or an allocentric place strategy subsequent to posterior DMS DA depletion, a significantly higher proportion of animals utilized an egocentric response (83%) compared with SHAM controls (50%) early in testing. During later phases of learning no differences were observed between groups. As it is more common for animals to utilize a place strategy during early training and transition into the response strategy following continued training, the inference was that DA-depleted animals exhibited a deficit in allocentric performance during the phase of acquisition when place learning normally dominates. No overall learning deficit was observed in that both groups learned the task; only the strategy used initially differed.

Differences between mazes may explain the lack of effect in the current study for place learning compared with the Lex et al. (2011) study. The T-maze is more rudimentary than the CWM, making it easier to solve. While the T-maze gives a choice between two strategies, the CWM and MWM are configured such that only one strategy or the other is effective. The CWM is tested under infrared light eliminating spatial cues, and animals do not develop an egocentric learning strategy in the MWM using the testing protocol herein (Morris, 1981). While animals in the Lex et al. (2011) study resorted to a response strategy over a place learning strategy in the T maze, animals in the present study learned at the same rate as controls when given only the option of allocentric learning in the MWM but had deficits when given only the option of egocentric learning in the CWM. Because the CWM is a more complex egocentric learning task, it was able to uncover the involvement of DA in the DMS for this type of learning.

It is unlikely that greater DA loss would have resulted in a MWM allocentric learning impairment. Allocentric learning deficits in the MWM require a threshold of about 60% neostriatal DA depletion (Whishaw and Dunnett, 1985, Lindner et al., 1999, Miyoshi et al., 2002,
Da Cunha et al., 2003, Mura and Feldon, 2003, De Leonibus et al., 2007, Braun et al., 2012), a level of reduction exceeded in the present experiments. Since DA loss in the DLS and DMS lesioned groups surpassed this level of reduction, it suggests that more widespread neostriatum DA loss is necessary before allocentric learning deficits are observed rather than greater subregional loss. In agreement with this, genetically DA-deficient mice unable to show allocentric MWM learning exhibit a restoration of learning following DA supplementation to either the DMS or DLS; this also indicates that allocentric learning does not depend on DA signaling in a single striatal subregion (Darvas and Palmiter, 2010). Taken together with previous studies, it appears that allocentric learning deficits following DMS lesions require a lesion of more than DA alone.

Limitations to the present study include: attempts to deplete DA further without causing 6-OHDA damage to other regions were unsuccessful; that we tested only CWM egocentric learning and it is possible that other tasks might show different effects; that navigation is undoubtedly the product of complex interactions among different neurotransmitters and receptors in different regions, such that isolating only the role of DA in two neostriatal subregions is necessarily artificial; and test order may have contributed to the findings since there may have been positive transfer from the CWM to the MWM which, if it occurred, would have benefited MWM performance and reduced apparent effects on allocentric navigation. This is unlikely though, as mice lacking neostriatal DA are impaired in strategy-switching, and excitotoxic lesions of the DMS increase perseverative behavior in both rats and marmoset monkeys (Rogers et al., 2001, Clarke et al., 2008, Castane et al., 2010, Darvas and Palmiter, 2010). Future studies are needed, however, to further clarify each of these points.
While neostriatal DA has been shown to be involved in egocentric navigation (Anguiano-Rodriguez et al., 2007, Braun et al., 2012), this is the first experiment to implicate both DLS and DMS DA as modulatory factors in egocentric route-based navigation. This study is also the first to directly implicate the DMS in route-based learning. Taken together with our previous data where DA was depleted throughout the neostriatum (Braun et al., 2012), the findings support the view that neostriatal DA involvement in allocentric learning requires contributions from both the DLS and DMS. Conversely, the DLS and DMS can each influence egocentric learning independently.


Whishaw IQ, Dunnett SB (1985) Dopamine depletion, stimulation or blockade in the rat disrupts spatial navigation and locomotion dependent upon beacon or distal cues. BehavBrain Res 18:11-29.


### Table 1
Dorsolateral striatal DA metabolite levels and turnover rate after DLS 6-OHDA injection

<table>
<thead>
<tr>
<th></th>
<th>DOPAC (pg/mg)</th>
<th>HVA (pg/mg)</th>
<th>DOPAC/DA ratio</th>
<th>HVA/DA ratio</th>
<th>(DOPAC + HVA)/DA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLS 6-OHDA lesion</td>
<td>499.9 ± 92.4 ***</td>
<td>232.4 ± 49.7 ***</td>
<td>0.18 ± 0.01 ***</td>
<td>0.08 ± 0.01</td>
<td>0.25 ± 0.02 **</td>
</tr>
<tr>
<td>Sham lesion</td>
<td>1308.2 ± 85.9</td>
<td>721.7 ± 60.8</td>
<td>0.11 ± 0.004</td>
<td>0.06 ± 0.005</td>
<td>0.17 ± 0.008</td>
</tr>
</tbody>
</table>

** & *** denotes significance between surgery groups ( ** p ≤ 0.01; *** p ≤ 0.001)
Table 2
Dorsomedial striatal DA metabolite levels and turnover rate after DMS 6-OHDA injection

<table>
<thead>
<tr>
<th></th>
<th>DOPAC (pg/mg)</th>
<th>HVA (pg/mg)</th>
<th>DOPAC/DA ratio</th>
<th>HVA/DA ratio</th>
<th>(DOPAC + HVA)/DA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS 6-OHDA lesion</td>
<td>695.4 ± 111.8 **</td>
<td>346.9 ± 29.85 *</td>
<td>0.25 ± 0.03 *</td>
<td>0.12 ± 0.01 ***</td>
<td>0.38 ± 0.04 **</td>
</tr>
<tr>
<td>Sham lesion</td>
<td>1324.8 ± 114.2</td>
<td>482.2 ± 36.7</td>
<td>0.18 ± 0.009</td>
<td>0.06 ± 0.004</td>
<td>0.24 ± 0.01</td>
</tr>
</tbody>
</table>

*, **, *** denotes significance between surgery groups ( * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001)
Figure 1. Immunohistochemistry. Unilateral DLS VEH injections did not alter TH levels (A). Unilateral DLS 6-OHDA injections on the contralateral side selectively destroyed TH neurons only in the DLS region of the striatum (B). DMS VEH injection the striatum in a different animal did not affect TH levels (C). TH loss was limited to the DMS following unilateral 6-OHDA injections in the DMS (C).
Figure 2. DLS Cincinnati water maze latency and errors. There was a main effect of lesion: DLS lesioned animals had a longer latency and made more errors across than SHAMs (A). Across days, DLS lesioned animals had significantly longer latencies during blocks 3-8 (B) and made significantly more errors during blocks 3-6 and block 8, compared with SHAMs. N = 12/group. *p ≤ 0.05, **p ≤ 0.01.
Figure 3. DLS Morris water maze latency. 6-OHDA injected in the DLS did not have a significant effect on latency (main effect or day x lesion interaction) to find the hidden platform compared with SHAMs.
**Figure 4. DLS Monoamine levels.** 6-OHDA injection in the DLS significantly decreased DA levels in the DLS by 75% (A), with no change in NE (B), or 5-HT (C) DLS levels, compared with SHAMS. DMS DA (D), NE (E), and 5-HT (F) levels were not altered following DLS DA depletion. ***p ≤ 0.001.
Figure 5. DMS Cincinnati water maze latency and errors. (A) Animals with DA depletion in the DMS had significantly longer latencies to find the platform, and a trend towards significantly making more errors compared to controls over the duration of testing. DMS lesioned animals were not significantly different than SHAMs in latency across time (B), but made significantly more errors than SHAM controls on blocks 4-6 and block 9 (C) across time. N = 7/6-OHDA; 9/SHAM. +p ≤ 0.1, *p ≤ 0.05
Figure 6. DMS Morris water maze latency. 6-OHDA injected in the DMS did not have a significant effect on latency (main effect or day x lesion interaction) to find the hidden platform compared with SHAMs.
Figure 7. DMS Monoamine levels. 6-OHDA injection in the DMS significantly decreased DA levels in the DMS by 62% (A), with no change in NE (B), or 5-HT (C) DMS levels, compared with SHAMS. DLS DA (D), NE (E), and 5-HT (F) levels were not altered following DMS DA depletion. ***p ≤ 0.001.
CHAPTER 4:
Dopamine depletion in the nucleus accumbens impair egocentric and allocentric learning in rats
As part of:

Abstract

The nucleus accumbens (Nacc) is involved in learning and receives dopamine innervation from the ventral tegmental area. Rats with 6-hydroxydopamine (6-OHDA) induced dopamine reductions in the Nacc were tested for egocentric and allocentric learning. Nacc dopamine depletion resulted in allocentric learning and memory deficits in the Morris water maze (MWM) on acquisition, reversal trials, and probe trials. MWM cued performance was also affected but straight channel swim times and swim speed during hidden platform trials in the MWM were not. Lesioned animals were also significantly impaired in egocentric Cincinnati water maze (CWM). Dopamine depleted animals tested in the CWM in a reverse path were not significantly affected but showed a trend towards slower learning. 6-OHDA injections directed at the Nacc resulted in 60% dopamine reductions in the Nacc and 20% off-target reductions in the dorsal striatum. The data suggest that Nacc dopamine is a modulatory factor in both allocentric and egocentric spatial learning.
Introduction

Successful navigation can be accomplished through utilizing egocentric or allocentric search strategies. An allocentric strategy involves using spatial cues independent of body orientation (object-to-object relations) to create an external map of the environment, whereas egocentric strategies involves internal movement and directional heading, and proximal cues to navigate (Byrne, 1982, Aguirre and D'Esposito, 1999, Garber, 2000). Egocentric learning can be divided into path integration and route-based navigation. Route-based navigation is a self-oriented (subject-to-object relations) representation of space connected by “nodes” or choice points representing successive navigational decision points (Byrne, 1982, Aguirre and D'Esposito, 1999, Ma et al., 2012), whereas path integration involves vector addition allowing short-cuts to goals instead of retracing previous routes. Within the context of this paper, egocentric navigation refers to route-based navigation, not path integration.

Dopamine (DA) has been shown to be an important modulator of both egocentric and allocentric learning (Whishaw and Dunnett, 1985, Lindner et al., 1999, Mura and Feldon, 2003, De Leonibus et al., 2007, Braun et al., 2012). DA neurons, originating primarily in the substantia nigra pars compacta (SN) and the ventral tegmental area (VTA) in the midbrain, project to regions involved in cognition, reward, and motor control (Tzschentke, 2001). The SNc projects DA neurons primarily to the dorsomedial striatum (DMS) and dorsolateral striatum (DLS) whereas dopaminergic neurons in the VTA project primarily to the prefrontal cortex (PFC), hippocampus, amygdala, and nucleus accumbens (Nacc), with lesser projections to the DMS (Bjorklund and Dunnett, 2007). The striatum and PFC are the major dopaminergic targets for the VTA and SNc and have been implicated in both egocentric and allocentric learning (Kesner et al., 1989, McDonald and White, 1994, Tritsch and Sabatini, 2012). We have shown
that dorsal striatal (DMS + DLS, i.e., dStr) DA reductions of 80% impair egocentric learning in the Cincinnati water maze (CWM) and allocentric learning in the Morris water maze (MWM) (Braun et al., 2012). Furthermore, DA loss to either the DLS or DMS results in CWM, but not MWM deficits (Braun et al., 2014). However, the role of DA in the Nacc has not been studied in relation to egocentric learning.

Besides innervation from the VTA, the Nacc also receives glutamatergic input from the hippocampus, amygdala, and PFC (Grigoryan et al., 1996). Electrolytic lesions, excitotoxic lesions, and pharmacological glutamatergic manipulations of Nacc function result in allocentric memory deficits in the MWM, radial arm maze (RAM), T-maze, spatial object recognition, and hole-board maze (Annett et al., 1989, Cools et al., 1993, Ploeger et al., 1994, Smith-Roe et al., 1999, Coccurello et al., 2000, Sargolini et al., 2003, Ferretti et al., 2005, Tirado-Santiago et al., 2006, Nelson et al., 2010). Studies examining the specific role of Nacc DA in allocentric learning have mixed findings. For example, no impairments in MWM learning were observed following DA depletion of 70% after 6-hydroxydopamine (6-OHDA) injections into the Nacc (Hagan et al., 1983, Grigoryan et al., 1996), whereas spatial discrimination impairments in a T-maze were observed following 88% DA depletion in the Nacc (Taghzouti et al., 1985). Administration of D₁ and D₂ receptor antagonists and agonists into the Nacc consistently show a role for DA in the MWM as D₁ and D₂ receptor antagonists impair MWM learning and the D₂ receptor agonist quinpirole enhances MWM learning (Cools et al., 1993, Ploeger et al., 1994, Setlow and McGaugh, 1998, Coccurello et al., 2000, Nelson et al., 2010). The role of the Nacc in egocentric learning has not been examined as fully as in allocentric learning, however it is implicated in this type of learning. In one study, both egocentric and allocentric frames of
reference in a spatial object recognition task were impaired following intra-Nacc injections of AP-5 (De Leonibus et al., 2005). The role of DA in the Nacc on egocentric learning is unknown.

The present study examined the role of DA reduction in the Nacc on egocentric learning in the CWM and allocentric learning in the MWM. Motivation to escape water and swimming performance were analyzed using straight channel swimming trials and visible platform trials in the MWM. Nacc DA has been implicated in both strategy switching and reversal learning, and therefore in order to address this, two cohorts of rats were prepared with Nacc 6-OHDA injections (Taghzouti et al., 1985). In one cohort, rats were tested in the CWM on two paths, S-G and G-S (Fig. 1). We previously showed that path S-G is impaired by DA depletion in the dStr or in DMS or DLS subregions (Braun et al., 2012, Braun et al., 2014). Herein, animals were tested in the both the S-G and G-S paths, the latter requiring animals to solve the maze by a somewhat different strategy than for the S-G path. The second cohort of rats was tested in the MWM for both acquisition and reversal learning and memory on probe trials. DA receptors have been implicated in strategy switching, therefore both tasks employed methods that required the rats to switch strategies (Bubser and Schmidt, 1990, Rich and Shapiro, 2009).

Methods

Animals

Adult male Sprague-Dawley CD IGS rats (225-250 g) were purchased from Charles River Laboratories, Raleigh, NC (Strain 001). The vivarium is a barrier, pathogen free, facility using a Modular Animal Caging System (Alternative Design, Siloam Spring, AR) with HEPA filtered air (Alternative Design, Siloam Spring, AR) at 30 air changes/h. Reverse osmosis filtered water was provided ad libitum. The vivarium (21 ± 1°C) was maintained on a 14 h light-dark cycle (lights on at 600 h). Each cage (polysulfonate cages 26 x 48 cm and 20 cm tall) had
ad libitum NIH-07 diet, woodchip bedding, and a semicircular stainless steel enclosure to provide partial environmental enhancement (Vorhees et al., 2008). Animals for behavior were pair-housed for at least one week prior to surgery. All procedures were in compliance with the Institutional Animal Care and Use Committee and the vivarium is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Stereotaxic Surgery

Prior to Nacc surgery, equal numbers of animals were assigned using a random numbers table to be tested for egocentric or allocentric learning. Twenty two animals were administered 6-OHDA in the Nacc and 18 animals received vehicle (VEH) injections. Of these, 4 Nacc 6-OHDA animals were removed from the analysis because they had DA depletion less than 50% or lesions outside the Nacc. One control was removed because of a sample error for HPLC assay. To reduce effects of 6-OHDA on norepinephrine (NE), rats were pretreated with the NE reuptake inhibitor desipramine (s.c.; 15 mg/kg in 3 mL/kg dosing volume; Sigma, St. Louis, MO) 30 min prior to surgery (Bubser and Schmidt, 1990). Anesthesia was induced and maintained by continuous inhalation of isoflurane (IsoThesia; Butler Animal Health Supply, Dublin OH) via nose cone throughout surgery. Animals were placed in a computer-controlled stereotaxic apparatus (StereoDrive, Stoelting Co., Wood Dale, IL), see (Braun et al., 2012, Braun et al., 2014). A 26 gauge 10 µL Hamilton Gastight syringe (Reno, NV) was used for injections. Coordinates were based on the Paxinos and Watson brain atlas (Paxinos et al., 1985). Nacc lesioned animals were given bilateral injections of 6 µg/µL 6-OHDA hydrobromide (Sigma, St. Louis, MO) in 0.4% ascorbic acid at a volume of 2 µL/site at the following coordinates: from bregma: AP: + 1.8 mm, ML: ± 1.45 mm; from dura: DV: -6.6 mm. Each injection was made over 10 min with the syringe left in place for 5 min following injection. Control animals
(SHAM) received an identical volume of vehicle (VEH; 0.4% ascorbic acid) using the same procedure and coordinates as above. Following surgery, 0.1 mL buprenorphine hydrochloride was given s.c. to minimize pain and animals were given 2 weeks recovery prior to testing.

**Immunohistochemistry**

Using the same surgical procedures, separate animals were given the same injection of 6-OHDA in the Nacc unilaterally with VEH injection on the contralateral side (N = 3/lesion). Two weeks after surgery animals were perfused transcardially with 4% paraformaldehyde, and the brains dissected, postfixed, and sunk in sucrose overnight. Brains were sectioned (at 30-µm) on a microtome, and the free-floating sections processed for tyrosine hydroxylase (TH) immunohistochemistry as described (Braun et al., 2014, Hemmerle et al., 2014) using mouse monoclonal anti-TH primary antibody (MAB318, diluted 1:8000; EMD Millipore, Telecuma, CA), biotinylated horse anti-mouse IgG secondary antibody (BA-2000, diluted 1:200; Vector Laboratories, Burlingame, CA), and ABC Elite Kit reagents (Vector Laboratories) with diaminobenzidine as chromagen. Nacc immunostaining for TH was examined for the regional specificity of 6-OHDA injections as indicated by TH depletion. Sections were viewed and scanned at 20X on the Aperio AT2 slide scanner and uploaded to Aperio eSlide Manager (Leica Biosystems, Buffalo Grove, IL).

**Cohort 1: Effects of Nacc DA depletion on CWM route-based learning**

**Straight Channel**

One day pre- and one day post-CWM testing, animals were tested for swimming in a 244 cm long x 15 cm wide x 51 cm high water filled (38 cm deep) straight channel for 4 consecutive trials (2 min limit/trial) (Herring et al., 2008; Vorhees et al., 2008). Straight channel swimming served three functions: (a) water acclimation, (b) teaching that escape was possible by climbing
on a submerged platform at the opposite end of the channel, and (c) to determine if animals had comparable swimming speeds.

*Cincinnati water maze – Path S-G*

CWM testing started 14 days post-surgery. The apparatus is a nine-unit multiple T maze (Vorhees, 1987, Vorhees et al., 1991, Vorhees et al., 2008) see Fig. 1. Animals had to locate a submerged platform in a room that was illuminated only with infrared lighting in order to eliminate visual cues. Two trials/day (5 min limit/trial) were given for 18 days. If an animal failed to find the escape within 5 min on trial-1 of each day, it was given 5 min of rest before trial-2. If an animal found the escape on trial-1 in less than 5 min, trial-2 was given immediately. Latency to escape and number of errors (defined as head and shoulder entry in a stem or arm of a T that was not on the path to the goal) were recorded. To correct for animals that stopped searching for the full 5 min, animals not reaching the goal were assigned an error score equal to the number of errors made by the animal making the most errors while finding the platform + 1. Data were analyzed in 2-day (4 trials) blocks to match 4-trial blocks used for MWM data.

*Cincinnati water maze – Path G-S*

Following completion of Path S-G, rats were placed in the maze at G with the platform located at S for 5 additional days.

*Tissue Collection and Monoamine Assessment*

Tissue collection was performed as in our previous experiments (Braun et al., 2012, Braun et al., 2014). Animals were brought to an adjacent suite and decapitated.Brains were removed and the dStr was dissected using a brain block with an initial cut at the decussation of the optic chiasm and another cut 2 mm rostral to the first cut (Williams et al., 2007). The Nacc was dissected from another block of tissue 1 mm rostral to the dStr and frozen for later assay.
Monoamines were assayed by high performance liquid chromatography with electrochemical detection (HPLC-ECD). Frozen tissues were weighed, thawed, and sonicated in appropriate volumes of 0.1 N perchloric acid (Fisher Scientific, Pittsburgh, PA). Samples were centrifuged for 14 min at 13,000 RCF at 4 °C. The supernatant sample was transferred to a new vial for injection onto a Supelco Supelcosil™ LC-18 column (150 × 4.6 mm, 3 µm; Sigma-Aldrich Co., St. Louis, MO). The HPLC system consisted of a Waters 717plus autosampler (Waters Corp., Milford, MA), ESA 584 pump, and Coulochem III electrochemical detector. The potential settings were -150 mV for E1 and +250 mV for E2, with a guard cell potential set at +350 mV. MD-TM mobile phase (ESA, Inc., Chelmsford, MA) was used and consisted of 75 mM sodium dihydrogen phosphate (monohydrate), 1.7 mM 1-octanesulfonic acid sodium salt, 100 µL/L triethylamine, 25 µM EDTA, and 10% acetonitrile, with a final pH of 3.0. The pump flow rate was set at 0.7 mL/min, and the samples were run at 28 °C. Standards for DA, 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), NE, 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) (all obtained from Sigma-Aldrich Co., St. Louis, MO) were prepared in 0.1 N perchloric acid. All neurotransmitters were run on a single chromatogram.

**Cohort 2: Effects of Nacc DA depletion on MWM allocentric learning**

**Straight Channel**

One day prior to beginning MWM acquisition testing, animals were tested in the straight channel as above.

**Morris water maze – Acquisition**

Animals were placed in a 244 cm diameter tank filled half-way with water (21 ± 1 °C) to find a fixed submerged, camouflaged 10 cm diameter platform in the SW position quadrant. Start positions were pseudo-randomized between cardinal and ordinal positions around the
perimeter of the tank. Rats were given 4 trials/day for 6 days with a 2 min trial limit and an ITI of 15 s on the platform. If a rat failed to find the platform within the time limit, it was placed on the platform for the ITI. On the 7th day, a 45 s probe trial was given from a novel start position with the platform removed. Data were collected using video tracking software (AnyMaze, Stoelting Co., Wood Dale, IL); dependent measures on platform trials were: latency, path length, and average swim speed. On probe trials the dependent measures were: average swim speed and average distance to the former platform site.

**Morris water maze – Reversal**

The day after acquisition, the platform was placed in a new position diagonal from the acquisition position (NE quadrant) and was slightly smaller (7 cm in diameter). Using new start positions, the same procedure as acquisition was used for the 6 days of platform trials followed by a 45 s probe trial on the 7th day.

**Morris water maze – Cued**

Cued MWM testing began the day following reversal. Curtains were closed around the tank to minimize distal cues, and a yellow plastic ball was attached to the top of a brass rod mounted in the center of the submerged platform (10 cm diameter) to mark its location. On each of two days, animals were given 4 trials with the locations of the platform and start positions randomized (ITI of 15 s on the platform plus 15-20 s to reposition the platform with the animal placed in a holding cage outside the curtains). Latency was recorded.

**Tissue Collection and Monoamine Assessment**

Tissue collection was identical to cohort 1 above.

**Statistical Analysis**
Data were analyzed using mixed linear ANOVA models (SAS v9.3, SAS Institute, Cary, NC). The covariance matrix for each dataset was tested for best fit statistics (AICC method). In most cases, the best fit was to the autoregressive-1 covariance structure. Kenward-Rodger adjusted degrees of freedom were used. Measures taken repetitively on the same animal, such as day, or block, were within-subject factors. Significant interactions were analyzed using slice-effect ANOVAs (SAS ProcMixed option) at each level of the repeated measure factor. HPLC data were analyzed with cohorts combined by two-way ANOVA with cohort and lesion as factors for experiment 1 and by t-test for independent samples for experiment 2. Significance was set at $p \leq 0.05$. Data are presented as least square (LS) mean $\pm$ LS SEM.

Results

Immunohistochemistry

A representative section from an animal that received unilateral Nacc 6-OHDA injections is shown in Fig. 2. As can be seen, TH immunoreactivity (IR) was severely reduced in the Nacc along with some attendant loss in the DMS (Fig. 2A) compared with the contralateral side (Fig. 2B).

Pre-Maze Straight Channel

Straight channel performance was not altered following 6-OHDA injection into the Nacc compared with SHAM-treated rats (mean $\pm$ SEM across trials: Nacc 6-OHDA: 19.19 $\pm$ 2.03 s, SHAM: 18.88 $\pm$ 2.11 s (N = 18/Nacc 6-OHDA; 17/SHAM).

Cincinnati water maze –Path S-G

One animal was removed from analysis because it never found the goal on any trial. Analysis of all successful animals showed no main effect of Nacc 6-OHDA treatment for latencies or errors but there were treatment x block interactions. The interaction for latency was
significant ($F(8,116) = 2.27$, $p < 0.05$; [Fig. 3A]) as it was for errors ($F(8,116) = 2.26$, $p < 0.05$; [Fig. 3B]). Slice-effect ANOVAs showed significant differences on blocks 6-9. On these blocks, Nacc 6-OHDA-treated animals had significantly increased latencies and errors compared with SHAM controls. N=10/Nacc 6-OHDA; 7/SHAM.

**Cincinnati water maze – Path G-S**

Nacc DA depletion did not cause a significant change in latency ([Fig. 3C]) or errors committed ([Fig. 3D]) compared with SHAMs, but there was an evident trend for the 6-OHDA-treated rats to perform worse.

**Post-Maze Straight Channel**

Nacc 6-OHDA exposure did not alter swim latency compared with SHAMs (Nacc 6-OHDA: 10.33 ± 1.4 s, SHAM: 9.34 ± 1.6 s).

**Morris water maze acquisition**

Latency was increased in Nacc 6-OHDA-treated animals compared with SHAM-treated controls (main effect: $F(1,15.4) = 5.94$, $p < 0.05$; [Fig. 4A]); this was also reflected in the lesion x day interaction ($F(5,55.7) = 4.14$, $p < 0.01$) with Slice-effect ANOVA differences on days 2, 5, and 6. Nacc 6-OHDA-treated animals similarly had increased path lengths to find the platform on days 2 and 5 (lesion x day interaction ($F(5,52.8) = 2.51$, $p < 0.05$; [Fig. 4B])) compared with SHAM-treated controls. Average swim speed was not altered ([Fig. 4C]). In order to ensure that animals did not start out differently, Day 1 data were analyzed separately trial-by-trial. There was no effect of Nacc 6-OHDA treatment (Nacc 6-OHDA: 87.96 ± 9.54 s, SHAM: 78.98 ± 8.99 s). During the probe trial, Nacc 6-OHDA-treated animals had increased average distance to the platform site compared with SHAM controls ($t(15) = 2.50$, $p < 0.05$; [Fig. 4D]). Average swim speed on the probe trial was not affected (not shown), N=8/Nacc 6-OHDA; 9/SHAM.
Morris water maze reversal

The treatment main effect was significant for latency (F(1,15) = 12.42, p < 0.01; Fig. 5A) and path length (F(1,15) = 10.95, p < 0.01; Fig. 5B); Nacc 6-OHDA-treated animals had longer latencies and path lengths compared with SHAM-treated controls. Average swim speed was not different between groups (Fig. 5C). During the reversal probe trial, there was no difference between groups on average distance from the platform site (Fig. 5D) or average swim speed.

Morris water maze cued

Nacc 6-OHDA-treated animals had a significant increase in latency to find the visible platform compared with SHAM-treated controls (F(1, 15) = 10.04; p < 0.01; Nacc 6-OHDA: 46.96 ± 4.85 s, SHAM: 25.84 ± 4.57 s).

Monoamines

For monoamines analyses, there were no interactions between cohort and lesion. DA concentrations in Nacc of 6-OHDA-treated animals was significantly decreased by an average of 60% (F(1,3) = 18.17, p < 0.001; Fig. 6A) compared with SHAM-treated controls. Significant decreases were also seen in Nacc lesioned animals for DOPAC (F(1,3) = 26.37, p < 0.001; Nacc 6-OHDA: 1859.8 ± 142.9 pg/mg, SHAM: 929.3 ± 123.4 pg/mg) and HVA (F(1,3) = 29.27, p < 0.001; Nacc 6-OHDA: 644.4 ± 53.87, SHAM: 303.6 ± 33.7 pg/mg). Nacc NE was decreased by 60% (F(1,3) = 1.78, p < 0.05; Fig. 6B) in lesioned animals compared with SHAM controls. Nacc 5-HT was not altered (Fig. 6C).

6-OHDA treatment in the Nacc reduced DA in the dStr by 20% (F(1,3) = 5.82, p < 0.05; Fig. 6D). dStr DOPAC was also decreased (F(1,3) = 6.67, p < 0.05; Nacc 6-OHDA: 791.4 ± 45.1 pg/mg, SHAM: 998.6 ± 75.0 pg/mg) while HVA was not altered. NE (Fig. 6E) and 5-HT
(Fig. 6F) levels in the dStr were also not different in lesioned 6-OHDA-treated animals compared with SHAM-treated controls (N = 18/Nacc 6-OHDA; 16/SHAM).

Discussion

The effect of Nacc DA reduction was tested for egocentric (Cohort 1) and allocentric (Cohort 2) learning and memory. Motor skills and motivation to escape were also assessed. Six weeks after surgery Nacc 6-OHDA treatment reduced DA levels in this region by 60% and impaired egocentric CWM Path S-G learning (increased latency and errors), and caused similar trends in Path G-S performance. Allocentric acquisition and reversal in the MWM were also impaired by Nacc DA reductions (increased latency and path length), as was MWM proximal learning in the cued version of the MWM. Nacc DA is known to be involved in reward processes (Koob, 1992, Berridge and Robinson, 1998), however DA involvement in allocentric learning has not been associated with Nacc reward pathways (Coccurello et al., 2000). We found that the learning deficits were independent of motivation or performance factors in that no differences in straight channel swim latency prior to MWM and CWM testing were found, nor when retested following CWM assess. Similarly, no swim speed difference in the MWM was obtained.

In addition to affecting DA, Nacc 6-OHDA treatment affects NE. Pretreatment with desipramine was used to limit the effects of 6-OHDA on NE, but this was only partially effective in that NE levels were still decreased by 60%. Others have shown that desipramine and other NE reuptake inhibiting pretreatments fail to provide complete protection to NE neurons (Bubser, 1994). Therefore, the present experiment cannot rule out a potential contribution of Nacc NE depletion for the learning and memory changes observed here. However, preferential NE reductions in this region do not result in allocentric MWM deficits (Hagan et al., 1983, Selden et
al., 1990), supporting a Nacc DA-specific role in such learning. The role of Nacc NE in egocentric learning has not been explored, and cannot be discounted as playing some role in the CWM learning deficits we found. No effects on Nacc 5-HT were found.

6-OHDA Nacc injections caused some off-target effects, i.e., a 20% reduction in dStr DA. This reduction was verified by decreased TH-IR in the DMS following unilateral 6-OHDA into the Nacc. We previously showed that DA reductions in the dStr impair egocentric and allocentric learning but require large reductions. The 20% reduction seen here is below the threshold of DA loss required to cause the present learning deficits (Braun et al., 2012). Previous studies have observed that dStr DA decreases of less than 60% do not alter allocentric learning (Miyoshi et al., 2002, Da Cunha et al., 2003). We have similar findings in which even a 52% DA loss in the DMS did not cause egocentric CWM or allocentric MWM deficits (unpublished).

Electrolytic and excitotoxic lesions in the Nacc impair allocentric learning in the MWM and radial-arm maze (RAM) (Annett et al., 1989, Cools et al., 1993, Ploeger et al., 1994, Smith-Roe et al., 1999, Coccurello et al., 2000, Sargolini et al., 2003, Ferretti et al., 2005, Tirado-Santiago et al., 2006, Nelson et al., 2010) however, to the best of our knowledge, this is the first study to show allocentric MWM learning deficits following Nacc DA loss. These deficits were observed in latency and path length, as well as increased average distance to the platform site on the probe trial. Further evidence for the specificity of the effect is that the Nacc 6-OHDA-treated animals did not begin the MWM with preexisting performance differences since a detailed analysis of day 1 trial-by-trial did not show group differences during the early phase of testing as animals were learning the basic task requirements. Together with the absence of effects on
straight channel performance and swim speed in the MWM this indicates that the deficits are attributable to impaired learning rather than being secondary to performance effects.

Some studies investigating the role of Nacc DA in MWM have not found impaired learning after Nacc 6-OHDA treatment (Hagan et al., 1983, Grigoryan et al., 1996), but there are differences between those studies and ours. Hagan et al. (1983) used a maze about half the size of the one we used but with a similarly-sized platform. The pool to platform area ratio in Hagan et al. (1983) was 218:1 cm² whereas in our experiment it was 595:1 cm². It may be that the smaller maze made the test less sensitive to changes following Nacc 6-OHDA administration. Grigoryan et al. (1996) also had a smaller maze compared with ours (400:1 cm²), and they used a different protocol. Our study had 4 trials/day for 6 days with a 15 s ITI on the platform whereas Grigoryan et al. had 2 trials/day for 15 days with a 10 min ITI. Animals undergoing distributed trials often learn at a more efficient rate than those undergoing massed trials (Commins et al., 2003). However, this is not always the case and depends on the experimental conditions. We, for example, have found that rats learning the MWM perform only slightly better when given a 15 min ITI compared with a 15 s ITI (Vorhees and Williams, 2014), but there were a number of other differences between our experiment and that of Commins et al. (2003) besides trial spacing.

While this is the first study to show MWM deficits following Nacc DA reduction, other studies support a role of Nacc DA in allocentric learning. Spatial discrimination in a T-maze was impaired following Nacc DA reduction (Taghzouti et al., 1985). Nacc injections of the D₂ agonist quinpirole or the D₂ antagonist sulpiride enhanced or impaired MWM learning and RAM learning, respectively (Packard and White, 1991, Cools et al., 1993, Setlow and McGaugh, 1998). Since Nacc DA is involved in reward, Coccurello et al. (2002) used a non-associative
allocentric test with no explicit reward following intra-Nacc injections of sulpiride or the D_{1} receptor antagonist SCH 23390 (Coccurello et al., 2000). Both antagonists decreased reactivity to spatial changes in an object placement arena test (Coccurello et al., 2000).

We also found MWM reversal learning to be impaired following Nacc 6-OHDA administration. Spatial reversal learning has been linked to the Nacc. Both acquisition and reversal learning in a T-maze were impaired in rodents following Nacc ibotenic acid or 6-OHDA treatment (Taghzouti et al., 1985, Annett et al., 1989). It is suggested that since T-maze acquisition and reversal are impaired following Nacc lesions the deficit is not limited to reversal but is a generalized learning impairment (Annett et al., 1989). This would suggest impaired learning from the very beginning of testing, but here, we found no differences on day 1 between Nacc 6-OHDA-treated animals and SHAM-treated controls in a trial-by-trial analysis indicating that lesioned animals did not begin the test with preexisting deficits. Rather, 6-OHDA-treated animals’ deficits only emerged over days as the controls improved more rapidly than did the treated animals.

MWM cued performance was reduced in Nacc 6-OHDA-treated animals. It is unlikely that this was from an inability to perform the task because they found the platform and because they had experience finding the hidden platform during acquisition and reversal. A more likely explanation is that after having learned the hidden platform task, the 6-OHDA-treated rats were simply slower to adapt to the visible platform procedure where the start and platform were moved on every trial. Since we gave few cued trials, the 6-OHDA-treated animals may not have had sufficient time to switch strategies. We note that MWM cued deficits are not typically found following Nacc DA loss or electrolytic lesions to this region (Hagan et al., 1983, Sutherland and Rodriguez, 1989). Since Nacc DA is implicated in switching behavior, the present cued deficit
seems most parsimoniously attributed to an impairment in DA-mediated strategy switching (Taghzouti et al., 1985).

This is the first study to link Nacc DA to egocentric navigation. There is precedence for the Nacc to be involved in egocentric learning inasmuch as intra-Nacc injections of the glutamate antagonist AP-5 impair egocentric learning (De Leonibus et al., 2007). The mechanism behind Nacc DA-mediated allocentric and egocentric navigation remains unknown but DA involvement is inexorably implicated. The Nacc has been hypothesized to maintain associations between locations, actions, and goals to implement navigational learning strategies (Mogenson et al., 1980, Redish and Touretzky, 1997). Afferent connections from the hippocampus and PFC support an overarching role for the Nacc in both types of navigation (Morris et al., 1982, de Bruin et al., 1997, de Bruin et al., 2001). It has been proposed that DA in the Nacc modulates these processes by regulating the flow of information between different brain regions (Floresco, 2007, Goto and Grace, 2008).

This study has several limitations. The CWM was the only egocentric task used; it is possible that another egocentric task might yield different results. Only one dose of 6-OHDA was given and it is currently unknown if the effects would be different with DA depletions greater or less than 60% in the Nacc. While it is unlikely that NE contributed to the deficits, some NE contribution cannot be ruled out since NE was reduced by our treatment regimen. Navigation is the product of complex interactions among different neurotransmitters, receptors, regions, and their interacting circuitry, hence, isolating the role of DA in the Nacc is never complete and was not herein either but the data do support an important role for Nacc DA in egocentric learning and memory.
Reference List


Whishaw IQ, Dunnett SB (1985) Dopamine depletion, stimulation or blockade in the rat disrupts spatial navigation and locomotion dependent upon beacon or distal cues. Behav Brain Res 18:11-29.

**Figure 1. Cincinnati water maze.** The path shown from S-G represents the most efficient route from the start (S) to the goal (G) where a hidden platform is located. When the path is reversed it is designated G-S. The test is conducted in a dark room under infrared lighting with an infrared camera above connected to a closed circuit monitor outside the room where the experimenter could monitor performance.
Figure 2. Immunohistochemistry. 6-OHDA injection into the Nacc on one side decreased TH-IR in the Nacc and to a lesser extent in the DMS (A). Contralateral injection with VEH did not alter striatal TH-IR (B).
Figure 3. Nacc Cincinnati water maze. Nacc 6-OHDA-treated animals had significantly increased latency (A) and errors (B) to find the hidden platform compared with SHAM-treated controls tested in path S-G. When the path was reversed (G-S), Nacc 6-OHDA-treated animals showed a trend toward longer latencies (C) and more errors than SHAM-treated controls (D), but the effect was not significant. N = 10/6-OHDA; 7/SHAM. *p < 0.05, **p < 0.01.
Figure 4. Nacc Morris water maze: Acquisition. Nacc 6-OHDA-treated animals had increased latency to find the hidden platform (A) and path length (B) compared with SHAM-treated controls. Average speed (C) was not altered by treatment. On the probe trial, 6-OHDA-treated animals had increased average distance from the platform site (D) compared with the SHAM-treated controls. N = 8/6-OHDA; 9/SHAM. *p < 0.05; **p < 0.01.
Figure 5. Nacc Morris water maze: Reversal. Nacc 6-OHDA-treated animals had increased latency (A) and path length (B) to the platform compared with SHAM-treated controls. Average swim speed was not altered by treatment (C). On the probe trial, 6-OHDA-treated animals did not differ significantly in average distance from the platform site (D). **p < 0.01.
Figure 6. Nacc Monoamine levels. 6-OHDA injection into the Nacc decreased DA (A) and NE levels (B), but not 5-HT (C) 6 weeks post-surgery. dStr DA (D), but not NE (E), or 5-HT (F) levels were altered after Nacc 6-OHDA injections compared with SHAM-treated controls. N = 18/Nacc 6-OHDA; 16/SHAM. * p<0.05; *** p < 0.001.
Chapter 5:
Injection of 6-hydroxydopamine into the medial prefrontal cortex does not impair egocentric or allocentric navigational learning in rats
As part of:
6-Hydroxydopamine injections in the nucleus accumbens, but not the medial prefrontal cortex, impair egocentric and allocentric learning and memory in rats.

Abstract
The medial prefrontal cortex (mPFC) has been implicated in egocentric learning and memory; however the role of mPFC dopamine in egocentric learning has yet to be determined. This experiment tested whether bilateral 6-hydroxydopamine (6-OHDA) injections directly into the mPFC impaired long-term egocentric and/or allocentric learning and memory. Two weeks following 6-OHDA injections, rats began testing in the Cincinnati water maze (CWM) followed by the Morris water maze (MWM) for egocentric and allocentric learning, respectively. Lesioned animals were not impaired in CWM, with no difference in latency or find the platform or number of errors. mPFC dopamine depletion did not impair acquisition MWM learning; no difference was observed in path length to the platform or average distance from the platform site during the probe trial between groups. Motivation to escape the water and motor function were also unaltered following dopamine depletion in the mPFC. Dopamine levels in the mPFC after 6-OHDA injections were decreased by 88%, while norepinephrine levels were decreased by 59% (even though desipramine was injected prior to 6-OHDA). These findings suggest that CWM and MWM learning are not dependent on or modulated by mPFC dopamine.
Introduction

Successful navigation can be accomplished utilizing egocentric or allocentric strategies. An allocentric strategy involves using spatial cues independent of body orientation (object-to-object relations) to create a map-like representation of the distal environment, whereas an egocentric strategy involves internal movement, directional heading, and proximal cues to navigate (Byrne, 1982, Aguirre and D'Esposito, 1999, Garber, 2000). Egocentric learning is sometimes divided into path integration and route-based navigation. Route-based navigation is a self-oriented (subject-to-object relations) representation of space connected by “nodes” representing successive decision points (Byrne, 1982, Aguirre and D'Esposito, 1999, Ma et al., 2012), whereas path integration involves vector addition allowing short-cuts to goals instead of retracing of previous routes. Within the context of this paper, egocentric navigation refers to route-based navigation, not path integration.

Dopamine (DA) has been shown to be an important modulator of both egocentric and allocentric learning (Whishaw and Dunnett, 1985, Lindner et al., 1999, Mura and Feldon, 2003, De Leonibus et al., 2007, Braun et al., 2012). DA neurons, originating primarily in the substantia nigra pars compacta (SNC) and the ventral tegmental area (VTA) in the midbrain, project to regions involved in cognition, reward, and motor control (Tzschtentke, 2001). The SNC projects DA neurons primarily to the dorsomedial striatum (DMS) and dorsolateral striatum (DLS) whereas dopaminergic neurons in the VTA project primarily to the prefrontal cortex (PFC), hippocampus, amygdala, and nucleus accumbens (Nacc), with lesser projections to the DMS (Bjorklund and Dunnett, 2007).

The medial PFC (mPFC) in rodents and corresponding dorsolateral PFC (dIPFC) in humans and nonhuman primates is a major target for VTA dopaminergic input, and the mPFC
has been implicated in egocentric and allocentric learning (Semmes et al., 1963, Butters et al., 1972, Pohl, 1973, Kesner et al., 1989, Bubser and Schmidt, 1990, Kolb et al., 1994, de Bruin et al., 1997, de Bruin et al., 2001, Ethier et al., 2001, Ragozzino and Kesner, 2001, Ma et al., 2003, Ma et al., 2004, Velazquez-Zamora et al., 2011, Gonzalez-Burgos et al., 2012, Ma et al., 2012). The role of the mPFC in egocentric navigation is well-established (Kesner et al., 1989) but which neurotransmitters are involved is unclear. Human subjects with frontal cortex damage and nonhuman primates with dlPFC aspiration lesions are impaired in egocentric learning, but do not show allocentric impairments (Butters et al., 1972; Ma et al., 2003; Pohl, 1973; Semmes et al., 1963). In rodents, aspiration, electrolytic, and pharmacological lesions of the mPFC impair learning in an adjacent arm RAM task and in an egocentric version of the MWM (Kesner et al., 1989, Kolb et al., 1994, de Bruin et al., 1997, de Bruin et al., 2001, Ethier et al., 2001). Unlike the allocentric MWM, in the egocentric version the hidden and start position for each trial are moved but the spatial relationship between the two are held constant.

There is conflicting evidence for mPFC involvement in allocentric learning. While some studies have shown impairment in MWM following aspiration mPFC lesions, other studies have shown no deficit following electrolytic or excitotoxic mPFC lesions (Kolb et al., 1982, Poucet, 1989, Bubser and Schmidt, 1990, Poucet, 1990, de Bruin et al., 1994, Maaswinkel et al., 1996, de Bruin et al., 2001, Ethier et al., 2001, Lacroix et al., 2002, Rawson et al., 2010). Studies using different allocentric tasks have failed to show an impairment following electrolytic mPFC lesions or mPFC DA depletion, supporting the idea that VTA DA projections to the mPFC are not necessary for allocentric learning but questions remain (Kesner et al., 1989, Poucet, 1989, Bubser and Schmidt, 1990, Poucet, 1990, King and Corwin, 1992, Bubser, 1994, Rawson et al., 2010). Moreover, the role of mPFC DA in egocentric navigation has yet to be determined.
The present study examined the role of DA reduction in the mPFC on egocentric learning in the Cincinnati water maze (CWM) and allocentric learning in the Morris water maze (MWM). Motivation to escape water and swimming performance were analyzed using straight channel swimming trials and visible platform trials in the MWM. Animals were tested for egocentric learning prior to allocentric learning as we have done previously in other lesion models (Braun et al., 2012, Braun et al., 2014).

Methods

Animals

Adult male Sprague-Dawley CD IGS rats (225-250 g) were purchased from Charles River Laboratories, Raleigh, NC (Strain 001). The vivarium is a barrier, pathogen free, facility using a Modular Animal Caging System (Alternative Design, Siloam Spring, AR) with HEPA filtered air (Alternative Design, Siloam Spring, AR) at 30 air changes/h. Reverse osmosis filtered water was provided ad libitum. The vivarium (21 ± 1°C) was maintained on a 14 h light-dark cycle (lights on at 600 h). Each cage (polysulfonate cages 26 x 48 cm and 20 cm tall) had ad libitum NIH-07 diet, woodchip bedding, and a semicircular stainless steel enclosure to provide partial environmental enhancement (Vorhees et al., 2008). Animals for behavior were pair-housed for at least one week prior to surgery. All procedures were in compliance with the Institutional Animal Care and Use Committee and the vivarium is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Surgery

To reduce effects of 6-OHDA on norepinephrine (NE), rats were pretreated with the NE reuptake inhibitor desipramine (s.c.; 15 mg/kg in 3 mL/kg dosing volume; Sigma, St. Louis, MO) 30 min prior to surgery (Bubser and Schmidt, 1990). Anesthesia was induced and
maintained by continuous inhalation of isoflurane (IsoThesia; Butler Animal Health Supply, Dublin OH) via nose cone throughout surgery. Animals were placed in a computer-controlled stereotaxic apparatus (StereoDrive, Stoelting Co., Wood Dale, IL), see (Braun et al., 2012, Braun et al., 2014). A 26 gauge 10 µL Hamilton Gastight syringe (Reno, NV) was used for injections. Lesioned rats received bilateral injections of 6 µg/µL 6-hydroxydopamine hydrobromide (6-OHDA; Sigma, St. Louis, MO) in 0.2% ascorbic acid saline solution (VEH) in the mPFC. Control animals received equivalent bilateral mPFC injections of VEH (SHAM). Two infusion sites per hemisphere were made using a 26 gauge 10 µL Hamilton Gastight syringe (Reno, NV) with solutions infused at a volume of 1 µL over 4 min. The needle was left in place for 1 min after each injection. The following stereotaxic coordinates were used (Paxinos et al., 1985) from bregma: AP: +3.0 mm; ML: ± 0.8 mm; DV: -4.5 mm and DV: -3.5 mm. Twelve animals were administered 6-OHDA into the mPFC and 10 given VEH. Three lesioned animals were removed from analysis following incomplete or misplaced lesions; a SHAM animal was removed following incorrect mPFC dissection for monoamine analysis. Following surgery, animals were given 0.1 ml buprenorphine hydrochloride to minimize pain and allowed to recover for 2 weeks before the beginning of cognitive testing.

Immunohistochemistry

Using the same surgical procedures, separate animals were given the same injection of 6-OHDA in the mPFC unilaterally with VEH injection on the contralateral side (N = 3/lesion). Two weeks after surgery animals were perfused transcardially with 4% paraformaldehyde, and the brains dissected, postfixed, and sunk in sucrose overnight. Brains were sectioned (at 30-µm) on a microtome, and the free-floating sections processed for tyrosine hydroxylase (TH) immunohistochemistry as described (Braun et al., 2014, Hemmerle et al., 2014) using mouse
monoclonal anti-TH primary antibody (MAB318, diluted 1:8000; EMD Millipore, Telecuma, CA), biotinylated horse anti-mouse IgG secondary antibody (BA-2000, diluted 1:200; Vector Laboratories, Burlingame, CA), and ABC Elite Kit reagents (Vector Laboratories) with diaminobenzidine as chromagen. Nacc or mPFC immunostaining for TH was examined for the regional specificity of 6-OHDA injections as indicated by TH depletion. Sections were viewed and scanned at 20X on the Aperio AT2 slide scanner and uploaded to Aperio eSlide Manager (Leica Biosystems, Buffalo Grove, IL).

**Straight Channel**

One day prior to CWM testing, animals were tested in a 244 cm long x 15 cm wide x 51 cm high water filled (38 cm deep) straight channel for 4 consecutive trials with a maximum time limit of 2 min/trial (Herring et al., 2008; Vorhees et al., 2008). Straight channel swimming served three functions: (a) swimming acclimation, (b) to teach that escape was possible by climbing on the submerged platform at the opposite end of the channel, and (c) to determine if all animals had comparable swimming ability.

**Cincinnati water maze**

CWM testing started 14 days post-surgery. The apparatus is a nine-unit multiple T maze (Vorhees, 1987, Vorhees et al., 1991, Vorhees et al., 2008). Animals had to locate a submerged platform in a room that was illuminated only with infrared lighting in order to eliminate visual cues. Two trials/day (5 min limit/trial) were given for 18 days. If an animal failed to find the escape within 5 min on trial-1 of each day, it was given 5 min of rest before trial-2. If an animal found the escape on trial-1 in less than 5 min, trial-2 was given immediately. Latency to escape and number of errors (defined as head and shoulder entry in a stem or arm of a T that was not on the path to the goal) were recorded. To correct for animals that stopped searching for the full 5
min, animals not reaching the goal were assigned an error score equal to the number of errors made by the animal making the most errors while finding the platform + 1. Data were analyzed in 2-day (4 trials) blocks to match 4-trial blocks used for MWM data.

*Morris water maze -- Acquisition*

Animals were placed in a 244 cm diameter tank filled half-way with water (21 ± 1 °C) to find a fixed submerged, camouflaged 10 cm diameter platform in the SW position quadrant. Start positions were pseudo-randomized between cardinal and ordinal positions around the perimeter of the tank. Rats were given 4 trials/day for 6 days with a 2 min trial limit and an ITI of 15 s on the platform. If a rat failed to find the platform within the time limit, it was placed on the platform for the ITI. On the 7th day, a 45 s probe trial was given from a novel start position with the platform removed. Data were collected using video tracking software (AnyMaze, Stoelting Co., Wood Dale, IL); dependent measures on platform trials were: latency, path length, and average swim speed. On probe trials the dependent measures were: average swim speed and average distance to the former platform site.

*Morris water maze – Cued*

Cued MWM testing began the day following acquisition. Curtains were closed around the tank to minimize distal cues, and a yellow plastic ball was attached to the top of a brass rod mounted in the center of the submerged platform (10 cm diameter) to mark its location. On each of two days, animals were given 4 trials with the locations of the platform and start positions randomized (ITI of 15 s on the platform plus 15-20 s to reposition the platform with the animal placed in a holding cage outside the curtains). Latency was recorded.

*Tissue Collection and Monoamine Assay*
Following behavioral testing, animals were brought to an adjacent suite and decapitated. Brains were removed and dissected and the mPFC and neostriatum were frozen as previously described for monoamine assay (Williams et al., 2007). Monoamines were assayed via high performance liquid chromatography with electrochemical detection (HPLC-ECD). Frozen tissues were weighed, thawed, and sonicated in appropriate volumes of 0.1 N perchloric acid (Fisher Scientific, Pittsburgh, PA). Samples were centrifuged for 14 min at 13,000 RCF at 4°C. The supernatant sample was transferred to a new vial for injection onto a Supelco Supelcosil™ LC-18 column (150 × 4.6 mm, 3 µm; Sigma-Aldrich Co., St. Louis, MO). The HPLC system consisted of a Waters 717plus autosampler (Waters Corp., Milford, MA), ESA 584 pump, and Coulochem III electrochemical detector. The potential settings were -150 mV for E1 and +250 mV for E2, with a guard cell potential set at +350 mV. MD-TM mobile phase (ESA, Inc., Chelmsford, MA) was used and consisted of 75 mM sodium dihydrogen phosphate (monohydrate), 1.7 mM 1-octanesulfonic acid sodium salt, 100 µl/l triethylamine, 25 µM EDTA, and 10% acetonitrile, with a final pH of 3.0. The pump flow rate was set at 0.7 ml/min, and the samples were run at 28°C. Standards for DA, 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), NE, 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) (all obtained from Sigma-Aldrich Co., St. Louis, MO) were prepared in 0.1 N perchloric acid. All neurotransmitters were run on a single chromatogram.

Statistical Analysis

Data were analyzed using mixed linear ANOVA models. The covariance matrix for each dataset was checked using best fit statistics. In most cases, the best fit was to the autoregressive-1 covariance structure. Kenward-Rodger adjusted degrees of freedom were used. Measures taken repetitively on the same animal, such as week, day, or block, were within-subject factors.
Significant interactions were analyzed using simple-effect slice ANOVAs at each level of the repeated measure factor. Biochemical data were analyzed using two-tailed t-tests. Significance was set at $p \leq 0.05$, trends at $p \leq 0.10$. Data are presented as least squared (LS) mean ± LS SEM.

**Results**

*Immunohistochemistry*

A representative section from an animal that received unilateral mPFC 6-OHDA injections is shown in **Fig. 1**. As can be seen, TH immunoreactivity (IR) was severely reduced in the mPFC (**Fig. 1A**) compared with the contralateral side (**Fig. 1B**).

*Straight Channel*

No difference in time to swim the straight channel was observed across trials between 6-OHDA-lesioned and SHAM animals (LS mean ± LS SEM across trials: 6-OHDA: 14.61 ± 2.03 s; SHAM: 14.77 ± 2.03 s).

*Cincinnati water maze*

No significant main effect of lesion or interaction effect of lesion with block was observed in CWM testing for either latency to find the platform (**Fig 2A**) or number of errors made (**Fig 2B**) compared with SHAM animals.

*Morris water maze – Acquisition*

Compared with SHAM animals, path length, latency, and cumulative distance to the platform, as well as average and initial heading error were not affected by DA depletion in the mPFC (**Fig 3A**). Mean speed was also not altered by DA depletion (**Fig 3B**) compared with SHAM animals. During the probe trial, mean speed, and mean distance from the platform (6-OHDA: 1.55 ± 0.44 m; SHAM: 1.22 ± 0.40 m) did not differ in lesioned animals compared with SHAM controls.
Morris water maze – cued testing

No significant difference between 6-OHDA injected animals and SHAM controls was seen in latency to find the platform (6-OHDA: 21.91 ± 3.56 s; SHAM: 27.21 ± 3.56 s).

Monoamine assessment

Five lesioned animals had DA levels that were under the range of detection in the mPFC and were therefore given a value of 1 pg/mg for analysis. mPFC DA was significantly reduced by 88% in 6-OHDA lesioned animals compared with SHAM controls (t(15) = 6.17, p ≤ 0.001; 6-OHDA: 5.87 ± 2.67 pg/mg; SHAM: 47.03 ± 5.80 pg/mg). NE in the mPFC was significantly decreased by 59% in 6-OHDA treated animals compared with SHAM animals (t(15) = 5.10, p ≤ 0.001; 6-OHDA: 92.56 ± 19.28 pg/mg; SHAM: 226.84 ± 17.89 pg/mg). No differences in 5-HT levels were observed in 6-OHDA lesioned animals compared with SHAM controls, although it approached significance (t(15) = 1.88, p = 0.07; 6-OHDA: 541.99 ± 58.27 pg/mg; SHAM: 670.98 ± 38.75 pg/mg). Striatal catecholamines were unaffected by mPFC 6-OHDA lesions (DA 6-OHDA: 10,694.27 ± 618.01 pg/mg; SHAM: 10,451.71 ± 1019.83 pg/mg; NE 6-OHDA: 132.24 ± 44.80 pg/mg; SHAM: 165.32 ± 49.94 pg/mg).

Discussion

This study evaluated the role of mPFC DA in allocentric and egocentric learning. Motor skills and motivation to escape were also assessed. 6-OHDA administration into the mPFC did not alter egocentric learning in the CWM or allocentric learning in the MWM. mPFC DA was decreased by 88%. Monoamine loss in this region was not limited to DA since NE levels were also decreased by 59% even with desipramine treatment. Others have also shown that desipramine or other NE reuptake inhibitors prior to 6-OHDA injection does not offer complete protection to NE neurons (Bubser, 1994). At the level of reduction seen here in both DA and NE
together with no change in egocentric or allocentric learning suggests no direct role for mPFC catecholamines in these forms of learning and memory. mPFC TH staining was consistent with the monoamine changes showing marked TH-IR reduction after 6-OHDA. Striatal monoamine levels were not altered from mPFC 6-OHDA injections. Motivational and performance based factors (as measured in the straight channel, swim speed in MWM, and cued platform MWM) were not altered from 6-OHDA injection. All animals tested learned both tasks, as evidenced by a significant learning curve across blocks and days on all measured indices.

While some studies have observed MWM deficits following aspiration lesions of the mPFC (Kolb et al., 1982, Sutherland et al., 1982, Kolb and Whishaw, 1983), others have not observed this effect following electrolytic or excitotoxic mPFC lesions (de Bruin et al., 1994, Maaswinkel et al., 1996, de Bruin et al., 2001, Lacroix et al., 2002). These differences are most likely the result of the method of lesion induction since some are selective for specific neurotransmitters and some simply ablate all cells in a target region. Procedural differences may also be important. In some studies that found differences, lesioned animals that did not find the platform were not placed on it during the ITI thereby limiting access to distal cue information whereas in studies that found no differences, animals were placed on or guided to the platform if they did not locate it within the time limit (de Bruin et al., 1994). In the current study, animals were placed on the platform if they were unable to locate it within the time limit and we found no MWM deficits following mPFC DA depletion. Other studies using different allocentric tests have also failed to show impairments following mPFC electrolytic lesions and selective DA depletion, supporting the notion that the mPFC does not play a role in this type of learning (Kesner et al., 1989, Poucet, 1989, Bubser and Schmidt, 1990, Poucet, 1990, King and Corwin, 1992, Bubser, 1994, Rawson et al., 2010).
While mPFC DA may not be involved in egocentric learning and memory, this brain region, as well as the dorsal striatum are (Braun et al., 2012; Butters et al., 1972; de Bruin et al., 1997; de Bruin et al., 2001; Ethier et al., 2001; Kesner et al., 1989; Ma et al., 2012; Ma et al., 2003; Ma et al., 2004; Pohl, 1973; Semmes et al., 1963). In rodents, excitotoxic, electrolytic, and aspiration lesions of the mPFC impair egocentric learning in a response version of the MWM (de Bruin et al., 1997, de Bruin et al., 2001, Ethier et al., 2001). Impaired learning following mPFC lesions, but not parietal cortex lesions, is also observed in an egocentric version of the RAM that requires visits to adjacent arms (Kesner et al., 1989, Kolb et al., 1994). Hence, integrity of the mPFC appears necessary for egocentric learning and memory but does not depend on DA, or most probably, NE.

This study has several limitations. The CWM was the only egocentric task used; it is possible that another egocentric task might yield different results. Only one dose of 6-OHDA was given and it is currently unknown if the effects would be different with DA depletions greater or less than 88% in the mPFC, respectively. Navigation is the product of complex interactions among different neurotransmitters, receptors, regions, and their interacting circuitry, hence, isolating the role of DA in the mPFC is never complete and was not herein either but the data do not support a necessary or modulatory role for mPFC DA or NE in egocentric and allocentric learning.
Reference List


King VR, Corwin JV (1992) Spatial deficits and hemispheric asymmetries in the rat following unilateral and bilateral lesions of posterior parietal or medial agranular cortex. BehavBrain Res 50:53-68.


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Figure 1. Immunohistochemistry. Unilateral mPFC 6-OHDA administration decreased TH-IR (A). No TH-IR reduction was seen in the contralateral mPFC following VEH injections.
Figure 2. Cincinnati water maze. 6-OHDA lesion of the mPFC did not have a significant effect on latency to find the platform (A), or on the number of errors committed (B) compared with SHAMs. N = 9/group.
Figure 3. **Morris water maze.** 6-OHDA lesion of the mPFC did not have a significant effect on path length (A) to find the hidden platform or on overall mean speed (B) compared with SHAMs.
CHAPTER 6: Discussion

The results of these studies indicate that striatal DA is an important modulator in egocentric and allocentric learning. In regard to egocentric learning, DA projections to each striatal subregion investigated showed independent contributions. In Chapters 3 and 4, regional DA depletion in the DLS, DMS, and Nacc resulted in egocentric CWM learning deficits. This appears to be specific to the striatum, as mPFC DA depletion (Chapter 5) did not affect CWM learning. The results from Chapter 5 are interesting since the mPFC is involved in egocentric learning, however DA in this region does not appear to be important (de Bruin et al., 1997). The role of striatal DA in allocentric learning is slightly different than that of egocentric learning. Both Nacc and dStr DA modulate allocentric learning. Data from Chapter 3 however showed that DA depletion in only the DMS or DLS was not sufficient to impair allocentric learning, supporting the view that dStr DA involvement in this learning requires contributions from both the DLS and DMS. mPFC DA is not involved in allocentric learning. It has long been accepted that the dStr (with the exception of the DMS and its indirect connectivity with the hippocampus) and hippocampus operate through separate spatial learning networks. These studies indicate both a divergence and convergence between the two regions that appear to be more complex than originally hypothesized.

Striatal Dopamine and Spatial Navigation

Behavioral, anatomical, and electrophysiological evidence have shown that the dStr, and its subregions the DLS and DMS, are involved in spatial learning (Braun et al., 2012; Cook and Kesner, 1988; Devan and White, 1999; Devan et al., 1999; Jog et al., 1999; McDonald and White, 1994; McGeorge and Faull, 1989; Mizumori et al., 2004; Mizumori et al., 2009; Packard et al., 1989; Packard, 2009; Penner and Mizumori, 2012; Potegal, 1969; Potegal, 1972; Ragozzino et al., 1989; Packard, 2009; Penner and Mizumori, 2012; Potegal, 1969; Potegal, 1972; Ragozzino et al.,
Electrolytic lesions of the dStr impair egocentric learning in the RAM as well as in an egocentric right-left discrimination task (Potegal, 1969, Cook and Kesner, 1988). AP-5 injections into the dStr also impair egocentric information consolidation, implicating dStr glutamate (Glu) in egocentric learning (De Leonibus et al., 2005). dStr 5-HT depletion facilitates egocentric learning in a modified MWM through modulation of DA; increased egocentric learning was not observed in dStr 5-HT depleted rats following D₁ and D₂ receptor blockade (Anguiano-Rodriguez et al., 2007). Lesions of the dStr do not normally result in allocentric learning deficits; however dStr DA does appear to modulate allocentric learning (McDonald and White, 1993, 1994, Oliveira et al., 1997, De Leonibus et al., 2007). It is currently unknown why dStr DA-specific lesions, but not ablation of the dStr, result in allocentric deficits. It has been hypothesized that dStr DA may be essential for choosing the correct sensorimotor subsystems necessary for allocentric learning to occur, and is not involved in retaining the location of objects or plotting the correct allocentric path (Whishaw and Dunnett, 1985). Hippocampal processes are necessary for retaining the location of objects and can most likely compensate for dStr ablation, so learning can still occur when only the hippocampal navigational learning system is active, but not after dStr DA loss when both dStr and hippocampal learning systems are still active (Morris, 1981, Whishaw and Dunnett, 1985).

We have shown that an 80% DA reduction in the dStr results in both allocentric MWM and egocentric CWM deficits (Braun et al., 2012). Monoamines in the PFC were unaffected by 6-OHDA striatal treatment, however NE levels were decreased in the hippocampus. Route-based navigation is thought to be independent of hippocampal function (Devan et al., 1999; Devan and White, 1999; McDonald and White, 1993; McDonald and White, 1994),
supporting the hypothesis that dStr DA loss results in CWM egocentric deficits. A role for hippocampal NE in the MWM learning deficit cannot be completely discounted. Reversible functional inactivation of the locus coeruleus via lidocaine injection throughout MWM testing does disrupt acquisition learning (Khakpour-Taleghani et al., 2009). Allocentric deficits in the MWM are not observed after 6-OHDA injections into the dorsal noradrenergic bundle (Hagan et al., 1983, Selden et al., 1990). As 6-OHDA mediated NE decreases do not result in allocentric MWM deficits, the deficits seen herein are most likely a result of dStr DA reductions.

The observed learning deficits were also independent of motivational factors (no difference in straight channel or cued platform MWM ability between groups). While slightly slower swim speeds were observed in animals exposed to 6-OHDA in the dStr, the swim speed was not responsible for the observed learning deficits. The allocentric deficit observed is consistent with previous data showing that when dStr DA levels are depleted by at least 60%, allocentric MWM learning is impaired (De Leonibus et al., 2007b; Lindner et al., 1999; Mura and Feldon, 2003; Whishaw and Dunnett, 1985b), whereas smaller reductions do not produce this effect (Miyoshi et al., 2002, Da Cunha et al., 2003).

Egocentric learning was impaired in the CWM following 80% DA depletion in the dStr (Braun et al., 2012). This finding is consistent with data that adult exposure to drugs of abuse such as methamphetamine, that target DA systems and produce decreases in DA levels, affect CWM learning, while drugs of abuse that preferentially target 5-HT systems do not produce CWM deficits (Herring et al., 2008; Herring et al., 2010; Vorhees et al., 2010b).

Methamphetamine exposure has effects on a number of systems and brain regions, while dStr 6-OHDA is DA specific. Ours was the first study to indicate a direct modulatory role for dStr DA in egocentric navigation.
The dStr is a heterogeneous structure with two anatomical subregions (DMS, DLS) with different connections and functions that contribute independently to egocentric learning. We observed that 6-OHDA injections yielding 62% DA depletion in the DMS or 75% depletion in the DLS resulted in egocentric CWM deficits but not allocentric MWM deficits. These impairments were independent of motivational or motoric deficits. The finding that DLS DA is involved in egocentric, but not allocentric learning is supported by previous studies. Allocentric deficits have not been observed following ablation of the DLS, regardless of lesion type (Cook and Kesner, 1988, Devan et al., 1999). Electrolytic DLS lesions produced a preference for utilizing a place strategy in a MWM cued-place competition task (Devan and White, 1999). During the MWM competition test rodents are trained to find a stationary visible and hidden platform across 10 days (Devan and White, 1999). The last day of testing involves moving the visible platform to the opposite quadrant and observing if the rodent spends more time at the visible platform (indicative of cued learning) or where the hidden platform was located during earlier testing (indicative of place learning). Following electrolytic DLS lesions this resulted in lesioned animals exhibiting a preference towards place learning (Devan and White, 1999). Their study supports a DLS role in egocentric learning. Our study is the first to observe a direct effect of DLS DA on egocentric navigation.

The DMS has been implicated in both egocentric and allocentric learning. Electrolytic and excitotoxic DMS lesions impair allocentric MWM performance (Whishaw et al., 1987, Devan et al., 1999, Devan and White, 1999). DMS NMDA receptor blockade with propyl-1-phosphonic acid, at similar levels to NMDA blockade in the hippocampus, impairs RAM and MWM performance (Holahan et al., 2005). Pharmacological inhibition of the DMS in rhesus monkeys reduced route-based learning, but not allocentric learning (Etienne et al., 2012).
Excitotoxic lesions of either the DMS or DLS impair egocentric procedural learning in a 14-unit T-maze (Pistell et al., 2009). The role of DA in the DMS in egocentric and allocentric learning was only studied in a T-maze prior to our studies. DA depletion in the posterior DMS did not result in an overall learning impairment in the T-maze, but lesioned animals preferentially used an egocentric strategy over an allocentric strategy early in the testing paradigm. Control animals used an allocentric strategy early in testing, implying a role for posterior DMS DA in allocentric place strategy (Lex et al., 2011). During later phases of learning no differences in strategy were observed between groups. As it is more common for animals to utilize a place strategy during early training and transition into a response strategy after prolonged training, the inference was that DA-depleted animals exhibited a deficit in allocentric performance during the phase of acquisition when place learning normally dominates. No overall learning deficit was observed in that both groups learned the task; only the strategy used initially differed.

The differences between the T-maze, MWM, and CWM may explain some of the above discrepancies. Both the MWM and CWM are harder to solve than T-mazes and require an allocentric or an egocentric spatial learning strategy, respectively, to find the goal. While animals in the Lex et al. (2011) study resorted to a response strategy over a place learning strategy in the T-maze, animals in the present study learned at the same rate as controls when given only the option of allocentric learning in the MWM. Deficits were observed when DA-depleted animals were given only the option of egocentric learning in the CWM. Because the CWM is a more complex egocentric learning task, it uncovered involvement of DA in the DMS for this type of learning.

DA reductions over 60% in the DLS and DMS each resulted in egocentric CWM deficits. Both the DMS and DLS have separate cortical pathways that can contribute to the neuronal
requirements for egocentric learning. The “sensorimotor” circuit connecting the DLS to sensory and motor cortical regions likely provides the DLS with necessary proprioceptive information for egocentric orientation in space (Figure 1) (Penner and Mizumori, 2012). The DMS connects to an “associative” circuit with the prefrontal cortex and parietal association cortex, both regions involved in egocentric orientation and learning (Figure 1) (Penner and Mizumori, 2012). It has been hypothesized that indirect connections between the DMS and hippocampus are responsible for the observed role of the DMS in allocentric learning (Devan and White, 1999). These connections do not appear to depend on DMS DA.

It is likely that DA in the DMS and DLS have separate and independent roles in modulating egocentric learning as shown through observed differences in cortical networks. DA loss in the DMS or DLS does not cause allocentric learning deficits; dStr DA-mediated allocentric learning appears to require contributions from both the DLS and DMS. This could be, in part, due to the larger role of hippocampal processes in allocentric learning. The hippocampus most likely can compensate for DA loss in either the DMS or DLS, but cannot compensate for DA loss of greater than 60% spread throughout the dStr.

Both allocentric and egocentric navigational learning deficits were observed following 60% depletion of Nacc DA. These impairments were independent of motivational or motoric deficits since basic swimming speed was unaffected. In addition, Nacc NE was decreased by 60%, however NE loss from 6-OHDA injections have not been associated with allocentric MWM deficits (Hagan et al., 1983, Selden et al., 1990), supporting a dopaminergic role in allocentric learning rather than a noradrenergic one. The role of Nacc NE in egocentric learning has not been adequately explored, but its role in the present results cannot be completely discounted until experiments designed to specifically target its possible involvement.
For the Nacc 6-OHDA injections, it was found that off-target DA in the dStr was decreased by 20%. Decreased TH-IR was observed in the DMS, specifically. This is below the 60% needed for a dStr-mediated spatial (egocentric or allocentric) deficit. We have similar findings in which even a 52% DA loss in the DMS did not cause egocentric CWM or allocentric MWM deficits (unpublished). Therefore, it is unlikely that off-target DA effects contributed to the CWM differences found here. The role of Nacc DA in egocentric learning had not been previously examined, although Nacc Glu has been implicated in egocentric learning. Intra-Nacc injections of AP-5 (an NMDA antagonist) into the Nacc impaired both egocentric and allocentric reference frames in a modified spatial object recognition task implicating glutamatergic NMDA receptors in both types of learning (De Leonibus et al., 2005). As noted in the Introduction, unlike the traditional spatial object recognition test where all animals have a consistent starting point use an allocentric frame of reference for detecting spatial change, in the modified test some animals are placed in random start positions across testing thus diminishing the ability to use distal cues to recognize spatial change and encouraging an egocentric reference frame for spatial displacement detection (De Leonibus et al., 2005).

The Nacc is involved in allocentric learning. In particular, Glu has been most consistently implicated in the contribution of the Nacc to allocentric learning. Excitotoxic and electrolytic lesions of the Nacc result in allocentric learning deficits in both the MWM and RAM (Annett et al., 1989, Cools et al., 1993, Ploeger et al., 1994, Smith-Roe et al., 1999, Coccurello et al., 2000, Sargolini et al., 2003, Ferretti et al., 2005, Tirado-Santiago et al., 2006, Nelson et al., 2010). Previous data regarding the role of Nacc DA in allocentric learning are conflicting. Some studies have not found allocentric MWM deficits following 6-OHDA injections in the Nacc, however procedural differences between our study and previous work may explain the
differences (Hagan et al., 1983, Grigoryan et al., 1996b). In Hagan et al. the maze diameter was almost half the size of the maze used here, with similarly sized platforms, making the search ratio much smaller than what we used. Hagan et al.’s tank search area to platform area was 218:1 cm² whereas ours was 595:1 cm². It is likely that the search ratio made the Hagan task not as sensitive as the one we used. Grigoryan et al. also had a smaller maze than ours with a search ratio of 400:1 cm²; not as small as Hagen’s but not as challenging as ours, ours still being 50% high in search ratio compared to Grigoryan’s. In addition, Grigoryan et al. used a different learning protocol than we did. We used 4 trials/day for 6 days with a 15 s ITI on the platform between trials. Grigoryan et al. had 2 trials/day for 15 days with a 10 min ITI in a holding cage. Animals undergoing distributed trials learn at different rates than those undergoing massed in some versions of the MWM (Commins et al., 2003). We have previously showed that rats learn the MWM slightly faster when given a 15 min ITI compared to a 15 s ITI (Vorhees and Williams, 2014) but the effect was not significant in a large maze. However, giving 2 trials per day also distributes learning out over a much large time span than in our procedure with 4 trials per day and this may have helped Grigoryan’s animals consolidate and retain what they learned each day better and thereby minimize group differences. Through these procedural and maze size differences we were able to uncover a role for Nacc DA in allocentric MWM learning. Multiple types of pharmacological manipulations of DA signaling in the Nacc have consistently produced allocentric impairments (Cools et al., 1993, Ploeger et al., 1994, Setlow and McGaugh, 1998, Coccurello et al., 2000, Nelson et al., 2010) that also support a role for Nacc DA in allocentric learning.

The mechanisms behind the role of Nacc DA in allocentric and egocentric learning are currently unknown. The Nacc is hypothesized to maintain associations between locations,
actions, and goals during learning (Mogenson et al., 1980, Redish and Touretzky, 1997).

Through the “limbic” circuit, the Nacc network extends through the parahippocampal area, hippocampus, and the prefrontal cortex, all areas necessary for allocentric (parahippocampal area and hippocampus) and egocentric (prefrontal cortex) learning (Figure 1) (Morris et al., 1982, de Bruin et al., 1997, de Bruin et al., 2001, Mizumori et al., 2009). These connections support an overarching role for the Nacc in both navigational types. It has been proposed that Nacc DA regulates the information from these and other regions (Floresco, 2007, Goto and Grace, 2008). Nacc DA projections arise from the VTA and the VTA projects to the mPFC and hippocampus, regions involved in egocentric and allocentric learning.

The dStr, with the DMS and DLS individually, and the Nacc contribute independently to egocentric CWM learning. The differences in cortical networks between the Nacc, DMS, and DLS that all receive necessary information regarding egocentric learning are likely behind these independent roles. Nacc and dStr DA depletion each result in allocentric MWM deficits. It is hypothesized that each region contributes to allocentric learning independently through different mechanisms; via maintaining the correct associations between locations, actions and goals or choosing the correct sensorimotor systems, respectively.

**Medial Prefrontal Cortex Dopamine and Navigation**

The mPFC has been shown to be necessary for egocentric navigation (de Bruin et al., 1997, Ethier et al., 2001). Its role in allocentric navigation has been debated but the current hypothesis suggests that it does not play a role (de Bruin et al., 1994, de Bruin et al., 2001). The mPFC receives input from somatosensory and motor cortices that likely provide the proprioceptive information necessary for egocentric learning (Ragozzino and Kesner, 2001). The parietal cortex plays an important role in learning specific motor movements and it has
efferents to the mPFC (Ragozzino and Kesner, 2001). The mPFC may be critical for holding information from afferents in order to execute the correct movement. Glu is an important mPFC modulatory influence in egocentric learning, since excitotoxic mPFC lesions disrupt learning in an egocentric MWM test (Ethier et al., 2001); for example, during egocentric MWM testing the task is configured with start site and platform position changed on every trial in such a way that the direct path to the goal remains consistent across trials (de Bruin et al., 1997) and under these circumstances mPFC excitotoxic lesions impair performance.

Acetylcholine (ACh) projections to the mPFC also exert an effect on egocentric learning; intra-mPFC injections of the muscarinic ACh receptor antagonist scopolamine induce deficits in the egocentric version of the MWM (Nieto-Escamez et al., 2002). A role of mPFC 5-HT in egocentric and allocentric learning has also been documented. 6-OHDA injections in the hippocampus result in MWM learning deficits with no effect on performance-based factors (Gasbarri et al., 1996). From the present data, it can only be concluded that mPFC DA and NE do not appear to be involved in CWM egocentric learning since in Chapter 5, 6-OHDA lesions of the mPFC caused an 88% loss of DA and a 59% loss of NE, with no effect on CWM or MWM learning.

**Hippocampal Role in Allocentric Navigation**

The hippocampus is a vital region for allocentric learning and is considered the focal region for allocentric navigation (Morris et al., 1982, Mizumori et al., 2005, Penner and Mizumori, 2012). Ablation of the hippocampus impairs allocentric learning in the MWM (Morris et al., 1982). The role of hippocampal DA in allocentric learning has also been documented. 6-OHDA injections in the hippocampus result in MWM learning deficits with no effect on performance-based factors (Gasbarri et al., 1996). Hippocampal Glu-DA receptor
interactions are known to be necessary for LTP, which is an established cellular correlate of allocentric learning (O'Carroll and Morris, 2004, Stramiello and Wagner, 2008).

Electrolytic hippocampal lesions do not result in egocentric deficits in most tasks (Devan et al., 1996, Devan and White, 1999, de Bruin et al., 2001). The role of hippocampal DA in egocentric learning has not yet been tested. However, considering the low percentage of DA projections into the hippocampus from the VTA and that complete ablation of the hippocampus does not affect egocentric learning ability, it is expected that loss of hippocampal DA would have a minimal or no effect on egocentric learning in the CWM. It cannot be completely discounted however that there could be a role for hippocampal DA in egocentric learning since this was not tested here.

**Implications**

Two of the most important implications regarding the present studies relate to a better understanding of the convergence and divergence between egocentric and allocentric learning networks and the validation of the CWM as a test of egocentric route-based learning. It has been accepted that the hippocampal network (including the entorhinal cortex and subiculum) is sufficient for allocentric learning, whereas the mPFC/dStr network is necessary for egocentric learning (Potegal, 1972, Morris et al., 1982, Cook and Kesner, 1988, de Bruin et al., 1997, Mizumori et al., 2005, Penner and Mizumori, 2012). Convergence of these networks was observed when it was found that DMS and Nacc lesions impaired allocentric learning (Annett et al., 1989, Devan and White, 1999). The role of the DMS and Nacc in allocentric learning has been attributed to the hippocampal connections to both of these regions that are destroyed by excitotoxic and electrolytic lesions. Further support for allocentric learning network convergence was seen in our studies that showed that dStr DA is necessary for optimal
allocentric learning, but it is also clear that DA is only part of the story since reducing DA did not eliminate egocentric learning but merely impaired it. This along with our findings that the DMS and Nacc DA are also involved in egocentric learning illustrates the complexity of these networks. The evolutionary need to navigate has developed into a multifaceted, intertwined network in that egocentric systems overlap with allocentric systems to allow for compensation when one system is disrupted and the other system partially compensates for it.

The present studies solidified the CWM as a complex test of egocentric route-based learning that should prove useful in other contexts. The CWM was developed as a test of complex learning (Vorhees, 1987), but whether it measured egocentric or allocentric learning was unknown at first. This was in part due to the fact that testing was run under white light such that rats could use external cues to find the escape, or proprioceptive self-movement cues, or both, making it difficult to isolate the strategy involved. By contrast, animals tested under complete darkness are forced to use only egocentric learning strategies by eliminating access to distal cues. Since introducing this change, we have observed a separation of deficits between the CWM and MWM following developmental and adult drug and genetic manipulations (Herring et al., 2008, Vorhees et al., 2011, Schaefer et al., 2012, Vorhees et al., 2012). These studies illustrate that the CWM and MWM test different learning strategies. The studies herein offer the first lesion-based validation of the CWM as a test of route-based egocentric navigation. The CWM offers some advantages compared with other egocentric tests. Firstly, other tasks of egocentric learning are either not specific for egocentric learning, are too simple, or both. For example, the T-maze is the most frequently used test of egocentric learning, however at best it can only reveal a preference for allocentric versus egocentric learning (Penner and Mizumori, 2012). Oftentimes there is no overall learning deficit in the T-maze, but simply a change in
strategy preference (Packard and McGaugh, 1996, Packard and Knowlton, 2002, Mizumori et al., 2009, Lex et al., 2011). The CWM offers a way to selectively study egocentric learning. It is also similar to human virtual reality egocentric learning tasks used in brain imaging studies. While these virtual reality mazes are not tested exactly as we run the CWM they do prevent the use of allocentric cues to perform the tasks. Importantly, the human studies implicate the same regions with virtual egocentric tasks as we report here, thereby supporting the utility of the CWM as a readout for egocentric ability.

Regions and neuronal pathways required for navigation between rodents and humans are generally homologous but not identical. Other forms of human learning and memory, such as declarative, procedural, and episodic memory, are also tied to the same regions as those found in rodents. Developing analogous tests for both types of spatial learning that can be performed by, and compared across species, such as the MWM and CWM enhance animal models that seek to understand human memory-related diseases, such as Alzheimer’s and other neurodegenerative diseases.

**Potential Mechanisms Underlying Striatal Dopamine Modulated Spatial Learning**

**Striatal Dopamine Receptors**

Understanding the different role of striatal DA during learning has important implications for different disease states, such as Parkinson’s disease, Huntington’s disease, and schizophrenia that produce deficits in navigational learning, likely as at least partially a consequence of altered DA or DA receptor function. Receptor specific drugs that target these pathways may be able to correct networks involved in the cognitive impairments seen in these disorders, as well as have the potential to decrease side effects that less specific drugs have.
Striatal DA mediated effects act mainly through $D_1$ and $D_2$ receptors located in the striatonigral “direct” and striatopallidal “indirect” pathways, respectively (Parent and Hazrati, 1995, Tritsch and Sabatini, 2012). Both networks contribute to DA-mediated learning. dStr $D_1$ and $D_2$ receptors are expressed differently after 6-OHDA lesions, with $D_1$ receptor expression decreased, and $D_2$ receptor expression increased, thereby increasing overall dStr activity (Gerfen et al., 1990). As described in the Introduction, striatonigral $D_1$ neurons have direct inhibitory axon collaterals to the basal ganglia output regions, the entopenduncular nucleus and SN pars reticulata; with decreased $D_1$ expression decreasing the inhibitory signals to these two regions (Gerfen et al., 1990, Parent and Hazrati, 1995, Pan et al., 2010, Surmeier et al., 2011). Striatopallidal $D_2$ neurons have indirect excitatory axon collaterals to these output regions, such that increased $D_2$ expression increase excitatory signaling from the dStr (Gerfen et al., 1990, Parent and Hazrati, 1995, Pan et al., 2010, Surmeier et al., 2011). This imbalance of DA receptor expression increases dStr excitatory signaling to the entopenduncular nucleus and SN pars reticulata, which in turn increase inhibitory signals to cortical projections (Gerfen et al., 1990). It appears that differential receptor expression and activity significantly alter striatum efferent firing and projection activity from these regions. Further research is needed to determine exactly how striatal $D_1$ and $D_2$ receptors and their opposing signaling pathways separately and in tandem contribute to egocentric and allocentric learning.

Information regarding differences between dStr striatonigral and striatopallidal MSNs is limited with regard to learning. Generation of in vivo models that separate these two populations has only recently begun to develop. Functional differences between DLS and DMS $D_1$ and $D_2$ MSNs are observed for motor learning; an effect not due to different proportions of striatopallidal or striatonigral MSNs in each region (Durieux et al., 2012). When $D_1$ receptor
striatonigral MSNs or D₂ receptor striatopallidal MSNs were selectively knocked out in the dStr, rotorod proficiency was decreased either overall or initially, respectively. A difference in involvement in motor learning was observed in the DMS and DLS. DLS D₁ receptor MSN ablation decreased rotarod learning, whereas DMS ablation of this neuronal population did not affect performance. DMS D₂ receptor MSN knockout impaired rotarod performance early in testing, however DLS D₂ receptor MSN knockout did not affect motor performance (Durieux et al., 2012). After extensive rotarod training all groups reached control levels indicating motor impairments were not present. Taken together, such data indicate a functional dissociation between DMS and DLS striatopallidal and striatonigral roles in motor training that may also extend to spatial learning.

The role of D₁ and D₂ receptors in Nacc-mediated allocentric learning has been studied in greater detail than for the dStr. Both D₁ and D₂ receptors are involved in allocentric learning, although the D₁ receptor is thought to be more selective for allocentric learning than the D₂ receptor (Packard and White, 1991, Cools et al., 1993, Setlow and McGaugh, 1998, Coccurello et al., 2000). In the spatial object recognition task intra-Nacc injections of D₁ receptor antagonist SCH 23390 impaired reactivity to spatial change using an allocentric frame of reference, with only a slight impairment in non-spatial change reactivity and no locomotor change. In contrast, intra-Nacc injections of sulpiride (a D₂ antagonist) impaired reactivity to spatial change, as well as non-spatial change, and increased locomotor activity (Coccurello et al., 2000).

Both the D₁ and D₂ receptors in the dStr (including the DMS and DLS separately) and Nacc likely play a role in striatal DA-modulated egocentric and allocentric learning. Differences in DMS and DLS D₁ and D₂ receptor activation during motor learning suggest a separation of receptor function in addition to the dStr subregion functional separation during learning. The
relationship between these receptors to either egocentric or allocentric learning has not yet been explored extensively. Nacc D₁ receptors appear to be more involved in allocentric learning than D₂ receptors but the evidence is insufficient to draw definitive conclusions.

Other DA receptors are not expressed as prominently as the D₁ and D₂ receptors in the striatum. For example, the D₄ receptor is not expressed in the striatum (Noain et al., 2006), and there is low, but widespread, D₅ receptor expression (Diaz et al., 1995, Rivera et al., 2002). Mice lacking whole brain D₅ expression did not perform differently than controls in the MWM (Holmes et al., 2001), suggesting that striatal D₅ receptors are not involved. The ventral striatum is the only part of the striatum that expresses the D₃ receptor (Diaz et al., 1995, Rivera et al., 2002). Whole brain exposure to a D₃ antagonist such as U-991949A, SB-277011, or RCH-1756 or the partial agonist BP-897 did not have an effect on learning a spatial labyrinth maze (not specified or able to determine if it was allocentric or egocentric) (Laszy et al., 2005). When D₃ drugs were given in conjunction with the memory impairing drug scopolamine or FG-7142, all D₃ receptor ligands improved memory (Laszy et al., 2005). Aged D₃ receptor knockout mice performed better than aged-matched controls in the MWM and no difference between young adult knockouts and WT mice (Xing et al., 2010). Based on available D₃ agonists, it seems unlikely that Nacc D₃ drugs in the absence of a learning impairment would have an effect on MWM or CWM learning compared with actions of D₁ and D₂ drugs. A role for the Nacc D₃ receptor in allocentric or egocentric learning cannot be discounted until more specific pharmacological drugs or an inducible Nacc D₃ receptor knockout mouse line becomes available. Current evidence suggests that the D₁ and D₂ receptors in the striatum play the largest role, and future studies should focus on determining the exact role they play in egocentric and allocentric
learning. Both DA receptors exhibit functional differences in the DMS and DLS that should also be explored.

**Striatal Dopamine-Glutamate Receptor Interactions**

One of the ways striatal DA modulates basal ganglia function, including learning is through interactions with Glu. Lesions of DA and Glu in the Nacc individually result in allocentric and egocentric deficits and egocentric deficits if lesions are in the dStr and DLS (Potegal, 1969, Cook and Kesner, 1988, Annett et al., 1989, Cools et al., 1993, Ploeger et al., 1994, Devan and White, 1999, Smith-Roe et al., 1999, Coccurello et al., 2000, Sargolini et al., 2003, Ferretti et al., 2005, Tirado-Santiago et al., 2006, Nelson et al., 2010). Striatal MSNs receive convergent dopaminergic and corticolimbic (including thalamic, hippocampal, and prefrontal) glutamatergic inputs (Coccurello et al., 2012). Striatal DA modulates glutamatergic synaptic activity (Surmeier et al., 2011). Multiple DA and Glu receptor subtypes have functional interactions for the modulation of MSN activity. The interactions between DA and Glu are receiving increased attention in regard to schizophrenia and Parkinson’s disease (Breysse et al., 2003, Bonsi et al., 2007, De Leonibus et al., 2009, Wang et al., 2012). Damage to Glu systems during disease is often concurrent with altered DA activity (Bonsi et al., 2007). Glu-targeted drugs ameliorate Glu-mediated and DA-mediated symptoms and side effects from DA drugs used to treat disorders schizophrenia, Parkinson’s, and Huntington’s diseases consistent with effects on both neurotransmitters.

Electrophysiological and behavioral learning tests have shown how interactions between DA and Glu receptors occur. In striatonigral MSNs, D₁ receptor activation increases the subcellular localization of NMDA and AMPA receptors (Dunah and Standaert, 2001, Berke et al., 2004, Berke, 2009). Conversely NMDA receptor activation slows D₁ receptor diffusion,
increases the number of D1 positive spines and D1 cell-surface expression (Scott et al., 2002, 
Scott et al., 2006, Kruusmagi et al., 2009). Activation of D1 receptors, but not D2 receptors, is 
required for striatal NMDA receptor-dependent LTP (Kerr and Wickens, 2001). While D1 
receptor activation affects AMPA receptor expression, it is thought that D1 and NMDA exhibit 
stronger interactions than D1 and AMPA receptors, and D2 and AMPA receptors have a stronger 
relationship than D2 and NMDA receptors (Wang et al., 2012). In striatopallidal neurons, the D2 
receptor consistently shows a profile of negatively regulating NMDA and AMPA receptors as 
well as Glu release (Cepeda et al., 1993, Hernandez-Lopez et al., 2000, Surmeier et al., 2011). In 
dStr tissue slices, quinpirole-induced D2 activation decreases AMPA currents and AMPA subunit 
GluR1 phosphorylation, thus inhibiting AMPA activation (Cepeda et al., 1993, Hernandez-Lopez 
et al., 2000, Hakansson et al., 2006, Surmeier et al., 2011). D2 signaling promotes AMPA 
receptor trafficking out of the synaptic membrane (Hakansson et al., 2006, Surmeier et al., 2011). 
These electrophysiological data indicate testing of NMDA/D1 receptor and AMPA/D2 receptor 
interactions may be an avenue for determining the role of Glu-DA receptor interactions in 
learning.

Functional testing of these receptor interactions in allocentric or egocentric learning has 
not yet been done in the dStr or its subregions. Available behavioral data in the Nacc support the 
electrophysiological data for allocentric learning. Intra-Nacc infusions of the DA1 antagonist 
SCH23390 and the NMDA antagonist AP-5, at doses that individually do not produce spatial 
deficits, selectively impaired the allocentric ability to detect spatial change in the spatial object 
recognition task (Coccurello et al., 2012). This effect was also observed after infusion with the 
AMPA receptor antagonist DNQX and the D2 receptor antagonist sulpiride at sub-threshold 
doses. When DA and Glu receptor antagonists were given at identical doses with the

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combination of AP-5/sulpiride and DNQX/SCH23390 no difference in spatial recognition was observed, providing evidence of preferential NMDA/D₁ and AMPA/D₂ receptor interactions in allocentric learning (Coccurello et al., 2012). These data support the electrophysiological data that NMDA and D₁ receptors have greater effects than AMPA and D₁ receptor interactions in allocentric learning, as do D₂ and AMPA receptor interactions.

Ionotropic Glu receptors are not the only receptors that interact with striatal DA systems. Other Glu receptors also interact with DA to influence allocentric learning. For example, low doses of the metabotropic Glu receptor mGluR5 antagonist 2-Methyl-6-(phenylethynyl)pyridine (MPEP) have value for the motor and cognitive deficits observed in Parkinson’s disease where DA levels are low (Breysse et al., 2003, Bonsi et al., 2007, De Leonibus et al., 2009). Acute and systematic exposure to low doses of MPEP that do not alter spatial recognition in rats diminished, but did not eliminate, the allocentric-based deficit in ability to detect spatial change in the spatial object reaction test caused by bilateral dStr 6-OHDA lesions in rats (De Leonibus et al., 2009). The exact mechanism behind MPEP compensation for striatal 6-OHDA lesion-induced cognitive deficits is unknown. Following striatal 6-OHDA lesions, striatal Glu hyperactivity is observed (Blandini et al., 2000). Increased dStr MPEP binding is also observed in 6-OHDA lesioned rodents, which decreases mGlu5 activity and synaptic Glu levels, thus partially reducing Glu hyperactivity (Thomas et al., 2001, Pellegrino et al., 2007, De Leonibus et al., 2009). This compensation for Glu hyperactivity may in part explain the benefits of MPEP exposure following striatal DA loss. This suggests that mGluR5 blockade could help ameliorate allocentric and egocentric deficits observed following human striatal DA depletion.

Pharmacological and electrophysiological data indicate striatal NMDA/D₁ receptors and AMPA/D₂ receptors preferentially interact as noted above. Behavioral evidence supports these
findings and implicates them in allocentric learning. Following dStr 6-OHDA induced lesions MPEP exposure alters mGluR5 receptor activity and partially alleviates allocentric deficits from dStr DA loss. These data indicate that DA-Glu receptor interactions differentially contribute to learning that may also extend to striatal DA-mediated egocentric learning.

**Striatal Dopamine-Acetylcholine Interactions**

ACh is another striatal neurotransmitter involved with LTP processes (Lovinger, 2010). Striatal ACh interneurons interact with DA and Glu projection neurons and bi-directionally modulate release of each other (Havekes et al., 2011). During stimulus-response learning, behavioral flexibility, and egocentric tests, striatal ACh activity increases, and striatal ACh lesions impair egocentric learning (Kobayashi and Iwasaki, 2000, Vetreno et al., 2008, Deiana et al., 2011). Therefore, the interaction between striatal ACh and DA release should be further explored.

Previous data have shown complicated and in some cases contradictory data regarding the DA-ACh relationship. Both D\textsubscript{1} and D\textsubscript{2} receptors are located on striatal cholinergic interneurons, and data indicate that D\textsubscript{1} receptors facilitate ACh release and D\textsubscript{2} receptors restrict release (Grigoryan et al., 1996a, Havekes et al., 2011). Nicotinic ACh receptors located presynaptically on the terminals of DA neurons directly facilitate or inhibit DA release depending on tonic DA release versus DA burst firing, respectively (De Belleruche et al., 1979, Zhou et al., 2001, Rice and Cragg, 2004, Zhang and Sulzer, 2004, Havekes et al., 2011). Presynaptic muscarinic ACh receptors modulate DA release, although there is conflicting evidence as to whether it facilitates or inhibits release (Giorguieff et al., 1977, De Belleruche et al., 1979, Hernandez-Lopez et al., 1992, Kudernatsch and Sutor, 1994, Havekes et al., 2011). The role of ACh in Parkinson’s disease is an area of increasing attention. Inhibiting ACh
signaling can partially rescue the motor deficits seen in Parkinson’s disease (Pisani et al., 2003). However, cholinergic drugs often impair cognition as ACh is necessary for learning and memory, attention, and cognitive shifting (Calabresi et al., 2006). Treatment with acetylcholinesterase inhibitors results in attention and cognitive improvement in Parkinson’s disease, as well as decreases the neuropsychiatric symptoms (Calabresi et al., 2006). It has been hypothesized that the ACh-DA imbalance in Parkinson’s disease from degeneration to both neurotransmitters underlies the positive effect of acetylcholinesterase inhibitors on cognition (Calabresi et al., 2006). The effect of striatal ACh on DA-mediated allocentric or egocentric learning has not yet been tested.

**Future Directions**

**Striatum**

It is possible that whole striatal DA depletion would result in a larger learning impairment than dStr and Nacc DA depletion alone, potentially eliminating or greatly reducing egocentric learning ability. Animals with whole striatal DA loss of at least 60% could be tested in the CWM and MWM along with dStr or Nacc DA-depleted animals, and the corresponding SHAM groups, to allow for a direct comparison between groups for differences in egocentric and allocentric learning.

Future research could attempt to separate the effect of DA in the Nacc shell versus core on egocentric and allocentric learning, as these areas have dopaminergic functional heterogeneity (Nelson et al., 2010). In regard to allocentric learning, Nacc shell DA is associated with allocentric place recognition, whereas Nacc core DA is implicated in object recognition in the object place recognition test (Nelson et al., 2010). However, the role of Nacc core and shell DA has not been investigated in egocentric learning and the results could be instructive.
As noted, striatal DA receptors play an important role in striatal DA-mediated learning. Functional D₁ and D₂ receptor heterogeneity exists in the DMS and DLS subregions as well but the details of how these relate to allocentric and egocentric learning are unknown. During short-term allocentric learning Nacc D₁ receptors are more selective for allocentric learning than D₂ receptors (Coccurello et al., 2000). The role of these receptors in the Nacc during longer-term allocentric and egocentric learning has not yet been explored. Discrete injections directly in the dStr, DMS, DLS, or Nacc of the D₁ receptor agonist SKF38393, D₂ receptor agonist quinpirole, D₁ receptor antagonist SCH23390, or the D₂ receptor antagonist sulpiride during CWM and MWM testing may help elucidate the regional function of striatal DA₁ and DA₂ receptors in these forms of learning and memory.

dStr D₁ receptor striatonigral MSNs may contribute to egocentric learning, with DLS striatonigral MSNs contributing more than DMS striatonigral MSNs (Durieux et al., 2012). DStr D₂ receptor striatopallidal MSNs may be involved early in egocentric learning, but not during later phases, with the majority of this contribution occurring in the DMS striatopallidal MSNs (Durieux et al., 2012). It is difficult to predict the exact role of dStr DA receptors as they pertain to allocentric learning. Nacc D₁ and D₂ receptors may also contribute to egocentric navigation as they do with allocentric learning.

Based on the data regarding striatal DA-Glu interactions, the role of D₁ and D₂ receptors interacting with the ionotropic AMPA and NMDA receptors, as well as the metabotropic mGluR5 receptor could be explored within the context of long-term allocentric and egocentric learning. While allocentric information processing in the Nacc has been investigated (Coccurello et al., 2012), the role of these interactions in egocentric or allocentric long-term learning in the Nacc and other striatal subregions has yet to be adequately explored. Using doses
that individually do not alter either spatial learning type, the combination of AP-5/SCH23390 and DNQX/sulpiride could be used in the Nacc or dStr while animals are tested in the CWM and MWM. If a learning deficit is observed in either task following dStr treatment, DMS and DLS regions could be investigated separately. It is hypothesized that these drugs injected in the Nacc will produce deficits in both egocentric and allocentric learning. DStr DA-mediated allocentric learning is likely independent of Glu since ablation of the dStr does not result in allocentric deficits, but does impair egocentric learning (Potegal, 1969, Cook and Kesner, 1988). It is more likely that the mechanism behind the modulation of allocentric learning is independent of Glu, while DA-mediated egocentric learning is linked with Glu receptor signaling. In regard to the DMS and DLS, the available data suggest the role of DA in these regions is restricted to egocentric learning, however other egocentric tests and increased DA loss in these subregions is needed to confirm this finding. Altered DA-Glu interactions within these subregions following 6-OHDA lesions may contribute to the observed egocentric deficits. Glu in the DMS is involved in allocentric learning (Devan et al., 1999, Devan and White, 1999). Glu is likely involved in DMS-mediated allocentric learning from an independent mechanism compared to that for Glu-DA receptor interactions in DMS and DLS-mediated egocentric learning. Chronic exposure to MPEP, AP-5, or DNQX following 6-OHDA injections in these regions could be investigated to determine if improvement in learning occurs due to decreased Glu hyperactivity following striatal 6-OHDA lesions.

Future projects could focus on the interactions between striatal ACh and DA. Treatment with acetylcholinesterase inhibitors decreases the cognitive deficits associated with Parkinson’s disease (Calabresi et al., 2006). However, the direct effect of ACh on dStr DA-mediated allocentric and egocentric learning has not yet been explored. Following dStr or 6-OHDA
lesions, chronic exposure to acetylcholinesterase inhibitors throughout CWM and MWM testing may attenuate the increased ACh levels that likely affect dStr-specific DA-depleted allocentric and egocentric learning. An identical approach with the Nacc could also be done. If increased ACh levels affect dStr or Nacc DA-mediated allocentric or egocentric learning following 6-OHDA lesions, pharmacological manipulations of either striatal muscarinic or nicotinic receptor systems could be performed following dStr or Nacc 6-OHDA lesions. The nonspecific muscarinic ACh receptor agonist pilocarpine or nicotinic ACh receptor agonist nicotine could be given in discrete injections into the dStr or Nacc during CWM and MWM testing to determine if there is any alleviation of 6-OHDA-mediated spatial learning deficits. If either type of spatial learning change is found following dStr lesions and pharmacological manipulations, the DMS and DLS could be examined separately. The effect of ACh agonists on egocentric learning has not been tested; however striatal ACh lesions impair egocentric learning indicating it is likely these agonists would improve the egocentric deficit from dStr and Nacc 6-OHDA lesions (Deiana et al., 2011). It is likely that both dStr and Nacc exposure to ACh receptor agonists would also partially alleviate the allocentric deficits.

Another direction would be to determine the role of the ACh-DA receptor interactions outside of DA depletion in allocentric and egocentric learning. Using doses that do not affect allocentric and egocentric learning, combinations of the muscarinic ACh receptor antagonist scopolamine or nicotinic ACh receptor antagonist mecamylamine with SCH23390, or sulpiride could be injected directly into the dStr or Nacc during MWM and CWM testing. Combinations that alter learning could be investigated in the DMS and DLS individually. D1 and D2 receptors each affect ACh release, facilitating and restricting release respectively (Grigoryan et al., 1996a, Havekes et al., 2011).
Muscarinic and nicotinic ACh receptors modulate DA release, and can increase or inhibit release (Giorguieff et al., 1977, De Belleroche et al., 1979, Hernandez-Lopez et al., 1992, Kudernatsch and Sutor, 1994, Havekes et al., 2011). It is likely that the combination of the D₁ antagonist SCH23390 and muscarinic ACh antagonist scopolamine will inhibit ACh and DA release (Grigoryan et al., 1996a, Havekes et al., 2011), thereby impairing CWM and MWM learning. As nicotinic ACh receptors restrict striatal DA burst firing it is likely the combination of mecamylamine, which will decrease this inhibition, coupled with the D₂ receptor antagonist sulpiride decreasing the inhibition of ACh release (Grigoryan et al., 1996a, Havekes et al., 2011), will result in increased DA burst firing and ACh release and may improve CWM and MWM learning. The combinations of SCH23390 and mecamylamine or sulpiride and scopolamine may not be worth testing as they are predicted to cancel each other out.

Less is known about the interaction of ACh and DA receptors in the DMS and DLS. DMS ACh extracellular output is not altered during a response acquisition task, but is increased during response reversal training indicating that DMS ACh may be involved in behavioral flexibility more so than egocentric learning (Deiana et al., 2011). DMS ACh is an important modulator of learning a novel response in allocentric learning in order to learn the most efficient spatial route, also indicative of a role in behavioral flexibility (Deiana et al., 2011). As DMS DA does not affect allocentric learning, the combination of ACh and DA pharmacological manipulations at sub-threshold doses would not likely affect allocentric learning. It is possible that DMS DA/ACh pharmacological manipulations would not affect egocentric learning, however DLS DA/ACh pharmacological manipulations would affect egocentric learning in a similar manner to that in the dStr. These studies of striatal ACh and DA as they pertain to
egocentric and allocentric learning could elucidate the complex interactions of these neurotransmitter systems.

Chronic treatment of receptor agonists and antagonists can cause side effects, and are not completely specific for the intended receptor making them not always optimal for long-term testing. Regarding the striatum, it can be safely assumed that learning differences due to drugs that mainly target D1 and D2 receptors can be interpreted as these receptors influencing learning and not due to off-target effects on other receptors. If different receptor agonist and antagonists affect motor or motivational processes when given prior to testing, post-training injections could be used instead. The use of DA receptor knockout mice would most likely not elucidate the role of DA receptors in egocentric learning. Mice do not learn the CWM. Frequently they stop searching, even early in testing. Despite different pre-testing and training regimens we have not been able to find an adequate protocol to address the issues mice have with the CWM. As genetic modification of rats becomes more viable, egocentric learning in the CWM will be able to use genetic tools to address these issues.

**Medial Prefrontal Cortex**

Large-scale ablation of mPFC DA levels (88% DA loss, 59% NE loss) did not affect egocentric learning. The relationship between mPFC function and DA has been hypothesized to be an inverted U-shaped function in that deficits arise after too little or too much D1 receptor stimulation (Arnsten, 1997; Granon et al., 2000; Rinaldi et al., 2007; Zahrt et al., 1997). Following large catecholamine decreases in the mPFC (80% DA and 30% NE neuronal loss), but not moderate decreases (60% DA neuronal loss and no NE neuronal loss), extracellular DA levels were similar to controls under basal and stress-evoked conditions (Venator et al., 1999). This normalization of extracellular mPFC DA levels following large-scale cellular catecholamine loss
is not seen following large DA loss in the nigrostriatal DA system (Abercrombie et al., 1990; Venator et al., 1999). It has been suggested that the partial loss of mPFC NE neurons reduces uptake of extracellular DA through decreases in the number of available NE transporters, and increased activity of the remaining DA neurons maintains synaptic DA at normal levels (Venator et al., 1999). Extracellular DA levels similar to controls would result in normal D₁ receptor stimulation, while a more moderate DA depletion and/or spared NE integrity would not show a compensatory effect, and therefore D₁ stimulation would be altered. Future research could conduct a dose-response analysis of 6-OHDA injections in the mPFC to see if such predictions alter egocentric learning as expected.

**Conclusion**

These studies illustrated the importance of striatal DA in allocentric and egocentric learning. They are the first to show a role for dStr, DMS, DLS, and Nacc DA in egocentric learning. They also established the CWM as an egocentric route-based learning task that unmasks egocentric learning effects that very simple egocentric tests do not. While it has been theorized that the hippocampal network controls allocentric learning and the striatal networks dominates egocentric learning, there is much more overlap between these two systems than previously thought. Greater understanding of this complex relationship between these two forms of learning will allow for better development of animal models of human conditions where these functions are compromised and further increase our understanding of core learning and memory system in the brain.
References


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Figure 1. Circuit of Striatal Connections as Pertains to Dopamine-Modulated Allocentric and Egocentric Learning. Black lines represent DA connections with the VTA and SN to Striatal subregions and the mPFC. The purple line designates the Nacc “limbic” loops with the ventromedial PFC. The DMS “association” loop with the mPFC and Parietal Association Cortex is represented in orange lines. The DLS “sensorimotor” loop with the sensorimotor cortices is represented in green. The hippocampus has direct connections with the Nacc, and indirect connections with the DMS that also likely contribute to allocentric learning. Dashed lines indicate lighter projections than solid lines.