Mesolimbic Dopamine, Its Receptors and Social Learning

by

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This thesis investigated the role of dopamine (DA) in the social transmission of food preferences (STFP) in male and female mice. A study conducted by Choleris et al., 2011 using systemic treatments found a role for DA D1-type receptors (D1, D5) in social learning, and a role for DA D2-type receptors (D2, D3, D4) in feeding behavior in the STFP in mice. The potential brain region(s) of action underlying these effects are only now being investigated. The ventral tegmental area (VTA) has dopaminergic projections to limbic structures including the hippocampus and nucleus accumbens (NAc). Previous work done in our lab finds that infusing the DA D1-type receptor antagonist SCH23390 into the dorsal hippocampus blocks social learning in male and female mice (Matta et al., 2017). This thesis found that infusing the DA D2-type receptor antagonist Raclopride into the dorsal hippocampus blocks social learning in female, but not male mice. This social learning impairment due to intrahippocampal Raclopride could not be explained by changes in total food intakes, exposure to the socially carried diet odor, or changes in olfactory discrimination between the two foods used during the choice test. Blocking hippocampal DA D2-type receptors also sex-dependently mediated the social interactions, whereby males had a greater reduction in agonistic-type behaviors, while females had a greater reduction in social investigatory-type behaviors. Using in vivo microdialysis, we further found that social learning in the STFP was associated with increased hippocampal DA...
release for males, whereas social learning was associated with decreased hippocampal DA release for females. This sex difference in hippocampal DA release during the STFP could not be explained by differences in the exposures to the socially carried food odor. Lastly, infusing SCH23390 into the shell of the NAc did not affect social learning or feeding behavior in the STFP in either male nor female mice. However, there were effects of intra-NAc SCH23390 on the social interactions, including reductions in social behaviors such as social investigation and agonistic behaviors for both sexes. Thus, mesolimbic DA and its receptors sex-dependently mediate the STFP in mice.
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<th>Description</th>
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<tbody>
<tr>
<td>ADHD</td>
<td>attention deficit hyperactivity disorder</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analyses of variance</td>
</tr>
<tr>
<td>ASD</td>
<td>autism spectrum disorders</td>
</tr>
<tr>
<td>CA1</td>
<td>cornu ammonis 1 (hippocampal subregion)</td>
</tr>
<tr>
<td>CA2</td>
<td>cornu ammonis 2 (hippocampal subregion)</td>
</tr>
<tr>
<td>CA3</td>
<td>cornu ammonis 3 (hippocampal subregion)</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CIN</td>
<td>1% ground cinnamon</td>
</tr>
<tr>
<td>COC</td>
<td>2% ground cocoa</td>
</tr>
<tr>
<td>CREB</td>
<td>cyclicAMP response element binding protein</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DAT</td>
<td>dopamine transporter</td>
</tr>
<tr>
<td>DAT KO</td>
<td>dopamine transporter knockout mice</td>
</tr>
<tr>
<td>DEM</td>
<td>demonstrator mouse</td>
</tr>
<tr>
<td>ER</td>
<td>estrogen receptor</td>
</tr>
<tr>
<td>FSCV</td>
<td>fast-scan cyclic voltammetry</td>
</tr>
<tr>
<td>GC-D</td>
<td>guanylyl cyclase (in the rodent olfactory epithelium)</td>
</tr>
<tr>
<td>GFP</td>
<td>green fluorescent protein</td>
</tr>
<tr>
<td>GPR30</td>
<td>G-protein-coupled estrogen receptor</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>LTD</td>
<td>long-term depression</td>
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</table>
LTP.................................................................long-term potentiation
mPFC..............................................................medial prefrontal cortex
NAc..............................................................nucleus accumbens
NDI..............................................................novel diet investigation
NMDA..........................................................N-methyl-D-aspartate
NON-DEM......................................................non-demonstrated
OBS..............................................................observer mouse
ODT..............................................................olfactory discrimination task
OFC..............................................................orbitofrontal cortex
PBS..............................................................phosphate buffered saline
PFC..............................................................prefrontal cortex
s.c..............................................................subcutaneous
STFP.........................................................social transmission of food preferences
STIM..........................................................stimulus mouse
VTA..............................................................ventral tegmental area
WT..............................................................wild type mice
CHAPTER 1: General Introduction

What is Social Learning?

Social learning is found in many species. While different definitions can be found in the literature on social learning (Brown & Laland, 2002; Humle et al., 2009; Laland & Hoppitt, 2003; Lonsdorf & Bonnie, 2010), here we define social learning simply as “learning that is influenced by observation of, or interaction with, another animal (typically a conspecific) or its products” (such as odor cues; Box, 1984; Galef, 1988a; Heyes, 1994). Social learning can be a highly adaptive form of learning, so much so, that it occurs in a wide range of organisms, including mice, rats, ants, bees, birds, monkeys, whales, and humans (Hoppitt & Laland, 2013). For example, chimpanzees learn from other chimps how to find and use precisely shaped stones to crack nuts (Biro et al., 2006), guppies copy the foraging behaviors of other highly successful foragers (Kendal et al., 2004), and humans prefer specific body shapes in accordance to their cultural norms (Markey et al., 2002). Its ability to facilitate both an animal’s reproduction and survival, by guiding an assortment of behaviors such as predator avoidance, tool use, and mate selection, makes social learning a biologically significant type of learning. Indeed, in many cases social learning can help animal’s exploit the ‘expertise of others’ (Russon, 1997), and avoid/circumvent the potentially costly trial-and-error individual learning.

Social Learning Strategies.

Throughout this thesis I will be referring to the conspecific that possesses diet relevant social information as the “demonstrator” (DEM) and the conspecific acquiring that information as the “observer” (OBS).
It would be overly simplistic to assume that social learning is inherently adaptive, and that it *always* allows animals to bypass the costs associated with trial and error. As modeled by Rogers 1988, there are various instances where individual learning may be more beneficial than social learning and vice versa. For example, social learners have higher fitness than individual learners when copying is uncommon. The reason for this is that the OBS, that has engaged in social learning, can avoid the costs associated with accurately sampling the environment that the DEM has already incurred. However, on the other end, if everyone is copying one another, and no one is sampling the environment, then individual learners will have higher fitness than social learners, because socially derived information may have no value if it is out of date (e.g., multiple generations old; Boyd & Richerson, 1985; Rogers 1988). Thus, as suggested by the cultural evolution literature, blindly using the information provided by others *may not always* be a recipe for success (Boyd & Richerson, 1985, 1995; Giraldeau et al., 2002; Rogers, 1988).

Instead, studies suggest that for individuals to increase their payoffs, they must be “savvy in their approach,” such that they must use a combination of both social *and* individual learning, i.e., they must know when to copy others, and when to directly sample the environment. Indeed, according to Galef, 1995 “social learning might best be described as socially-biased individual learning.” For social learning to be adaptive, an individual must be strategic and selective, that is, for social learning to be profitable, it has to be done properly. This brings up the matter of social learning strategies, such as whom to copy (older adults, more experienced individuals etc.), when to copy, and when not to copy. Many such strategies have been investigated in various species, and numerous social learning processes have been described. In general, these studies suggest
that an animal should copy a DEM when there may be costs associated with individual learning. For example, sticklebacks prefer to eat from food patches that contain richer foods as based on public information provided by a DEM, and forgo engaging in individual exploration in open water since there are costs (predation) associated with this behavior (Coolen et al., 2003). An animal should also copy when unsure. For instance, naïve OBS rats fed standard non-flavored rodent chow are more likely to socially learn a food preference from a DEM conspecific fed a flavored food-type than experienced OBS rats that have had previous exposure to the flavored food fed to their DEM (see below; Galef, 1996, 2009). Lastly, an animal should copy those who are successful—such as in the case of bats that are unable to find food alone using the cues (excrements) associated with more successful individuals to locate a food source (Wilkinson, 1992; see also Heath et al., 2001; Kendal, Giraldeau & Laland, 2009; Rendell et al., 2010).

The Social Transmission of Food Preferences.

One benefit of living in groups (as many rodents do) is that animals foraging together can assist one another to locate and identify foods. In addition, burrows or colonies can serve as locations for information exchange about potential foods in the neighborhood. This exchange of information can therefore make a valuable aspect of group living among rodents, since individuals can transmit information to other colony members about whether certain foods are edible, or simply available. Using laboratory experiments, it was discovered that most rodents would consume the same food eaten by a conspecific, over another food option. This social transmission of food preferences
(STFP), was initially described by Posadas-Andrews and Roper, 1983, and then thoroughly investigated by Dr. Bennett Galef at McMaster University in Ontario, Canada.

The STFP laboratory test was designed to mimic a naturalistic context wherein a foraging mouse or rat would eat a food some distance away from the burrow. After returning to its burrow, the forager would then socially interact with a conspecific, often a burrow-mate. That animal could then use the food-related information derived from the forager when later deciding which foods to consume (Galef, 1988b).

The basic experiment conducted in the laboratory is as follows: (1) a DEM conspecific is allowed to eat a novel food. Afterwards, (2) an OBS has a short social interaction with the DEM. The OBS animal is then (3) presented with a choice between two novel foods: one is the same flavored food-type the DEM consumed, and the other is a non-demonstrated flavored food. If social learning of a food preference has occurred, the OBS will consume more of the food its DEM ate (Galef, Kennett & Wigmore, 1984).

The simplest explanation for an OBS’s preference to eat the same food fed to a DEM in the STFP is based on familiarity. That is, it is well established that rodents are hesitant to eat novel foods, so called “food neophobia” (Barnett, 1958). In the STFP, an OBS socially interacts with a DEM that has been fed ‘food A’; the OBS has a chance to smell cues associated with ‘food A’ and should therefore become more familiar with ‘food A’ versus an equally palatable (and unfamiliar) ‘food B.’ Thus, an OBS that interacts with an A-fed-DEM should subsequently eat more of ‘food A’ simply because they are familiar with that diet. It would therefore be expected that a familiar diet should be preferred over an unfamiliar diet, even without a social interaction. Dr. Bennett Galef has conducted many studies designed to test this “simple familiarity hypothesis” and
found that even the most straightforward predictions derived from this hypothesis did not hold up in the laboratory (Galef, 1989). For example, OBS rats still prefer to consume the diet fed to their same-sex DEM (following a 15-minute social interaction), even if they were previously allowed to consume, and hence, familiarize with, the two choice-test diets for two days ad libitum. On the other hand, pre-exposing a rat to one of two flavored diets for 24 hours without a social interaction with a DEM does not lead to a food preference (Galef, Kennett & Stein, 1985). Thus, the DEM’s influence on the OBS’s subsequent food choice in the STFP cannot be solely explained by an increased familiarity with the DEM’s diet (Galef, 1988b).

Another possibility is that the DEM’s presence delivers a “social context” within which experience with food-related cues guides an OBS’s later food preference (Galef, 1988b). This idea has been referred to as the “contextual hypothesis.” If this were the case, then one would expect that a “contextual diet” presented during a social interaction should be preferred over a “non-contextual” diet which was not affiliated with socially relevant information. Again, like the “simple familiarity hypothesis,” studies have also shown that this alternative hypothesis cannot explain why a food preference occurs within the STFP. For example, Choleris et al., 2011 found that OBS mice allowed to smell, but not taste, a flavored diet while socially interacting with a DEM that ate standard chow, do not show a diet preference (Choleris et al., 2011; see also Galef, Mason, Preti & Bean, 1988). Hence, the food preferences of OBS’s are not influenced by olfactory exposure to a flavored food while in a “social context” with a DEM that did not consume a flavored diet.
Other studies have also mixed social odors (soiled bedding from an unfamiliar conspecific) with a flavored food, and allowed mice to smell (but not taste) the combination. In a subsequent choice test, mice do not display a food preference when given the option between the food type that was mixed with the social odor versus another novel food choice. Such results suggest that socially relevant odor cues alone are not sufficient to elicit a food preference in mice, whereas interacting with a recently fed DEM conspecific is (Ryan et al., 2008).

On the basis of these investigations, it was thus concluded that an OBS’s preference for the DEM’s food in the STFP is a form of “true” social learning.

A Few Factors that can Modulate the STFP.

Factors such as the age and number of DEM’s can influence the STFP in rodent species such as the house mouse (*Mus domesticus*; Choleris et al., 1997). On the one hand, among rats, familiarity or relatedness to a DEM has little influence on the OBS’s subsequent food choices in the STFP (Galef et al., 1998). On the other hand, familiarity or relatedness has been found to influence the food preferences of Mongolian gerbils (*Meriones unguiculatus*; Choleris et al., 1998; Galef et al., 1998; see also Valsecchi et al., 2002). That is, OBS gerbils were more likely to develop a food preference if the DEM was either related to them, or familiar via previous exposure, though the preference only lasted for a short period of time (less than 24 hours). In the absence of both kinship and familiarity, there was no social learning under the same experimental conditions. On the other hand, in Norway rats (*Rattus norvegicus*), a food preference could be acquired among OBSs from an unfamiliar or unrelated DEM rat, and did in fact last a few days.
Thus, either kinship or familiarity appears to be necessary for the transfer of food preferences among interacting Mongolian gerbils, but not rats or mice. Interestingly, administering OBS gerbils with the anxiolytic compound chlordiazepoxide can facilitate social learning among male and female gerbils such that they will develop a food preference from an unfamiliar or unrelated DEM conspecific (Choleris et al., 1998). Thus, while procedural differences may often be a factor (see Galef et al., 1998), the ease at which an animal may acquire a food preference from another animal in the wild may be species specific, and may further depend on socially relevant anxiety levels.

**Brain Regions Involved in the STFP.**

Dr. Galef has established that for an OBS animal to develop a food preference, there must be a detection of the food scent mixed with carbon disulfide (CS₂), a semiochemical product of digestion, found on the breath of the DEM (Galef, 2012A, 2012B; Galef, 1990; Galef et al., 1988; Galef & Stein, 1985; Galef & Wigmore, 1983). An OBS’s ability to detect CS₂ involves guanylyl cyclase that is expressed on the olfactory-receptor neurons (GC-D receptors) found in the rodent olfactory epithelium (Arakawa et al., 2013; Munger et al., 2010). Thus, the olfactory system and oronasal investigation (i.e., examination of the mouth area) are necessary for both mice (Valsecchi & Galef, 1989) and rats (Galef & Stein, 1985) to learn a food preference in the STFP. Most rodents socially interact in a way that promotes this type of learning: when rodents meet one another, they engage in sniffing each others’ mouth and face areas. Acquiring a food preference in rodents is therefore canalized (Waddington, 1966): through evolution, a series of developmental processes have converged to create a somewhat fail-safe
system that ensures that mice or rats will consume the same foods as other colony members (Shettleworth, 2010).

It should be noted that other sensory systems have also been implicated in the STFP, such as auditory cues (ultrasonic vocalizations; Moles & D'Amato, 2000). However, the involvement of the auditory nervous system in the STFP needs further investigation.

Many brain regions have been found to be involved in social learning in the STFP in rodents. Among these, the basal forebrain (Berger-Sweeney et al., 2000) and frontal, piriform and orbitofrontal cortices (Ross & Eichenbaum, 2006; Ross, McGaughy, & Eichenbaum, 2005; Smith, Countryman, Sahuque, & Colombo, 2007; Winocur & Moscovitch, 1999) have been reported to play an important role.

The hippocampus has also been well established as an important brain structure for developing a food preference in the STFP in rodents (Bunsey & Eichenbaum, 1995; Clark et al., 2002; Countryman & Gold, 2007). For example, rats with lesions to either their dorsal hippocampal subregion (Winocur, 1990), or their entire hippocampus (Clark et al., 2002) do not show a socially learned food preference. Overall, studies suggest that the hippocampus is important in the early processing (Alverez et al., 2001; Bunsey & Eichenbaum, 1995), but not subsequent consolidation/retrieval stages (Winocur et al., 2001) of a socially learned food preference. Conversely, extrahippocampal brain sites, such as the orbitofrontal cortex (OFC) may play a greater role in the later maintenance of memories of a socially transmitted food preference (Lesburgueres et al., 2011).
Neurochemicals Involved in the STFP.

A few neurochemical systems have been found to regulate the STFP in rodents, including the oxytocin/arginine-vasopressin system (Popik & van Ree, 1993; see also Strupp et al., 1990), opioid system (Moles et al., 1999) and galanin system (Wrenn et al., 2003). Moreover, Nmethyl-D-aspartate (NMDA) receptors for glutamate (Roberts & Shapiro, 2002), downstream transcription factors such as the cyclicAMP response element binding protein (CREB; Countryman & Gold, 2007), and gonadal hormones such as estrogens (reviewed in Ervin et al., 2015a) have been shown to mediate the STFP.

The cholinergic system (Berger-Sweeney et al., 2000; Boix-Trelis et al., 2007; Carballo-Márquez et al., 2009A; Gold et al., 2011; Ricceri et al., 2004; Ross et al., 2005) is also involved in the STFP. A study done by Gold et al., 2011 used *in vivo* microdialysis to investigate the role of acetylcholine in the ventral hippocampus and prefrontal cortex in OBS rats socially learning from a DEM in the STFP. In this study, OBS’s were assigned to one of three groups: (1) OBS’s that were allowed to interact with a DEM that had consumed a flavored diet, (2) OBS’s that were allowed to interact with a rat that had consumed a non-flavored diet, and (3) rats that were only allowed to smell (but not eat) a novel diet. Gold et al., 2011 found that only OBS’s that were allowed to interact with a DEM that consumed a flavored diet showed increased acetylcholine levels (relative to baseline, and compared to the other two groups) in the hippocampus, but not in the prefrontal cortex. Thus, increased acetylcholine in the hippocampus accompanies social learning in the STFP in rats (Gold et al., 2011).

Research also finds that the neurotransmitter dopamine (DA) can mediate the STFP in mice (Choleris et al., 2011; Matta et al., 2017).
**Dopamine Pathways and its Receptors.**

The vast majority of DA neuronal projections stem from the base of mid-brain structures such as the ventral tegmental area (VTA) and the substantia nigra (Figure 1). DA cell bodies within the substantia nigra that project to the caudate putamen (dorsal striatum) are known as the nigrostriatal DA pathway. The two other DA pathways arise from the VTA. DA fibers from the VTA that project to the prefrontal cortex regions are known as the mesocortical DA pathway. Lastly, the mesolimbic DA pathway constitutes axons from the VTA that ascend to limbic sites such as the septum, amygdala, hippocampus and nucleus accumbens (NAc; Federoff et al., 2003; Wise, 2004; Zigmond & Stricker, 1972).

**Figure 1.** The nigrostriatal (A), mesocortical (B), and mesolimbic (C) dopamine pathways. From Meyer and Quenzer, 2005, pp 125.
There are five DA receptors: D1, D2, D3, D4, and D5. The D1 and D5 receptors constitute the “D1-type” receptor subfamily, whereas D2, D3 and D4 receptors constitute the “D2-type” receptor subfamily. These two subfamilies are primarily categorized based on the fact that DA D1- and D2-type receptors have opposite actions on the cyclic adenosine monophosphate (cAMP) pathway. Specifically, activation of DA D1-type receptors triggers adenylyl cyclase activity (via stimulative G-proteins), which ultimately leads to greater DA production. Instead, activating DA D2-type receptors blocks adenylyl cyclase activity (via inhibitory G-proteins), which leads to lower DA production (Beaulieu & Gainetdinov, 2011).

In the rodent brain, DA D1- and D2-type receptors are most abundant in the basal ganglia, such as the substantia nigra, as well as the midbrain VTA. There are also high densities in the forebrain caudate putamen and limbic NAc. There are also moderate amounts in the subcortical globus pallidus. On the other hand, structures such as the thalamus and hypothalamus contain very small amounts of DA D1- and D2-type receptors. Moreover, the olfactory bulbs express very small amounts of DA D1-type receptors, while the cerebral cortex and amygdala express very little DA D2-type receptors (Camps et al., 1990).

Recent studies employing male and female mice with DA D1 and D2 promotor-driven green fluorescent protein (GFP) expression have revealed the distributions of DA D1 and D2 receptors in the dorsal hippocampus (Wei et al., 2018). DA D1 receptors are mainly expressed in granule cells in the dentate gyrus, and there is less expression in the cornu ammonis 1 (CA1) and cornu ammonis 3 (CA3) sub-regions (Gangarossa et al.,
These findings are in line with older studies using classical immunohistochemical techniques, which find DA D1 receptors throughout CA1 to CA3 areas (stratum radiatum and stratum oriens), with a somewhat greater expression in the cornu ammonis 2 (CA2; Boyson et al., 1986; Camps et al., 1990; Dubois et al., 1986; Richfield et al., 1987). DA D2 receptors are primarily found in hilar mossy cells (Wei et al., 2018), although earlier studies report a high density of DA D2 receptors in dorsal hippocampus (stratum lacunosum moleculare), though there is less DA D2 receptor expression in the rest of the hippocampus (Charuchinda et al., 1987).

Within the ventral hippocampus, DA D1 receptors are found in the dentate gyrus (granule cells), and DA D2 receptors were found in the hilus (Wei et al., 2018). Different than the dorsal hippocampus, the ventral hippocampus actually shows high DA D1 and D2 receptor expression in the CA1 and subicular areas (Puighermanal et al., 2017; Wei et al., 2018).

DA D5 receptors are found in CA1 pyramidal neurons (Yao et al., 2008; Medin et al., 2011; Medin et al., 2013). Unfortunately, there is very little information available about the distribution of DA D3 and D4 receptors in the rodent hippocampus (Edelmann & Lessmann, 2018), however, pharmacological studies confirm their presence in the rat hippocampus (Sigala et al., 1997; Romo-Parra et al., 2005).

**Dopamine and Social Behavior.**

Pharmacological studies conducted on rodents using DA agonists and antagonists suggest that normal social behavior requires optimal brain DA levels (Miczek et al., 2002). For example, DA agonists can induce social withdrawal in mice (Campi et al.,
facilitate social dominance (Kooij et al., 2018) and attenuate social avoidance in rats (Maple et al., 2017). Instead, using a DA receptor antagonist inhibits prairie voles from forming a partner preference (Gingrich et al., 2000), reduces maternal care behaviors in rats (Byrnes et al., 2002), decreases aggression-related behaviors in dominant animals (de Almeida et al., 2005), and increases social approach after a defeat in mice (Campi et al., 2014).

Dopamine and STFP.

Studies have investigated the role of DA in the STFP by using dopamine transporter (DAT) knockout mice (DAT KO) as a model. The DAT is a membrane-spanning protein located on synaptic vesicles, where it is primarily involved in removing DA from the synapse. Once in the cytosol, DA is then either repackaged into synaptic vesicles, where it can be stored for later release, or is broken down by several enzymes (Beaulieu & Gainetdinov, 2011). DAT KO mice often display abnormal stereotypic behaviors and hyperactivity because without a functional DAT, DA builds up in the extracellular space, and as a result, DA receptors on the postsynaptic membrane are subject to supraphysiologic amounts of DA, which leads to activational effects on various behaviors (Giros et al., 1996; Jones et al., 1998, 1999).

DAT KO mice have been found to display cognitive impairments in many types of non-social learning tasks (Dzirasa et al., 2009; Li et al., 2010; Morice et al., 2007; Pogorelov et al., 2005; Rodriguiz et al., 2004). Additionally, DAT KO mice have been tested in the STFP. Rodriguiz et al., 2004 reports that DAT KO mice display a preference for the food not eaten by the DEM, when compared to wild type (WT) controls.
However, research done by a different group (Wong et al., 2012) reports that DAT KO mice have a social learning impairment in the STFP. However, both studies find that DAT KO mice had normal levels of food intake (Rodriguiz et al., 2004; Wong et al., 2012), and Rodriguiz et al., 2004 further reports that these mice have intact olfactory recognition.

Inconsistencies between Rodriguiz et al., 2004 and Wong et al., 2012 may be due to methodological differences (see Galef et al., 1998). It is also possible that the DAT KO mice used by Rodriguiz et al., 2004 had increased levels of aggression and formed unstable social hierarchies, which has been reported among DAT KO mice (Rodriguiz et al., 2004). Indeed, the mice used by Rodriguiz et al., 2004 may have had a more agonistic social interaction with their respective DEM, and may have consequently developed an aversion to the demonstrated diet (see Choleris et al., 1998; Rodriguiz et al., 2004).

As described by Wong et al., 2012, altered DA receptor actions may have played a role in the social learning impairment observed in the DAT KO mice since they have drastically lowered DA D1- and D2-type receptor expression (El-Ghundi et al., 1999; Fauchey et al., 2000; Giros et al., 1996; Jones et al., 1998).

A study conducted by Choleris et al., 2011 using female mice found that blocking DA D1-type receptors with systemic treatments using the DA D1-type receptor antagonist SCH23390 blocked social learning in the STFP. Furthermore, this social learning impairment could not be explained by any changes in food intake since total food consumption was not influenced by DA D1-type receptor blockade. Moreover, a flavor recognition task showed that mice treated with the effective dose of SCH23390 that blocked social learning could discriminate between the two foods used in the choice
test. Thus, the social learning impairment caused by SCH23390 could not be directly explained by an olfactory discrimination deficit. Lastly, the social learning impairment could also not be explained by a reduced exposure to the socially carried food odor found on the breath of the DEM conspecific since the dose of SCH23390 that blocked social learning did not influence oronasal investigation among OBS mice. Collectively, Choleris and colleagues (2011) suggested that the social learning impairment due to DA D1-type receptor antagonism might have been due to effects on learning and memory processes. Interestingly, in the same study, it was also found that blocking DA D2-type receptors with the DA D2-type receptor antagonist Raclopride did not influence social learning, however, the total amount of food consumed was drastically reduced. Thus, DA D2-type receptors mediate food intake in the STFP, but not social learning itself (Choleris et al., 2011).

The two DA receptor families also appear to have different involvements in the social interactions in the STFP. For example, while DA D2-type receptor blockade influenced both social (e.g., dominance hierarchies) and non-social behaviors (e.g., horizontal exploration), blocking DA D1-type receptors only influenced non-social behaviors. Thus, blocking DA D1-type receptors did not affect the OBS’s overall motivation to socially engage the DEM conspecific, but it did block the STFP, suggesting different dopaminergic regulations of social interactions and social learning (Choleris et al., 2011).

Thus, while there seems to be evidence for an involvement of the DAT and DA receptors in the STFP, the brain site(s) mediating these effects have yet to be fully examined.
**Hippocampal Dopamine and Learning and Memory.**

Studies suggest that hippocampal DA can regulate the acquisition of a spatial memory, but may be less involved in mediating already consolidated memories in the Morris water maze (O' Carroll et al., 2006), radial arm maze (Packard & White, 1991) and even memories for foods in a specific location (Bethus et al., 2010).

Different than studies on spatial learning, most research on hippocampal DA investigating non-spatial types of learning, including step-down inhibitory avoidance (Bernabeu et al., 1997), single-trial passive avoidance learning (Rezayof et al., 2007), and fear conditioning (Rossato et al., 2009) report effects on both memory acquisition and expression.

Thus, hippocampal DA effects on various phases of memory appear to depend upon the type of memory.

**Dorsal Hippocampal Dopamine Receptors and Learning and Memory.**

DA plays a role in regulating motivationally relevant behaviors via synaptic plasticity mechanisms in the CA1 of the dorsal hippocampus (Huang & Kandel, 2006; Lismann & Otmakhova, 2001; Swanson-Park et al., 1999). The dopaminergic projections from the VTA to the dorsal hippocampus, termed the “VTA-hippocampal DA loop” gates the detection of novel stimuli and the encoding of motivationally relevant information (Lisman & Grace, 2005; McNamara et al., 2014). Work done on humans using neuroimaging techniques provides converging evidence for the VTA-hippocampal loop (Adcock et al., 2006; Wittmann et al., 2005). This loop has been found to work through DA D1-type receptor actions in the dorsal CA1 (Alverez et al., 2001; reviewed in Hansen
& Manahan-Vaughan, 2014; Otmakhova & Lisman, 1996) to influence memory acquisition and consolidation processes (Rossato et al., 2009). Hippocampal DA D2-type receptors are also involved in the formation of memories and synaptic plasticity processes (Kulla & Manahan-Vaughan, 2003; Xing et al., 2010). For example, both long-term potentiation (LTP; Kulla & Manahan-Vaughan, 2003) and long-term depression (LTD; Chen et al., 1996) can be mediated by postsynaptic DA D2-type receptors in dorsal hippocampal synapses. Furthermore, DA D2-type receptors have both pre- (Anzalone et al., 2012; Bello et al., 2011; Rocchetti et al., 2014) and post-synaptic effects (Bonci & Hopf, 2005), while DA D1-type effects are mainly on postsynaptic neurons (Gangarossa et al., 2011). DA D2-type receptors can therefore have a multifaceted influence, and may have a more complex role than DA D1-type receptors on hippocampal-dependent learning and memory processes.

_Dorsal Hippocampal Dopamine D1-type Receptors and STFP._

During my MSc, I found a role for dorsal hippocampal DA D1-type receptors in the STFP (Matta et al., 2017). That is, similar to Choleris et al., 2011, blocking DA D1-type receptors in the dorsal hippocampus with SCH23390 impaired social learning in male and female mice, without affecting total food intake, olfactory discrimination abilities, or oronasal investigation. Moreover, males were somewhat more affected by hippocampal infusions of SCH23390 than females, since male social learning was blocked at the lowest and two highest doses of SCH23390, whereas female social learning was only blocked at the highest dose (Matta et al., 2017). Interestingly, within the context of the STFP, dorsal hippocampal DA D1-type receptor blockade mediated
social behaviors during the social interactions in a *sex-dependent* manner. For example, in males there was a reduction in those behaviors that males typically perform more than females, such as boxing, whereas behaviors that are usually more common in females, such as social investigation, were reduced in females. Hence, blocking hippocampal DA D1-type receptors primarily reduced the behaviors that each sex was *already* largely engaged in to begin with (Matta et al., 2017). These sex differences suggest that sex hormones may affect the hippocampal DA D1-type receptor mediation of social learning and social interactions. Such results are also in agreement with the estrogenic regulation of the DA system (Becker, 1990), and the STFP (Clipperton et al., 2008; Ervin et al., 2015a).

What remains to be investigated is the role of hippocampal DA D2-type receptors in the STFP in male and female mice.

*Dopamine, Food Intake, and Food Preferences.*

It is well established that DA is involved in food consumption and feeding-related behaviors (Ball et al., 2011; Kobayashi et al., 2004; Pecina et al., 2003; Sun & Rebec, 2005). Many pharmacological experiments show that DA plays a multifaceted role in feeding-related behaviors, since *both* activating and inhibiting DA receptors can affect feeding. For instance, administering either a DA D1- or D2-type receptor agonist blocks the consumption of powdered chow (Rusk & Cooper, 1989), regular rodent food (Rusk & Cooper, 1988), and a sucrose diet (Bednar et al., 1995). However, this effect can be reversed with either a DA D1- or D2-type receptor antagonist (Bednar, et al., 1995). Additionally, antagonizing DA D2-type (but not D1-type) receptors can reduce total food
intake on flavored powdered chow (Choleris et al., 2011) and regular rodent food (Clifton et al., 1991; Duarte et al., 2003; Heffner et al., 1977; Rusk & Cooper, 1994).

DA is also involved in regulating both socially (Choleris et al., 2011) and individually acquired (Sclafani et al., 2011) food preferences in rodents. The actions of DA D1-type versus D2-type receptors on individually acquired food preferences are (mostly) dissociable (see Sclafani et al., 2011 for a review). For example, administering a DA D1-type receptor agonist can facilitate the preference for a highly palatable sweet food over regular chow, and treatments with a DA D2-type receptor agonist can block this enhancement (Cooper & Al-Naser, 2006). Furthermore, studies using DA receptor antagonists show that both DA D1- and D2-type receptors can mediate the acquisition of a food preference for highly palatable foods (Yu et al., 2000; Baker et al., 2003), although DA D2-type receptors are also involved in its expression (Yu et al., 2000).

_Nucleus Accumbens Dopamine, Food Intake, and Food Preferences._

DA in the NAc has been implicated in feeding and food-seeking behaviors. For example, rats that are normally fed standard rodent chow exhibit increased extracellular DA release in the NAc when allowed to eat a novel and highly palatable food-type (Bassareo & Di Chiara, 1997). Conversely, rats that were not naïve to the palatable food (Bassareo & Di Chiara, 1997), or were allowed to eat the palatable food _ad libitum_ prior to measuring DA (Rada et al., 2005) display lower NAc DA release. Moreover, rats that were allowed to eat regular chow _ad libitum_, or intermittently, do not display a significant change in NAc DA release (Rada et al., 2005). Thus, NAc DA may have a greater role on feeding on palatable foods than regular chow. Such findings also suggest that NAc DA
may have a greater role in *food preferences* than actual consumption.

Work done on individually acquired food preferences find that both NAc DA D1- and D2-type receptors are not involved in the acquisition, but can regulate the expression and extinction of a fructose conditioned flavor preference in rats (Bernal et al., 2008). Additionally, DA D1-type receptors in the NAc can mediate the acquisition (though less so the expression) of a glucose conditioned flavor preference (Touzani et al., 2008). However, whether DA receptors in the NAc are involved in socially learning a food preference has not been investigated.

*Nucleus Accumbens Dopamine D1-type Receptors and Social Behavior.*

Dopaminergic transmission in the NAc has been strongly implicated in social behaviors (Tidey & Miczek, 1996; Trainor et al., 2011). In particular, DA D1-type receptors in the NAc are actively involved in mediating many social behaviors in rodents, including social play (Manduca et al., 2016), social dominance (Kooij et al., 2018), defeat-induced social withdrawal (Campi et al., 2014), and pair bonding behavior (Liu et al., 2010). Additionally, optogenetic studies conducted by Gunaydin et al., 2014 implicate a role for dopaminergic VTA projections to the NAc shell (outer substructure of the NAc) for processing socially relevant stimuli. That is, they found that optogenetically activating VTA to NAc shell DA projections increases social interaction behaviors in female mice towards another novel mouse, while this same effect was not found for novel object investigation. Moreover, it was shown that blocking NAc DA D1-type receptors with SCH23390 (but not Raclopride) could mitigate these prosocial optogenetic effects. Gunaydin and colleagues (2014) proposed that this VTA to NAc DA
circuit may be a specific sub-collection of neurons geared towards the detection of novel social stimuli in mice (Gunaydin et al., 2014). Whether DA D1-type receptors in the NAc shell could also regulate social learning and/or social interactions in the STFP in mice has yet to be determined.

*Dopamine and Gonadal Steroids.*

Estrogens can regulate the rate at which DA neurons fire, and can also directly affect the release and synthesis of DA (Xiao & Becker, 1994; Pasqualini et al., 1995; Becker, 1990a, 2000), to influence learning and memory processes (Almey et al., 2015). Our lab has also shown that estrogen receptors (ER) play a role in social learning in female mice in the STFP (Ervin et al., 2015a). For instance, ER-α inhibits, whereas ER-β can extend a socially learned food preference (Clipperton et al., 2008). Additionally, activating the G protein-coupled estrogen receptor (GPER), but not ER-α or β, rapidly enhances social learning in the STFP in female mice (Ervin et al., 2015b). Furthermore, while Matta et al., 2017 did not find an interaction between drug treatment and the estrous cycle on the STFP, the untreated proestrus females in Choleris et al., 2011 exhibited a preference for the demonstrated food that was longer than that of mice in estrus or diestrus (Choleris et al., 2011). Other studies have also found that only mice that were in proestrus on the day of the social interaction exhibited a food preference one day later (Sanchez-Andrade et al., 2005). Similarly, maternal status has been shown to influence the STFP, whereby pregnant Mongolian gerbils show better social learning than non-pregnant females (Choleris et al., 2012), and postpartum mothers that have high estrogens (as well as other hormones) show a greater food preference when compared to
virgin female rats (Fleming et al., 1994).

Estrogens can also regulate hippocampal synaptic plasticity (Phan et al., 2011, 2015; Woolley & McEwen, 1993), and such neuronal processes can vary as a function of the estrous cycle (Thompson & Moss, 1997; Woolley & McEwen, 1992). Estrogens have also been found to influence structural changes (such as dendritic spine density) in the NAc (Peterson et al., 2015).

Androgens may also interact with the DA system. For instance, anabolic androgenic steroid treatment leads to an increase in hippocampal DA (Tucci, et al., 2012), and can decrease dendritic spine density in the NAc shell of male rats (Wallin-Miller et al., 2016). However, some of the effects of testosterone may be attributed to its conversion to estradiol by the enzyme aromatase (Maclusky, Walters, Clark & Toran-Allerand, 1994).

What remains to be investigated is whether gonadal steroids interact with hippocampal DA D2-type receptors, hippocampal DA release, and NAc DA D1-type receptors to affect the STFP in mice.

**Objectives**

The purpose of this PhD thesis was to (1) investigate the role of DA D2-type receptors in the dorsal hippocampus in the STFP, (2) determine whether there are changes in dorsal hippocampal DA release in association with social learning in the STFP, and (3) explore the involvement of DA D1-type receptors in the NAc shell in the STFP.
For objective (1) we predicted that infusing the DA D2-type receptor antagonist Raclopride into the dorsal hippocampus would block social learning in the STFP. For objective (2) we predicted that social learning would be associated with changes in DA release in the dorsal hippocampus. For objective (3), we predicted that infusing the DA D1-type receptor antagonist SCH23390 into the NAc shell would block social learning in the STFP. These predictions are collectively based upon the results of Choleris et al., 2011 with systemic administrations, and intrahippocampal treatments of Matta et al., 2017, and research conducted on individually acquired food preferences and mesolimbic DA transmission (Lisman & Grace, 2005; Malkusz et al., 2012; Sclafani, et al., 2011).

For objectives (1) and (3), we administered drug treatments to adult female and male CD-1 mice prior to the social interactions in the STFP. For objective (2), we employed in vivo microdialysis and high performance liquid chromatography (HPLC) methods to detect DA levels in the dorsal hippocampus during the social interactions in the STFP.

To examine possible effects of drug treatment (Raclopride and SCH23390) and exposure manipulations (in vivo microdialysis) on various social and non-social behaviors, we performed a full ethological analysis on the social interactions for all experiments.

Given that gonadal steroids such as estrogens/progesterone (Thompson & Moss, 1997) and androgens (Tucci et al., 2012; Wallin-Miller et al., 2016) interact with DA, and that estrogens regulate social learning in the STFP in mice (Clipperton et al., 2008; Ervin et al., 2015b), we assessed for possible sex differences and monitored the female estrous cycles for all experiments. We predicted that gonadal hormones (estrogens, progesterone,
testosterone) would interact with drug treatment (for the Raclopride and SCH23390 experiments) and hippocampal DA (in vivo microdialysis experiment) to produce sex differences between gonadally intact female and male OBS mice in social learning and during the social interactions. Based on the sex differences found in Matta et al., 2017, and the established neuroprotective effects of estrogens/progesterone (Brann et al., 2007; Wise et al., 2005), we further predicted that females would be less susceptible to drug treatment (Raclopride and SCH23390) than males, whereby female social learning and social interactions would be less affected by dopaminergic manipulations.
CHAPTER 2: Sex Differences in Dorsal Hippocampal Dopamine D2-type Receptors Involvement in Social Learning, Social Interactions and Food Intake in Male and Female Mice
INTRODUCTION

The ‘expertise of others’ (Russon, 1997) can be exploited through social learning which can be defined as “learning that is influenced by observation of, or interaction with, another animal (typically a conspecific) or its products” (Box, 1984; Galef, 1988a; Heyes, 1994). Social learning can help numerous species (including mice, monkeys and even humans) avoid potentially harmful trial-and-error individual learning (Hoppitt and Laland, 2013). While social learning is a biologically significant and adaptive type of learning (Hoppitt and Laland, 2013), few studies have investigated its underlying neurobiological mechanisms.

One well-established form of social learning, which can be investigated in a laboratory setting, is the social transmission of food preferences (STFP; Galef et al., 1984), which involves a demonstrator (DEM) mouse first ingesting a novel diet, followed by a same-sex observer (OBS) mouse socially interacting with the recently fed DEM. Later, when given a choice test between two novel diets, OBS mice prefer to eat the same food fed to their respective DEM, which indicates that social learning has occurred during the social interaction (Galef et al., 1984). It is now well established that the OBS needs to detect the flavored food odor mixed with carbon disulfide (CS$_2$; chemical associated with digestion; Galef et al., 1988) found on the breath of the DEM. It has been further established that it is the guanylyl cyclase type D (GC-D) receptors on olfactory neurons which detect CS$_2$ (Arakawa et al., 2013; Munger et al., 2010). Hence, oronasal investigation, which involves sniffing the mouth/face regions of the DEM, is crucial for the STFP to occur in OBS mice (Valsecchi and Galef, 1989) and rats (Galef and Stein, 1985).
The neurobiological mechanisms underlying the STFP are slowly being unraveled. A few neurotransmitters (acetylcholine, glutamate, oxytocin/vasopressin, and opioid) and brain regions (orbitofrontal, frontal and piriform cortices, and basal forebrain) mediate social learning in the STFP (reviewed in Choleris et al, 2009; Ervin et al, 2015a; Matta et al, 2016). Studies have also found an involvement of the neurotransmitter dopamine (DA) in the STFP (Choleris et al, 2011; Matta et al., 2017; Rodriguiz et al, 2004; Wong et al, 2012). For example, DA transporter (DAT) knock out (KO) OBS mice either show an opposite food preference (Rodriguiz et al, 2004), or no socially acquired food preference (Wong et al, 2012) in the STFP. Furthermore, using systemic treatments prior to the social interactions, we found that the DA D1-type receptor (D1, D5) antagonist SCH23390 blocked social learning, whereas the DA D2-type receptor (D2, D3, D4) antagonist Raclopride reduced total food intake in female mice in the STFP (Choleris et al, 2011). The brain region(s) of action underlying these effects are only now being investigated.

The ventral tegmental area (VTA) sends direct dopaminergic projections to numerous limbic structures, such as the amygdala, nucleus accumbens, and hippocampus (McNamara et al, 2014; Wise, 2004). The hippocampus has been established as necessary for the initial encoding/acquisition of the STFP (Bunsey and Eichenbaum, 1995; Clark et al, 2002; Countryman and Gold, 2007). The VTA-hippocampal DA loop (Lisman & Grace 2005) regulates reward related memory processing, as well as registering novel stimuli (Brankack et al., 1996; Floresco et al., 2003). We have found that administration of the DA D1-type receptor antagonist SCH23390 into the dorsal hippocampus blocks social learning in both male and female mice (Matta et al., 2017). Furthermore, these
effects on social learning could not be accounted for by changes in total food intake, olfactory discrimination, or exposure to the socially carried food odor (Matta et al., 2017). However, the role of dorsal hippocampal DA D2-type receptors in the STFP has yet to be elucidated.

DA D2-type receptors can regulate both long-term potentiation (LTP; Kulla & Manahan-Vaughan, 2003) and long-term depression (LTD; Chen et al., 1996). Furthermore, DA D2-type receptors mediate hippocampal dependent information processing (Rocchetti et al., 2014), and DA D2-type receptors found in other limbic brain regions (such as in the amygdala) have been implicated in individually acquired food preferences (Malkusz et al., 2012). Moreover, DA D2-type receptors are involved in the regulation of many social behaviors including affiliative social bonding (Gingrich et al., 2000), aggressive behavior (Aguilar et al., 1994), observational fear learning (Kim et al., 2014), and social hierarchies (Yamaguchi et al., 2017). However, studies investigating the role of DA D2-type receptors specifically in the dorsal hippocampus in the context of social learning are lacking. Given the role of DA D2-type receptors in social behavior and individually acquired food preferences, it can be hypothesized that like hippocampal DA D1-type receptors (Matta et al., 2017), dorsal hippocampal DA D2-type receptors may regulate the STFP.

The purpose of this study was to investigate whether infusing a DA D2-type receptor antagonist (Raclopride) directly into the dorsal hippocampus prior to the social interactions would block social learning in the STFP. We also investigated the possible effects of Raclopride on total food intake and olfactory discrimination. Additionally, a full ethological analysis (Clipperton et al, 2008) was conducted on the social interactions
(where social learning occurs), to assess drug effects on numerous social and nonsocial behaviors including oronasal investigation. Given that our previous DA work finds clear sex differences in DA D1-type receptor involvement in the STFP (Matta et al., 2017), we used both males and females throughout. Lastly, since estrogens regulate the STFP in mice (Clipperton et al., 2008; Ervin et al., 2015a), and estrogens/progesterone mediate the mesolimbic DA system (Thompson and Moss, 1997), we also attended to possible effects of the estrous cycle on social learning.

**MATERIALS AND METHODS**

*Animals*

Male and female CD-1 mice (*Mus musculus*; Charles Rivers, St. Constant, QC, Canada) that were 2-3 months old (young adults) and were experimentally naïve were used in all experimental procedures. Upon arrival to the colony room, mice were triple housed (with the same sex) in clear polyethylene cages (26 x 16 x 12 cm³). All cages had *ad libitum* standard rodent food (Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Teklad, WI) and water. Cages also contained environmental enrichment (paper cups and paper nesting material) and corncob bedding. All mice had at least one week to adjust to the colony room upon arrival before starting any experiments. Mice were later double housed with a same-sex animal for the STFP (see below) or singly housed for the olfactory discrimination task (ODT) (see below) in the same polyethylene cages and conditions as mentioned above. The colony room temperature was set to 21±1 °C, with 40–50% humidity, and was fixed on a reversed light/dark cycle (12:12 hours; lights off at 08:00). All experimental procedures were approved by the University of Guelph.
Institutional Animal Care and Use Committee, and were in agreement with the guidelines of the Canadian Council on Animal Care.

**General Surgical Procedures**

All DEM’s in the STFP were gonadectomized to make certain that any effects on the OBS’s social learning in the STFP was due to the drug manipulations, and not due to the hormonal status of the DEM during the social interactions. OBS’s and ODT mice had bilateral guide cannulas implanted, but were left gonadally intact to investigate any potential hormonal actions on the STFP (see Choleris et al., 2011; Clipperton et al., 2008; Sanchez-Andrade et al., 2005) and diet odor discrimination. DEM mice were reused (approximately 10 to 12 times) while OBS’s tested in the STFP, and mice tested in the ODT were always experimentally naïve.

All surgeries involved mice first receiving an injection of carprofen at 50mg/kg (analgesic/anti-inflammatory agent, subcutaneous [s.c.] injection; Rimadyl, Pfizer Canada Inc, Kirkland, QC, Canada). Thirty minutes later, mice were anaesthetized with isoflurane (Benson Medical Industries, Markham, ON), and received a local anaesthetic (2 to 3 drops; mix of 0.67% lidocaine [Alveda Pharmaceuticals, Toronto, ON, Canada] and 0.17% bupivacaine [Hospira, Inc., Montreal, QC, Canada]) for all incision areas (shaved and cleaned beforehand). At the end of each surgery, all mice were given a rehydrating 0.5mL intraperitoneal (i.p.) injection of warm saline solution (0.9% NaCl). All incisions for DEM castrations/ovariectomies (see below) were closed via MikRon wound clips (Autoclip, 9mm; MikRon Precision Inc., Gardena, CA). All mice were allowed to recover for (at least) 7 days (singly housed in a clean cage, *ad libitum* food
and water access, same housing parameters as stated above) before being pair housed for the STFP, or tested in the ODT.

**Ovariectomy Surgery**

This surgery is described in detail in Clipperton-Allen et al., 2011A. Briefly, for all adult anaesthetized DEM females, a 2cm dorsal incision was made on the skin of the lower back. Two more bilateral incisions (1cm each) were made on the muscles covering both ovaries, which were then pulled out, the fallopian tubes were clamped, and the ovaries were removed. The fallopian tubes were then reinserted into the muscles. The skin incision area was then closed using 1-2 wound clips (see above; Clipperton-Allen et al., 2011A).

**Castration Surgery**

Adult DEM males were first anesthetized, and then placed on their backs. A small ventral incision (1cm) was then made on the midline of the scrotum. Next, the skin was pulled back, and the lateral walls of the tunica were cut on the left and right sides (0.5cm each). The testes were then pulled out, the spermatic cords were ligated (using hot sterile hemostatic clamps), and the testes were removed. The tubules and various blood vessels were then put back into the tunica, and the skin incision site was closed with 1-2 wound clips (see above; Matta et al., 2017).

**Cannulation Surgery**

See Phan et al., 2015 for detailed procedures of this surgery. The skin on top of the skull of anesthetized OBS and ODT mice was removed, and via a stereotaxic frame (David Kopf instruments, CA) that held atraumatic mouse ear bars, 26 gauge bilateral guide cannulae (Plastics One, HRS Scientific, Anjou, QC, Canada) were implanted into
the skull aiming for the anterior dorsal hippocampus, with respect to Bregma (flat skull position; AP = -1.7mm; Lat. = 1.5mm; DV [below the skull surface] = -1.3mm).

Anatomical coordinates were based on the mouse brain atlas of Paxinos and Franklin 2001. Jewellers screws (1.6mm; Plastics One, HRS Scientific, Anjou, QC, Canada) were inserted into the skull, and dental cement (Central Dental Ltd, Scarborough, ON, Canada) was used to keep the guide cannulae fixed in place. At the end of the surgery, dummy cannulae (Plastics One, HRS Scientific, Anjou, QC, Canada) were inserted into the guides to prevent blockage or infection. Injectors (Plastics One, HRS Scientific, Anjou, QC, Canada) used during experimental procedures had a ventral coordinate of 2.3mm below the surface of the mouse skull.

**Materials**

Custom-made perforated steel cage dividers (25.8 x .2 x 10.8 cm³; see Matta et al., 2017 for details) were used to keep same-sex mice physically separated during the three-day pair housing period before testing in the STFP. On the morning of testing, DEM’s were placed into clean polyethylene cages (26 x 16 x 12 cm³), and with glass feeding jars (5cm H, 7.5cm W; Dyets, Bethlehem, PA) with stainless steel screw-on lids equipped with collars. Jar lids had a hole that was 2.5cm in diameter, and jars also contained a perforated stainless steel disk which was placed on top of the powdered food to thwart spillage and allow accurate intake measurements. All behavioral testing was recorded under infrared light using a JVC Everio camcorder (Mississauga, ON, Canada). Overhead recording was made possible using clear, glare-resistant, Plexiglas cage lids with air holes, which were used for all social interactions in the STFP and phases of the ODT. For choice testing in the STFP, OBS’s were placed into clean polyethylene cages.
(42.5 x 26.5 x 18.5cm³) with two separate openings leading to stainless steel tunnels protruding at the front of the cage, where one tunnel led to a removable Plexiglas container filled with CIN, while the other tunnel led to a container filled with COC (see Choleris et al, 2011 and Valsecchi & Galef, 1989 for a detailed description of this equipment; Tecniplast, Varese, Italy). A scale (Sartorius Analytical Balance, Sartorius Inc., United Kingdom) that was accurate to 0.01g was used to weigh all the DEM jars and OBS choice test feeding containers. All olfactory exposure phases for the ODT involved the use of glass mason jars (5.5cm H, 6.5cm diameter). To give mice access to the odor of the flavored diets without being able to actually ingest it, the ODT mason jars also had screw-on lids (7cm diameter) equipped with a 0.5cm fine mesh grid (5.5cm diameter; Matta et al., 2017). Following all behavioral testing, all equipments/materials were washed with baking soda and odorless Alconox detergent powder, and left to air dry to eliminate any possible odor cues from trial to trial.

**Flavored Diets**

We used 1% ground cinnamon (CIN; McCormick Ground Cinnamon, McCormick Canada, London, Canada) or 2% ground cocoa (COC; Fry’s Premium Cocoa, Cadbury, Mississauga, Canada) mixed with ground rodent chow. Published work from our lab (Choleris et al, 2011; Clipperton et al, 2008) suggests that these two diets are equally palatable to CD-1 mice.

**Drug**

The D2-type DA receptor antagonist Raclopride tartrate (Hall et al., 1988; Sigma-Aldrich, Oakville, ON, Canada) was dissolved in sterile saline solution (0.9% NaCl).

**Experimental Procedures**
All OBS mice were pair-housed (with a same-sex DEM) for at least three days prior to behavioral testing in the STFP. During this pair-housing period, OBS’s and DEM’s were separated using perforated cage dividers that split the home cage into two sections (lengthwise; see Matta et al., 2017). This was done to prevent the DEM from chewing off the OBS’s cannula and headcap, while still allowing OBS-DEM dyads to become familiar, and have sensory exposure to one another for the three-day period. The OBS’s side (right/left) was counterbalanced for each sex and dose throughout testing. Approximately 12-14 hours before testing, all mice (OBS’s and DEM’s) were food deprived overnight, and transferred into the experimental room. Early in the dark period on the morning of testing, half the DEM’s were allowed to eat CIN, and the other half ate COC for 1 hour in a clean cage, and the DEM’s food (CIN or COC) was counterbalanced for each sex/dose. Only OBS’s whose DEM consumed at least 0.1g of food were tested (no OBS’s were excluded based on this criteria). After eating, the DEM’s were returned to the home cage, and allowed to socially interact (without a cage divider) with their same-sex OBS cage mate for 30 minutes. Following the social interaction, OBS mice were immediately placed into the choice test cages where they had \textit{ad libitum} access to both CIN and COC diets (and water) for 8 hours. The side each food was on (left or right) was counterbalanced for each sex/treatment. Food containers were weighed at 2, 4, 6, and 8 hours into choice testing, to determine food preferences and total amounts of food consumed. 

With the use of a microinfusion pump (PHD 2000 injector, Harvard Apparatus, QC, Canada), OBS’s received a single intrahippocampal infusion of either saline solution
(0.9% NaCl) to serve as a control group, or one of four doses of Raclopride (at 10, 14, 18, or 20 µg/µL) ten minutes prior to the social interaction (where OBS’s socially learn a food preference; Figure 2). The doses selected were primarily based on our own pilot studies, as well as in vitro mouse studies that administered Raclopride within hippocampal or striatal brain slices (Chepkova et al., 2005; Etter & Krezel, 2014; Hamada et al., 2004; Yabuuchi et al., 2006), and behavioral mouse studies that infused Raclopride into the striatum (Gunaydin et al., 2014; Jung et al., 2013; Krzyosiak et al., 2010; Kurokawa et al., 2012; Presti et al., 2004; Young et al., 2014). The ten-minute delay period was chosen based on previous behavioral mouse studies that used Raclopride systemically (Choleris et al., 2011), in the NAc (Gunaydin et al., 2014; Jung et al., 2013; Krzyosiak et al., 2010; Kurokawa et al., 2012; Young et al., 2014A), and in the dorsolateral striatum (Presti et al., 2004). Mice were infused with 1.0µL (0.5µL per hemisphere) at a flow rate of 0.2µL per minute. The injector was left in the brain for one additional minute after the microinfusion ended to avoid any back-flow. Male and female OBS’s were always tested on the same day, and the order in which the treatment was administered was counterbalanced for each sex, from trial to trial.

Only the OBS’s behaviors during the social interactions were later scored by a trained researcher who was blind to the OBS’s experimental group (The Observer Video software, Noldus Information Technology, Wageningen, Netherlands). Various single behaviors (see Table 1 below for full list) including social investigative actions (e.g., oronasal investigation, which is necessary for the STFP), agonistic behaviors (e.g., ritualized aggression), and non-social active behaviors (e.g., horizontal and vertical exploration) were examined during the social interactions (Blanchard et al, 1993;
Choleris et al, 2003; Clipperton et al, 2008). Grouped behaviors were also calculated from a combination of the single behaviors to examine whether drug treatment had an influence on aspects such as overall activity levels and social hierarchies (see Table 2 below for full list).

![Timeline for the intra-hippocampal Raclopride social transmission of food preferences (STFP) experiment. “DEM” stands for demonstrator mouse; “OBS” stands for observer mouse.](image)

**Figure 2.** Timeline for the intra-hippocampal Raclopride social transmission of food preferences (STFP) experiment. “DEM” stands for demonstrator mouse; “OBS” stands for observer mouse.

**Olfactory Discrimination Task**

The purpose of this experiment was to determine whether the block of social learning by intrahippocampal Raclopride could be accounted for by changes in olfactory discrimination (see Figure 3).

Mice were transferred to the testing room and food deprived 12-14 hours the day before testing. Four habituation phases and one test phase were conducted consecutively in the home cage. During habituation, mice were presented with 2 mason jars (with meshed lids) that each contained 1 tablespoon (15g) of the same flavored food (both jars contained CIN, or both jars contained COC). During test, one jar contained CIN and the
other jar contained COC (i.e., one familiar, and one novel diet). The four habituation sessions and one test session were each 5 minutes, and there was 1 minute in between each session. Researchers stood 1 meter away holding two stopwatches to measure the amount of time mice spent investigating the meshed area of the jars (i.e., where the food odor was emitted). The flavored diet presented during the habituation phase, and the location of the novel diet (left/right) during the test phase were counterbalanced for each treatment group (Matta et al., 2017).

Ten minutes prior to the first habituation phase, female mice received an intrahippocampal microinfusion of Raclopride at either 18 µg/µL or 20 µg/µL (the two doses that blocked social learning), or saline solution (control). Microinfusion parameters (volume per hemisphere and infusion rate) were the same as the STFP experiment.

Procedures including the length of food deprivation, and time delay between drug microinfusion and behavioral testing were conducted to mimic the STFP procedures.

**Figure 3.** Timeline for the intra-hippocampal Raclopride olfactory discrimination task (ODT). “Hab” stands for habituation phase.
**Estrous Phase Determination**

To examine possible effects of the estrous cycle and potential interactions with drug treatment on social learning, OBS vaginal smears were taken directly after the choice test was complete in the STFP, and after the test session in the ODT. To confirm that the ovariectomy surgeries were successful (see Oksjoki et al., 1999), DEM vaginal smears were also taken following the end of the 30 minute social interactions. To do this, females were first gently restrained, then cotton-tipped swabs were moistened with room temperature saline solution (0.9% NaCl), inserted into the vagina, rotated 2-3 turns along the vaginal walls, and then applied onto clean glass microscope slides. To better visualize cells, microscope slides were later (at least 24 hours) stained with Giemsa (Sigma-Aldrich, Oakville, ON, Canada) and left to air-dry overnight in a fume hood, before being examined with a light microscope (3.2x magnification). Proestrus was determined as primarily nucleated epithelial cells, with very little to no leukocytes or cornified epithelial cells present. Estrus was determined by great amounts of cornified epithelial cells, though a few nucleated epithelial cells may have been present. Lastly, diestrus was determined as numerous leukocytes, with some occasional cornified or nucleated epithelial cells being present (Byers et al., 2012; Caligioni, 2009; Clipperton et al., 2008).

**Histology**

Following all behavioral testing, cannulated mice received an intrahippocampal infusion of 1% Chicago blue dye in phosphate buffered saline (PBS) solution, under the same microinfusion parameters as during behavioral testing (see above). Brains were then extracted 40 minutes after initiating dye infusions. This time point was chosen based on the time period between infusion and when OBS mice were given the choice test. Brains
were initially placed into 4% paraformaldehyde for 10-14 days at 4°C. Following this, brains were then transferred into 30% sucrose in PBS solution for 3-5 days, or until the brains sank to the bottom of the tubes (also at 4°C). Brains were then kept at -80 °C before being sectioned with a cryostat (coronal slices at 30µm; Leica CM 1850, Leica Microsystems, Richmond Hill, ON). Slices were mounted onto gelatin-coated glass microscope slides, and were later coverslipped with DPX mounting solution. We found that the dye was mostly localized to the dorsal hippocampus (Figure 6), which is consistent with our past intrahippocampal studies (Lymer et al., 2017; Matta et al., 2017; Phan et al, 2015). Brains with incorrectly placed cannulas (as examined with a light microscope at 3.2x magnification) within the hippocampus were removed from all analyses (n = 13 mice removed across all groups; Paxinos & Franklin, 2001).

Data Handling

To determine whether social learned occurred in the STFP, a percent of CIN diet was calculated \[\frac{\text{CIN}}{\text{CIN} + \text{COC}} \times 100\] for every OBS, at each time point (2, 4, 6 and 8 hours). A CIN preference score towards 0% implies that OBS’s preferred to eat COC, while a CIN preference score towards 100% implies that OBS’s preferred to eat CIN. A statistically significant difference between the CIN preference scores of OBS’s that had a CIN DEM versus OBS’s that had a COC DEM (at a particular time point) denotes a socially learned food preference (for that treatment group). The total amount of food consumed (CIN + COC) was also calculated for each OBS, at every time point.

To compare the strength of food preferences across different treatment groups, we also calculated a percent of DEM diet \[\frac{\text{DEM}}{\text{DEM} + \text{NONDEM}} \times 100\], for every OBS, at each time point (2, 4, 6 and 8 hours). A DEM preference score around 50%
(chance levels) implies that OBS’s had no food preference, while a DEM preference score towards 100% implies that OBS’s preferred to eat their DEM’s food type (see Choleris et al., 2012).

A percent of CIN diet and percent of DEM diet ratio was calculated only if OBS’s ate at least 0.1g of food in total for each choice test time point. For this reason, there were often empty cells for certain time points, which decreased the sample size for the overall model. Accordingly, separate analyses were also conducted on the CIN and DEM preference scores at each time point separately (2, 4, 6 and 8 hours), in addition to the analyses including the full 8 hours.

For the ODT, a percent of novel diet investigation (NDI) value \([\text{(novel diet investigation time/total time investigating both diets)} \times 100]\), and a total investigation value (novel diet investigation time + familiar diet investigation time) was calculated for all phases, for each mouse. Additionally, an average value of the four habituation NDI scores was calculated for every mouse. This average value was expected to be around 50% (chance) since mice were investigating two jars containing the same diet. Since mice preferentially investigate novel over familiar stimuli (Choleris et al., 2003, 2011; Matta et al., 2017), it was expected that the NDI scores during test would be significantly higher than the average habituation NDI scores.

The homogeneity of variance assumption is violated by ratio data. Accordingly, all ratio data was arcsin-transformed prior to analysis. However, all graphs depict the original (non-transformed) ratio data.
**Statistical Analyses**

Mixed model analyses of variance (ANOVAs) were conducted on the CIN preference score data. The between groups factors in these models were: sex (male or female), DEM food (CIN or COC), treatment condition (saline, or Raclopride at 10, 14, 18, or 20 µg/µL), and the repeated measures factor was time (2, 4, 6 and 8 hours).

Mixed model ANOVAs were conducted on the DEM preference score and total food intake data. The between groups factors in these models were: sex (male or female), and treatment condition (saline, or Raclopride at 10, 14, 18, or 20 µg/µL), and the repeated measures factor was time (2, 4, 6 and 8 hours).

Food preferences within the STFP are mostly influenced by drug treatments within the early hours of testing (where the food preferences are most robust; Choleris et al., 2011; Clipperton et al., 2008; Ervin et al., 2015a; Matta et al., 2017). For this reason, mean comparisons were planned at these time points. Specifically, independent samples t-tests were conducted on the CIN preference scores of OBS’s that had a CIN fed DEM versus OBS’s that had a COC fed DEM at each time point, for each sex, and dose group. In doing so, we could address whether or not OBS’s showed a socially learned food preference. We also employed independent samples t-tests on the DEM preference scores between saline infused mice and Raclopride infused mice, at each time point, for each sex. Planned one-sample t-tests comparing to 50% (chance) were also conducted on the DEM preference scores at each time point, for each sex, and dose group. In doing so, we could compare the strength of socially learned food preferences across different treatment groups, and in comparison to chance.
Mixed model ANOVAs were conducted on the duration, frequency and latency data derived from the single and grouped behaviors from the 30 minute social interactions in the STFP. The between groups factors in these models were: sex (male or female) and treatment condition (saline, or Raclopride at 10, 14, 18, or 20 µg/µL). We also performed planned independent samples t-tests on the saline versus Raclopride groups for each of the single and grouped behaviors for both sexes. We used non-parametric analyses (Kruskal–Wallis and Mann–Whitney U) when normality could not be achieved by ln transforming the data. Given that the duration, frequency and latency data yield mostly similar results, we only report the duration results below, and report the frequency/latency results only when different than the duration results.

To assess baseline sex differences during the social interactions, we also performed planned independent samples t-tests on the saline treated males versus saline treated females for all single and grouped behavioral data.

Mixed model ANOVAs were conducted on the NDI scores and ODT total investigation data. Additionally, planned paired samples t-tests were conducted comparing the averaged habituation NDI scores versus the test NDI scores. A significant difference between these two values indicates that mice were able to discriminate between the two flavored diets. For the ODT, the between groups factor was treatment (saline, or Raclopride at 18 or 20 µg/µL), and the repeated measures factor was time (habituation to test).

The phase of the estrous cycle (proestrus, estrus, and diestrus) was one additional between groups factor that was applied to all models involving only female mice. This
was done to establish whether or not the phases of the estrous cycle interacted with drug treatment to influence social learning.

Overall ANOVAs were conducted for all models to reduce the risk of committing type I errors, and all binary mean comparisons throughout were planned *a priori* to attain greater power and help reduce the risk of committing type II errors. *Post-hoc* multiple comparisons (e.g., Bonferroni) were not used here because they are very conservative, and cause type II errors above acceptable levels (see Moran, 2003; Nakagawa, 2004; Rothman, 1990). Indeed, many published neuroscience articles take the same statistical approach (see Bales et al., 2007; Cushing et al., 2004; Cushing & Wynne-Edwards, 2006; Litvin et al., 2011; Murakami et al., 2011; Ribeiro et al., 2009).

We applied the Greenhouse–Geisser correction for repeated measures. Statistical significance was set at *p* < .05. Non-significant values were not reported, except in the case of meaningful results or values, and a clear statistical trend (*T* = .05 < *p* < 0.1). All analyses were executed on SPSS version 20 (IBM Corp, Armonk, NY).
**Table 1.** Single behaviors scored for the 30 minute social interactions in the STFP study (from Clipperton et al., 2008, based on Grant and Mackintosh, 1963).

<table>
<thead>
<tr>
<th>Social Behaviors</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Following the DEM</strong></td>
<td>OBS actively follows, or pursues and chases the DEM; reciprocal to avoid.</td>
</tr>
<tr>
<td><strong>Dominant Behavior</strong></td>
<td>The OBS is in control; includes pinning of DEM, aggressive grooming, crawling over or on top, and mounting attempt.</td>
</tr>
<tr>
<td><strong>Attacks Delivered</strong></td>
<td>Physical attacks, including dorsal/ventral bites. Only frequency of attacks was measured.</td>
</tr>
<tr>
<td><strong>Boxing</strong></td>
<td>Physical attacks which include box/wrestle, offensive and defensive postures, lateral sideways threats and tail rattle.</td>
</tr>
<tr>
<td><strong>Open Aggression</strong></td>
<td>Physical attacks with a locked fight including tumbling, kick-away and counterattack where the attacker cannot be identified.</td>
</tr>
<tr>
<td><strong>Avoidance of DEM</strong></td>
<td>OBS withdraws and runs away from DEM while DEM is following.</td>
</tr>
<tr>
<td><strong>Submissive Behavior</strong></td>
<td>DEM is in control; includes crawl under, supine posture (ventral side exposed), prolonged crouch, and any other behavior in which the DEM is dominant (e.g., DEM pins, aggressively grooms, etc, OBS).</td>
</tr>
<tr>
<td><strong>Attacks Received</strong></td>
<td>Physical attacks including bites to dorsal/ventral regions. Only frequency of attacks was measured.</td>
</tr>
<tr>
<td><strong>Defensive Upright Posturing</strong></td>
<td>Species-typical defensive behavior; upright with the head tucked and the arms ready to push away.</td>
</tr>
<tr>
<td><strong>Social Inactivity</strong></td>
<td>Includes sit/lie/sleep together.</td>
</tr>
<tr>
<td><strong>Oronasal Investigation</strong></td>
<td>Active sniffing of DEM’s oronasal area.</td>
</tr>
<tr>
<td><strong>Body Investigation</strong></td>
<td>Active sniffing of DEM’s body.</td>
</tr>
<tr>
<td><strong>Anogenital investigation</strong></td>
<td>Active sniffing of DEM's anogenital region.</td>
</tr>
<tr>
<td><strong>Stretched Approach</strong></td>
<td>Risk assessment behavior; back feet do not move and front feet approach DEM. Only frequency of stretched approaches was measured.</td>
</tr>
<tr>
<td><strong>Approaching and/or Attending to the DEM</strong></td>
<td>Often from across the cage; OBS's attention is focused on DEM, head tilted toward DEM and movements toward DEM; this becomes 'Follow Opponent' once along the tail or sniff.</td>
</tr>
<tr>
<td><strong>Non-social Behaviors</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Horizontal Exploration</strong></td>
<td>Movement around the cage; includes active sniffing of air and ground.</td>
</tr>
<tr>
<td><strong>Vertical Exploration</strong></td>
<td>Movement to investigate upwards, both front feet off the ground; includes sniffing, wall leans and lid chews (less than 3).</td>
</tr>
<tr>
<td><strong>Digging</strong></td>
<td>Rapid stereotypical movement of forepaws in the bedding.</td>
</tr>
<tr>
<td><strong>Abnormal Stereotypies</strong></td>
<td>“Strange” behaviors, including spinturns, repeated jumps/lid chews/head shakes (more than 3).</td>
</tr>
<tr>
<td><strong>Solitary Inactivity</strong></td>
<td>No movement; includes sit, lie down and sleep.</td>
</tr>
<tr>
<td><strong>Self-Grooming</strong></td>
<td>Rapid movement of forepaws over facial area and along body.</td>
</tr>
</tbody>
</table>
**Table 2.** Grouped behaviors scored for the 30 minute social interactions in the STFP study (from Clipperton et al., 2008).

<table>
<thead>
<tr>
<th>Total Activity</th>
<th>All behaviors involving activity, both social and non-social. Excluded from this group are Inactive Alone, Inactive Together, and Self-Groom.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Social Behavior</strong></td>
<td>This composite behavior does not indicate whether the social interactions are affiliative or agonistic. It includes: Follow DEM, Dominant Behavior, Attack Delivered, Boxing, Open Aggression, Avoid DEM, Submissive Behavior, Attack Received, Defensive Upright Posture, Inactive Together, Oronasal Investigation, Body Investigation, Anogenital investigation, Stretched Approach, and Attend to/approach DEM.</td>
</tr>
<tr>
<td><strong>Agonistic Behavior Delivered</strong></td>
<td>Follow Demonstrator, Dominant Behavior, and Attack Delivered.</td>
</tr>
<tr>
<td><strong>Agonistic Behavior Received</strong></td>
<td>Avoid DEM, Submissive Behavior, Attack Received, and Defensive Upright Posture.</td>
</tr>
<tr>
<td><strong>Total Agonistic Behaviors</strong></td>
<td>This composite behavior does not indicate the direction of the agonistic behavior (i.e., whether agonistic behavior is directed toward OBS or toward DEM). It includes Agonistic Behavior Delivered and Received plus Open Aggression, and Boxing.</td>
</tr>
<tr>
<td><strong>Dominance Score</strong></td>
<td>Total agonistic behavior delivered minus total agonistic behavior received. A negative score indicates that the OBS was the submissive animal in the pair, while a positive score signifies that the OBS was the dominant animal.</td>
</tr>
<tr>
<td><strong>Social Investigation</strong></td>
<td>Oronasal Investigation, Body Investigation, Anogenital investigation, Stretched Approach, and Attend to/approach DEM.</td>
</tr>
<tr>
<td><strong>Non-social Behaviors</strong></td>
<td>Horizontal Exploration, Vertical Exploration, Dig, Stereotypies, Inactive Alone, and Self-Groom.</td>
</tr>
<tr>
<td><strong>Non-social Locomotor Behaviors</strong></td>
<td>Horizontal Exploration, Vertical Exploration, and Dig.</td>
</tr>
<tr>
<td><strong>Non-social Non-locomotor Behaviors</strong></td>
<td>Inactive and Self-Groom.</td>
</tr>
</tbody>
</table>
RESULTS

Mouse Brain Cannula Placements for the STFP and ODT Intrahippocampal Raclopride Experiments

For the STFP (Figure 4) and ODT (Figure 5) intrahippocampal Raclopride experiments, most injectors were found in the anterior dorsal hippocampus (between -1.70mm and -1.82mm), however some were more posterior (-2.46mm) though still within the dorsal hippocampus. The locations of the cannula placements did not appear to differ between groups (for either sex).
Figure 4. All mouse brain cannula placements for the intrahippocampal Raclopride social transmission of food preferences (STFP) experiment. Open circles indicate the location of the injector tip in the mouse dorsal hippocampus. Numbers on the side (in mm) refer to the coronal section posterior to Bregma. Most injectors were found in the anterior dorsal hippocampus (between -1.70mm and -1.82mm), however some were more posterior (-2.46mm) though still within the dorsal hippocampus. The locations of the cannula placements did not appear to differ between groups. Images adapted from Paxinos & Franklin 2001.
Figure 5. All mouse brain cannula placements for the intrahippocampal Raclopride olfactory discrimination task. Open circles indicate the location of the injector tip in the mouse dorsal hippocampus. Numbers on the side (in mm) refer to the coronal section posterior to Bregma. All injectors were found in the anterior dorsal hippocampus (between -1.70mm and -1.82mm). The locations of the cannula placements did not appear to differ between groups. Images adapted from Paxinos & Franklin 2001.
**Figure 6.** Photomicrograph (at 3.2x magnification) of a coronal slice of the mouse dorsal hippocampus following an infusion of 1% Chicago blue dye in phosphate buffered saline. Arrow signifies the site of the microinjection.
Social Learning Study: Effects of Intrahippocampal Raclopride on Social Learning and Food Intake

Analyses conducted on the percent of CIN diets revealed that intrahippocampal Raclopride blocked social learning in females infused with 18 µg/µL and 20 µg/µL (Figure 7G and I). Further analyses on the percent of DEM diets showed that females infused with Raclopride at 18 µg/µL and 20 µg/µL had a weaker food preference than saline infused control mice (Figure 8G and I). The analyses on the CIN and DEM preference scores showed that male social learning was unaffected by all doses (Figure 7 and 8). There were no overall treatment effects of intrahippocampal Raclopride on total food intake (Figure 9) for either sex. The estrous cycle may have interacted with intrahippocampal Raclopride to influence female social learning.

The RM ANOVA conducted on the percent of CIN diets of all OBS mice revealed a significant time x DEM food interaction \[ F(3, 396) = 4.35, \ p = .005 \], significant main effect of DEM food \[ F(1, 132) = 25.93, \ p < .001 \], significant main effect of treatment \[ F(4, 132) = 2.36, \ p = .05 \], and a trend towards a main effect of sex \[ F(1, 132) = 3.33, \ p = .070 \] for the full 8 hours.

Separate ANOVAs conducted on the percent of CIN diets of all OBS’s at individual time points further revealed a significant main effect of treatment at 2 hours \[ F(4, 197) = 3.42, \ p = .010 \], 4 hours \[ F(4, 201) = 2.38, \ p = .05 \], and 6 hours \[ F(4, 184) = 2.46, \ p = .047 \]. There was also a significant main effect of DEM food at 2 hours \[ F(1, 197) = 90.11, \ p < .001 \], 4 hours \[ F(1, 201) = 19.13, \ p < .001 \], 6 hours \[ F(1, 184) = 12.21, \ p = .001 \], and 8 hours \[ F(1, 153) = 9.02, \ p = .003 \]. Furthermore, there was a trend towards a treatment x DEM food interaction at 2 hours \[ F(4, 197) = 2.22, \ p = .068 \], and a
trend towards a sex x DEM food interaction at 2 hours [\(F(1, 197) = 2.89, p = .091\)] and 8 hours [\(F(1, 153) = 2.96, p = .087\)] for all OBS mice. Interestingly, there was a significant main effect of sex at 8 hours [\(F(1, 153) = 3.90, p = .05\)]. Thus, intrahippocampal Raclopride affected the percent of CIN diets of OBS mice in a time- and sex-dependent manner, and in relation to the flavored food their DEM ate.

Separate ANOVAs conducted on the percent of CIN diets of females and males revealed a significant main effect of DEM food for males [\(F(1, 66) = 20.67, p < .001\)] and females [\(F(1, 66) = 7.29, p = .009\)] for the full 8 hours. There was also a significant time x DEM food interaction for males [\(F(3, 198) = 2.92, p = .035\)] and a trend towards significance for females [\(F(3, 198) = 2.57, p = .055\)] for the full 8 hours. Furthermore, there was a significant main effect of DEM food at 2 hours [\(F(1, 80) = 14.31, p < .001\)] and a trend towards significance at 4 hours [\(F(1, 79) = 2.81, p = .098\)] for females, whereas males showed a significant main effect of DEM food at all time points [2 hours: \(F(1, 98) = 61.47, p < .001\); 4 hours: \(F(1, 104) = 9.55, p = .003\); 6 hours: \(F(1, 86) = 11.12, p = .001\); 8 hours: \(F(1, 78) = 11.74, p = .001\)]. Moreover, ANOVAs revealed a significant main effect of treatment at 2 hours [\(F(4, 80) = 3.28, p = .015\)] and 4 hours [\(F(4, 79) = 3.25, p = .016\)] for females, and for males at 6 hours [\(F(4, 86) = 2.99, p = .023\)] and 8 hours [\(F(4, 78) = 2.70, p = .037\)], while there was only a trend towards significance for males at 2 hours [\(F(4, 98) = 2.19, p = .076\)]. Hence, intrahippocampal Raclopride influenced the percent of CIN diets of both male and female OBS’s for various time points during the choice test, and as a function of their DEMs food-type.

Independent samples t-tests conducted on the percent of CIN diets of OBS mice that interacted with a CIN DEM versus OBS mice that interacted with a COC DEM
revealed a social learning impairment for females infused with Raclopride at 18 µg/µL and 20 µg/µL (Figure 7). Specifically, at 2 hours there was a socially acquired food preference indicated by a significant difference between the percent of CIN diets of OBS mice that interacted with a CIN DEM versus COC DEM for both females \( t(19) = 5.01, p < .001 \) and males \( t(18) = 3.15, p = .006 \) infused with saline, females \( t(21) = 2.43, p = .024 \) and males \( t(17) = 5.51, p < .001 \) infused with Raclopride at 10 µg/µL, females \( t(19) = 2.59, p = .018 \) and males \( t(21) = 3.38, p = .003 \) infused with Raclopride at 14 µg/µL, and males infused with Raclopride at 18 µg/µL \( t(21) = 2.62, p = .016 \) and 20 µg/µL \( t(21) = 3.02, p = .006 \). Furthermore, at 4 hours there was a significant difference between the percent of CIN diets of OBS mice that interacted with a CIN DEM versus COC DEM for females infused with saline \( t(20) = 2.42, p = .025 \) and Raclopride at 14 µg/µL \( t(19) = 2.26, p = .036 \), and males infused with Raclopride at 20 µg/µL \( t(21) = 2.26, p = .035 \), while males infused with Raclopride at 10 µg/µL \( t(19) = 1.94, p = .067 \) and 18 µg/µL \( t(22) = 1.79, p = .086 \) showed a trend towards significance at this time point. Additionally, at 6 hours there was a significant difference between the percent of CIN diets of OBS mice that interacted with a CIN DEM versus COC DEM for males infused with saline \( t(19) = 2.14, p = .045 \), while males infused with Raclopride at 10 µg/µL \( t(15) = 1.99, p = .065 \) and 14 µg/µL \( t(16) = 1.79, p = .092 \) trended towards significance at this time point. Lastly, at 8 hours there was a significant difference between the percent of CIN diets of OBS mice that interacted with a CIN DEM versus COC DEM for males infused with Raclopride at 14 µg/µL \( t(18) = 2.61, p = .018 \), while there was only a trend towards significance for females infused with Raclopride at 14 µg/µL \( t(14) = 1.84, p = .088 \) at this time point. Importantly, there was no significant
difference between the percent of CIN diets of OBS mice that interacted with a CIN DEM versus COC DEM at any time points during the choice test for females infused with Raclopride at 18 µg/µL and 20 µg/µL, which indicates that intrahippocampal Raclopride blocked social learning at these doses (Figure 7G and I).

Interestingly, the RM ANOVA conducted on the percent of CIN diets of females for the full 8 hours revealed a significant main effect of the estrous phase \[F(2, 50) = 3.82, p = .029\] and a significant time x estrous phase x DEM food interaction \[F(6, 150) = 2.73, p = .015\]. Separate ANOVAs also showed a significant estrous phase x treatment interaction at 2 hours \[F(8, 80) = 2.34, p = .026\], and a significant estrous phase x DEM food interaction at 6 hours \[F(2, 80) = 3.42, p = .038\]. Hence, the estrous phase may have affected the female percent of CIN diets for certain time points during the choice test, as a function of their treatment group and the food their DEM ate. However, mean comparisons for each treatment group broken down by estrous phase did not yield significances, likely due to the small number of females for each phase, per treatment group (Table 5).
Figure 7. Percent of cinnamon (CIN) diet (CIN food consumed divided by the total amount of food consumed) for observer (OBS) female (A, C, E, G, I) and male (B, D, F, H, J) mice that received a single intrahippocampal microinfusion of either saline vehicle (A, B), or the dopamine (DA) D2-type receptor antagonist Raclopride at 10 µg/µL (C, D), 14 µg/µL (E, F), 18 µg/µL (G, H) or 20 µg/µL (I, J). Infusions were administered 10 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground CIN (white squares) or 2% ground cocoa (COC; black circles) flavored diet. Percent of CIN diets are displayed at 2, 4, 6 and 8 hours into the OBS choice test. The n’s represent the number of OBS mice in each group. Data are
presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, T = 0.05 < p < 0.1, in
comparison between OBS mice that socially interacted with a DEM that consumed CIN versus
OBS mice that socially interacted with a DEM that consumed COC.

The RM ANOVA conducted on the percent of DEM diets of all OBS mice
revealed a significant main effect of time [F(3, 429) = 4.30, p = .005] for the full 8 hours.
Separate ANOVAs also showed a trend towards a main effect of sex at 8 hours [F(1, 164)
=3.39, p = .068] for all OBS mice. RM ANOVAs further revealed a significant main
effect of time for both males [F(3, 213) = 2.89, p = .036] and females [F(3, 216) = 2.70, p
= .047] for the full 8 hours. However, ANOVAs did not yield any significant main effects
of treatment, or any treatment interactions with other variables for the full 8 hours, or at
separate time points. Notably, independent samples t-tests conducted on the percent of
DEM diets comparing control mice infused with saline versus mice infused with
Raclopride revealed that female mice infused with Raclopride at 18 µg/µL [t(40) = 2.35,
p = .024] and 20 µg/µL [t(41) = 2.05, p = .047] had a significantly lower percent of DEM
diet than saline infused females at 2 hours into the choice test. One-sample t-tests
comparing the percent of DEM diets to 50% (chance) further showed that females [t(20)
= 4.86, p < .001] and males [t(19) = 3.03, p = .007] infused with saline, females [t(22) =
2.49, p = .021] and males [t(18) = 5.08, p < .001] infused with Raclopride at 10 µg/µL,
females [t(20) = 2.59, p = .017] and males [t(22) = 3.19, p = .004] infused with
Raclopride at 14 µg/µL, and males infused with Raclopride at 18 µg/µL [t(22) = 2.64, p =
.015] and 20 µg/µL [t(22) = 3.09, p = .005] had a significantly higher percent of DEM
diet than 50% (chance) at 2 hours, while females infused with Raclopride at 20 µg/µL
only showed a trend towards significance [t(21) = 1.75, p = .095] at this time point.
Moreover, at 4 hours females infused with saline \([t(21) = 2.52, p = .02]\) and Raclopride at 14 µg/µL \([t(20) = 2.32, p = .031]\), and males infused with Raclopride at 20 µg/µL \([t(22) = 2.32, p = .03]\) showed a significantly higher percent of DEM diet than 50%, whereas males infused with Raclopride at 10 µg/µL \([t(20) = 1.89, p = .073]\) and 18 µg/µL \([t(23) = 1.84, p = .08]\) were only trending towards significance at this time point. Additionally, males infused with saline \([t(20) = 1.94, p = .066]\) and Raclopride at 10 µg/µL \([t(16) = 1.88, p = .079]\) showed a trend towards a higher percent of DEM diet when compared to 50% at 6 hours. Lastly, at 8 hours only males infused with Raclopride at 14 µg/µL \([t(19) = 2.65, p = .016]\) had a significantly higher percent of DEM diet than 50%. Notably, the percent of DEM diets for females infused with Raclopride at 18 µg/µL and 20 µg/µL were not significantly different than 50% (chance) at any time points during the choice test. Hence, female OBS mice infused with Raclopride at 18 µg/µL and 20 µg/µL had a weaker percent of DEM diet than saline infused control mice, and were not significantly different than chance levels (50%), which confirms that the STFP was blocked in females at these doses (Figure 8G and I).

Notably, the RM ANOVA conducted on the percent of DEM diets of females revealed a significant time x estrous phase interaction \([F(6, 189) = 2.81, p = .012]\) for the full 8 hours. There was also a trend towards a main effect of estrous phase at 6 hours \([F(2, 93) = 2.64, p = .076]\). Hence, the estrous phase may have influenced the female percent of DEM diets in a time dependent manner. However, mean comparisons did not yield any significances, likely due to the small number of females for each phase, per treatment group (Table 5).
Figure 8. Percent of demonstrator (DEM) diet (DEM food consumed divided by the total amount of food consumed) for observer (OBS) female (A, C, E, G, I) and male (B, D, F, H, J) mice that received a single intrahippocampal microinfusion of either saline vehicle (white circles; A, B), or the dopamine (DA) D2-type receptor antagonist Raclopride at 10 µg/µL (black triangles; C, D), 14 µg/µL (black circles; E, F), 18 µg/µL (black squares; G, H) or 20 µg/µL (black diamonds; I, J). Infusions were administered 10 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex DEM mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Percent of DEM diets are displayed at 2, 4, 6 and 8 hours into the OBS
choice test. The n’s represent the number of OBS mice in each group. Data are presented as mean ± SEM. *p < 0.05, in comparison between OBS mice that received an infusion of saline vehicle versus OBS mice that received an infusion of Raclopride. #p < 0.05, ##p < 0.01, ###p < 0.001, T = 0.05 < p < 0.1, in comparison to 50% (chance).

The RM ANOVA conducted on the total intakes of all OBS mice revealed a significant main effect of time [F(3, 648) = 76.45, p < .001]. Separate RM ANOVAs conducted on the total food intakes of males and females analyzed separately (Figure 9) revealed a significant main effect of time for both sexes [males: F(3, 324) = 38.97, p < .001; females: F(3, 267) = 19.07, p < .001]. There were, however, no significant main effects of treatment or sex, and no treatment x sex interactions (or interactions with time) for the full 8 hours or at separate time points for either sex. Hence, the social learning impairment found in this study is not secondary to changes in feeding behavior.

Interestingly, the RM ANOVA conducted only on female total intake showed a trend towards a time x estrous phase x treatment interaction [F(24, 267) = 1.52, p = .061] for the full 8 hours. Separate ANOVAs also showed a trend towards an estrous phase x treatment interaction at 2 hours [F(8, 89) = 1.98, p = .057] and 6 hours [F(8, 89) = 1.84, p = .079]. Further mean comparisons did not reveal any significances, likely due to the small number of mice for each phase, per treatment group (Table 5).
Figure 9. Total food intake (1% ground cinnamon [CIN] + 2% ground cocoa [COC]) for observer (OBS) female (A) and male (B) mice that received a single intrahippocampal microinfusion of either saline vehicle (white circles; \( n = 23 \) for females and \( n = 24 \) for males), or the dopamine (DA) D2-type receptor antagonist Raclopride at 10 µg/µL (black triangles; \( n = 24 \) for females and \( n = 23 \) for males), 14 µg/µL (black circles; \( n = 23 \) for females and \( n = 24 \) for males), 18 µg/µL (black squares; \( n = 24 \) for females and \( n = 24 \) for males) or 20 µg/µL (black diamonds; \( n = 24 \) for females and \( n = 23 \) for males). Infusions were administered 10 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a CIN or COC flavored diet. Total food intakes are displayed at 2, 4, 6 and 8 hours into the OBS choice test. There were no significant effects of intrahippocampal Raclopride on total food intakes for either sex. Data are presented as mean ± SEM.
Social Learning Study: Effects of Intrahippocampal Raclopride on Behavior During the Social Interactions

While total activity was only reduced for female mice, total social behavior was reduced for both sexes (Figure 10). This was paralleled by an increase in the amount of time spent engaging in non-social non-locomotor behaviors (such as solitary inactivity) for both females and males (Figure 15).

Male overt agonistic behaviors (e.g., boxing and agonistic behavior delivered; Figure 11) and female social investigatory behaviors (e.g., body and anogenital investigation; Figure 12) were reduced by Raclopride (i.e., the behavior that each sex performs more of, in general was reduced). In addition, intrahippocampal Raclopride led to a reduced dominance score for both males (Figure 11B) and females (Figure 13C), which indicates that they were both more subordinate to their DEM during the social interactions (Table 3 and 4).

Notably, the social learning impairment found in females due to intrahippocampal Raclopride was not secondary to any changes in the time spent engaging in oronasal investigation (Figure 12C). Hence, the social learning impairment found in this study could not be explained by any changes in the amount of exposure to the socially learned food odor emitted by the DEM.

ANOVAs revealed a significant main effect of sex for total activity [F(1, 226) = 5.601, p = .019]. This could be partially explained by the significant main effect of sex for non-social locomotor behaviors [F(1, 226) = 4.191, p = .042]. The effects on non-social locomotor behaviors could be partially accounted for by the significant main effect of sex for digging [F(1, 226) = 4.709, p = .031]. There was also a trend towards a sex x
drug interaction for vertical exploration [F(4, 226) = 2.036, p = .09]. Additionally, a trend towards a main effect of sex for horizontal exploration [F(1, 226) = 3.629, p = .058] was found.

There were various sex-mediated effects on agonistic behaviors. Specifically, there was a significant main effect of sex [F(1, 226) = 28.878, p < .001] and a trend towards a sex x drug interaction [F(4, 226) = 2.063, p = .087] for the frequency of total agonistic behaviors. This effect could be partially explained by the significant main effect of sex [F(1, 226) = 42.693, p < .001] and sex x drug interaction [F(4, 226) = 2.377, p = .05] for boxing. Moreover, there was a significant main effect of sex for open aggression [F(1, 226) = 19.046, p < .001]. ANOVAs also revealed a trend towards a main effect of sex for dominant behavior [F(1, 226) = 3.431, p = .065]. Additionally, there was a significant main effect of sex [F(1, 226) = 39.695, p < .001] and a trend towards a sex x drug interaction [F(4, 226) = 2.315, p = .058] for the frequency of agonistic behavior delivered. In line with this, ANOVAs revealed a significant main effect of sex [F(1, 226) = 43.414, p < .001] and a trend towards a sex x drug interaction [F(4, 226) = 2.151, p = .075] for the frequency of attacks delivered. There was also a significant main effect of sex for agonistic behavior received [F(1, 226) = 16.672, p < .001], and avoid frequency [F(1, 226) = 5.472, p = .02]. Additionally, ANOVAs showed a significant main effect of sex [F(1, 226) = 16.118, p < .001] and sex x drug interaction [F(4, 226) = 2.451, p = .047] for submissive behavior. As a result, these effects led to a significant main effect of sex for dominance score [F(1, 226) = 17.411, p < .001]. Specifically, total agonistic behaviors were greater for males than females, whereby males spent more time engaging in boxing, open aggression, and dominant behavior than females. Males also more
frequently delivered agonistic behaviors (they attacked more often) than females. Conversely, females received more agonistic behaviors, and were more submissive than males. The dominance score was therefore greater for males than females. Hence, males engaged in agonistic-type behaviors to a greater extent than females (for full results, see social learning study investigating baseline sex differences in mice infused with saline; Figure 16).

There were also many sex-biased social-investigatory effects. For example, ANOVAs revealed a significant main effect of sex \( F(1, 226) = 5.744, p = .017 \) and sex x drug interaction \( F(4, 226) = 2.825, p = .026 \) for social investigation. This could be partly accounted for by the significant main effect of sex \( F(1, 226) = 9.707, p = .002 \) and sex x drug interaction \( F(4, 226) = 3.243, p = .013 \) for body investigation. There was also a significant main effect of sex \( F(1, 226) = 4.144, p = .043 \) and sex x drug interaction \( F(4, 226) = 2.818, p = .026 \) for anogenital investigation. Furthermore, there was a trend towards a main effect of sex for approaching and/or attending to the DEM \( F(1, 226) = 3.429, p = .065 \). Additionally, ANOVAs showed a significant sex x drug interaction for the frequency of following the DEM \( F(4, 226) = 3.557, p = .008 \). There was also a significant main effect of sex for the frequency of social inactivity \( F(1, 226) = 4.018, p = .046 \). Specifically, females spent longer performing social investigation, whereby they spent more time engaging in body and anogenital investigation, and more frequently followed the DEM than males. Thus, social-investigatory behaviors were greater for female mice (for full results, see social learning study investigating baseline sex differences in mice infused with saline; Figure 16).
ANOVAs revealed a significant main effect of sex for non-social non-locomotor behaviors \([F(1, 226) = 8.423, p = .004]\). This could be partly explained by the significant main effect of sex for solitary inactivity \([F(1, 226) = 8.110, p = .005]\).

Separate ANOVAs run on only females or males revealed that female total activity was significantly reduced \([F(4, 113) = 4.26, p = 0.003; \text{Figure 10A}]\) by all doses of Raclopride \(10 \mu g/\mu L: t(45) = 3.332, p = 0.002; 14 \mu g/\mu L: t(44) = 2.606, p = 0.012; 18 \mu g/\mu L: t(45) = 3.827, p < .001; 20 \mu g/\mu L: t(45) = 3.163, p = 0.003\]. This could be partially explained by the finding that female total social behavior was significantly reduced \([F(4, 113) = 4.158, p = 0.004; \text{Figure 10C}]\) by all doses of Raclopride \(10 \mu g/\mu L: t(45) = 2.205, p = 0.033; 14 \mu g/\mu L: t(44) = 2.922, p = 0.005; 18 \mu g/\mu L: t(45) = 3.497, p = 0.001; 20 \mu g/\mu L: t(45) = 3.15, p = 0.003\).

While male total activity was unaffected by drug treatment \([\text{Figure 10B}]\), male total social behavior was significantly reduced \([F(4, 113) = 7.485, p < .001; \text{Figure 10D}]\) by all doses of Raclopride \(10 \mu g/\mu L: t(45) = 4.687, p < .001; 14 \mu g/\mu L: t(46) = 4.454, p < .001; 18 \mu g/\mu L: t(46) = 2.835, p = 0.007; 20 \mu g/\mu L: t(45) = 4.14, p < .001\).
Figure 10. Active behaviors (in sec) for observer (OBS) female [A, C] and male [B, D] mice that received a single intrahippocampal microinfusion of either saline vehicle (n = 23 for females and n = 24 for males), or the dopamine (DA) D2-type receptor antagonist Raclopride at 10 µg/µL (n = 24 for females and n = 23 for males), 14 µg/µL (n = 23 for females and n = 24 for males), 18 µg/µL (n = 24 for females and n = 24 for males) or 20 µg/µL (n = 24 for females and n = 23 for males). Infusions were administered 10 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Intrahippocampal Raclopride effects on female [A] and male [B] total activity, and female [C] and male [D] total social behavior are shown. There were no significant effects of intrahippocampal Raclopride on male total activity [B]. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, in comparison to saline infused control animals.

It is interesting to note that for both females and males, all non-social locomotor behaviors (horizontal exploration, vertical exploration, and dig) were unaffected by intrahippocampal Raclopride.
The frequency of male total agonistic behaviors was significantly reduced \[F (4, 113) = 2.602, p = 0.04; \text{Figure } 11A\] by the two highest doses of Raclopride \[18 \mu g/\mu L: t(46) = 2.087, p = 0.042; 20 \mu g/\mu L: t(45) = 2.604, p = 0.012\]. An overall reduction in agonistic behaviors delivered, and an increase in submissive behaviors and agonistic behaviors received resulted in a significantly reduced male dominance score \[F (4, 113) = 7.163, p < 0.001; \text{Figure } 11B\] by all doses of Raclopride \[10 \mu g/\mu L: t(45) = 4.907, p < 0.001; 14 \mu g/\mu L: t(46) = 5.215, p < 0.001; 18 \mu g/\mu L: t(46) = 3.912, p < 0.001; 20 \mu g/\mu L: t(45) = 4.163, p < 0.001\]. Specifically, male dominant behavior was significantly reduced \[\chi^2(4) = 19.106, p = 0.001; \text{Figure } 11C\] by all doses of Raclopride \[10 \mu g/\mu L: U = 118.5, z = -3.355, p = 0.001; 14 \mu g/\mu L: U = 113.5, z = -3.602, p < 0.001; 18 \mu g/\mu L: U = 127.5, z = -3.317, p = 0.001; 20 \mu g/\mu L: U = 117, z = -3.386, p = 0.001\]. Additionally, male boxing was significantly reduced \[F (4, 113) = 2.413, p = 0.05; \text{Figure } 11D\] by 20 \mu g/\mu L \[t(45) = 2.434, p = 0.019\] while Raclopride at 18 \mu g/\mu L only trended towards significance \[t(46) = 1.807, p = 0.077\]. Furthermore, male submissive behavior was significantly increased \[F (4, 113) = 2.585, p = 0.041; \text{Figure } 11E\] by all doses of Raclopride \[10 \mu g/\mu L: t(45) = -2.609, p = 0.012; 14 \mu g/\mu L: t(46) = -2.841, p = 0.007; 18 \mu g/\mu L: t(46) = -2.737, p = 0.009; 20 \mu g/\mu L: t(45) = -2.824, p = 0.007\]. In line with this, male agonistic behavior received was significantly increased \[\chi^2(4) = 20.101, p < 0.001; \text{Figure } 11F\] by all doses of Raclopride \[10 \mu g/\mu L: U = 383, z = 2.285, p = 0.022; 14 \mu g/\mu L: U = 454.5, z = 3.441, p = 0.001; 18 \mu g/\mu L: U = 422.5, z = 2.786, p = 0.005; 20 \mu g/\mu L: U = 482.5, z = 4.399, p < 0.001\]. Moreover, male agonistic behavior delivered was significantly reduced \[\chi^2(4) = 24.111, p < 0.001; \text{Figure } 11G\] by all doses of Raclopride \[10 \mu g/\mu L: U = 98, z = -3.788, p < 0.001; 14 \mu g/\mu L: U = 85, z = -4.186, p < 0.001; 18 \mu g/\mu L: U = 122, z = -3.423, p = 0.001;\]
20 µg/µL: \( U = 90, z = -3.958, p < .001 \). The frequency of male attacks delivered \([F (4, 113) = 2.157, p = 0.078]\) and open aggression \([F (4, 113) = 2.136, p = 0.081]\) also trended towards a decrease. Collectively, these results suggest that intrahippocampal Raclopride reduced many male-typical agonistic-related behaviors during the social interactions.
Figure 11. Agonistic behaviors for observer (OBS) male mice that received a single intrahippocampal microinfusion of either saline vehicle \((n = 24)\), or the dopamine (DA) D2-type receptor antagonist Raclopride at \(10 \mu g/\mu L \ (n = 23)\), \(14 \mu g/\mu L \ (n = 24)\), \(18 \mu g/\mu L \ (n = 24)\) or \(20 \mu g/\mu L \ (n = 23)\). Infusions were administered 10 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Intrahippocampal Raclopride effects on male total agonistic behaviors [A], dominance score [B], dominant behavior [C], boxing [D], submissive behavior [E], agonistic behavior received [F], and agonistic behavior delivered [G] are shown. Data are presented as mean + SEM. *\(p < 0.05\), **\(p < 0.01\), ***\(p < 0.001\), T = 0.05 < \(p < 0.1\), in comparison to saline infused control animals.
In addition to agonistic behaviors, other male social behaviors were influenced by Raclopride. That is, male social inactivity, the social behavior that involves the least amount of locomotion, was significantly reduced [F (4, 113) = 2.727, p = 0.033] by 10 µg/µL of Raclopride [t(45) = 2.672, p = 0.01]. Moreover, male social investigation was significantly reduced [F (4, 113) = 3.319, p = 0.013; Figure 12B] by the two lowest doses [10 µg/µL: t(45) = 2.273, p = 0.028; 14 µg/µL: t(46) = 3.393, p = 0.001], while the highest dose of Raclopride trended towards significance [t(45) = 1.737, p = 0.089]. This could be partially explained by the finding that male body investigation was significantly reduced [F (4, 113) = 3.005, p = 0.021; Figure 12F] by the two lowest doses of Raclopride [10 µg/µL: t(45) = 2.479, p = 0.017; 14 µg/µL: t(46) = 3.393, p = 0.001]. Furthermore, male anogenital investigation was significantly reduced [F (4, 113) = 4.51, p = 0.002; Figure 12H] by the two lowest doses, and the highest dose of Raclopride [10 µg/µL: t(45) = 2.214, p = 0.032; 14 µg/µL: t(46) = 4.202, p < .001; 20 µg/µL: t(45) = 2.967, p = 0.005]. In line with these findings, the frequency of males following the DEM also trended towards a reduction [F (4, 113) = 2.174, p = 0.076]. Thus, intrahippocampal Raclopride decreased social inactive and various social-investigatory behaviors for males.

The general reduction in total social behavior in females may be partly accounted for by the significant decrease in female social investigation [F (4, 113) = 7.618, p < .001; Figure 12A] by all doses of Raclopride [10 µg/µL: t(45) = 3.261, p = 0.002; 14 µg/µL: t(44) = 3.865, p < .001; 18 µg/µL: t(45) = 4.814, p < .001; 20 µg/µL: t(45) = 4.197, p < .001]. This could be partially explained by the finding that female body investigation was significantly reduced [F (4, 113) = 8.64, p < .001; Figure 12E] by all doses of Raclopride [10 µg/µL: t(45) = 3.338, p = 0.002; 14 µg/µL: t(44) = 3.924, p <
Moreover, female anogenital investigation was significantly reduced \([F (4, 113) = 7.911, p < .001; Figure 12G]\) by all doses of Raclopride \([10 \mu g/\mu L: t(45) = 3.317, p = 0.002; 14 \mu g/\mu L: t(44) = 3.155, p = 0.003; 18 \mu g/\mu L: t(45) = 5.115, p < .001; 20 \mu g/\mu L: t(45) = 3.998, p < .001]\). Following the DEM for females was also significantly reduced \([F (4, 113) = 4.774, p = 0.001; Figure 12I]\) by all doses of Raclopride \([10 \mu g/\mu L: t(45) = 2.08, p = 0.043; 14 \mu g/\mu L: t(44) = 2.615, p = 0.012; 18 \mu g/\mu L: t(45) = 4.152, p < .001; 20 \mu g/\mu L: t(45) = 2.844, p = .007]\). Therefore, drug treatment decreased many social-investigatory behaviors for female mice.

Importantly, female \([Figure 12C]\) and male \([Figure 12D]\) oronasal investigation durations were not affected by intrahippocampal Raclopride. This implies that the social learning blockade found in females was not due to changes in the time exposed to the socially carried food odor transferred by the DEM.
Figure 12. Social investigatory behaviors (in sec) for observer (OBS) female [A, C, E, G, I] and male [B, D, F, H, J] mice that received a single intrahippocampal microinfusion of either saline vehicle \((n = 23\) for females and \(n = 24\) for males), or the dopamine (DA) D2-type receptor antagonist Raclopride at 10 µg/µL \((n = 24\) for females and \(n = 23\) for males), 14 µg/µL \((n = 23\) for females and \(n = 24\) for males), 18 µg/µL \((n = 24\) for females and \(n = 24\) for males) or 20 µg/µL \((n = 24\) for females and \(n = 23\) for males). Infusions were administered 10 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Intrahippocampal Raclopride
effects on female and male social investigation [A, B], oronasal investigation [C, D], body investigation [E, F], anogenital investigation [G, H], and following the DEM [I, J] are shown. There were no significant effects of intrahippocampal Raclopride on either female [C] or male [D] oronasal investigation, or following the DEM for males [J]. Data are presented as mean + SEM. *p < 0.05, **p < 0.01, ***p < 0.001, T = 0.05 < p < 0.1, in comparison to saline infused control animals.

In addition to social-investigatory behaviors, intrahippocampal Raclopride influenced agonistic behaviors for females. Female total agonistic behaviors trended towards a decrease [F (4, 113) = 1.995, p = 0.1]. Notably, reduced dominant behavior and agonistic behavior delivered resulted in the female dominance score to be significantly reduced [F (4, 113) = 5.241, p = 0.001; Figure 13C] by all doses of Raclopride [10 µg/µL: t(45) = 3.726, p = 0.001; 14 µg/µL: t(44) = 2.028, p = 0.049; 18 µg/µL: t(45) = 3.403, p = 0.001; 20 µg/µL: t(45) = 2.752, p = 0.009]. This is likely explained by the finding that female agonistic behavior delivered was significantly reduced [F (4, 113) = 6.677, p < .001; Figure 13A] by all doses of Raclopride [10 µg/µL: t(45) = 2.88, p = 0.006; 14 µg/µL: t(44) = 2.437, p = 0.019; 18 µg/µL: t(45) = 4.616, p < .001; 20 µg/µL: t(45) = 3.981, p < .001]. Furthermore, female dominant behavior was significantly reduced [F (4, 113) = 4.278, p = 0.003; Figure 13B] at the lowest, and two highest doses of Raclopride, while 14 µg/µL only approached significance [10 µg/µL: t(45) = 2.395, p = 0.021; 14 µg/µL: t(44) = 1.842, p = 0.072; 18 µg/µL: t(45) = 3.789, p < .001; 20 µg/µL: t(45) = 3.408, p = .001]. In addition, female submissive behavior [F (4, 113) = 2.023, p = 0.096] and agonistic behavior received [F (4, 113) = 2.001, p = 0.099] trended towards an
increase. Thus, various agonistic-related behaviors were reduced by drug treatment in females.

Figure 13. Agonistic behaviors (in sec) for observer (OBS) female mice that received a single intrahippocampal microinfusion of either saline vehicle \((n = 23)\), or the dopamine (DA) D2-type receptor antagonist Raclopride at 10 µg/µL \((n = 24)\), 14 µg/µL \((n = 23)\), 18 µg/µL \((n = 24)\) or 20 µg/µL \((n = 24)\). Infusions were administered 10 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Intrahippocampal Raclopride effects on female agonistic behavior delivered [A], dominant behavior [B], and dominance score [C] are shown. Data are presented as mean + SEM. 

* \(p < 0.05\), ** \(p < 0.01\), *** \(p < 0.001\), \(T = 0.05 < p < 0.1\), in comparison to saline infused control animals.

Non-social behaviors were significantly increased in females \([F (4, 113) = 3.262, p = 0.014; \text{Figure 14A}]\) by all doses of Raclopride \([10 \, \mu g/\mu L: t(45) = -2.569, p = 0.014; 14 \, \mu g/\mu L: t(44) = -2.93, p = 0.005; 18 \, \mu g/\mu L: t(45) = -3.293, p = 0.002; 20 \, \mu g/\mu L: t(45) = -2.558, p = 0.014]\), and males \([F (4, 113) = 4.913, p = 0.001; \text{Figure 14B}]\) by all doses of Raclopride \([10 \, \mu g/\mu L: t(45) = -4.318, p < .001; 14 \, \mu g/\mu L: t(46) = -2.939, p = 0.005; 18 \, \mu g/\mu L: t(46) = -2.389, p = 0.021; 20 \, \mu g/\mu L: t(45) = -3.561, p = 0.001]\).
intra-hippocampal microinfusion of either saline vehicle \((n = 23\) for females and \(n = 24\) for males), or the dopamine (DA) D2-type receptor antagonist Raclopride at \(10 \, \mu g/\mu L\) \((n = 24\) for females and \(n = 23\) for males), \(14 \, \mu g/\mu L\) \((n = 23\) for females and \(n = 24\) for males), \(18 \, \mu g/\mu L\) \((n = 24\) for females and \(n = 24\) for males) or \(20 \, \mu g/\mu L\) \((n = 24\) for females and \(n = 23\) for males). Infusions were administered 10 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Data are presented as mean ± SEM. *\(p < 0.05\), **\(p < 0.01\), ***\(p < 0.001\), in comparison to saline infused control animals.

Female non-social non-locomotor behaviors were significantly increased [F (4, 113) = 4.911, \(p = 0.001\); Figure 15A] by all doses of Raclopride \(10 \, \mu g/\mu L\): \(t(45) = -3.731, p = 0.001\); \(14 \, \mu g/\mu L\): \(t(44) = -2.517, p = 0.016\); \(18 \, \mu g/\mu L\): \(t(45) = -3.726, p = 0.001\); \(20 \, \mu g/\mu L\): \(t(45) = -3.127, p = 0.003\)]. This effect could be partly explained by the finding that female solitary inactivity was significantly increased [F (4, 113) = 10.191, \(p < .001\); Figure 15C] by all doses of Raclopride \(10 \, \mu g/\mu L\): \(t(45) = -5.243, p < .001\); \(14 \, \mu g/\mu L\): \(t(44) = -4.469, p < .001\); \(18 \, \mu g/\mu L\): \(t(45) = -6.065, p < .001\); \(20 \, \mu g/\mu L\): \(t(45) = -6.069, p < .001\)]. In addition, female self-grooming trended towards a reduction [F (4, 113) = 3.975, \(p = 0.001\); Figure 15B].
Thus, the overall decrease in female total activity was paralleled by an increase in non-social non-locomotor behaviors.

Similarly, male non-social non-locomotor behaviors were significantly increased [F (4, 113) = 2.414, p = 0.05; Figure 15B] by the three highest doses of Raclopride [14 µg/µL: t(46) = -2.023, p = 0.049; 18 µg/µL: t(46) = -2.269, p = 0.028; 20 µg/µL: t(45) = - 2.992, p = 0.004]. These results could be partly explained by the finding that male solitary inactivity was significantly increased [χ²(4) = 28.755, p < .001; Figure 15D] by all doses of Raclopride [10 µg/µL: U = 462, z = 3.958, p < .001; 14 µg/µL: U = 472, z = 3.794, p < .001; 18 µg/µL: U = 457, z = 3.485, p < .001; 20 µg/µL: U = 505, z = 4.873, p < .001]. Moreover, male self-grooming was significantly reduced [F (4, 113) = 3.06, p = 0.02; Figure 15F] by all doses of Raclopride [10 µg/µL: t(45) = 2.48, p = 0.017; 14 µg/µL: t(46) = 2.215, p = 0.032; 18 µg/µL: t(46) = 2.366, p = 0.022; 20 µg/µL: t(45) = 2.474, p = 0.017]. Thus, drug treatment resulted in an increase in the time spent engaging in non-social non-locomotor behaviors in males.
Figure 15. Non-social non-locomotor behaviors (in sec) for observer (OBS) female [A, C, E] and male [B, D, F] mice that received a single intrahippocampal microinfusion of either saline vehicle (n = 23 for females and n = 24 for males), or the dopamine (DA) D2-type receptor antagonist Raclopride at 10 µg/µL (n = 24 for females and n = 23 for males), 14 µg/µL (n = 24 for females and n = 23 for males), 18 µg/µL (n = 24 for females and n = 24 for males) or 20 µg/µL (n = 24 for females and n = 23 for males). Infusions were administered 10 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Intrahippocampal Raclopride effects on female and male non-social non-locomotor behaviors [A, B], solitary inactivity [C, D], and self-grooming [E, F] are shown. There were no significant effects of intrahippocampal Raclopride on female self-grooming [E]. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, in comparison to saline infused control animals.
No other significances were found. Notably, intrahippocampal Raclopride did not produce any abnormal stereotypies.

No estrous cycle effects were found on the behaviors during the social interactions. This was likely due to the small number of females in each phase, per treatment group (Table 5).
**Table 3.** Summarized intrahippocampal Raclopride effects on grouped behaviors during the 30min social interactions for the STFP experiment.

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
<th>Saline treated sex difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Activity</td>
<td>↓</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total Social Behavior</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
</tr>
<tr>
<td>Agonistic Behavior Delivered</td>
<td>↓</td>
<td>↓</td>
<td>[F] Females &lt; Males</td>
</tr>
<tr>
<td>Agonistic Behavior Received</td>
<td>↑</td>
<td>↑</td>
<td>Females &gt; Males</td>
</tr>
<tr>
<td>Total Agonistic Behaviors</td>
<td>↓</td>
<td>[F]↓</td>
<td>Females &lt; Males</td>
</tr>
<tr>
<td>Dominance Score</td>
<td>↓</td>
<td>↓</td>
<td>Females &lt; Males</td>
</tr>
<tr>
<td>Social Investigation</td>
<td>↓</td>
<td>↓</td>
<td>Females &gt; Males</td>
</tr>
<tr>
<td>Non-social Behaviors</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Non-social Locomotor Behaviors</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Non-social Non-locomotor Behaviors</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
</tbody>
</table>

— no significant drug effect; ↓ significant decrease; ↑ significant increase; ↓ trend towards decrease; ↑ trend towards increase. Unless otherwise specified with an “F” (which indicates frequency effect), all effects are on duration.
Table 4. Summarized intrahippocampal Raclopride effects on single behaviors during the 30min social interactions for the STFP experiment.

<table>
<thead>
<tr>
<th>Social Behaviors</th>
<th>Females</th>
<th>Males</th>
<th>Saline treated sex difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Following the DEM</td>
<td>↓ [F]</td>
<td>↓ [F]</td>
<td>[F] Females &gt; Males</td>
</tr>
<tr>
<td>Dominant Behavior</td>
<td>↓</td>
<td>↓</td>
<td>[T] Females &lt; Males</td>
</tr>
<tr>
<td>Attacks Delivered</td>
<td>—</td>
<td>[F]</td>
<td>[F] Females &lt; Males</td>
</tr>
<tr>
<td>Boxing</td>
<td>—</td>
<td>↓</td>
<td>Females &lt; Males</td>
</tr>
<tr>
<td>Open Aggression</td>
<td>— [F]</td>
<td>↓</td>
<td>Females &lt; Males</td>
</tr>
<tr>
<td>Avoidance of DEM</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Submissive Behavior</td>
<td>↑</td>
<td>↑</td>
<td>Females &gt; Males</td>
</tr>
<tr>
<td>Attacks Received</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Defensive Upright Posturing</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Social Inactivity</td>
<td>—</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Oronasal Investigation</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Body Investigation</td>
<td>↓</td>
<td>↓</td>
<td>Females &gt; Males</td>
</tr>
<tr>
<td>Anogenital investigation</td>
<td>↓</td>
<td>↓</td>
<td>[T] Females &gt; Males</td>
</tr>
<tr>
<td>Stretched Approach</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Approaching and/or Attending to the DEM</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Non-social Behaviors</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Horizontal Exploration</td>
<td>—</td>
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<td></td>
</tr>
<tr>
<td>Vertical Exploration</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Digging</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Abnormal Stereotypies</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Solitary Inactivity</td>
<td>↑</td>
<td>↑</td>
<td>[F][T] Females &gt; Males</td>
</tr>
<tr>
<td>Self-Grooming</td>
<td>↓</td>
<td>↓</td>
<td></td>
</tr>
</tbody>
</table>

— no significant drug effect; ↓ significant decrease; ↑ significant increase; ↓ trend towards decrease; ↑ trend towards increase; “T” indicates statistical trend (.05 < p < 0.1).

Unless otherwise specified with an “F” (which indicates frequency effect), all effects are on duration.
Baseline Sex Differences Among Saline Infused Mice During the Social Interactions in the STFP

Males were more agonistic than females, as they boxed more, delivered more attacks, and had a higher dominance score (Figure 16; Table 3 and 4). On the other hand, social investigation type behaviors were greater for female mice, as they engaged in more body and anogenital investigation than males (Figure 16; Table 3 and 4).

Males had significantly greater \([t(45) = -2.38, p = 0.022; \text{Figure 16A}]\) total agonistic behaviors than females. This could be partly explained by the finding that males spent significantly more time boxing \([t(45) = -3.163, p = 0.003; \text{Figure 16A}]\), engaged in open aggression \([t(45) = -2.5, p = 0.016; \text{Figure 16A}]\), and trended towards spending more time engaging in dominant behavior \([t(45) = -1.846, p = 0.072]\) than females. Moreover, the frequency of agonistic behavior delivered was significantly greater \([t(45) = -3.302, p = 0.002; \text{Figure 16B}]\) for males than females. Specifically, the frequency of attacks delivered was significantly greater \([U = 414, z = 3.833, p < .001; \text{Figure 16B}]\) for males. On the other hand, females spent significantly more time engaging in submissive behavior \([U = 77.5, z = -4.348, p < .001; \text{Figure 16A}]\), and received significantly more agonistic behaviors \([U = 83, z = -4.114, p < .001; \text{Figure 16A}]\) than males.

Correspondingly, males had a significantly higher \([t(45) = -3.369, p = 0.002; \text{Figure 16A}]\) dominance score than females. Hence, males displayed more agonistic-related behaviors than females.

Females spent significantly \([t(45) = 2.772, p = 0.008; \text{Figure 16A}]\) longer performing social investigation than males. This could be partially accounted for by the finding that females spent significantly more time engaging in body investigation \([t(45) = \ldots\]
3.203, \( p = 0.002 \); Figure 16A], and trended towards spending more time engaging in anogenital investigation \([t(45) = 1.957, \ p = 0.057]\) than males. Moreover, the frequency of following the DEM was significantly greater \([t(45) = 2.518, \ p = 0.015; \text{Figure 16B}]\) for females than males. There was also a trend towards females more frequently engaging in solitary inactivity \([t(45) = 1.772, \ p = 0.083]\). Hence, saline treated females displayed more social-investigatory type behaviors than saline treated males.
Figure 16. Baseline sex differences for observer (OBS) mice that received a single intrahippocampal microinfusion of saline vehicle \( (n = 23 \text{ for females } \text{[black bars] and } n = 24 \text{ for males [white bars]}) \). Infusions were administered 10 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Sex differences are shown for various grouped and single behaviors for duration [A] and frequency [B] data.

“Ago Rec” stands for agonistic behavior received; “Tot Ago” stands for total agonistic behaviors; “Dom Score” stands for dominance score; “Soc Inves” stands for social investigation; “Dominant Behav” stands for dominant behavior; “Open Aggr” stands for open aggression; “Submissive” stands for submissive behavior; “Body Inves” stands for body investigation; “Anogenital Inves” stands for anogenital investigation; “Ago Del” stands for agonistic behavior delivered; “Follow” stands for following the DEM; “Attacks Del” stands for attacks delivered; “Sol Inactivity” stands for solitary inactivity. Data are presented as mean + SEM. \( *p < 0.05, \ **p < 0.01, \ ***p < 0.001, \ T = 0.05 < p < 0.1, \text{ saline infused females versus saline infused males.} \)
Olfaction Control Study: Effects of Intrahippocampal Raclopride on Female Olfactory Discrimination

The results of the NDI scores suggest that female mice infused with Raclopride at 18 µg/µL and 20 µg/µL, the two doses that blocked the STFP, could discriminate between the two foods used during the STFP choice test (Figure 17A). Hence, the social learning impairment found in this study due to intrahippocampal Raclopride cannot be directly explained by changes in olfactory discrimination.

The RM ANOVA run on the NDI scores for the average of the four habituation sessions and test session revealed a significant main effect of time [F(1, 32) = 51.457, p < .001], but there was no significant time x treatment interaction, and no main effect of treatment. Notably, planned paired samples t-tests conducted on the NDI scores of the average of the four habituation sessions versus test session further revealed that the NDI scores during test were significantly higher than during habituation for all groups tested [saline: t(11) = -5.232, p < .001; Raclopride at 18 µg/µL: t(10) = -2.592, p = .027; Raclopride at 20 µg/µL: t(11) = -5.458, p < .001; Figure 17A]. Thus, females infused with the two highest doses of Raclopride (that blocked the STFP) could discriminate between the two foods used during the choice test in the social learning study.

The RM ANOVA conducted on the total investigation data revealed no significant time x treatment interaction, and no main effect of treatment, but there was a significant main effect of time [F(4, 128) = 8.793, p < .001]. Independent samples t-tests further revealed that during the first habituation session, saline infused animals had significantly higher investigation times than mice infused with Raclopride at 18 µg/µL [t(21) = 2.619, p = .016; Figure 17B] and 20 µg/µL [t(22) = 3.516, p = .002; Figure
Mice infused with Raclopride at 20 µg/µL also trended towards a longer investigation time than saline infused animals during the third habituation session \[t(22) = -1.985, p = .06\]. Since olfactory discrimination was unaffected by drug treatment for all groups, these treatment effects on total investigation seem to be negligible.

There were no effects of the estrous cycle for the NDI scores or total investigation data from habituation to test (or at separate phases), likely due to the small number of mice per phase, for each treatment group (Table 5).

**Figure 17.** Intrahippocampal Raclopride olfactory discrimination task (ODT). Female mice received a single intrahippocampal microinfusion of either saline vehicle (\(n = 12\)), or the dopamine (DA) D2-type receptor antagonist Raclopride at 18 µg/µL (\(n = 11\)) or 20 µg/µL (\(n = 12\)). Infusions were administered 10 minutes prior to four habituation phases and one test phase. During the habituation phases (black bars) mice were exposed to two jars (covered with wire mesh to prevent consumption) containing the same food odors [i.e., two jars containing 1% ground cinnamon (CIN), or two jars containing 2% ground cocoa (COC)]. For the test session (gray bars) one of the formerly presented familiar diets was exchanged for a novel diet (i.e., mice were exposed to both CIN and COC). Each phase was five minutes in duration, and was administered consecutively in the home cage. For graph A: intrahippocampal Raclopride effects on female percent of novel diet investigation (NDI) scores ((time investigating novel diet / total...
time investigating both diets] x100); data are presented as mean ± SEM; *p < 0.05, ***p < 0.001, averaged NDI scores of the four habituation phases versus test NDI scores. For graph B: intrahippocampal Raclopride effects on female total investigation [time investigating familiar diet + time investigating novel diet]; data are presented as mean ± SEM; *p < 0.05, **p < 0.01, T = 0.05 < p < 0.1, in comparison to saline infused control animals.

**Table 5.** Sample sizes (n’s) for each estrous phase, per treatment group, for the social transmission of food preferences (STFP) and olfactory discrimination task (ODT) intrahippocampal Raclopride experiments.

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<td><strong>Olfactory discrimination task (ODT)</strong></td>
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<td>Raclopride at 20 µg/µL</td>
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DISCUSSION

*Effects of Intrahippocampal Raclopride on Social Learning and Food Intake.*

This study found that blocking dorsal hippocampal DA D2-type receptors with the DA D2-type receptor antagonist Raclopride impaired social learning in female, but not male mice (Figure 7 and 8), without affecting feeding behavior (Figure 9). A comprehensive ethological assessment of the 30 minute social interactions revealed that drug treatment had no effects on the time spent engaging in oronasal investigation (for either sex; Figure 12C and D), indicating that the STFP impairment could not be directly explained by a reduced exposure to the socially relevant food odor transmitted by the DEM. Lastly, an olfactory discrimination task showed that female mice infused with the two doses of Raclopride, which blocked social learning, could still distinguish between COC and CIN (Figure 17A).

The effects of intrahippocampal Raclopride on socially acquired food preferences found here are consistent with those of previous work that finds a role for other mesocorticolimbic brain regions in individually acquired food preferences. That is, Raclopride infused into the amygdala (Malkusz et al., 2012) or medial prefrontal cortex (mPFC; Malkusz et al., 2015) impairs the acquisition of a fructose-conditioned flavor preference, while infusions into the NAc shell (Bernal et al., 2008) or medial orbital frontal cortex (MOFC; Malkusz et al., 2015) regulates its expression in rats. Hence, our results extend those findings also to hippocampal DA D2-type receptor activity in the mediation of socially acquired food preferences.
Hippocampal DA D2-type receptors have been implicated in non-social types of learning, including spatial learning (Wilkerson & Levin, 1999), expression of conditioned place preference (Haghparast et al., 2013), and state-dependent learning (Zarrindast et al., 2010). Thus, both non-social and social forms of learning involving the hippocampus may be mediated via similar dopaminergic actions.

The social learning impairment found in this study may have been due to effects on learning and memory processes. The VTA-to-hippocampal dopaminergic loop is involved in regulating novel information signaling associated with goal directed behavior (Lisman and Grace, 2005). It is possible that female mice in the STFP encountered a behaviorally salient stimulus, in this case, a novel food odor found on the breath of the DEM, which resulted in mild stimulation of the VTA inputs to the hippocampus (Schultz 1999; Lisman and Grace 2005). This signal may have resulted in (mostly) low frequency tonic firing from the VTA to the hippocampus. It has been established that tonic firing of DA neurons ascending to the hippocampus results in constant (but low levels) of extracellular DA, which primarily (if not exclusively) activates high affinity DA D2-type receptors that actively bind extracellular DA with higher affinity than other DA receptors (Edelmann & Lessmann, 2018; Hsu, 1996; van Wieringen et al., 2013). Thus, socially learning about a food preference for female mice may depend on tonic firing of DA neurons, where the low levels of extracellular DA in the hippocampus may activate DA D2-type receptors (which processes socially-derived novel odors), and contribute to hippocampus dependent engram formation and/or stabilization (Edelmann & Lessmann, 2018; Lisman & Grace, 2005). See General Discussion (Chapter 5) for more details on potential mechanisms of action.
The effects of intrahippocampal Raclopride on social learning and food intake found in this study are opposite to the results with systemic treatments obtained by Choleris et al., 2011, which found that females injected with Raclopride were not impaired in social learning, although the total amount of food consumed was reduced (Choleris et al., 2011). Here, we found that female social learning was impaired, without any effects on food intake. The difference in results between these two studies may be explained by the fact that the Choleris et al., 2011 study used systemic treatments of Raclopride, whereas here we infused Raclopride directly into the dorsal hippocampus.

Dorsal hippocampal DA D2-type receptors regulated social learning (Figure 7 and 8), but not feeding behavior (Figure 9), in the STFP. These results are in agreement with our previous intrahippocampal results (Matta et al., 2017) showing no effects of SCH23390 on feeding behavior in the STFP. Hence, neither D1- nor D2-type DA receptors in the dorsal hippocampus mediate food intake in the STFP. This indicates that the food intake results obtained by Choleris et al., 2011 using Raclopride injections are mediated by another brain region (e.g., hypothalamus; Pawłowski et al., 2001; Stuber & Wise, 2016).

Analyses on the social interactions (where social learning occurs) showed that intrahippocampal Raclopride did not influence oronasal investigation durations for any dose, for either sex (Figure 12C and D). This implies that the social learning blockade found in female mice were not secondary to changes in the exposure to the socially transmitted food odor found on the breath of the DEM. Interestingly, these results are different than the results of the systemic experiment conducted by Choleris et al., 2011, which found that Raclopride injections reduced oronasal investigation durations for all
doses tested (Choleris et al., 2011). These differences may be explained the fact that Choleris et al., 2011 used systemic treatments, whereas this study employed direct intrahippocampal microinfusions. The results of Choleris et al., 2011 may have been due to actions of another brain site (or multiple sites), while this study was only limited to the dorsal hippocampus. Indeed, this assumption is supported by the fact that the results of this study are in line with the intrahippocampal results of Matta et al., 2017, which also found no effects of SCH23390 on oronasal investigation durations for any dose, for either sex. Thus, antagonizing either dorsal hippocampal D1- or D2-type DA receptors does not affect female or male oronasal investigations.

An olfaction control study revealed that female mice infused with the two highest doses of Raclopride, that also impaired social learning, could still discriminate between the two flavored diets used in the STFP (Figure 17A). Hence, the social learning blockade found in the STFP was not secondary to changes in olfactory discrimination. Such findings are in line with the systemic study of Choleris et al., 2011, and the intrahippocampal results of Matta et al., 2017, which found that mice treated with the highest dose of SCH23390 that blocked social learning, could still discriminate between CIN and COC (the two foods used during the choice test). Hence, blocking either dorsal hippocampal D1- or D2-type DA receptors does not affect diet odor discrimination.

The analyses on the CIN (Figure 7G and I) and DEM (Figure 8G and I) preference scores showed that female social learning was impaired at the two highest doses of Raclopride, whereas male social learning was unaffected by all doses (Figure 7 and 8). The sex difference in social learning found here is surprising given that our previous intrahippocampal study actually finds somewhat different results. That is, while
Matta et al., 2017 found that blocking DA D1-type receptors impaired social learning in both sexes, male social learning was impaired by the lowest and two highest doses of drug treatment, whereas female social learning was only impaired by the highest dose. Hence, male mice in the Matta et al., 2017 study were actually more sensitive to the drug manipulation, while female mice were less affected. Given our previous (Matta et al., 2017) and current hippocampal results, we conclude that female social learning is mediated by both hippocampal DA D1- and D2-type receptors, whereas male social learning is only mediated by hippocampal DA D1-type receptors.

The finding that Raclopride affected female but not male social learning suggests an involvement of gonadal steroids (estrogens/progesterone and testosterone) in the dopaminergic regulation of social learning through hippocampal DA D2-type receptors. Indeed, our lab finds clear effects of estrogens on the STFP in mice (Clipperton et al., 2008; Ervin et al., 2015a). Additionally, the estrous cycle can mediate both the STFP (Choleris et al., 2011) and mesolimbic DA activity (Thompson and Moss, 1997). Consistently, this study did find effects of the estrous cycle, and interactions of the estrous cycle with Raclopride treatment on food preferences. However, further mean comparisons for each treatment group broken down by estrous phase did not yield any significances. This was likely due to the small number of females for each estrous phase, per treatment group (Table 5). Given that male social learning was unaffected by drug treatment in this study, it is possible that testosterone was having neuroprotective effects on males (see Siddiqui et al., 2016). There is evidence from studies conducted on Alzheimer's disease, and mild cognitive impairment (e.g., dementia) to support these assumptions, however, testosterone may not always protect against pharmacological
manipulations of the DA system (Bialek et al., 2004). Clearly, further research assessing the involvement of gonadal hormones in the dopaminergic mediation of social learning is needed.

In summary, we found that antagonizing hippocampal DA D2-type receptors blocks the STFP in female, but not male mice. This sex-dependent social learning impairment could not be explained by any secondary effects of drug treatment on feeding behavior, oronasal investigation, or olfactory discrimination. Hence, hippocampal DA D2-type receptors may be only regulating female social learning. Further studies examining the role of hippocampal DA D2-type receptors and possible interactions with gonadal steroids would be valuable. Collectively, this study may have implications for the treatment of various neurological social disorders associated with altered DA transmission that are more prevalent in females, such as anxiety disorders (Maeng & Milad, 2015; Martin et al., 2009) and depression (Marcus et al., 2005; Nolen-Hoeksema, 1987; Weissman et al., 1996).

Effects of Intrahippocampal Raclopride on Behavior during the Social Interactions.

We found that total activity was reduced for female (Figure 10A) but not male mice (Figure 10B), although both sexes showed a parallel increase in time spent engaging in solitary inactivity (Figure 15C and D). Both sexes showed a reduction in total social behavior (Figure 10C and D), whereby males and females both showed a reduction in social investigatory (Figure 12) and agonistic behaviors (Figure 11 and 13). However,
reductions in agonistic-type behaviors were more pronounced in males (Figure 11), while reductions in social investigatory-type behaviors were more pronounced in females (Figure 12). There was a reduction in the dominance score for both sexes (Figure 11B and Figure 13C). Importantly, oronasal investigations were unaffected by intrahippocampal Raclopride (Figure 12C and D), which implies that the social learning impairment could not be explained by a decreased exposure to the socially transferred diet odor.

Drug treatment reduced total social behavior for both males (Figure 10D) and females (Figure 10C). However, there were sex-dependent effects of drug treatment on agonistic and investigatory-related behaviors. For male social behaviors, there was a decrease in total agonistic behaviors (Figure 11A), whereby agonistic behaviors delivered were reduced (Figure 11G), including dominant behavior (Figure 11C) and boxing (Figure 11D). In parallel with this, there was an increase in agonistic behaviors received (Figure 11F), including submissive behaviors (Figure 11E). This consequently resulted in a reduced dominance score for males (Figure 11B). There was also an overall reduction in male non-agonistic social investigation (Figure 12B), including body (Figure 12F) and anogenital (Figure 12H) investigation. Hence, while male agonistic behaviors and social investigatory behaviors were both affected by drug treatment, effects on agonistic behaviors were greater (as compared to effects on social investigatory behaviors), likely because males display more agonistic-type behaviors (as compared to social investigatory behaviors) to begin with (see results on baseline sex differences, Figure 16).

Female social behaviors were also affected by intrahippocampal Raclopride. Drug treatment reduced female total social investigation (Figure 12A), including body (Figure
12E) and anogenital (Figure 12G) investigation, as well as following the DEM (Figure 12I). Importantly, female (and even male) oronasal investigation (Figure 12C and D) was unaffected by drug treatment, which is not surprising given that this is a reciprocal behavior which the DEM animal initiates often. Interestingly, intrahippocampal Raclopride affected certain female agonistic behaviors (Figure 13). Females are generally less aggressive than males (Anton, 1969; Edwards, 1970; Jacobson-Pick et al., 2013; Miczek et al., 2001), and instead of engaging in male-typical overt aggressive behaviors (e.g., boxing), females will use a more non-attack agonistic approach, such as aggressive grooming and pushing down (Clipperton et al., 2008; Clipperton-Allen et al., 2010, 2011B; Grant and Mackintosh, 1963). In this study, female agonistic behavior delivered was reduced (Figure 13A), including dominant behavior (Figure 13B), which resulted in a reduced dominance score (Figure 13C). Hence, intrahippocampal Raclopride affected many female social behaviors, including mainly social investigatory behaviors, as well as specific agonistic behaviors.

DA D2-type receptors have been implicated in a number of social behaviors such as social play (Wang et al., 2016), maternal behavior (Zhu et al., 2011), aggressive behavior (Aguilar et al., 1994) and partner preference formation (Young et al., 2014B). Unfortunately, few studies have investigated the specific brain regions involved in the DA D2-type receptor mediation of social behaviors. One study found that DA D2 receptor levels increase in the mouse hippocampus following a social defeat (Montagud-Romero et al., 2016). Studies conducted on other mesocorticolimbic brain regions have found that non-aggressive mice show lower DA D2 receptor expression throughout the NAc, as compared to aggressive mice (Couppis et al., 2008). Additionally, wild
subordinate male rats have increased DA D2 receptor density in the NAc and dorsolateral caudate putamen as compared to dominant animals (Lucas et al., 2004). The results of this study are in line with the literature above, where now hippocampal DA D2-type receptors have been shown to mediate social behaviors during the social interactions in the STFP.

The dominance scores of both males (Figure 11B) and females (Figure 13C) infused with Raclopride were negative values, which implies that OBS mice of both sexes were the subordinate animals of the pair during the social interactions. These dorsal hippocampal results are consistent with the systemic results of Choleris et al., 2011 which found that Raclopride injections resulted in a reduced dominance score at all doses (Choleris et al., 2011). These results are also in line with the intrahippocampal results of Matta et al., 2017, which found that males and females infused with SCH23390 were more subordinate towards the DEM. Our results are also consistent with a study which found that administering a DA D2-type receptor antagonist to socially dominant mice (but not subordinate animals) attenuates social dominance, and leads to more stable social hierarchies (Yamaguchi et al., 2017). Hence, hippocampal DA D2-type receptors are also involved in social dominance-type behaviors. Interestingly, research has shown that social status can affect social learning. For example, as shown by Clipperton et al., 2008, OBS mice treated with an estrogen receptor (ER)-β selective agonist that were more submissive towards their DEM, received more agonistic behaviors and therefore had reduced dominance scores actually maintained a longer food preference for the DEM diet during the subsequent choice test in the STFP (Clipperton et al., 2008). Similarly, studies conducted on deer mice have shown that a subordinate OBS will show better social
learning from a DEM than a dominant OBS (Kavaliers et al., 2005). Indeed, a successful dominant DEM may be a more useful source of high quality food information than a less successful subordinate DEM conspecific. Hence, these studies highlight that social dynamics (in this case social hierarchies) can have effects on social learning. Thus, future studies investigating the dopaminergic system and possible modulatory effects of social status on socially acquired food preferences would be valuable.

This study found that blocking hippocampal DA D2-type receptors resulted in a reduction in total activity for female (Figure 10A), but not male (Figure 10B) mice. This overall decrease in female total activity was paralleled by an increase in time spent engaging in non-social non-locomotor behaviors (Figure 15A), including solitary inactivity (Figure 15C). Similarly, male non-social non-locomotor behaviors (i.e., solitary inactivity) were increased (Figure 15B and D), and in addition, male self-grooming was reduced (Figure 15F). Intrahippocampal Raclopride also led to an increase in non-social behaviors in both sexes (Figure 14). Given the role of DA in regulating locomotion (Boutrel, 2008), such reductions in activity may be expected with DA receptor antagonists (Choleris et al., 2011; Matta et al., 2017). Since female total activity levels were similar across all Raclopride doses (Figure 10A), and because Raclopride reduced total activity at all doses, even the doses that did not impair social learning, it is unlikely that the reduction in total activity could directly account for the social learning impairment found in this study. Additionally, we did not observe any abnormal stereotypic behaviors, which can be found at higher doses of drug treatment (Cooper and Al-Naser, 2006). Furthermore, oronasal investigation was unaffected by any dose, for either sex (Figure 12C and D), which suggests that the social learning impairment could
not be explained by a reduced exposure to the socially transferred diet odor. Intrahippocampal Raclopride also had no effects on feeding at any dose, for either sex (Figure 9). Hence, performing a full ethological analysis allowed us to tease apart effects on the STFP versus other effects.

The sex differences in various social behaviors found in this study suggest that the hippocampus is a structure that sex-dependently regulates social interactions through DA D2-type receptors. These results are similar to our previous hippocampal DA work, which found sex differences in social behaviors in response to DA D1-type receptor antagonism (Matta et al., 2017). The exact mechanisms of action underlying how gonadal hormones may be interacting with DA receptors to mediate sex-dependent effects on social behaviors is unclear. Further studies examining the interactions of gonadal hormones such as estrogens, progestins and androgens with the hippocampal DA system in the mediation of social behavior are therefore needed.

In conclusion, hippocampal DA D2-type receptors sex-dependently mediate social learning and social interactions, but not food intake in the STFP. Blocking hippocampal DA D2-type receptors affected both social and non-social behaviors in males and females. While there were effects of drug treatment on social investigatory and agonistic behaviors in both sexes, males showed a greater reduction in agonistic-type behaviors, whereas females showed a greater reduction in social investigatory-type behaviors. This study warrants further research on the hippocampal DA system in the regulation of social behaviors.
CHAPTER 3: Examination of Dorsal Hippocampal Dopamine
Release in Association with Social Learning in Male and Female
Mice
INTRODUCTION

Social learning is “learning that is influenced by observation of, or interaction with, another animal (typically a conspecific) or its products” (Box, 1984; Galef, 1988a; Heyes, 1994). This type of learning can directly facilitate many animal behaviors relating to survival and reproduction, which makes it a biologically adaptive type of learning for various social species (Hoppitt and Laland, 2013). Most research conducted on the neurobiology of learning and memory has focused on individual learning processes. In contrast, few studies have investigated the neurotransmitters and brain regions underlying social learning (reviewed in Matta et al., 2016). Indeed, little is known about the ‘social brain’.

The social transmission of food preferences (STFP) is a robust test of social learning in rats and mice (Galef et al., 1984). This involves a naïve observer (OBS) animal socially interacting with a familiar demonstrator (DEM) animal of the same sex that has just consumed a novel flavored diet. Later, the OBS is given a test between the diet the DEM ate, and an alternative novel food. If the OBS acquired a socially learned food preference, they will eat more of the DEM diet (Galef et al., 1984).

Socially learned food preferences are mediated by carbon disulfide (CS₂; Galef et al., 1988), a metabolic by-product of digestion found on the exhaled breath of the DEM. The guanylyl cyclase D (GC-D) sensory neurons located in the posterior region of the olfactory bulbs respond to the socially relevant CS₂ signal (Arakawa et al., 2013; Munger et al., 2010). The STFP therefore requires contact through oronasal investigation (sniffing mouth and face area) in both rats (Galef and Stein, 1985) and mice (Valsecchi and Galef, 1989).
Neurotransmitters and neuromodulators such as oxytocin/vasopressin, opioids, cholinergic, and glutamatergic systems are involved in social learning in the STFP (reviewed in Choleris et al., 2009; Ervin et al., 2015a; Matta et al., 2016). Studies have also found a role for the catecholamine dopamine (DA). For example, mice lacking the DA transporter (DAT) do not develop a socially acquired food preference (Wong et al., 2012), or (paradoxically) prefer to eat the non-demonstrated diet (Rodriguiz et al., 2004). Additionally, a study conducted by our lab using systemic treatments found a role for DA D1-type receptors (D1, D5) in social learning, and DA D2-type receptors (D2, D3, D4) in feeding behavior in the STFP in mice (Choleris et al., 2011).

Brain regions including the basal forebrain and orbitofrontal, frontal and piriform cortices are involved in the regulation of the STFP (reviewed in Choleris et al., 2009; Ervin et al., 2015a; Matta et al., 2016). In addition, the hippocampus has been found to play a strong role in the early stages of socially learned food preferences. For example, lesions to the hippocampus impair the acquisition of the STFP (Alvarez et al., 2001, 2002; Bunsey and Eichenbaum, 1995; Clark et al., 2002; Winocur, 1990; Winocur and Moskovitch, 1999; Winocur et al., 2001). Some studies (Berger-Sweeney et al., 2000; Carballo-Marquez et al., 2009B; Gold et al., 2011; Matta et al., 2017) have further investigated the hippocampal neurotransmitters of action mediating the STFP.

Hippocampal DA plays a role in various different types of learning including object recognition (Yang et al., 2017), spatial learning (Kempadoo et al., 2016), and novelty detection (Lisman and Grace, 2005), presumably by strengthening the encoding of memories based on the salience of stimuli (Russo and Nestler, 2013). Indeed, hippocampal dependent memories triggered by novel stimuli are established and
stabilized by the VTA-hippocampal dopaminergic loop (Lisman and Grace, 2005). In our previous pharmacological studies, we have found that infusing a DA D1-type receptor antagonist into the dorsal hippocampus impairs social learning in the STFP in both males and females (Matta et al., 2017). Additionally, we have found that infusing a DA D2-type receptor antagonist into the dorsal hippocampus impairs social learning in female, but not male mice (see Chapter 2). Thus, DA receptors in the dorsal hippocampus mediate social learning in a sex-dependent manner.

DA in the hippocampus is mostly released via axon terminals that originate from the ventral tegmental area (VTA; Frey et al., 1990; McNamara et al., 2014; Wise, 2004). Hence, hippocampal DA release may mediate the sex-dependent effects on social learning, however, this has yet to be investigated. Indeed, whether socially learned food preferences involve changes in DA levels in the hippocampus without pharmacological manipulations is unknown.

The purpose of this study was to examine dorsal hippocampal DA release in association with social learning in the STFP in mice. In addition, in order to tease apart the various components of the STFP, we also investigated hippocampal DA release in association with novel diet odor investigation, a social interaction that did not involve social learning, investigation of a demonstrated (socially learned) diet odor, and food intake during the choice test. We used both male and female mice in this study since there are sex differences in the dopaminergic mediation of social learning in the STFP (Matta et al., 2017, see also chapter 2). We also performed an ethological analysis (Clipperton et al., 2008) throughout to examine possible effects of each exposure/manipulation on many social and nonsocial behaviors. Additionally, the estrous
cycle was monitored since estrogens are highly involved in the STFP in mice (Clipperton et al., 2008; Ervin et al., 2015a), and estrogens/progesterone interact with mesolimbic DA (Thompson and Moss, 1997).

**MATERIALS AND METHODS**

*Animals*

Young adult (2-3 month old) male and female CD-1 mice (*Mus musculus*) were used. Upon arrival from Charles River (St Constant, QC, Canada) mice were housed in same-sex trios in clear polyethylene cages (26 x 16 x 12 cm³) that had *ad libitum* access to rodent chow (Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Teklad, Madison, WI) and tap water, as well as environmental enrichment (corncob bedding, nest material and plastic houses). Mice were given at least a 1 week acclimatization period to the colony room before starting any surgeries. Seven days after surgery, mice were triple housed for at least 3 days (same housing parameters as above) prior to behavioral testing. All OBS mice were experimentally naïve, while DEM and stimulus (STIM) mice were reused (11–12 times). The colony room (21 ± 1°C, humidity of 40 – 50%) lights went off at 0800 h, and mice were maintained on a reversed 12:12 h light/dark cycle. All procedures followed the Canadian Council on Animal Care guidelines, and approved by the University of Guelph Institutional Animal Care and Use Committee.

*General Surgical Procedures*

Similar to Matta et al., 2017, OBS mice underwent a unilateral hippocampal cannulation surgery, but remained gonadally intact to investigate possible interactions of gonadal hormones and DA release in relation to the STFP (Choleris et al, 2011; Clipperton et al, 2008; Matta et al, 2017; Sanchez-Andrade et al, 2005). On the other
hand, all DEM and STIM mice were gonadectomized to eliminate possible effects of the DEM’s and STIM’s gonadal hormones on the OBS.

All surgical procedures involved a subcutaneous (s.c.) injection of the anti-inflammatory drug carprofen (50mg/kg; Rimadyl, Pfizer Canada Inc, Kirkland, QC, Canada) 30 minutes before mice were initially anaesthetized via isoflurane (Benson Medical Industries, Markham, ON) at 5%, then maintained at 1.5% for the remainder of the surgery. Before incision, surgical regions were shaved and disinfected/cleaned with Germi-Stat soap, alcohol and antiseptic tincture. A local anaesthetic comprised of 0.67% lidocaine (Alveda Pharmaceuticals, Toronto, ON, Canada) and 0.17% bupivacaine (Hospira, Inc., Montreal, QC, Canada) was applied (2 to 3 drops) to all incision sites. Closing sites of incision (gonadectomies) involved using 2-3 surgical staples (MikRon Autoclip; 9mm; MikRon Precision Inc., Gardena, CA). Mice were rehydrated via 0.5mL intraperitoneal (i.p.) injection of saline solution (0.9% NaCl) following all surgical procedures. Animals were allowed a recovery period of at least 1 week in clean cages (singly housed, in same housing conditions as previously stated above), before being triple housed for at least 3 days to become familiar with other same-sex mice prior to behavioral testing (Matta et al., 2017).

**Ovariectomy Surgery**

The fur on the lower back of anesthetized DEM and STIM mice was shaved off before making a 1cm midline dorsal incision on the skin. Each ovary was pulled out of a small incision made on the back muscles above the ovaries, the fallopian tubes were clamped, each ovary was removed, and the dorsal skin incision was stapled shut. See Clipperton-Allen et al, 2011A for detailed description of this surgery.
**Castration Surgery**

The fur on the scrotum of anesthetized DEM and STIM mice was shaved off before making a 1cm ventromedial scrotal incision. Each testicle was pulled out of a small incision made on both sides of the lateral walls of the tunica, the spermatic cords were ligated via hot hemostatic clamps, and each testicle was removed. Spermatic cords were tucked back into the tunica, and the scrotum was stapled shut (as in Matta et al., 2017).

**Cannulation Surgery**

Similar to the surgery described in detail in Phan et al., 2015, OBS mice were anesthetized and placed into atraumatic ear bars attached to a stereotaxic frame (David Kopf instruments, CA). The skin on the skull was removed and a unilateral guide cannula (AG-1.3, Eicom Corporation, San Diego, CA, USA) was implanted (one per mouse, counterbalanced per hemisphere) aiming for the anterior dorsal hippocampus (Paxinos and Franklin, 2001). Brain coordinates were calculated from Bregma (flat skull; 1.7mm posterior; 1.5mm lateral; 1.3mm ventral). Dental cement (Central Dental Ltd, Scarborough, ON, Canada) was used to anchor the guide cannula to three jeweller’s screws (1.6mm; Plastics One, HRS Scientific, Anjou, QC, Canada), which were fixed to the skull. A dummy cannula (AD-1.3, Eicom Corporation, San Diego, CA, USA) was inserted into the guide cannula to prevent blockage, and a cap nut (AC-5, Eicom Corporation, San Diego, CA, USA) was used to fasten the dummy cannula to the guide cannula. When inserted, the microdialysis probe membrane protruded 2mm past the tip of the guide cannula. The final ventral coordinate of the microdialysis probe was therefore 3.3mm below the skull surface.
**Materials**

Steel cage dividers (25.8 x 2 x 10.8 cm$^3$; see Matta et al., 2017 for details) were used during the three-day familiarization period to separate OBS mice from their same-sex DEM and STIM. The home cage was divided lengthwise into two separate compartments, and there were perforations throughout the dividers to allow for olfactory exposure. This was done to ensure that DEM and STIM mice do not chew off their OBS’s cannula. On the morning of testing, DEM and STIM mice were placed into clean polyethylene cages (26 x 16 x 12 cm$^3$) that contained glass jars (5cm H, 7.5cm W; Dyets, Bethlehem, PA) filled with powdered food. These feeding jars were equipped with stainless steel lids that had a hole (2.5cm diameter) to allow DEM and STIM mice access to the food. An additional perforated stainless steel ring was placed over the powdered food inside the jar to minimize spillage. All odor and social exposures were recorded from above (under infrared light) using a JVC Everio camcorder (Mississauga, ON, Canada). The choice test consisted of placing flavoured food into smaller porcelain jars (8cm diameter x 4cm H; ON, Canada), which sat inside larger porcelain jars (11.5cm diameter x 4cm H; ON, Canada) to catch any spillage. A scale that was precise to 0.01g (Sartorius Analytical Balance, Sartorius Inc., United Kingdom) was used to weigh the DEM/STIM feeding jars and OBS choice test jars. Plastic microdialysis chambers (35 x 17 x 47 cm$^3$) had a square cut out on the back wall (17.1cm H, 11cm W) to allow the counter-balanced lever arm (SMCLA, 9cm; Instech Laboratories, Plymouth Meeting, PA, USA) to move freely. Flavored food, STIM and DEM mice were placed into clear Plexiglas cylinders (7cm diameter x 32 cm H; detailed description in Choleris et al., 2006) for all exposures; cylinders were perforated on the bottom to allow for olfactory
cues to be emitted. To eliminate odor cues throughout the experiment, all equipment used for behavioral testing was hand washed with odorless detergent (Alconox) and baking soda and then air-dried.

**Diets**

We used ground rodent chow mixed with either 1% ground cinnamon (CIN; McCormick Ground Cinnamon, McCormick Canada, London, Canada) or 2% powdered cocoa (COC; Fry’s Premium Cocoa, Cadbury, Mississauga, Canada). Our previous work (Choleris et al., 2011; Clipperton et al., 2008) suggests that these two flavored diets are equivalently palatable to CD-1 mice obtained from Charles River (Quebec).

**Experimental Procedures**

At least three days before behavioral testing, same-sex OBS, STIM and DEM mice were triple housed into 1 cage. Using a steel cage divider, a single STIM mouse and a single DEM mouse were placed on one side, and a single OBS mouse was placed on the other side (sides were counterbalanced).

Males and females were always tested on the same day, and in the dark phase of the light cycle, when mice are most active.

The night before behavioral testing, STIM and DEM mice were fully food deprived, while OBS mice were left with approximately five grams of food. On the morning of testing, OBS mice were placed into the microdialysis chambers with no food, corncob bedding and water *ad libitum*. No food was included in the chambers to increase motivation to investigate experimental stimuli.

STIM mice were allowed to eat unflavored powdered chow for 1 hour directly before the social interaction with their respective OBS (NON-DEM social interaction).
Similarly, DEM mice were allowed to eat either COC or CIN for 1 hour directly before the social interaction with their respective OBS (STFP interaction). DEM mice had to eat at least 0.1g of food for their OBS to be included in the final analysis ($n = 1$ OBS removed). Powdered food, STIM and DEM mice were placed into Plexiglas cylinders for all exposures to ensure OBS mice could not actually ingest the powdered food presented during the odor exposures, and to prevent STIM and DEM mice from chewing on the OBS’s microdialysis probes/lines during the social interactions. Unpublished data from our lab finds that OBS mice readily acquire a food preference from a DEM contained in these Plexiglas cylinders. For all exposures, a Plexiglas cylinder was stacked on top of another Plexiglas cylinder to prevent OBS mice from crawling on top and escaping. Additionally, for the NON-DEM social and STFP exposures, a Plexiglas ceiling (7cm diameter, 0.3cm thickness) was placed on the bottom of the higher cylinder to confine STIM and DEM mice to the bottom of the cylinders where the perforations are located. All STIM and DEM mice were habituated to the Plexiglas cylinders before behavioral testing. A full ethological analysis was conducted on the social interactions to assess various social (e.g., oronasal investigation) and non-social behaviors (e.g., horizontal exploration, see Table 6; The Observer Video software, Noldus Information Technology, Wageningen, Netherlands). A researcher blind to the experimental conditions scored only the OBS mouse behaviors (Clipperton et al., 2008).

For both the NON-DEM food odor and DEM food odor exposures, approximately 7.5 grams of flavored food was placed into the cylinders. The amount of time (in sec) OBS mice spent investigating the perforations (within 1-2mm) of the cylinders (where
the food odor was emitted) was recorded with a stopwatch by trained researchers standing 1 metre away.

The experimental procedures below (1 to 6; see Figure 18) were conducted consecutively in the microdialysis chamber. There was a downtime period between each exposure (and after the choice test) in which OBS mice were not exposed to any experimental stimuli. Each exposure and downtime period was 40 minutes. Following any exposure, it was expected that during the downtime periods DA levels would return to relatively stable/normal levels (Cui et al., 2014; Ferris et al., 2014; Lohani et al., 2018; Shen et al., 2004; Taylor et al., 2015). Samples were collected every 20 minutes. Artificial cerebrospinal fluid (aCSF (in mM): 147 NaCl, 2.8 KCl, 1.2 MgCl₂, 1.2 CaCl₂; pH 7.2-7.4) was continuously perfused (Pump 11 Elite, Harvard Apparatus, QC, Canada) through the microdialysis probes (AZ-1.3-02, Eicom Corporation, San Diego, CA, USA) at a flow rate of 0.6 µL/min. Microdialysis probe membranes were made of artificial cellulose (50 kDa molecular weight cutoff; average mass of dopamine is 153.18 Da), where the membrane outer diameter was 0.22mm, and the membrane length was 2mm. Counter-balanced lever arms for mice attached with single channel plastic swivels (25ga, 375/25PS, Instech Laboratories, Plymouth Meeting, PA, USA) were used for all behavioral procedures.

(1) Baseline. OBS mice were restrained, cap nuts and dummy cannulae were removed, and microdialysis probes were gently inserted into the guide cannulae and secured in place with a cap nut, and mice were placed in the microdialysis chamber. Baseline sample collection started 2 hours after the probes were inserted into the guide cannulae, so that the mice would recover from the restraint stress associated with probe
insertion, and allow for more stable baseline samples. During baseline, mice were not exposed to any experimental stimuli.

(2) NON-DEM diet odor. OBS mice received olfactory exposure to the flavored food the STFP DEM did not eat (see below). For example: if the STFP DEM ate CIN, during this session the OBS was exposed to COC.

(3) NON-DEM social. This exposure involved a social interaction between the OBS and a same-sex STIM animal that recently ate non-flavored rodent chow.

(4) STFP. OBS mice were presented with a same-sex DEM mouse that recently consumed either a CIN or COC flavored diet. This social interaction provided the opportunity to acquire a food preference.

(5) DEM diet odor. OBS’s received olfactory exposure to the same flavored food that their respective STFP DEM ate.

(6) Choice test. OBS mice were given a two-hour choice test where they could eat both the CIN and COC diet. Two tablespoons (30 g) of flavored food was placed into each choice test jar, which were weighed at 1 and 2 hours into the choice test.

**Figure 18.** Timeline for the in vivo microdialysis experiment. “DEM” stands for demonstrator mouse; “OBS” stands for observer mouse; “STIM” stands for stimulus mouse. “NON-DEM diet odor” refers to the OBS receiving olfactory exposure to the flavored food the social transmission of food preferences (STFP) DEM did not eat. “NON-DEM social” refers to the social interaction between the OBS and STIM where the STIM animal recently ate non-flavored rodent chow.
“STFP” refers to the social interaction between the OBS and DEM where the DEM animal recently ate either a cocoa (COC) or cinnamon (CIN) flavored food. “DEM diet odor” refers to the OBS receiving olfactory exposure to the same flavored food that their respective STFP DEM ate. “Downtime” refers to the time period in which the OBS is not exposed to any experimental stimuli.

All samples were collected, frozen with dry ice, and then stored in a -80 °C freezer until high-performance liquid chromatography (HPLC) methods were conducted (see below).

**Estrous Phase Determination**

The estrous phase of OBS mice was identified to examine whether there were any main effects of the estrous cycle or interactions with the STFP. Vaginal smears were also collected from DEM and STIM mice to confirm that the ovariectomies were complete (Oksjoki, et al, 1999). All vaginal smears were taken directly after behavioral testing. Specifically, females were gently restrained, saline solution (0.9% NaCl) soaked cotton tipped swabs were gently inserted into the vagina, twisted softly, and cells were rolled onto clean microscope slides, which were later stained with Giemsa (Sigma-Aldrich, Oakville, ON, Canada) and examined with a light microscope (3.2x magnification). A detailed description of how each phase was identified can be found in Choleris et al, 2011 and Clipperton et al, 2008. Briefly, proestrus was characterized by numerous nucleated epithelial cells, and scarce leukocytes or cornified epithelial cells. A large amount of cornified epithelial cells with some infrequent nucleated epithelial cells was defined as estrus. Diestrus was characterized by many leukocytes, and scarce nucleated or cornified epithelial cells (Byers et al., 2012; Caligioni, 2009).
**Histology**

Following behavioral testing, brains were extracted, put into 4% paraformaldehyde in phosphate buffered saline (PBS) for 2 weeks, then moved into 30% sucrose in PBS for 3-5 days (at 4°C), and then stored in a −80°C freezer. Brains were then coronally-sectioned with a cryostat (30µm thickness; Leica CM 1850, Leica Microsystems, Richmond Hill, ON), stained with 0.5% cresyl violet (pH 3.9), dehydrated, then coverslipped with DPX mounting solution. Slices were then examined with a light microscope (3.2x magnification) to verify probe placements. All probes were located in the dorsal hippocampus (see Figures 19 and 20); hence no mice were removed from the analyses based on probe placements (Paxinos & Franklin, 2001).

**HPLC Detection of Dopamine**

The dialysates were analyzed for DA using the Eicom HTEC-510 HPLC/ECD system (Eicom, USA). Each sample was loaded on a C-18 reverse-phase column (PP-ODS II, 4.6 x 30 mm, Eicom, USA) using a manual injection port (Rheodyne 9725i; 20 µL loop). The column was maintained at a temperature of 25 °C with a mobile phase (0.1 M Phosphate buffer pH 5.4 including 1.5% methanol, 500 mg/L Decansulfonate sodium salt [DSS] and 50 mg/L 2Na-EDTA) set at a flow rate of 500 µL/min. DA was electrochemically detected using a graphite electrode (WE-3G, Eicom, USA) maintained at a potential of +400 mV relative to an Ag/AgCl reference electrode (RE-500, Eicom, USA). The concentration of DA in the dialysates were converted to percent baseline (determined by the mean pg/µL of the four baseline readings).
Data Handling

A percent change in DA release from basal levels was calculated for each mouse for all samples collected. This entailed calculating an average of the four samples taken during the baseline period, and expressing each sample as the percent change from that average \([\text{sample/average of the four baseline samples } \times 100]\). This data was then ln transformed to achieve normality, however, graphs show the original ratio data (for clarity). Values that were 2SDs ± mean were excluded (8.5% of cells for males, 6.7% of cells for females).

For all OBS mice, a CIN preference ratio \([\frac{\text{CIN}}{\text{CIN}+\text{COC}} \times 100]\), DEM preference ratio \([\frac{\text{DEM food consumed}}{\text{total food consumed}} \times 100]\) and total food intake amount \((\text{CIN} + \text{COC})\) was calculated for both time points during the choice test. CIN preference ratios were used to assess whether or not social learning occurred, and DEM preference ratios were used to compare social learning across groups (see Choleris et al., 2012 for a detailed explanation and interpretation of the two calculations). A CIN preference ratio towards 100% indicates a preference for CIN, and a value towards 0% indicates a preference for COC. A DEM preference ratio towards 100% indicates a preference for the DEM food, and a value around 50% indicates no food preference. CIN and DEM preference ratios were only calculated for choice test time points where the OBS mouse ate at least 0.1g of food. Arcsin-transformations were conducted on the CIN and DEM preference ratio data since ratios violate the homogeneity of variance assumption, however graphs display the original ratio data.

Statistical Analyses

Mixed model analyses of variance (ANOVAs) were conducted on DA levels for
all samples collected. In addition, separate mixed model ANOVAs were run on DA levels for each separate exposure (and their associated downtime), including baseline, NON-DEM diet odor, NON-DEM social, STFP, DEM diet odor, and choice test. The between groups factors in these models were: sex (male or female), the within groups factor was condition (baseline, NON-DEM diet odor, NON-DEM social, STFP, DEM diet odor, and choice test), and the repeated measures factor was time (sample 1 to sample 28).

In view of the sex differences we found in DA receptor involvement in the STFP (Matta et al., 2017; see also Chapter 2 in this thesis), planned independent sample t-tests were used to compare male versus female DA release for all samples collected. Paired samples t-tests were also planned to compare each exposure to baseline samples, and across selected exposures (for each sex). Across exposure comparisons entailed comparing samples collected during the NON-DEM diet odor versus DEM diet odor, and NON-DEM social versus STFP.

Mixed model ANOVAs were used to analyze CIN preference ratio data. The between groups factors in these models were: sex (male or female), DEM food (CIN or COC), and the repeated measures factor was time (1 and 2 hours). In addition, planned independent samples t-tests were conducted on both hours of the choice test comparing the CIN preference ratios of OBS mice that interacted with a CIN DEM versus OBS mice that interacted with a COC DEM. A statistically significant difference between these two groups indicates social learning has occurred.

Mixed model ANOVAs were used to analyze the DEM preference ratio data and total food intake data. The between groups factors in these models were: sex (male or
female), and the repeated measures factor was time (1 and 2 hours). Additionally, planned independent samples $t$-tests were conducted comparing the DEM preference ratios of males versus females at each time point. Planned one-sample $t$-tests were also used to compare the DEM preference ratios to 50% (chance) at each time point. A DEM preference ratio significantly higher than 50% indicates social learning has occurred.

The criterion that mice had to eat at least 0.1g of food at each time point for a CIN and DEM preference score to be calculated led to some mice having empty cells for certain time points, which consequently meant that data corresponding to such mice were completely removed when running the RM ANOVA. Hence, mixed model ANOVAs were also conducted on the CIN and DEM preference ratios at the two time points separately.

Binary mean comparisons were conducted on the duration, frequency and latency data derived from the NON-DEM social and STFP videos. Planned independent samples $t$-tests were conducted comparing male versus female behaviors during the NON-DEM social and STFP exposures. Planned paired samples $t$-tests comparing behaviors during the NON-DEM social versus STFP exposures were also conducted. Similarly, planned independent samples $t$-tests were conducted comparing male versus female NON-DEM diet odor and DEM diet odor investigation durations. Planned paired samples $t$-tests comparing the investigation durations during the NON-DEM diet odor versus DEM diet odor exposures were also conducted. Additionally, paired samples $t$-tests were employed comparing social investigation (STIM and DEM) versus non-social stimuli investigation (NON-DEM diet odor and DEM diet odor) for each sex. The between groups factor in these models were: sex (male or female), and the within groups factor was condition.
(NON-DEM diet odor, NON-DEM social, STFP, and DEM diet odor). If the normality assumption was still violated after performing a ln transformation, data was analyzed with non-parametric tests (Mann–Whitney U). Unless otherwise different, only duration results are reported (duration, frequency and latency data produce largely analogous results).

The phase of the estrous cycle was an additional between-groups variable for all analyses conducted only on females.

*Post hoc* multiple comparisons (e.g., Bonferroni) were not conducted here as they increase the risk of type II errors beyond an acceptable range (Nakagawa, 2004). Hence, to reduce the risk of type II errors (and even attain greater power), binary mean comparisons were planned throughout. The risk of type I errors was reduced by running an overall ANOVA. For a more detailed description of the logic behind this statistical analysis decision, see Matta et al., 2017 (see also Choleris et al., 2011 and Clipperton et al., 2008).

The Greenhouse-Geisser RM correction was used throughout. Statistical significance was set to $p < .05$. Statistically significant results, statistical trends ($T = .05 < p < 0.1$), and meaningful non-significant results are reported. SPSS (v.20; IBM, Armonk, NY) was used for all analyses.
Table 6. Single and grouped behaviors scored for the 40 minute social interactions in the microdialysis study (based on Grant and Mackintosh, 1963).

<table>
<thead>
<tr>
<th>Social Behaviors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Social Inactivity</strong></td>
<td>Includes sit/lie/sleep together.</td>
</tr>
<tr>
<td><strong>Oronasal Investigation</strong></td>
<td>Active sniffing of STIM/DEM’s oronasal area.</td>
</tr>
<tr>
<td><strong>Body Investigation</strong></td>
<td>Active sniffing of STIM/DEM’s body.</td>
</tr>
<tr>
<td><strong>Anogenital investigation</strong></td>
<td>Active sniffing of STIM/DEM's anogenital region.</td>
</tr>
<tr>
<td><strong>Stretched Approach</strong></td>
<td>Risk assessment behavior; back feet do not move and front feet approach STIM/DEM. Only frequency of stretched approaches was measured.</td>
</tr>
<tr>
<td><strong>Approaching and/or Attending to the DEM</strong></td>
<td>Often from across the cage; OBS's attention is focused on STIM/DEM, head tilted toward STIM/DEM and movements toward STIM/DEM.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-social Behaviors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Horizontal Exploration</strong></td>
<td>Movement around the cage; includes active sniffing of air and ground.</td>
</tr>
<tr>
<td><strong>Vertical Exploration</strong></td>
<td>Movement to investigate upwards, both front feet off the ground; includes sniffing, wall leans and lid chews (less than 3).</td>
</tr>
<tr>
<td><strong>Digging</strong></td>
<td>Rapid stereotypical movement of forepaws in the bedding.</td>
</tr>
<tr>
<td><strong>Abnormal Stereotypies</strong></td>
<td>“Strange” behaviors, including spinturns, repeated jumps/lid chews/head shakes (more than 3).</td>
</tr>
<tr>
<td><strong>Solitary Inactivity</strong></td>
<td>No movement; includes sit, lie down and sleep.</td>
</tr>
<tr>
<td><strong>Self-Grooming</strong></td>
<td>Rapid movement of forepaws over facial area and along body.</td>
</tr>
<tr>
<td><strong>Cylinder Investigation</strong></td>
<td>Active sniffing of the non-perforated areas of the Plexiglas cylinder that contains the STIM/DEM.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grouped Behavior</th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Social Investigation</strong></td>
<td>Composite behavior that includes active sniffing of all perforated areas of the Plexiglas cylinder that contains the STIM/DEM. This includes: Oronasal Investigation, Body Investigation, and Anogenital investigation.</td>
</tr>
</tbody>
</table>
RESULTS

Mouse Brain Probe Placements for the Hippocampal Microdialysis Experiment

Unilateral probe placements for the hippocampal microdialysis study were all found in the anterior dorsal hippocampus (between -1.70mm and -1.82mm), and did not appear to differ between groups (Figure 19).

Figure 19. Brain placements for the hippocampal microdialysis experiment. Black lines indicate the location of the microdialysis probes in the mouse dorsal hippocampus. Numbers on the side (in mm) refer to the coronal section posterior to Bregma. Probe placements were all found in the anterior dorsal hippocampus (between -1.70mm and -1.82mm), and did not appear to differ between groups. Images adapted from Paxinos & Franklin 2001.
**Figure 20.** Photomicrograph (at 3.2x magnification) of a Nissl-stained coronal slice of the mouse dorsal hippocampus. Arrow signifies the site of the microdialysis probe.
Dorsal Hippocampal DA Release in Association with Aspects of Social Learning

Independent samples $t$-tests showed that hippocampal DA release during the NON-DEM diet odor, NON-DEM social, and STFP exposure was greater for males than females, while there were no sex differences in hippocampal DA release during the DEM diet odor exposure or choice test (Figure 21). Paired samples $t$-tests comparing to baseline samples revealed that female hippocampal DA release decreased during the NON-DEM diet odor, NON-DEM social, and STFP exposures, but did not change during the DEM diet odor exposure or choice test. On the other hand, paired samples $t$-tests comparing to baseline samples showed that male hippocampal DA release increased during the NON-DEM diet odor exposure, and trended towards an increase during the NON-DEM social, STFP exposure, and choice test, but did not change during the DEM diet odor exposure. Additionally, males showed greater DA release in association with the NON-DEM diet odor exposure versus the DEM diet odor exposure (Figure 21).

While the overall RM ANOVA conducted on all 28 samples for both males and female revealed no main effect of time or sex, and no time x sex interaction, the RM ANOVA conducted on the NON-DEM diet odor exposures and the two successive downtimes revealed a significant main effect of sex [$F(1, 13) = 4.77, p = .048$] and a trend towards a time x sex interaction [$F(3, 39) = 2.677, p = .06$]. Furthermore, there was a significant main effect of sex [$F(1, 14) = 7.846, p = .014$] and a trend towards a time x sex interaction [$F(3, 42) = 2.318, p = .089$] for the NON-DEM social exposures and the two successive downtimes. Notably, there was also a significant main effect of sex [$F(1, 14) = 5.422, p = .035$] for the STFP exposures and the two successive downtimes. RM
ANOVA conducted on only males or females separately revealed no significant main effects of time for all 28 samples, or at separate exposures.

Independent samples t-tests comparing DA release of males versus females (Figure 21) revealed that males had significantly higher DA release during the first \[t(15) = -3.25, p = .005\] and second \[t(16) = -2.922, p = .01\] downtime of the NON-DEM diet odor exposure, while the first half of the NON-DEM diet odor exposure only approached statistical significance \[t(17) = -1.832, p = .085\]. Furthermore, male DA release was significantly greater than females during the first \[t(17) = -2.526, p = .022\] and second \[t(15) = -2.642, p = .018\] half of the NON-DEM social exposure. Notably, males also had significantly higher DA release than females during the first \[t(16) = -2.851, p = .012\] and second \[t(16) = -2.442, p = .027\] half of the STFP exposure, as well as the second downtime of the STFP exposure \[t(17) = -2.138, p = .047\]. There were no significant differences between male versus female DA release during the DEM diet odor exposures, or during the choice test. Hence, hippocampal DA release associated with the processing of novel food odors, social stimuli, and social learning was greater for males than females, however, there were no sex differences in hippocampal DA release in response to food odors previously encountered on the DEM’s breath or actual food intake (Figure 21).

Paired samples t-tests conducted on females comparing to the baseline samples (Figure 21) revealed that DA release was significantly lower for the first downtime of the NON-DEM diet odor exposure than the first \[t(8) = 2.375, p = .045\] and third \[t(8) = 2.531, p = .035\] baseline sample, while comparisons to the second baseline sample only trended towards significance \[t(8) = 1.947, p = .087\]. Similarly, female DA release was
significantly lower than the first \( t(8) = 2.45, p = .04 \) and third \( t(8) = 2.805, p = .023 \) baseline sample for the second half of the NON-DEM social exposure, while the first half of the NON-DEM social exposure only trended towards a reduction when compared to the first \( t(8) = 1.95, p = .087 \), second \( t(8) = 2.039, p = .076 \) and third \( t(8) = 1.858, p = .1 \) baseline sample. Notably, DA release for female mice was significantly lower than the first, second and third baseline sample for the first half [versus first baseline: \( t(8) = 2.461, p = .039 \); versus second baseline: \( t(8) = 2.776, p = .024 \); versus third baseline: \( t(8) = 2.422, p = .042 \)] and second half of the STFP exposure [versus first baseline: \( t(8) = 2.599, p = .032 \); versus second baseline: \( t(8) = 2.414, p = .042 \); versus third baseline: \( t(8) = 2.669, p = .028 \)]. Additionally, the second downtime of the STFP showed a trend towards a reduction when compared to the third baseline sample \( t(9) = 1.901, p = .09 \). There were no significant changes in female DA release during the DEM diet odor exposures, or during the choice test as compared to baseline. Hence, female hippocampal DA release decreased in response to novel food odors, social stimuli, and social learning, but did not change in response to food odors previously encountered on the DEM’s breath, or actual food intake (Figure 21).

Paired samples \( t \)-tests conducted on males (Figure 21) revealed significantly higher DA release than the third baseline sample \( t(8) = -2.512, p = .036 \) for the second downtime of the NON-DEM diet odor exposure, and there was a trend towards an increase when compared to the first baseline sample \( t(8) = -1.93, p = .09 \). Furthermore, there was a trend towards an increase in DA release for the first downtime of the NON-DEM diet odor exposure when compared to the first \( t(7) = -1.912, p = .097 \) and third baseline sample \( t(7) = -1.922, p = .096 \). There was also a trend towards higher male DA
release as compared to the third baseline sample for the first half of the NON-DEM diet odor exposure \([t(8) = -1.971, p = .084]\), first half of the NON-DEM social exposure \([t(9) = -1.938, p = .085]\), first half of the STFP exposure \([t(8) = -1.885, p = .096]\), and second sample into the choice test \([t(8) = -2.102, p = .069]\). There were no significant changes in male DA release during the DEM diet odor exposures as compared to baseline. Hence, male hippocampal DA release increased in response to novel food odors, and may have increased in response to social stimuli, social learning, and actual food intake, but did not change in response to food odors previously encountered on the DEM’s breath (Figure 21).

Paired samples \(t\)-tests conducted on males across exposures showed that DA release was significantly higher \([t(7) = 3.306, p = .013]\) during the first downtime of the NON-DEM diet odor exposure than the first downtime of the DEM diet odor exposure (Figure 21). Hence, male hippocampal DA release was greater when mice were presented with a novel diet odor over a diet odor previously encountered on the breath of the DEM.
Figure 21. Dopamine release in the dorsal hippocampus expressed as percent of basal levels for observer (OBS) female (black circles; \(n=10\)) and male (white squares; \(n=10\)) mice for the *in vivo* microdialysis experiment. After baseline samples were collected, OBS mice received olfactory exposure to the flavored food the social transmission of food preferences (STFP) demonstrator (DEM) did not eat. Next, there was a social interaction between the OBS and a same-sex stimulus (STIM) animal that recently ate non-flavored rodent chow. Afterwards, OBS mice had a social interaction where they had the opportunity to acquire a food preference from a same-sex DEM mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. The OBS’s then received olfactory exposure to the same flavored food that their respective STFP DEM ate. Lastly, OBS mice were given a 2 hour choice test where they could eat both the CIN and COC diet. There was a downtime period between each
exposure (and after the choice test) in which the OBS was not exposed to any experimental stimuli. Each exposure and downtime period was 40 minutes. Samples were collected every 20 minutes. Data are presented as mean ± SEM. *p < .05, **p < .01, T = .05 < p < 0.1, in comparison between males and females; @p < .05, t = .05 < p < 0.1, in comparison to baseline sample #1 (for each sex); ąp < .05, + = .05 < p < 0.1, in comparison to baseline sample #2 (for each sex); #p < .05, † = .05 < p < 0.1, in comparison to baseline sample #3 (for each sex); &p < .05, in comparison between the first downtime of the NON-DEM diet odor exposure versus the first downtime of the DEM diet odor exposure for males.
Social Learning and Food Intake

The CIN (Figure 22) and DEM (Figure 23) preference scores revealed that both male and female mice acquired a socially learned food preference. There were no sex differences in total food intakes (Figure 24), and no effects of the estrous cycle.

The RM ANOVA conducted on the CIN preference scores for all OBS’s revealed a significant time x sex interaction \[F(1, 16) = 5.57, p = .031\], significant main effect of DEM food \[F(1, 16) = 12.47, p = .003\], and a trend towards a main effect of sex \[F(1, 16) = 3.37, p = .085\]. Furthermore, the RM ANOVA conducted on the CIN preference scores of only males revealed a significant main effect of DEM food \[F(1, 8) = 8.81, p = .018\], and a trend towards a main effect of time \[F(1, 8) = 4.69, p = .062\]. Moreover, mixed-model ANOVAs for all OBSs showed a significant main effect of DEM food in the first \[F(1, 16) = 17.83, p = .001\] and second \[F(1, 16) = 4.78, p = .044\] hour, and main effect of sex \[F(1, 16) = 6.36, p = .023\] only in the second hour of the choice test. Mixed-model ANOVAs conducted on males also revealed a significant main effect of DEM food \[F(1, 9) = 13.45, p = .006\] in the first hour, while there was only a trend towards significance \[F(1, 9) = 4.78, p = .094\] for females at this time point. Hence, the OBS’s CIN preference scores (for certain time points during the choice test) were sex-dependently influenced by the food fed to their DEM’s.

Independent samples t-tests revealed a significant difference between the CIN preference scores of OBS mice that had a CIN fed DEM versus a COC fed DEM for both female \[t(8) = 2.49, p = .037; \text{Figure 22A}\] and male \[t(8) = 3.67, p = .006; \text{Figure 22B}\] OBS mice in the first hour of the choice test. Hence, both male and female OBS mice acquired a socially learned food preference.
Figure 22. Percent of cinnamon (CIN) diet (CIN food consumed divided by the total amount of food consumed) for observer (OBS) female (A) and male (B) mice for the in vivo microdialysis experiment. After baseline samples were collected, OBS mice received olfactory exposure to the flavored food the social transmission of food preferences (STFP) DEM did not eat. Next, there was a social interaction between the OBS and a same-sex stimulus (STIM) animal that recently ate non-flavored rodent chow. Afterwards, OBS mice had a social interaction where they had the opportunity to acquire a food preference from a same-sex DEM mouse that recently consumed either a 1% ground CIN (white squares) or 2% ground cocoa (COC; black circles) flavored diet. The OBS’s then received olfactory exposure to the same flavored food that their respective STFP DEM ate. Lastly, OBS mice were given a 2 hour choice test where they could eat both the CIN and COC diet. There was a downtime period between each exposure (and after the choice test) in which the OBS was not exposed to any experimental stimuli. Each exposure and downtime period was 40 minutes. Samples were collected every 20 minutes. Percent of CIN diets are displayed at 1 and 2 hours into the OBS choice test. The n’s represent the number of OBS mice in each group. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, in comparison between OBS mice that socially interacted with a DEM that consumed CIN versus OBS mice that socially interacted with a DEM that consumed COC.
Overall ANOVAs conducted on the DEM preference scores did not reveal any main effects, or interactions for the full 2 hours, or at separate time points. However, one-sample t-tests revealed that the DEM preference scores were significantly higher than 50% (chance) in the first hour of the choice test for both females \([t(9) = 2.49, p = .034; \text{Figure 23A}]\) and males \([t(9) = 3.10, p = .013; \text{Figure 23B}]\), and a trend towards a higher DEM preference score (versus 50%) for female mice in the second hour of the choice test \([t(9) = 1.95, p = .083; \text{Figure 23A}]\). Thus, both female and male mice developed a STFP.

**Figure 23.** Percent of demonstrator (DEM) diet (DEM food consumed divided by the total amount of food consumed) for observer (OBS) female (A) and male (B) mice for the *in vivo* microdialysis experiment. After baseline samples were collected, OBS mice received olfactory exposure to the flavored food the social transmission of food preferences (STFP) DEM did not eat. Next, there was a social interaction between the OBS and a same-sex stimulus (STIM) animal that recently ate non-flavored rodent chow. Afterwards, OBS mice had a social interaction where they had the opportunity to acquire a food preference from a same-sex DEM mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. The OBS’s then received olfactory exposure to the same flavored food that their respective STFP DEM ate. Lastly, OBS mice were given a 2 hour choice test where they could eat both the CIN and COC diet. There was a downtime period between each exposure (and after the choice test) in which the OBS was not exposed to any experimental
stimuli. Each exposure and downtime period was 40 minutes. Samples were collected every 20 minutes. Percent of DEM diets are displayed at 1 and 2 hours into the OBS choice test. The n’s represent the number of OBS mice in each group. Data are presented as mean ± SEM. *p < 0.05, T = 0.05 < p < 0.1, in comparison to 50% (chance).

The RM ANOVA conducted on total food intake for all OBS’s revealed a main effect of time [F(1, 16) = 17.12, p = .001], and a significant main effect of time [F(1, 8) = 37.35, p < .001] for males analyzed separately. There was no significant main effect of sex, or a sex x time interaction for feeding behavior. Hence, other than effects of time, there were no sex differences in food intake (Figure 24).

![Figure 24. Total food intake (1% ground cinnamon [CIN] + 2% ground cocoa [COC]) for observer (OBS) female (black diamonds) and male (white circles) mice for the in vivo microdialysis experiment. After baseline samples were collected, OBS mice received olfactory exposure to the flavored food the social transmission of food preferences (STFP) demonstrator (DEM) did not eat. Next, there was a social interaction between the OBS and a same-sex stimulus (STIM) animal that recently ate non-flavored rodent chow. Afterwards, OBS mice had a social interaction where they had the opportunity to acquire a food preference from a same-sex DEM mouse that recently consumed either a CIN or COC flavored diet. The OBS’s then received olfactory exposure to the same flavored food that their respective STFP DEM ate. Lastly, OBS mice were given a 2 hour choice test where they could eat both the CIN and COC diet. There was a downtime period between each exposure (and after the choice test) in which the OBS was not exposed to any experimental stimuli. Each exposure](image-url)
and downtime period was 40 minutes. Samples were collected every 20 minutes. Total food
intakes are displayed at 1 and 2 hours into the OBS choice test. There were no significant effects
on total food intakes for either sex. The n’s represent the number of OBS mice in each group.
Data are presented as mean ± SEM.
Behavior during NON-DEM Diet Odor, NON-DEM Social, STFP, and DEM Diet Odor

Exposures

Analyses on the NON-DEM social and STFP exposures revealed that males were generally more investigatory towards the STIM than females during the NON-DEM social exposure (Figure 25). Importantly, there were no differences between males and females for any behaviors during the STFP exposure (Figure 25), which indicates that the sex differences in hippocampal DA release during the STFP found in this study could not be explained by differences in the amount of time spent engaging in oronasal investigation. Comparisons between the food exposures revealed that both female and male mice spent more time investigating the DEM diet odor over the NON-DEM diet odor (Figure 26).

Paired samples t-tests conducted on female behaviors during the NON-DEM social versus STFP exposure revealed a trend towards greater social investigation during the STFP \([t(9) = -2.047, p = .071]\). These results could be partly explained by the finding that female anogenital investigation was significantly higher during the STFP \([t(9) = -2.351, p = .043]\). Hence, female social investigatory behaviors were moderately greater during the STFP than during the NON-DEM social exposure (Figure 25).

Independent samples t-tests comparing male versus female behaviors during the NON-DEM social exposure (Figure 25) revealed that males engaged in social investigation for significantly longer \([t(18) = -2.235, p = .038]\). These results are explained by the significantly greater male anogenital investigation \([t(18) = -2.283, p = .035]\), and trend towards greater oronasal investigation \([t(18) = -1.936, p = .069]\) and body investigation \([t(18) = -1.765, p = .094]\). Hence, males were generally more
investigatory towards the STIM social stimuli in the NON-DEM social exposure than females (Figure 25).

Notably, independent samples t-tests comparing male versus female behaviors during the STFP revealed no significances for any behaviors. Importantly, there was no significant difference between male versus female oronasal investigation durations during the STFP (Figure 25). This suggests that the sex differences in dorsal hippocampal DA release found during the STFP could not be directly explained by differences in the amount of exposure to the socially carried diet odor emitted by the DEM.

![Figure 25. Total duration (in sec) investigating the non-demonstrated (NON-DEM) social (black bars) and social transmission of food preferences (STFP; gray bars) same-sex stimulus (STIM) or DEM animals for observer (OBS) female (n = 10) and male (n = 10) mice for the in vivo microdialysis experiment. After baseline samples were collected, OBS mice received olfactory exposure to the flavored food the STFP DEM did not eat. Next, there was a social interaction between the OBS and a same-sex STIM animal that recently ate non-flavored rodent chow. Afterwards, OBS mice had a social interaction where they had the opportunity to acquire a food preference from a same-sex DEM mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. The OBS’s then received olfactory exposure to the same flavored food that their respective STFP DEM ate. Lastly, OBS mice were given a 2 hour choice test where they could]
eat both the CIN and COC diet. There was a downtime period between each exposure (and after the choice test) in which the OBS was not exposed to any experimental stimuli. Each exposure and downtime period was 40 minutes. Samples were collected every 20 minutes. Data are presented as mean + SEM. “Soc Inves” stands for social investigation; “Oronasal Inves” stands for oronasal investigation; “Body Inves” stands for body investigation; “Anogenital Inves” stands for anogenital investigation. *p < 0.05, † = 0.05 < p < 0.1, in comparison between behaviors during NON-DEM social versus STFP exposure (for female mice). #p < 0.05, † = 0.05 < p < 0.1, in comparison between behaviors for males versus females during the NON-DEM social exposure.

Independent samples t-tests comparing male versus female time investigating the NON-DEM and DEM diet odors revealed no significances. However, planned paired samples t-tests revealed that both females [t(9) = -5.444, p < .001] and males [t(9) = -5.248, p = .001] spent significantly more time investigating the DEM diet odor than the NON-DEM diet odor (Figure 26).

**Figure 26.** Total duration (in sec) investigating the non-demonstrated (NON-DEM; black bars) and DEM (gray bars) diet odors for observer (OBS) female (n = 10) and male (n = 10) mice for the in vivo microdialysis experiment. After baseline samples were collected, OBS mice received olfactory exposure to the flavored food the social transmission of food preferences (STFP) DEM did not eat. Next, there was a social interaction between the OBS and a same-sex STIM animal that recently ate non-flavored rodent chow. Afterwards, OBS mice had a social interaction where they had the opportunity to acquire a food preference from a same-sex DEM mouse that recently consumed
either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. The OBS’s then received olfactory exposure to the same flavored food that their respective STFP DEM ate.

Lastly, OBS mice were given a 2 hour choice test where they could eat both the CIN and COC diet. There was a downtime period between each exposure (and after the choice test) in which the OBS was not exposed to any experimental stimuli. Each exposure and downtime period was 40 minutes. Samples were collected every 20 minutes. Data are presented as mean + SEM. **p < 0.01, ***p < 0.001, in comparison between NON-DEM and DEM diet odor investigation (for each sex).

Paired samples t-tests did not reveal a significant difference in time spent socially investigating the DEM during the STFP versus the DEM diet odor for either females or males. However, paired samples t-tests did show that both female \( t(9) = -3.818, p = .004 \) and male \( t(9) = -8.543, p < .001 \) mice spent significantly more time socially investigating the STIM mouse (Figure 25) than the NON-DEM diet odor (Figure 26). Thus, both sexes were more interested in investigating social stimuli over a food odor.

No other significant effects were found. Notably, there were no abnormal stereotypies observed during any of the exposures.

In all analyses performed, no effects of the estrous cycle were found, likely due to the small number of animals per phase \( n = 3 \) Proestrus, \( n = 4 \) Estrus, \( n = 3 \) Diestrus).
DISCUSSION

Both male and female mice acquired a socially learned food preference (Figure 22 and 23), and there were no sex differences in total food intakes (Figure 24). Hippocampal DA release (Figure 21) was greater for males than females during social learning in the STFP, as well as in association with a non-learned novel food odor, and a social interaction (no social learning). Interestingly, female hippocampal DA release decreased (relative to baseline) in association with social learning, a non-learned novel diet odor, and a social interaction (no social learning), whereas male hippocampal DA release increased during these exposures. There were no differences between male and female oronasal investigation durations during the STFP (Figure 25), which indicates that the sex differences in hippocampal DA release during the STFP could not be explained by differences in the exposure to the socially transmitted diet odor found on the breath of the DEM. Female and male mice both spent more time investigating the DEM (learned) diet odor over the NON-DEM diet odor (Figure 26). Both sexes also spent more time socially investigating the STIM mouse (Figure 25) than the NON-DEM diet odor (Figure 26).

In this study, hippocampal DA release primarily changed in accordance to novel stimuli in a sex dependent manner, such that in males novelty was associated with increased DA release, while in females novelty was associated with decreased DA release (Figure 21). Specifically, hippocampal DA release during the NON-DEM diet odor, NON-DEM social, and STFP exposure was greater for males than females, and there were no sex differences in hippocampal DA release during the DEM diet odor exposure or during the choice test. As compared to baseline, female hippocampal DA release declined during the NON-DEM diet odor, NON-DEM social, and STFP exposures, but
did not change during the DEM diet odor exposure or during the choice test. On the other hand, male hippocampal DA release increased during the NON-DEM diet odor exposure, and trended towards an increase during the NON-DEM social, STFP exposure, and choice test, but did not change during the DEM diet odor exposure. In addition, males showed greater DA release in association with the NON-DEM diet odor exposure as compared to the DEM diet odor exposure. It can be argued that the food odors were no longer novel during the DEM diet odor exposure since OBSs had previously encountered that odor on the breath of the DEM earlier. Similarly, COC and CIN were not novel during the choice test since OBS mice had already encountered both of these foods during the odor exposures beforehand. Thus overall, these results show that dorsal hippocampal DA processes novel food odors associated with aspects of the STFP.

The changes in hippocampal DA release primarily in association with novelty found in this study is consistent with a model proposed by Lisman and Grace, 2005 of the role of DA in novelty detection. It is well established that presentation of novel environmental stimuli results in strong increases in hippocampal activity (Fyhn et al., 2002; Nitz & McNaughton, 2004), and dorsal hippocampal DA is released in male mice in association with novel contextual information (Moreno-Castilla et al., 2017) and being placed in a novel environment (Ihalainen et al., 1999). Indeed, DA neurons produce rapid bursts of spikes in response to novelty or reward that cease as stimuli become more familiar (Ljungberg et al., 1992; Steinfels et al., 1983). Our results (primarily with male mice) are in accordance with these studies supporting a role for hippocampal DA in novelty detection, where we extend the mechanisms also to socially transmitted novel odor detection.
Interestingly, a study conducted by Gold et al., 2011 found an increase in ventral hippocampal acetylcholine release during the STFP in male rats, which is highly consistent with the results of this study. However, different from this study, Gold et al., 2011 found only a small increase in hippocampal acetylcholine in OBS rats interacting with a rat that ate non-flavored chow (no social learning), and no changes in hippocampal acetylcholine in rats presented with a novel odor (Gold et al., 2011). While these differences may be explained by differences in regions sampled (ventral versus dorsal hippocampus) or species used (rats versus mice), they may also highlight the distinct role of hippocampal DA (and not acetylcholine) in processing novelty, and the specificity of acetylcholine in the STFP.

It has been shown that female rats have more VTA DA neurons than males (Dewing et al., 2006; McArthur et al., 2007; Murray et al., 2003). It is therefore possible that the sex differences in DA release in association with novelty detection found in this study may be explained by sex differences in the number of VTA DA neurons. However, as suggested by McArthur et al., 2005, 2007, an increase in the number of VTA DA neurons and their associated dopaminergic innervations does not actually translate into an increase in extracellular DA levels in target structures. In fact, a larger VTA DA neuron population may actually lead to greater pre-synaptic auto-inhibitory DA D2-type receptor activity, and consequently more stable DA levels in target structures (Beaulieu & Gainetdinov, 2011). Indeed, activating presynaptic DA D2-type autoreceptors results in a decrease in DA release, since these receptors are involved in the negative feedback mechanism that regulates DA synthesis, release, and the rate at which DA neurons fire (Beaulieu & Gainetdinov, 2011). Thus, it is possible that in the current study, male mice
showed a rise in hippocampal DA release in association with novelty detection because they have fewer VTA DA neurons (and less presynaptic DA D2-type autoreceptor activity), while females showed reduced hippocampal DA release in association with novelty detection because they have more VTA DA neurons which may be (more so) inhibited by presynaptic DA D2-type autoreceptors (Beaulieu & Gainetdinov, 2011). These assumptions are supported by our pharmacological work, which finds that female (but not male) mice show a social learning impairment in response to dorsal hippocampal infusions of a DA D2-type receptor antagonist (see Chapter 2).

Sex differences in tonic and phasic DA signaling may explain why we found that social learning in the STFP was associated with increased hippocampal DA release for males, whereas social learning was associated with decreased hippocampal DA release for females. Tonic and phasic DA signals have been shown to be involved in maintaining and stabilizing/consolidating pathways activated during learning and memory (Bromberg-Martin et al., 2010; Durstewitz et al., 2000; Floresco et al., 2003; Grace et al., 2007). Tonic responses of DA neurons (slower mode of DA signaling) is associated with mild (low frequency) stimulation, which results in constant, though low, levels of DA, which only activates high affinity DA receptors (i.e., D2-type receptors; Edelmann & Lessmann, 2018; van Wieringen et al., 2013). On the other hand, additional short bursts of phasic responses of DA neurons associated with strong stimulation results in fast rising (though short-lived) higher levels of DA (Schultz, 1999; Lisman and Grace, 2005), which activates even low affinity DA receptors (i.e., D1-type receptors; Edelmann & Lessmann, 2018; Hsu, 1996). We propose that female mice encountering the STFP DEM may have resulted in more tonic firing of hippocampal DA neurons, which may explain the
decreased, though stable DA levels found in this study. Males encountering the STFP
DEM, on the other hand, may have resulted in more phasic firing, which may explain the
increased DA levels. This interpretation of our findings is in agreement with our
pharmacological studies, which find that male social learning is mediated by only
hippocampal DA D1-type receptors (Matta et al., 2017), whereas female social learning
is mediated by both hippocampal D1- and D2-type DA receptors (Matta et al., 2017, see
also chapter 2). Hence, socially acquired food preferences are associated with
dopaminergic transmission, which may involve both tonic and phasic neurotransmission,
where these two modes may act on a continuum (Bromberg-Martin et al., 2010; Floresco
et al., 2003; Grace et al., 2007) to mediate DA dependent social learning in the STFP. See
General Discussion for more details on potential mechanisms.

The CIN (Figure 22) and DEM (Figure 23) preference ratio results showed that
males and females acquired a food preference, indicating that social learning occurred in
both sexes, and there were no sex differences in total food intakes (Figure 24). These
results show that mice can acquire a food preference without having a free social
interaction. Indeed, as long as mice are able to make enough physical contact to engage
in oronasal investigation (Valsecchi and Galef, 1989), in this case through perforations in
a cylinder, they can readily acquire a food preference. These results are consistent with
previous work showing that Mongolian gerbils (Choleris et al., 1998; Valsecchi et al.,
1996) and rats (Galef & Stein, 1985) can acquire a socially learned food preference
through a mesh screen.

Analyses on the NON-DEM social and STFP exposures (Figure 25) revealed that
female social investigatory behaviors (i.e., anogenital investigation) were greater during
the STFP than during the NON-DEM social exposure. Interestingly, males were
generally more investigatory towards the STIM during the NON-DEM social exposure
than females, whereby males engaged in social investigation for longer, which was driven
by greater anogenital investigation. This sex difference could be explained by the fact
that males are generally more aggressive than females (Anton, 1969; Edwards, 1970;
Jacobson-Pick et al., 2013; Miczek et al., 2001), and anogenital investigation can be
considered the most agonistic of the social investigatory-type behaviors (Clipperton-
Allen et al., 2010, 2011B; Grant and Mackintosh, 1963). Notably, analyses on the social
interactions revealed no sex differences in any behaviors displayed during the STFP.
Importantly, there were no differences in oronasal investigation durations between males
and females during the STFP (Figure 25). This indicates that the sex differences in
hippocampal DA release during the STFP found in this study could not be explained by
differences in the exposures to the socially carried food odor found on the breath of the
DEM.

Both sexes spent more time investigating the DEM (learned) diet odor than the
NON-DEM diet odor (Figure 26). While these results may simply be explained by
increased hunger during the DEM diet odor exposure (which was presented later during
testing), these results may also be a reflection of reduced novel food neophobia (Neath et
al., 2010) as indicated by greater interest in the odor affiliated with social learning, over
another food odor. Moreover, while there was no difference in time spent socially
investigating the DEM during the STFP versus the DEM diet odor for either sex (likely
due to ceiling effects; see Figure 25 and 26), both females and males spent more time
socially investigating the STIM mouse (Figure 25) than the NON-DEM diet odor (Figure
These results are consistent with the well-established effect that rodents preferentially investigate social stimuli over non-social stimuli (in this case a food odor; Crawley, 2004).

The sex differences in hippocampal DA release found in this study suggest a possible modulatory role for gonadal hormones such as estrogens/progesterone and testosterone. The estrous cycle has been shown to regulate the STFP in mice (Choleris et al., 2011) and mesolimbic DA levels (Thompson and Moss, 1997) in rats, and estrogens were shown to mediate the STFP in mice (Clipperton et al., 2008; Ervin et al., 2015a). Unfortunately, in the current study we did not find an interaction between the estrous cycle and hippocampal DA release, which can be attributed to the small number of females for each phase of the estrous cycle. Further research on the potential interactions between gonadal steroids and hippocampal DA release in association with social learning is therefore warranted.

To the best of our knowledge, this is the first study to show that hippocampal DA is released in association with various components of the STFP in mice. This study highlights the importance of studying both males and females, as we have shown that there are clear sex differences in hippocampal DA release in response to novel odors, social stimuli, and social learning in the STFP. Dorsal hippocampal DA therefore plays an important and sex-dependent role in socially relevant novelty processing.
CHAPTER 4: The Role of Nucleus Accumbens Dopamine D1-type Receptors on Social Learning, Social Interactions and Food Intake in Male and Female Mice
INTRODUCTION

Social learning occurs in a variety of species (Hoppitt & Laland, 2013). One of the most recognized definitions of social learning (as developed by Heyes, 1994) is “learning that is influenced by observation of, or interaction with, another animal (typically a conspecific) or its products” (Box, 1984; Galef, 1988a; Heyes, 1994). Learning new information from others can be beneficial and may even result in a biologically relevant change in behavior (Hoppitt and Laland, 2013). For instance, young and adult rodents can learn about nearby food sources by sampling those sources on the breath of another animal. This socially transmitted food preference (STFP) can reduce the chances of eating potentially toxic substances, which make this social behavior critical for a rodent’s survival. An animal’s tendency to prefer the food they found on the breath of a conspecific can be experimentally manipulated as a test of social learning in a laboratory setting (Galef et al., 1984). This paradigm is composed of three main stages: (1) a demonstrator (DEM) mouse is allowed to eat a flavored diet, (2) a naïve observer (OBS) of the same sex is allowed to socially interact with the DEM for a short period of time (often 30 minutes) whereby the OBS can collect odor information derived from the DEM’s breath, and (3) when presented with a choice between two novel diets, the OBS mouse usually shows a preference for the food the DEM has eaten, signifying that it socially learned during the social interaction that this food is safe to eat.

Oronasal investigation in mice (Valsecchi and Galef, 1989) and rats (Galef and Stein, 1985) is a fundamental social behavior that must occur for an OBS to acquire the STFP. Only OBS’s that detect the diet odor simultaneously with carbon disulfide (CS₂; volatile compound exhaled by the DEM following food digestion) will develop a food
preference (Galef et al., 1988). Furthermore, CS₂ must activate a special subpopulation of olfactory sensory neurons expressing the receptor guanylyl cyclase type D (GC-D) for mice to socially acquire these food preferences (Arakawa et al., 2013; Munger et al., 2010).

Brain regions reported to regulate social learning in the STFP include the hippocampus, basal forebrain, as well as the frontal, orbitofrontal and piriform cortices (reviewed in Choleris et al., 2009; Ervin et al, 2015a; Matta et al, 2016). Neurochemical systems implicated in a socially learned food preference include the cholinergic system, galanin system, vasopressin/oxytocin system, opioid system, glutamatergic system (reviewed in Choleris et al, 2009; Ervin et al, 2015a; Matta et al, 2016) and dopaminergic (DA) system (Choleris et al., 2011; Matta et al., 2017; Rodriguiz et al., 2004; Wong et al, 2012).

The DA transporter (DAT) and DA receptors mediate socially acquired food preferences. Behavioral studies using DAT knockout (KO) mice have shown that these mice show a food preference for the NON-DEM diet (Rodriguiz et al., 2004). However, follow-up research done by another group shows that when compared to wild-type (WT) animals, the DAT KO mice are impaired in the STFP (Wong et al., 2012). Additionally, experiments with systemic treatments conducted in our lab have demonstrated that antagonizing DA D1-type receptors (D1, D5) blocks social learning but does not affect feeding behavior, whereas antagonizing DA D2-type receptors (D2, D3, D4) does not affect social learning but reduces food intake in female mice (Choleris et al., 2011). Follow-up experiments designed to examine the functional contribution of DA dependent brain regions underlying these effects have since been conducted.
Mesolimbic DA cells of the ventral tegmental area (VTA) project broadly throughout the brain to limbic structures including the amygdala, hippocampus, medial prefrontal cortex (mPFC), and nucleus accumbens (NAc; Russo & Nestler, 2013; Wise, 2004). Among these, hippocampal DA was shown to mediate in the STFP in mice. Matta et al., 2017 showed that direct dorsal hippocampal infusions of the DA D1-type receptor antagonist SCH23390 blocks social learning in male and female mice. Furthermore, males were more sensitive to the hippocampal DA D1-type receptor antagonism than females, since male social learning was impaired at the lowest and two highest doses of drug treatment, whereas female social learning was only impaired at the highest dose (Matta et al., 2017). Additionally, as shown in Chapter 2 in this thesis, DA D2-type receptors in the dorsal hippocampus mediate female, but not male STFP. Moreover, Chapter 3 showed that dorsal hippocampal DA levels increase for males, and declines for females in association with social learning. Thus, the dorsal hippocampus is one site of action underlying the systemic effects reported by Choleris et al., 2011, however, there may be another brain region(s) involved.

DA D1-type receptors in the NAc have been strongly implicated in social behaviors. For example, NAc shell DA D1-type receptors are involved in pair bonding in male prairie voles (Liu et al., 2010), defeat-induced social withdrawal in female mice (Campi et al., 2014), and social play in male rats (Manduca et al., 2016). Additionally, optogenetic experiments find that VTA DA projections directly to the NAc shell regulate social interactions in female mice towards a novel same-sex conspecific, whereby this optogenetic effect is mediated specifically by NAc DA D1-type receptors (but not DA D2-type receptors; Gunaydin et al., 2014). Currently, it is unknown whether or not DA
D1-type receptors in the NAc shell are also involved in social learning and/or social interactions in the STFP in male and female mice.

In addition to their role in social behaviors, NAc DA D1-type receptors are involved in the expression of fructose conditioned flavor preferences (Bernal et al., 2008), and the acquisition of glucose conditioned flavor preferences (Touzani et al., 2008) in male rats. However, it is unknown whether the same NAc mechanisms mediate socially learned food preferences in mice.

The purpose of this study was to investigate the role of NAc DA D1-type receptors in the STFP. To do this, we microinfused a range of doses of the DA D1-type receptor antagonist SCH23390 into the NAc before social learning took place. Drug treatment was infused directly into the shell of the NAc since this subregion has been implicated in social behavior (Campi et al., 2014; Gunaydin et al., 2014; Liu et al., 2010; Manduca et al., 2016) and individually acquired food preferences (Bernal et al., 2008) in rodents. Hence, we predicted that infusing SCH23390 into the NAc shell would block social learning in the STFP. Given the sex differences in sensitivity to SCH23390 found by Matta et al., 2017, as well as the sex differences found in Chapter 2 and 3 in this thesis, we used both male and female adult mice in this study. We also examined possible secondary effects of intra-NAc SCH23390 on feeding behavior. Moreover, the social interactions, during which the social transmission of information occurs, were thoroughly analyzed for possible effects of drug treatment on a variety of social and non-social behaviors, including agonistic, investigatory, and solitary behaviors (Clipperton et al., 2008). Finally, possible effects of the estrous cycle on the STFP was assessed, since estrogens are involved in the STFP in mice (Clipperton et al., 2008; Ervin et al., 2015a)
and research finds that the mesolimbic DA system is regulated by estrogens/progesterone (Thompson & Moss, 1997).

**MATERIALS AND METHODS**

*Animals*

The subjects were experimentally naïve male and female CD-1 mice (*Mus musculus*; Charles Rivers, St. Constant, QC, Canada) that were 2 to 3 months old. Upon arrival, mice were housed in same-sex trios, and were allowed to adjust to the colony room for 1 week before initiating any procedures. Animals were housed in environmentally enriched (paper material for making nests and paper cups) clear polyethylene cages (26 x 16 x 12 cm$^3$) with corncob bedding and *ad libitum* access to rodent food (Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Teklad, WI) and water. Thereafter, same-sex mice were double housed (OBS and DEM dyads separated by a steel divider; same housing conditions as stated above) for at least three days (familiarization period) before behavioral testing. The colony room temperature was kept at 21±1 °C, and humidity was maintained at 40–50%. Animals were on a reversed 12:12 hour light/dark cycle (lights went off at 08:00 hours). All procedures were in accordance with the guidelines of the Canadian Council on Animal Care and approved by the University of Guelph Institutional Animal Care and Use Committee.

*General Surgical Procedures*

All DEM males and females were gonadectomized to eliminate potential hormonal actions on the OBS’s STFP. Instead, OBSs had bilateral intracerebral cannulae aimed at the NAc shell, and were left gonadally intact to investigate potential effects of gonadal hormones on the STFP (Choleris et al., 2011; Clipperton et al., 2008; Sanchez-
Andrade et al., 2005). All OBSs were experimentally naïve, while DEMs were reused around 9-12 times.

The anti-inflammatory carprofen (50mg/kg; Rimadyl, Pfizer Canada Inc, Kirkland, QC, Canada) was injected (subcutaneous [s.c.]) 30 minutes prior to mice being anaesthetized (isoflurane; Benson Medical Industries, Markham, ON). Two to three drops of a local anaesthetic (preparation of 0.67% lidocaine [Alveda Pharmaceuticals, Toronto, ON, Canada] and 0.17% bupivacaine [Hospira, Inc., Montreal, QC, Canada]) were applied to all incision sites, which had been appropriately shaved and cleaned (Germi-Stat soap, alcohol, antiseptic tincture). Castration and ovariectomy incision sites were closed using 2 to 3 wound clips (Autoclip, 9mm; MikRon Precision Inc., Gardena, CA). Saline (0.5mL; 0.9% NaCl) was injected (intraperitoneal [i.p.]) at the end of each surgery for rehydration. Mice were given a recovery period of at least 1 week (clean cages, singly housed, same housing conditions as mentioned above) before being pair-housed with a same-sex animal for at least 3 days before behavioral testing (see Matta et al., 2017 for greater details about surgical procedures).

**Ovariectomy Surgery**

The procedures of this surgery are described in great detail in Clipperton-Allen et al., 2011A. Adult DEM females were anaesthetized and a patch of fur on the lower back was shaved and the skin was appropriately cleaned. A 2cm incision was made on the skin, and two smaller 1cm lateral incisions were made on the back muscles overlaying exterior to the ovaries. Next, the ovaries were individually revealed and pulled out, the fallopian tubes were clamped, and the ovaries were removed. The fallopian tubes were then placed back into the muscles, and the skin incision was closed using wound clips.
**Castration Surgery**

Adult DEM males were anaesthetized, placed on their backs, the fur on the scrotum was shaved, and the skin was appropriately cleaned. A 1 cm midline incision was made on the ventral part of the scrotum. The tunica was then exposed and laterally pierced (0.5 cm) on both sides. Next, one at a time, the testes were pulled out, the spermatic cords connecting each testicle were ligated using sterile hemostatic clamps, and the testes were removed. The spermatic cords were then placed back into the tunica, and the skin incision was closed via wound clips (Matta et al., 2017).

**Cannulation Surgery**

Similar to Matta et al., 2017, OBS mice were anaesthetized and placed into a stereotaxic frame (David Kopf instruments, CA) that had atraumatic ear bars. The skin overlaying the mouse skull was shaved, cleaned and then removed. Bregma was visualized and membranes were removed by applying 3% hydrogen peroxide (H$_2$O$_2$) in saline solution (0.9% NaCl) to the skull. Bilateral intracerebral guide cannulae (26 gauge; Plastics One, HRS Scientific, Anjou, QC, Canada) were inserted into two holes that were drilled into the skull. Based on the mouse brain atlas of Paxinos and Franklin 2001, we aimed for the medial shell of the NAc (flat skull position and with respect to Bregma; Anterior = 1.25 mm; Lateral = 0.75 mm; Ventral = 4 mm). Jewellers screws (1.6 mm; Plastics One, HRS Scientific, Anjou, QC, Canada) were then placed into three additional holes that were drilled into the skull surrounding the guide cannulae. Next, dental cement (Central Dental Ltd, Scarborough, ON, Canada) was used to form a headcap. Dummy cannulae (Plastics One, HRS Scientific, Anjou, QC, Canada) were inserted into the guide cannulae to prevent blockage. Once inserted, injectors (Plastics One, HRS Scientific,
Anjou, QC, Canada) used before behavioral testing went 1mm past the end of the guide cannulae. The final ventral coordinate was therefore 5mm below skull surface.

**Materials**

Cage dividers made of steel (25.8 x .2 x 10.8 cm\(^3\); described in detail in Matta et al., 2017) were used to keep OBS and DEM mice separated during the three-day familiarization period. On the morning of behavioral testing, DEM’s were placed into clean polyethylene cages (26 x 16 x 12 cm\(^3\)), and had access to glass jars (5cm H, 7.5cm W; Dyets, Bethlehem, PA) that had a metal piece which sat over a flavored diet (to prevent spillage), and jars were sealed with screw on lids with a hole in the middle (2.5cm diameter) to allow access to the food. For overhead video recording of the 30 min social interactions, we utilized digital cameras that were able to record under infrared light (JVC Everio camcorders; Mississauga, ON, Canada), and clear Plexiglas cage lids equipped with air holes. The OBS was given a choice test in a large clean polyethylene cage (42.5 x 26.5 x 18.5cm\(^3\)) that had a wire lid allowing ad libitum water access, and the anterior face of the cage had two separate openings leading to steel tunnels that held clip-on Plexiglas containers each holding one flavored diet (equipment described in great detail in Choleris et al., 2011 and Valsecchi & Galef, 1989; Tecniplast, Varese, Italy). A scale accurate to 0.01g (Sartorius Analytical Balance, Sartorius Inc., United Kingdom) was used to weigh all DEM jars and OBS choice test containers. To appropriately remove odor cues and clean lab equipment after each trial, we used odorless detergent (Alconox) and baking soda.
Flavored Diets

Previous research from our lab (Choleris et al., 2011; Clipperton et al., 2008) using CD-1 mice sourced from Charles Rivers (St. Constant, QC, Canada) finds that 1% ground cinnamon (CIN; McCormick Ground Cinnamon, McCormick Canada, London, Canada) or 2% ground cocoa (COC; Fry’s Premium Cocoa, Cadbury, Mississauga, Canada) mixed with grounded rodent food are equally palatable.

Drug

We dissolved the DA D1-type receptor antagonist SCH23390 hydrochloride (Hyttel, 1983; Tocris Biosciences, Ellisville, MO) in sterile saline solution (0.9% NaCl).

Experimental Procedures

STFP

The following procedures (Figure 27) were adapted from Matta et al., 2017. At least three days before behavioral testing, OBS mice were pair-housed with a same-sex DEM for familiarization. The cage was separated into two lengthwise compartments with a perforated (to allow for sensory exposure) divider to prevent DEM’s from removing the OBS’s headcap. The compartment side of the home cage (right or left) the OBS was allotted was counterbalanced (for each sex and dose) throughout testing (Matta et al., 2017).

Approximately 12-14 hours before behavioral testing, OBS’s and DEM’s were fully food deprived. The next morning, early in the dark phase of the light cycle, DEM mice were placed into a clean cage and were allowed to eat a flavored diet for 1 hour out of glass jars. The DEM’s food (COC or CIN) was counterbalanced for each sex and treatment group. If the DEM ate at least 0.1g of their assigned food, their respective OBS
was allowed to be tested (no OBS mice were excluded based on this condition). After consuming the flavored diets, DEM mice were placed back into the home cage with their respective OBS without a cage divider and allowed to socially interact for a period of 30 minutes. Following the social interaction (where social learning occurs), OBS’s were put into choice test cages where they had unrestricted access to two feeder, one containing CIN and the other containing COC. The Plexiglas containers holding the flavored diets were weighed at 2, 4, 6 and 8 hours into the OBS’s choice test.

Fifteen minutes prior to the social interactions, OBS mice received a single bilateral intra-NAc microinfusion of either saline vehicle (0.9% NaCl) as a control group, or the DA D1-type receptor antagonist SCH23390 at 1, 2, 4 or 6 µg/µL. The doses and fifteen-minute delay period used here are primarily based on our own behavioral pilot studies, and behavioral mouse studies that infused SCH23390 into the NAc (Campi et al., 2014; Couppis and Kennedy, 2008; Gunaydin et al., 2014; Young et al., 2014). We used a microinfusion pump (PHD 2000 injector, Harvard Apparatus, QC, Canada) set to a flow rate of 0.2µL per minute and the infusion volume was 0.5µL per hemisphere. Injectors were left in the brain for 1 additional minute after the infusion was complete to minimize potential back-flow of the treatment. Throughout behavioral testing, the order of doses infused was counterbalanced from trial to trial (for each sex), and males and females were always tested on the same day.

The 30 minute social interactions were recorded and the OBS’s behaviors were later scored (see Tables 1 and 2 in Chapter 2) with The Observer Video software (Noldus Information Technology, Wageningen, Netherlands). A trained researcher blind to the drug condition scored single social behaviors such as following the DEM, agonistic
behaviors such as boxing, and social investigatory behaviors such as oronasal investigation, which is critical for the STFP to occur (Galef and Stein, 1985; Valsecchi and Galef, 1989). We also examined non-social behaviors, including horizontal/vertical exploration, self-grooming and digging. Various grouped behaviors were also calculated to assess possible generalized effects of drug treatment on features such as total activity and social investigation (Blanchard et al., 1993; Choleris et al., 2003; Clipperton et al., 2008).

**Figure 27.** Timeline for the intra-nucleus accumbens (NAc) SCH23390 social transmission of food preferences (STFP) experiment. “DEM” stands for demonstrator mouse; “OBS” stands for observer mouse.

_Estrous Phase Determination_

Following the eight-hour choice test for OBS mice, and after the 30 minute social interactions for DEM mice, vaginal smears were performed on females. This was done to determine possible interactions of the OBS’s estrous cycle with drug treatment on the STFP, and to verify that the DEM’s ovariectomy surgeries were complete (Oksjoki et al., 1999). To do this, cotton-tipped swabs were dipped in sterile saline solution (0.9% NaCl),
and lightly rotated in the vagina. Cells were then transferred onto glass microscope slides before being stained with Giemsa (Sigma-Aldrich, Oakville, ON, Canada) and examined with a light microscope (magnification at 3.2x). Proestrus was characterized by an overrepresentation of nucleated epithelial cells, and very few cornified epithelial cells or leukocytes (or none at all). Estrus was characterized by an abundance of cornified epithelial cells, however, a few nucleated epithelial cells could have been present. Diestrus was characterized by many leukocytes, although a few sporadic nucleated or cornified epithelial cells may have been on the slide (Byers et al., 2012; Caligioni, 2009; Clipperton et al., 2008).

**Histology**

OBS mice received an intra-NAc microinfusion of 1% Chicago blue dye in phosphate buffered saline (PBS), and brains were extracted approximately 45 minutes later. This time period was equivalent to the time period between the beginning of the drug microinfusion to the time at which OBS mice were given the choice test in the STFP (45 minutes). The procedures used for dye infusions were identical to the STFP infusion procedures outlined above. Brains were placed into 4% paraformaldehyde for 1.5-2 weeks (at 4°C), followed by 30% sucrose in PBS for 3-5 days (at 4°C). Next, brains were stored frozen at -80 °C before being sectioned with a cryostat (Leica CM 1850, Leica Microsystems, Richmond Hill, ON) at a thickness of 30µm. Coronal slices were placed onto gelatin-coated microscope slides, which were later cover slipped using DPX Mountant. A light microscope (magnification at 3.2x) was used to examine cannula placements. We found that the dye was mostly localized to the NAc (Figure 29).
with off target cannulas \((n = 15 \text{ mice removed across all groups})\) were removed from the data set (Paxinos & Franklin, 2001).

**Data Handling**

As a measure of social learning, a percent of CIN diet \(((\text{CIN}/\text{CIN+COC}) \times 100)\) was calculated for every mouse at 2, 4, 6 and 8 hours. A value towards 100% is indicative of an OBS preferring to eat CIN, while a value towards 0% is indicative of an OBS preferring to eat COC. A significant difference between the percent of CIN diet of OBS mice that had a CIN DEM vs. COC DEM at a choice test time point (for each sex) indicates a socially learned food preference has occurred (for that treatment group).

Additionally, a total food intake value was calculated \((\text{CIN+COC})\) for every mouse at 2, 4, 6 and 8 hours.

A percent of DEM diet \(((\text{DEM}/\text{DEM+NONDEM}) \times 100)\) was also calculated for every mouse at 2, 4, 6 and 8 hours. This calculation allows us to cross compare the strength of social learning between different treatment groups (but cannot be used instead of the percent of CIN diet; see Choleris et al., 2012 for discussion). A value towards 100% is indicative of an OBS preferring to eat more of the diet the DEM consumed. A value around 50% (chance) suggests that the OBS mouse had no food preference.

A food consumption threshold of at least 0.1g at each time point had to be met for a food preference ratio (percent of CIN and percent of DEM diet) to be calculated at any choice test time point for OBS’s. Similarly, DEM mice had the same threshold whereby only OBS’s whose same-sex DEM consumed at least 0.1g of food were tested. Some OBS mice did not eat a certain time points, which led to some empty cells, which caused the loss of all data from that mouse in the overall analyses on all time points for the full 8
hours. For this reason, analyses were conducted at each time point individually, as well as overall analyses (full 8 hours).

An arcsin transformation was applied to all data that involved a ratio calculation (percent of CIN and percent of DEM diet) because ratio data violate the homogeneity of variance assumption. However, all graphs depicted here display the originally calculated ratio data for clarity.

**Statistical Analyses**

Mixed model ANOVAs were conducted on the percent of CIN diet data. The between groups variables were sex (male or female), DEM food (CIN or COC) and treatment group (saline, or SCH23390 at 1, 2, 4 or 6 μg/μL). The repeated measures factor was time (2, 4, 6 and 8 hours).

Mixed model ANOVAs were conducted on the percent of DEM diet and total food intake data. The between groups variables for these data were sex (male or female) and treatment group (saline, or SCH23390 at 1, 2, 4 or 6 μg/μL). The repeated measures factor was time (2, 4, 6 and 8 hours).

STFP data from our lab (Choleris et al., 2011; Clipperton et al., 2008; Ervin et al., 2015a; Matta et al., 2017) has shown that drug treatment primarily affects food preferences in the early hours of the choice test. In accordance with these findings, binary mean comparisons were planned *a priori* for the early hours of testing. Specifically, independent samples *t*-tests (for each sex and treatment group) compared the percent of CIN diets of OBS’s with COC fed vs. CIN fed DEM’s, where a significant difference between these two groups indicates that a socially learned food preference has occurred. In addition, we conducted independent samples *t*-tests (for each sex) comparing the
percent of DEM diets for each dose of SCH23390 vs. saline infused control mice (at each time point). Furthermore, one-sample \( t \)-tests were conducted on the percent of DEM diets (for each sex) comparing to 50\% (chance) at each time point. Such tests on the percent of DEM diet data allowed us to compare the strength of food preferences between groups.

Single and grouped behaviors derived from the 30 minute social interactions were analyzed with mixed model ANOVAs. The between groups variables were sex (male or female) and treatment group (saline, or SCH23390 at 1, 2, 4 or 6 \( \mu \)g/\( \mu \)L). In addition, independent samples \( t \)-tests were employed to compare each dose of SCH23390 to saline treated mice (for each sex). Only duration data are reported here (unless frequency and latency data are different or meaningful). When the normality assumption was violated and could not be satisfied with a \( \ln \) transformation, we employed non-parametric tests, including Kruskal–Wallis and Mann–Whitney \( U \).

To analyze baseline sex differences during the 30 minute social interactions, we conducted independent sample \( t \)-tests on the single and grouped behaviors of saline infused males vs. saline infused females.

To investigate possible interactions of the estrous cycle with intra-NAc SCH23390 on the STFP, the estrous phase was an additional between groups factor added to all models involving only female mice.

As previously described in Matta et al., 2017, no post-hoc multiple comparisons were conducted. Instead, all binary mean comparisons were planned \textit{a priori} for all models. This allowed greater statistical power, reduced the risk of type II errors, and by greatly reducing the total number of comparisons and running overall ANOVAs
throughout, we were able to reduce the risk of type I errors (see Moran, 2003; Nakagawa, 2004; Rothman, 1990).

The Greenhouse–Geisser correction for repeated measures was implemented throughout. Statistical significance was defined as \( p < .05 \). We also reported statistical trends (\( T = .05 < p < 0.1 \)) and only non-significant results that were meaningful. All analyses were done with SPSS (version 20; IBM Corp, Armonk, NY).
RESULTS

*Mouse Brain Cannula Placements for the Intra-Nucleus Accumbens SCH23390 STFP Experiment*

Most injectors were found in the nucleus accumbens shell at 1.10mm anterior to Bregma, however, some were more posterior (0.98mm) and anterior (1.18mm) though still within the nucleus accumbens shell (Figure 28). Brain cannula placements did not appear to differ between groups.
Figure 28. All mouse brain cannula placements (only those that were retained) for the intra-nucleus accumbens SCH23390 social transmission of food preferences (STFP) experiment. Open circles indicate the location of the injector tip in the mouse nucleus accumbens shell. Numbers on the side (in mm) refer to the coronal section anterior to Bregma. Most injectors were found in the nucleus accumbens shell at 1.10mm anterior to Bregma, however, some were more posterior (0.98mm) and anterior (1.18mm) though still within the nucleus accumbens shell. The locations of the cannula placements did not differ between groups. Images adapted from Paxinos & Franklin 2001.
Figure 29. Photomicrograph (at 3.2x magnification) of a coronal slice of the mouse nucleus accumbens following an infusion of 1% Chicago blue dye in phosphate buffered saline. Arrow signifies the site of the cannula (microinjection 1mm below).
*Effects of Intra-Nucleus Accumbens SCH23390 on Social Learning and Food Intake*

Analyses on the CIN (Figure 30) and DEM (Figure 31) preference scores revealed that intra-NAc SCH23390 did not block social learning for either male or female mice. Drug treatment did not affect feeding behavior for either sex (Figure 32). The estrous cycle did not interact with SCH23390 to influence the STFP.

The RM ANOVA conducted on the CIN preference scores of all OBS’s for the full 8 hours revealed a significant sex x treatment \( [F(4, 193) = 3.662, p = .007] \), time x DEM food \( [F(3, 579) = 21.926, p < .001] \) and time x sex interaction \( [F(3, 579) = 2.899, p = .034] \), and main effect of DEM food \( [F(1, 193) = 50.653, p < .001] \) and sex \( [F(1, 193) = 6.463, p = .012] \), and trend towards a main effect of time \( [F(3, 579) = 2.560, p = .054] \).

ANOVA's conducted on the CIN preference scores of all OBS’s at individual time points revealed a significant main effect of DEM food at 2 hours \( [F(1, 211) = 108.893, p < .001] \), 4 hours \( [F(1, 217) = 71.181, p < .001] \) and 6 hours \( [F(1, 218) = 19.204, p < .001] \). There was also a trend towards a treatment x DEM food interaction at 2 hours \( [F(4, 211) = 2.161, p = .075] \). At 4 hours, the ANOVA revealed a significant sex x treatment \( [F(4, 217) = 3.384, p = .01] \) and sex x treatment x DEM food interaction \( [F(4, 217) = 3.305, p = .012] \). We also found a significant main effect of sex at 4 hours \( [F(1, 217) = 12.917, p < .001] \) and 6 hours \( [F(1, 218) = 4.753, p = .03] \). Hence, the CIN preference scores may have been affected by intra-NAc SCH23390 in a sex dependent manner for specific time points.

ANOVA's conducted on the CIN preference scores of both sexes separately for the full 8 hours revealed a significant main effect of treatment for females only \( [F(4, 97) = 2.510, p = .047] \) and a main effect of time for males only \( [F(3, 288) = 4.041, p = .008] \).
addition, there was a significant main effect of DEM food for males \( F(1, 96) = 19.152, p < .001 \) and females \( F(1, 97) = 34.057, p < .001 \), as well as a time x DEM food interaction for males \( F(3, 288) = 10.30, p < .001 \) and females \( F(3, 291) = 11.735, p < .001 \). ANOVAs conducted on separate time points revealed a significant main effect of treatment for males at 4 hours \( F(4, 109) = 2.489, p = .047 \) and a trend towards a main effect of treatment for females at 2 hours \( F(4, 104) = 2.212, p = .073 \). There was also a significant treatment x DEM food interaction for females at 2 hours \( F(4, 104) = 2.538, p = .044 \) and 4 hours \( F(4, 108) = 2.689, p = .035 \). ANOVAs further showed a significant main effect of DEM food for males at 2 hours \( F(1, 107) = 39.421, p < .001 \), 4 hours \( F(1, 109) = 33.346, p < .001 \) and 6 hours \( F(1, 108) = 9.784, p = .002 \). Similarly, there was a significant main effect of DEM food for females at 2 hours \( F(1, 104) = 80.066, p < .001 \), 4 hours \( F(1, 108) = 38.113, p < .001 \) and 6 hours \( F(1, 110) = 9.424, p = .003 \). Hence, there may have been effects of intra-NAc SCH23390 on food preferences at certain time points for both sexes.

Independent samples t-tests conducted on the CIN preference scores revealed that intra-NAc SCH23390 did not block social learning for either males or females (Figure 30). There was a significant difference between the CIN preference scores of OBS mice that interacted with a CIN fed DEM versus OBS mice that interacted with a COC fed DEM during the 2 hour time point for females \( t(22) = 4.857, p < .001 \) and males \( t(21) = 3.427, p = .003 \) infused with saline, females infused with SCH23390 at 1 µg/µL \( t(21) = 5.491, p < .001 \), females \( t(20) = 2.812, p = .011 \) and males \( t(22) = 3.968, p = .001 \) infused with SCH23390 at 2 µg/µL, males infused with SCH23390 at 4 µg/µL \( t(22) = 2.557, p = .018 \), and females \( t(20) = 5.478, p < .001 \) and males \( t(20) = 2.331, p = .03 \).
infused with SCH23390 at 6 µg/µL, while females infused with SCH23390 at 4 µg/µL only showed a trend towards significance at this time point \([t(21) = 1.778, p = .09]\). There was also a significant difference between the CIN preference scores of OBS mice that had a CIN DEM versus COC DEM at 4 hours for males infused with saline \([t(22) = 4.118, p < .001]\), females \([t(22) = 4.583, p < .001]\) and males \([t(22) = 2.114, p = .046]\) infused with SCH23390 at 1 µg/µL, females \([t(20) = 4.377, p < .001]\) and males \([t(22) = 2.78, p = .011]\) infused with SCH23390 at 2 µg/µL, females \([t(22) = 2.092, p = .048]\) and males \([t(21) = 2.899, p = .009]\) infused with SCH23390 at 4 µg/µL, and females infused with SCH23390 at 6 µg/µL \([t(22) = 2.489, p = .021]\). Additionally, there was a significant difference between the CIN preference scores of OBS’s that interacted with a CIN DEM versus COC DEM at 6 hours for males infused with saline \([t(22) = 2.481, p = .021]\), while females infused with SCH23390 at 2 µg/µL \([t(22) = 2.022, p = .055]\) and males infused with SCH23390 at 4 µg/µL \([t(21) = 1.749, p = .095]\) only trended towards significance at this time point. Hence, infusing SCH23390 into the NAc did not block social learning for any dose, for either sex (Figure 30).
Figure 30. Percent of cinnamon (CIN) diet (CIN food consumed divided by the total amount of food consumed) for observer (OBS) female (A, C, E, G, I) and male (B, D, F, H, J) mice that received a single intranucleus accumbens microinfusion of either saline vehicle (A, B), or the dopamine (DA) D1-type receptor antagonist SCH23390 at 1 µg/µL (C, D), 2 µg/µL (E, F), 4 µg/µL (G, H) or 6 µg/µL (I, J). Infusions were administered 15 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground CIN (white squares) or 2% ground cocoa (COC; black circles) flavored diet. Percent of CIN diets are displayed at 2, 4, 6 and 8 hours into the OBS choice test. The n’s represent the number of
OBS mice in each group. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, T = 0.05 < p < 0.1, in comparison between OBS mice that socially interacted with a DEM that consumed CIN versus OBS mice that socially interacted with a DEM that consumed COC.

Mixed-model ANOVAs conducted on the DEM preference scores of all OBS’s revealed a significant main effect of time [F(3, 609) = 21.693, p < .001], a trend towards a main effect of treatment at 2 hours [F(4, 221) = 2.194, p = .071], and a significant sex x treatment interaction at 4 hours [F(4, 227) = 2.97, p = .02]. ANOVAs conducted on both sexes analyzed separately revealed a significant main effect of time for females [F(3, 306) = 11.905, p < .001] and males [F(3, 303) = 9.943, p < .001]. There was also a significant main effect of treatment at 2 hours [F(4, 109) = 2.448, p = .05] and 4 hours [F(4, 113) = 2.607, p = .039] for females only. However, ANOVAs did not reveal any main effects of sex for the full 8 hours, or at separate time points. Notably, planned independent samples t-tests comparing the DEM preference scores of saline infused control animals versus animals infused with SCH23390 (Figure 31) revealed that at 2 hours, females infused with SCH23390 at 4 µg/µL had a DEM preference score significantly lower than saline infused control animals [t(45) = 2.282, p = .027], while males infused with SCH23390 at 1 µg/µL [t(45) = 1.932, p = .06] and females infused with SCH23390 at 2 µg/µL [t(44) = 1.701, p = .096] trended towards a lower DEM preference score than saline infused animals at this time point. At 4 hours, males infused with SCH23390 at 1 µg/µL [t(46) = 1.773, p = .083] and 6 µg/µL [t(46) = 1.719, p = .092] showed a trend towards a lower DEM preference score than saline infused controls. Interestingly, at 4 hours, females infused SCH23390 at 1 µg/µL had a DEM preference score significantly higher than saline infused control animals [t(46) = -2.313, p = .025],
and females infused with SCH23390 at 2 µg/µL showed a trend towards a higher DEM preference score than saline infused control animals \([t(44) = -1.983, p = .054]\) at this time point. At 6 hours, only males infused with SCH23390 at 1 µg/µL had a DEM preference score significantly lower than saline control animals \([t(46) = 2.218, p = .032]\).

Additionally, one-sample \(t\)-tests comparing the DEM preference scores to 50% (chance) revealed that DEM preference scores were significantly higher than 50% at 2 hours for females \([t(23) = 4.57, p < .001]\) and males \([t(22) = 3.445, p = .002]\) infused with saline, females infused with SCH23390 at 1 µg/µL \([t(22) = 5.609, p < .001]\), females \([t(21) = 2.871, p = .009]\) and males \([t(23) = 4.042, p = .001]\) infused with SCH23390 at 2 µg/µL, males infused with SCH23390 at 4 µg/µL \([t(23) = 2.587, p = .016]\), and females \([t(21) = 5.073, p < .001]\) and males \([t(21) = 2.317, p = .031]\) infused with SCH23390 at 6 µg/µL, while females infused with SCH23390 at 4 µg/µL \([t(22) = 1.831, p = .081]\) only trended towards a higher DEM preference score at this time point. At 4 hours, the DEM preference scores were significantly higher than 50% for males infused with saline \([t(23) = 3.588, p = .002]\), females \([t(23) = 4.58, p < .001]\) and males \([t(23) = 2.07, p = .05]\) infused with SCH23390 at 1 µg/µL, females \([t(21) = 4.427, p < .001]\) and males \([t(23) = 2.58, p = .017]\) infused with SCH23390 at 2 µg/µL, females \([t(23) = 2.119, p = .045]\) and males \([t(22) = 2.32, p = .03]\) infused with SCH23390 at 4 µg/µL, and females infused with SCH23390 at 6 µg/µL \([t(23) = 2.532, p = .019]\). At 6 hours, only males infused with saline \([t(23) = 2.499, p = .02]\) had a DEM preference score significantly higher than 50%, although females infused with SCH23390 at 2 µg/µL \([t(23) = 1.937, p = .065]\) showed a trend towards a higher DEM preference score at this time point. Collectively, these results suggest that females infused with SCH23390 at 1 µg/µL (Figure 31C) may have
had an enhancement of food preferences relative to control animals, however this was likely a result of the unusually low food preference score for the saline infused control animals at 4 hours. Furthermore, while females infused with SCH23390 at 4 µg/µL (Figure 31G) and males infused with SCH23390 at 1 µg/µL (Figure 31D) had a significantly weaker food preference than saline control animals, both groups of SCH23390 treated mice still showed food preferences significantly higher than chance levels. Thus, intra-NAc SCH23390 did not block social learning at any dose, for either sex (Figure 31).
Percent of demonstrator (DEM) diet (DEM food consumed divided by the total amount of food consumed) for observer (OBS) female (A, C, E, G, I) and male (B, D, F, H, J) mice that received a single intra-nucleus accumbens microinfusion of either saline vehicle (white circles; A, B), or the dopamine (DA) D1-type receptor antagonist SCH23390 at 1 µg/µL (black triangles; C, D), 2 µg/µL (black circles; E, F), 4 µg/µL (black squares; G, H) or 6 µg/µL (black diamonds; I, J). Infusions were administered 15 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex DEM mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Percent of DEM
diets are displayed at 2, 4, 6 and 8 hours into the OBS choice test. The n’s represent the number of OBS mice in each group. Data are presented as mean ± SEM. *p < 0.05, † = 0.05 < p < 0.1, in comparison between OBS mice that received an infusion of saline vehicle versus OBS mice that received an infusion of SCH23390.  

The RM ANOVA conducted on the total food intakes of all OBS mice showed a significant main effect of time [F(3, 690) = 119.984, p < .001]. Similarly, separate ANOVAs conducted on females [F(3, 345) = 71.482, p < .001] and males [F(3, 345) = 50.43, p < .001] revealed a significant main effect of time for total food intakes (Figure 32). No significant main effects of sex or treatment, or interactions with other variables were found. Thus, there were no effects of intra-NAc SCH23390 on feeding behavior for either sex (Figure 32).
Figure 32. Total food intake (1% ground cinnamon [CIN] + 2% ground cocoa [COC]) for observer (OBS) female (A) and male (B) mice that received a single intra-nucleus accumbens microinfusion of either saline vehicle (white circles; $n = 24$ for females and $n = 24$ for males), or the dopamine (DA) D1-type receptor antagonist SCH23390 at 1 µg/µL (black triangles; $n = 24$ for females and $n = 24$ for males), 2 µg/µL (black circles; $n = 24$ for females and $n = 24$ for males), 4 µg/µL (black squares; $n = 24$ for females and $n = 24$ for males) or 6 µg/µL (black diamonds; $n = 24$ for females and $n = 24$ for males). Infusions were administered 15 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a CIN or COC flavored diet. Total food intakes are displayed at 2, 4, 6 and 8 hours into the OBS choice test. There were no significant effects of intra-nucleus accumbens SCH23390 on total food intakes for either sex. Data are presented as mean ± SEM.

There were no main effects of the estrous cycle or interactions with intra-NAc SCH23390 on food preferences or food intakes for the full 8 hours, or at separate time intervals. This was likely due to the small number of females per phase for each treatment group (Table 7).
Effects of Intra-Nucleus Accumbens SCH23390 on Behavior During the Social Interactions

Total activity (Figure 33A and B) and total social behaviors (Figure 33C and D) were reduced by SCH23390 for male and female mice. The reduction in total activity was mirrored by an increase in time spent engaging in non-social non-locomotor behaviors, such as solitary inactivity (Figure 39). While intra-NAc SCH23390 reduced agonistic-type behaviors for both sexes (including dominance scores), drug treatment effects on agonistic behaviors were greater for males (Figure 35). Social investigatory behaviors (Figure 36), including oronasal, body and anogenital investigation were reduced by SCH23390 for both sexes (summarized in Table 8 and 9).

Overall ANOVAs that included both sexes revealed a significant sex x drug interaction for non-social locomotor behaviors [F(4, 230) = 2.766, p = .028]. This effect may be explained by the significant sex x drug interaction for vertical exploration [F(4, 230) = 2.765, p = .028]. Specifically, male mice engaged in non-social active behaviors (i.e., vertical exploration) more than female mice (for more details, see baseline sex-difference analyses conducted on saline-treated mice, Figure 40).

ANOVAs showed a significant main effect of sex for total social behavior [F(1, 230) = 4.944, p = .027] and non-social behaviors [F(1, 230) = 5.204, p = .023]. Hence, both social and non-social behaviors were regulated by sex.

ANOVAs showed a significant main effect of sex [F(1, 230) = 14.335, p < .001] and sex x drug interaction [F(4, 230) = 4.714, p = .001] for the frequency of total agonistic behaviors. These effects could be partially accounted for by the significant main effect of sex [F(1, 230) = 4.155, p = .043] and sex x drug interaction [F(4, 230) = 2.976, p
for agonistic behaviors delivered. Specifically, there was a significant main effect of sex \[F(1, 230) = 30.262, p < .001\] and sex x drug interaction \[F(4, 230) = 3.187, p = .014\] for boxing. Furthermore, there was a significant main effect of sex \[F(1, 230) = 28.583, p < .001\] and sex x drug interaction \[F(4, 230) = 3.436, p = .009\] for open aggression. Moreover, there was a significant sex x drug interaction for dominant behavior \[F(4, 230) = 2.442, p = .048\]. There was also a significant main effect of sex \[F(1, 230) = 40.851, p < .001\] and sex x drug interaction \[F(4, 230) = 5.977, p < .001\] for the frequency of attacks delivered. In parallel with these effects, there was a significant main effect of sex for agonistic behaviors received \[F(1, 230) = 23.912, p < .001\]. Specifically, there was a significant main effect of sex for submissive behavior \[F(1, 230) = 32.489, p < .001\]. Moreover, there was a significant main effect of sex \[F(1, 230) = 9.421, p = .002\] and sex x drug interaction \[F(4, 230) = 2.554, p = .04\] for defensive upright posturing. There was also a significant main effect of sex for the frequency of attacks received \[F(1, 230) = 4.264, p = .04\]. Collectively, these effects led to a significant main effect of sex \[F(1, 230) = 6.114, p = .014\] and sex x drug interaction \[F(4, 230) = 3.409, p = .01\] for the dominance score. Specifically, males had a higher total agonistic behavior and dominance score frequency than females, whereby the frequency of agonistic behaviors delivered (including attacks delivered) was higher for male mice. Males also engaged in more boxing and open aggression than females. Thus, males were overall more agonistic than females (for more details, see baseline sex-difference analyses conducted on saline-treated mice, Figure 40).

There was a trend towards a main effect of sex for the frequency of social investigation \[F(1, 230) = 3.22, p = .074\]. This could be partly accounted for by the
significant main effect of sex for the frequency of anogenital investigation \([F(1, 230) = 4.027, p = .046]\). Furthermore, there was a significant main effect of sex for body investigation \([F(1, 230) = 5.19, p = .024]\). There was also a significant main effect of sex for following the DEM \([F(1, 230) = 7.001, p = .009]\), frequency of stretched approaches \([F(1, 230) = 4.676, p = .032]\), and a trend towards a main effect of sex for the frequency of approaching and/or attending to the DEM \([F(1, 230) = 3.518, p = .062]\). Specifically, females more frequently engaged in social investigation, and spent more time approaching and/or attending to the DEM, engaging in body investigation, and following the DEM for longer than males. Thus, female mice displayed more social investigatory-related behaviors than male mice (for more details, see baseline sex-difference analyses conducted on saline-treated mice, Figure 40).

There was a trend towards a main effect of sex for non-social non-locomotor behaviors \([F(1, 230) = 3.62, p = .058]\). While there were no effects on the duration data, analyses on the frequency data revealed that there was a trend towards a sex x drug interaction for the frequency of self-grooming \([F(4, 230) = 1.996, p = .096]\).

ANOVA\(s\) conducted on each sex separately revealed that female total activity was significantly decreased \([F(4, 115) = 33.438, p < .001; \text{Figure 33A}]\) by SCH23390 at all doses \([1 \mu g/\mu L: t(46) = 7.315, p < .001; 2 \mu g/\mu L: t(46) = 10.747, p < .001; 4 \mu g/\mu L: t(46) = 9.205, p < .001; 6 \mu g/\mu L: t(46) = 10.932, p < .001]\). This effect could be partly explained by the finding that female total social behavior was significantly reduced \([F(4, 115) = 9.978, p < .001; \text{Figure 33C}]\) by drug treatment at all doses tested \([1 \mu g/\mu L: t(46) = 2.248, p = .029; 2 \mu g/\mu L: t(46) = 3.423, p = .001; 4 \mu g/\mu L: t(46) = 5.758, p < .001; 6 \mu g/\mu L: t(46) = 4.072, p < .001]\).
Similarly, male total activity was significantly decreased \(F(4, 115) = 52.848, p < .001\); Figure 33B] by SCH23390 at all doses \([1 \mu g/\mu L: t(46) = 8.788, p < .001; 2 \mu g/\mu L: t(46) = 10.66, p < .001; 4 \mu g/\mu L: t(46) = 15.757, p < .001; 6 \mu g/\mu L: t(46) = 15.396, p < .001\]. This effect could be partly accounted for by the significant reduction in male total social behavior \(F(4, 115) = 6.905, p < .001\); Figure 33D] by drug treatment at all doses tested \([1 \mu g/\mu L: t(46) = 3.066, p = .004; 2 \mu g/\mu L: t(46) = 2.478, p = .017; 4 \mu g/\mu L: t(46) = 4.599, p < .001; 6 \mu g/\mu L: t(46) = 4.304, p < .001\].

**Figure 33.** Active behaviors (in sec) for observer (OBS) female [A, C] and male [B, D] mice that received a single intra-nucleus accumbens microinfusion of either saline vehicle \((n = 24\) for females and \(n = 24\) for males), or the dopamine (DA) D1-type receptor antagonist SCH23390 at \(1 \mu g/\mu L\) \((n = 24\) for females and \(n = 24\) for males), \(2 \mu g/\mu L\) \((n = 24\) for females and \(n = 24\) for males), \(4 \mu g/\mu L\) \((n = 24\) for females and \(n = 24\) for males) or \(6 \mu g/\mu L\) \((n = 24\) for females and \(n = 24\) for males). Infusions were administered 15 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Intra-nucleus accumbens SCH23390 effects on female [A] and male [B] total activity, and female [C] and male [D] total social behavior.
social behavior are shown. Data are presented as mean + SEM. *p < 0.05, **p < 0.01, ***p < 0.001, in comparison to saline infused control animals.

The overall decrease in female total activity could be partially explained by the significant reduction in female non-social locomotor behaviors \([F(4, 115) = 4.202, p = .003; \text{Figure 34A}]\) by SCH23390 at 1 µg/µL \([t(46) = 2.811, p = .007]\), 2 µg/µL \([t(46) = 3.21, p = .002]\), and 4 µg/µL \([t(46) = 2.727, p = .028]\), while 6 µg/µL \([t(46) = 1.722, p = .092]\) only trended towards a decrease. This overall reduction in non-social locomotor behaviors was partly accounted for by the significant decrease in female vertical exploration \([F(4, 115) = 4.159, p = .003; \text{Figure 34C}]\) by drug treatment at all doses \([1 \text{ µg/µL}: t(46) = 2.919, p = .005; 2 \text{ µg/µL}: t(46) = 2.802, p = .007; 4 \text{ µg/µL}: t(46) = 2.785, p = .008; 6 \text{ µg/µL}: t(46) = 2.533, p = .015]\). Furthermore, female horizontal exploration was significantly reduced \([F(4, 115) = 7.098, p < .001; \text{Figure 34E}]\) by drug treatment at all doses tested \([1 \text{ µg/µL}: t(46) = 2.885, p = .006; 2 \text{ µg/µL}: t(46) = 3.266, p = .002; 4 \text{ µg/µL}: t(46) = 2.674, p = .01; 6 \text{ µg/µL}: t(46) = 5.49, p < .001]\). Moreover, female digging was significantly increased \([F(4, 115) = 7.639, p < .001; \text{Figure 34G}]\) by SCH23390 at the highest dose only \([t(46) = -3.269, p = .002]\). Hence, intra-NAc SCH23390 reduced both female total social behavior and non-social active behaviors.

Similarly, male non-social locomotor behaviors were significantly reduced \([\chi^2(4) = 35.066, p < .001; \text{Figure 34B}]\) by SCH23390 at all doses \([1 \text{ µg/µL}: U = 115, z = -3.567, p < .001; 2 \text{ µg/µL}: U = 98, z = -3.918, p < .001; 4 \text{ µg/µL}: U = 73, z = -4.433, p < .001; 6 \text{ µg/µL}: U = 142, z = -3.010, p = .003]\). This was partially reflected in the significant decrease in male vertical exploration \([\chi^2(4) = 34.777, p < .001; \text{Figure 34D}]\) by drug treatment at all doses tested \([1 \text{ µg/µL}: U = 99, z = -3.897, p < .001; 2 \text{ µg/µL}: U = 82, z = -
4.248, p < .001; 4 µg/µL: U = 53, z = -4.846, p < .001; 6 µg/µL: U = 94, z = -4, p < .001].

Additionally, male horizontal exploration was significantly reduced [F(4, 115) = 14.608, p < .001; Figure 34F] by SCH23390 at all doses [1 µg/µL: t(46) = 3.162, p = .003; 2 µg/µL: t(46) = 3.462, p = .001; 4 µg/µL: t(46) = 4.669, p < .001; 6 µg/µL: t(46) = 5.435, p < .001]. Moreover, digging for male mice was significantly decreased [F(4, 115) = 12.259, p < .001; Figure 34H] by SCH23390 at 1 µg/µL [t(46) = 3.014, p = .004], 2 µg/µL [t(46) = 3.529, p = .001], and 4 µg/µL [t(46) = 2.772, p = .008], and significantly increased at 6 µg/µL [t(46) = -3.003, p = .004]. Thus, intra-NAc SCH23390 reduced both male and female non-social active behaviors.
Figure 34. Non-social active behaviors (in sec) for observer (OBS) female [A, C, E, G] and male [B, D, F, H] mice that received a single intra-nucleus accumbens microinfusion of either saline vehicle ($n = 24$ for females and $n = 24$ for males), or the dopamine (DA) D1-type receptor antagonist SCH23390 at 1 µg/µL ($n = 24$ for females and $n = 24$ for males), 2 µg/µL ($n = 24$ for females and $n = 24$ for males), 4 µg/µL ($n = 24$ for females and $n = 24$ for males) or 6 µg/µL ($n = 24$ for females and $n = 24$ for males). Infusions were administered 15 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Intra-nucleus accumbens SCH23390 effects on female [A] and male [B] non-social locomotor behaviors, female [C] and male [D] vertical exploration, female [E] and male [F] horizontal exploration, and female [G] and male [H] digging are shown.
Data are presented as mean + SEM. *p < 0.05, **p < 0.01, ***p < 0.001, T = .05 < p < 0.1, in comparison to saline infused control animals.

Male total agonistic behaviors were significantly reduced [F(4, 115) = 5.948, p < .001; Figure 35A] by SCH23390 at the three highest doses [2 µg/µL: t(46) = 2.296, p = .026; 4 µg/µL: t(46) = 4.066, p < .001; 6 µg/µL: t(46) = 6.325, p < .001], while the lowest dose [1 µg/µL: t(46) = 1.691, p = .098] trended towards significance. In line with this, the dominance score of male mice was significantly reduced [χ²(4) = 46.038, p < .001; Figure 35B] by drug treatment at all doses [1 µg/µL: U = 137, z = -3.114, p = .002; 2 µg/µL: U = 63, z = -4.639, p < .001; 4 µg/µL: U = 28, z = -5.361, p < .001; 6 µg/µL: U = 56, z = -4.784, p < .001]. This reduced dominance score was partially accounted for by the finding that male agonistic behaviors delivered was significantly reduced [F(4, 115) = 14.406, p < .001; Figure 35C] by SCH23390 at all doses [1 µg/µL: t(46) = 2.315, p = .025; 2 µg/µL: t(46) = 4.367, p < .001; 4 µg/µL: t(46) = 6.259, p < .001; 6 µg/µL: t(46) = 5.508, p < .001]. In parallel with this, male agonistic behaviors received were significantly increased [F(4, 115) = 4.997, p = .001; Figure 35D] by drug treatment at all doses tested [1 µg/µL: t(46) = -2.304, p = .026; 2 µg/µL: t(46) = -3.169, p = .003; 4 µg/µL: t(46) = -4.28, p < .001; 6 µg/µL: t(46) = -3.884, p < .001]. Moreover, male boxing was significantly reduced [F(4, 115) = 3.22, p = .015; Figure 35E] by SCH23390 at the three highest doses [2 µg/µL: t(46) = 2.109, p = .04; 4 µg/µL: t(46) = 3.418, p = .001; 6 µg/µL: t(46) = 3.584, p = .001]. Furthermore, dominant behavior for male mice was significantly reduced [F(4, 115) = 10.334, p < .001; Figure 35G] by drug treatment at the three highest doses [2 µg/µL: t(46) = 3.57, p = .001; 4 µg/µL: t(46) = 5.355, p < .001; 6 µg/µL: t(46) = 4.736, p < .001], while the lowest dose [1 µg/µL: t(46) = 1.951, p = .057]
only trended towards a decrease. Additionally, male open aggression was significantly reduced \( F(4, 115) = 3.478, p < .001; \) Figure 35I by SCH23390 at 4 µg/µL \( t(46) = 2.89, p = .006 \) and 6 µg/µL \( t(46) = 3.902, p < .001 \), while 2 µg/µL \( t(46) = 1.781, p = .081 \) only trended towards a decrease. The frequency of male attacks delivered was also significantly reduced \( F(4, 115) = 6.016, p < .001; \) Figure 35J by drug treatment at all doses [1 µg/µL: \( t(46) = 2.126, p = .039 \); 2 µg/µL: \( t(46) = 2.727, p = .009 \); 4 µg/µL: \( t(46) = 4.382, p < .001 \); 6 µg/µL: \( t(46) = 4.132, p < .001 \)]. In parallel with these findings, male defensive upright posturing was significantly increased \( F(4, 115) = 2.969, p = .022 \); Figure 35F] by SCH23390 at the three highest doses [2 µg/µL: \( t(46) = -2.433, p = .019 \); 4 µg/µL: \( t(46) = -2.221, p = .031 \); 6 µg/µL: \( t(46) = -2.537, p = .015 \)], while the lowest dose [1 µg/µL: \( t(46) = -1.746, p = .088 \] only trended towards an increase. Male submissive behavior was also significantly increased \( \chi^2(4) = 25.101, p < .001 \); Figure 35H] by drug treatment at all doses tested [1 µg/µL: \( U = 426, z = 2.855, p = .004 \); 2 µg/µL: \( U = 483, z = 4.025, p < .001 \); 4 µg/µL: \( U = 488, z = 4.130, p < .001 \); 6 µg/µL: \( U = 463, z = 3.612, p < .001 \)]. Hence, intra-NAc SCH23390 reduced male agonistic behaviors, and led to an increase in submissive behaviors displayed, which collectively resulted in a reduced dominance score.
Figure 35. Agonistic behaviors for observer (OBS) male mice that received a single intra-nucleus accumbens microinfusion of either saline vehicle (n = 24), or the dopamine (DA) D1-type receptor antagonist SCH23390 at 1 µg/µL (n = 24), 2 µg/µL (n = 24), 4 µg/µL (n = 24) or 6 µg/µL (n = 24). Infusions were administered 15 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Intra-nucleus accumbens SCH23390 effects on male total agonistic behaviors [A], dominance score [B], agonistic behavior delivered [C], agonistic behavior received [D], boxing [E], defensive upright posturing [F],
dominant behavior [G], submissive behavior [H], open aggression [I], and attacks delivered [J] are shown. Data are presented as mean + SEM. *p < 0.05, **p < 0.01, ***p < 0.001, T = .05 < p < 0.1, in comparison to saline infused control animals.

Male non-agonistic social behaviors were also affected by drug treatment. That is, male social inactivity was significantly increased [F(4, 115) = 6.156, p < .001] by SCH23390 at all doses [1 µg/µL: t(46) = -2.07, p = .044; 2 µg/µL: t(46) = -4.17, p < .001; 4 µg/µL: t(46) = -2.55, p = .014; 6 µg/µL: t(46) = -4.96, p < .001]. Furthermore, males approaching and/or attending to the DEM was significantly increased [F(4, 115) = 5.633, p < .001] by drug treatment at all doses tested [1 µg/µL: t(46) = -2.54, p = .015; 2 µg/µL: t(46) = -2.994, p = .004; 4 µg/µL: t(46) = -3.162, p = .003; 6 µg/µL: t(46) = -4.461, p < .001]. The frequency of male stretched approaches were also significantly increased [F(4, 115) = 7.268, p < .001] by SCH23390 at the three highest doses [2 µg/µL: t(46) = -2.904, p = .006; 4 µg/µL: t(46) = -3.795, p < .001; 6 µg/µL: t(46) = -4.559, p < .001], while the lowest dose [1 µg/µL: t(46) = -1.878, p = .067] trended towards an increase. There were also effects of drug treatment on male social investigatory-type behaviors. Specifically, male social investigation was significantly reduced [F(4, 115) = 20.474, p < .001; Figure 36B] by SCH23390 at all doses tested [1 µg/µL: t(46) = 4.36, p < .001; 2 µg/µL: t(46) = 4.906, p < .001; 4 µg/µL: t(46) = 7.177, p < .001; 6 µg/µL: t(46) = 7.256, p < .001]. This was partly accounted for by the finding that male body investigation was significantly reduced [F(4, 115) = 20.479, p < .001; Figure 36F] by SCH23390 at all doses [1 µg/µL: t(46) = 4.858, p < .001; 2 µg/µL: t(46) = 4.997, p < .001; 4 µg/µL: t(46) = 7.917, p < .001; 6 µg/µL: t(46) = 6.597, p < .001]. Additionally, male anogenital investigation was significantly reduced [χ²(4) = 49.378, p < .001; Figure
36H] by drug treatment at all doses [1 µg/µL: \( U = 93, z = -4.021, p < .001 \); 2 µg/µL: \( U = 77, z = -4.351, p < .001 \); 4 µg/µL: \( U = 31, z = -5.299, p < .001 \); 6 µg/µL: \( U = 28, z = -5.361, p < .001 \)]. It is interesting to note that while male oronasal investigation was significantly reduced [\( F(4, 115) = 3.655, p = .008 \); Figure 36D] by SCH23390 at 6 µg/µL [\( t(46) = 3.936, p < .001 \)], this dose did not affect social learning. In line with these effects, males following the DEM was also significantly reduced [\( F(4, 115) = 20.369, p < .001 \); Figure 36J] by SCH23390 at all doses [1 µg/µL: \( t(46) = 2.591, p = .013 \); 2 µg/µL: \( t(46) = 4.957, p < .001 \); 4 µg/µL: \( t(46) = 5.468, p < .001 \); 6 µg/µL: \( t(46) = 4.923, p < .001 \)]. Thus, intra-NAc SCH23390 reduced many non-agonistic social behaviors for males.

Drug treatment affected many non-agonistic social behaviors in females. That is, female social inactivity was significantly increased [\( F(4, 115) = 6.924, p < .001 \)] by SCH23390 at 1 µg/µL [\( t(46) = -4.471, p < .001 \)], 2 µg/µL [\( t(46) = -3.871, p < .001 \)] and 6 µg/µL [\( t(46) = -6.182, p < .001 \)], while 4 µg/µL [\( t(46) = -1.804, p = .078 \)] only trended towards an increase. Additionally, females approaching and/or attending to the DEM was significantly increased [\( F(4, 115) = 5.436, p < .001 \)] by SCH23390 at the highest dose only [\( t(46) = -2.96, p = .005 \)]. Similarly, the frequency of female stretched approaches were significantly increased [\( F(4, 115) = 6.392, p < .001 \)] by drug treatment at 6 µg/µL [\( t(46) = -4.089, p < .001 \)], while 4 µg/µL [\( t(46) = -1.892, p = .065 \)] only trended towards an increase. The reduction in female total social behaviors may be partially accounted for by the significant decrease in female social investigation [\( F(4, 115) = 21.166, p < .001 \); Figure 36A] by SCH23390 at all doses tested [1 µg/µL: \( t(46) = 4.938, p < .001 \); 2 µg/µL: \( t(46) = 6.95, p < .001 \); 4 µg/µL: \( t(46) = 8.822, p < .001 \); 6 µg/µL: \( t(46) = 7.665, p < .001 \)].
This was reflected by a significantly reduction in female body investigation \[F(4, 115) = 20.671, p < .001; \text{Figure 36E}\] by all doses of drug treatment \[1 \mu\text{g}/\mu\text{L}: t(46) = 5.251, p < .001; 2 \mu\text{g}/\mu\text{L}: t(46) = 6.778, p < .001; 4 \mu\text{g}/\mu\text{L}: t(46) = 7.928, p < .001; 6 \mu\text{g}/\mu\text{L}: t(46) = 6.812, p < .001\]. Furthermore, female anogenital investigation was significantly reduced \[\chi^2(4) = 48.244, p < .001; \text{Figure 36G}\] by SCH23390 at all doses tested \[1 \mu\text{g}/\mu\text{L}: U = 58, z = -4.743, p < .001; 2 \mu\text{g}/\mu\text{L}: U = 37, z = -5.176, p < .001; 4 \mu\text{g}/\mu\text{L}: U = 9, z = -5.753, p < .001; 6 \mu\text{g}/\mu\text{L}: U = 33, z = -5.258, p < .001\]. It is interesting to note that oronasal investigation was significantly reduced \[F(4, 115) = 2.544, p = .043; \text{Figure 36C}\] by SCH23390 at 6 \mu\text{g}/\mu\text{L} \[t(46) = 4.158, p < .001\], and 4 \mu\text{g}/\mu\text{L} \[t(46) = 1.824, p = .075\] trended towards a decrease, however, female social learning was unaffected by drug treatment. In line with these results, following the DEM for females was significantly reduced \[F(4, 115) = 12.634, p < .001; \text{Figure 36I}\] by the three highest doses of drug treatment \[2 \mu\text{g}/\mu\text{L}: t(46) = 4.751, p < .001; 4 \mu\text{g}/\mu\text{L}: t(46) = 5.312, p < .001; 6 \mu\text{g}/\mu\text{L}: t(46) = 5.511, p < .001\] while the lowest dose \[1 \mu\text{g}/\mu\text{L}: t(46) = 1.961, p = .056\] only trended towards a reduction. Thus, various female non-agonistic social behaviors were affected by intra-NAc SCH23390.
Figure 36. Social investigatory behaviors (in sec) for observer (OBS) female [A, C, E, G, I] and male [B, D, F, H, J] mice that received a single intranucleus accumbens microinfusion of either saline vehicle \( (n = 24 \text{ for females and } n = 24 \text{ for males}) \), or the dopamine (DA) D1-type receptor antagonist SCH23390 at 1 µg/µL \( (n = 24 \text{ for females and } n = 24 \text{ for males}) \), 2 µg/µL \( (n = 24 \text{ for females and } n = 24 \text{ for males}) \), 4 µg/µL \( (n = 24 \text{ for females and } n = 24 \text{ for males}) \) or 6 µg/µL \( (n = 24 \text{ for females and } n = 24 \text{ for males}) \). Infusions were administered 15 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored...
diet. Intra-nucleus accumbens SCH23390 effects on female [A] and male [B] social investigation, female [C] and male [D] oronasal investigation, female [E] and male [F] body investigation, female [G] and male [H] anogenital investigation, and female [I] and male [J] following the DEM are shown. Data are presented as mean ± SEM. *p < 0.05, ***p < 0.001, T = .05 < p < 0.1, in comparison to saline infused control animals.

Female agonistic behaviors were also influenced by SCH23390. Specifically, female total agonistic behaviours were significantly reduced [F(4, 115) = 3.76, p = .007; Figure 37A] by SCH23390 at 6 µg/µL [t(46) = 3.534, p = .001], while 4 µg/µL [t(46) = 1.857, p = .07] only trended towards a decrease. In line with these findings, the dominance score of females was significantly reduced [χ²(4) = 48.283, p < .001; Figure 37B] by drug treatment at all doses tested [1 µg/µL: U = 68, z = -4.536, p < .001; 2 µg/µL: U = 32, z = -5.279, p < .001; 4 µg/µL: U = 15, z = -5.629, p < .001; 6 µg/µL: U = 22, z = -5.485, p < .001]. This could be partly explained by the finding that female agonistic behaviors delivered were significantly reduced [χ²(4) = 61.303, p < .001; Figure 37C] by SCH23390 at all doses [1 µg/µL: U = 91, z = -4.062, p < .001; 2 µg/µL: U = 59, z = -4.722, p < .001; 4 µg/µL: U = 14, z = -5.663, p < .001; 6 µg/µL: U = 5, z = -5.837, p < .001]. Specifically, female dominant behavior was significantly reduced [χ²(4) = 50.610, p < .001; Figure 37E] by all doses of drug treatment [1 µg/µL: U = 94, z = -4, p < .001; 2 µg/µL: U = 101, z = -3.860, p < .001; 4 µg/µL: U = 35, z = -5.229, p < .001; 6 µg/µL: U = 22, z = -5.493, p < .001]. In line with these findings, female agonistic behaviors received were significantly increased [F(4, 115) = 7.244, p < .001; Figure 37D] by drug treatment at all doses tested [1 µg/µL: t(46) = -3.997, p < .001; 2 µg/µL: t(46) = -5.304, p < .001; 4 µg/µL: t(46) = -5.595, p < .001; 6 µg/µL: t(46) = -4.514, p < .001].
This effect could be accounted for by the finding that female submissive behavior was significantly increased [$\chi^2(4) = 34.877, p < .001$; Figure 37F] by all doses of SCH23390 [1 µg/µL: $U = 489, z = 4.145, p < .001$; 2 µg/µL: $U = 525, z = 4.887, p < .001$; 4 µg/µL: $U = 519, z = 4.763, p < .001$; 6 µg/µL: $U = 469, z = 3.732, p < .001$]. Thus, intra-NAc SCH23390 reduced many female agonistic behaviors, and increased submissive behaviors displayed, which collectively led to a reduced dominance score.

**Figure 37.** Agonistic behaviors (in sec) for observer (OBS) female mice that received a single intra-nucleus accumbens microinfusion of either saline vehicle ($n = 24$), or the dopamine (DA) D1-type receptor antagonist SCH23390 at 1 µg/µL ($n = 24$), 2 µg/µL ($n = 24$), 4 µg/µL ($n = 24$) or 6 µg/µL ($n = 24$). Infusions were administered 15 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Intra-nucleus accumbens SCH23390 effects on female total
agonistic behaviors [A], dominance score [B], agonistic behavior delivered [C], agonistic behavior received [D], dominant behavior [E], and submissive behavior [F] are shown. Data are presented as mean + SEM. **p < 0.01, ***p < 0.001, T = .05 < p < 0.1, in comparison to saline infused control animals.

The overall decrease in female total activity was reflected by a significant increase in female non-social behaviors [F(4, 115) = 8.991, p < .001; Figure 38A] by SCH23390 at all doses [1 µg/µL: t(46) = -2.04, p = .047; 2 µg/µL: t(46) = -3.272, p = .002; 4 µg/µL: t(46) = -5.32, p < .001; 6 µg/µL: t(46) = -3.891, p < .001].

Likewise, male non-social behaviors were significantly increased [F(4, 115) = 6.207, p < .001; Figure 38B] by drug treatment at all doses tested [1 µg/µL: t(46) = -2.92, p = .005; 2 µg/µL: t(46) = -2.265, p = .028; 4 µg/µL: t(46) = -4.511, p < .001; 6 µg/µL: t(46) = -3.727, p = .001].

**Figure 38.** Non-social behaviors (in sec) for observer (OBS) female [A] and male [B] mice that received a single intranucleus accumbens microinfusion of either saline vehicle (n = 24 for females and n = 24 for males), or the dopamine (DA) D1-type receptor antagonist SCH23390 at 1 µg/µL (n = 24 for females and n = 24 for males), 2 µg/µL (n = 24 for females and n = 24 for males), 4 µg/µL (n = 24 for females and n = 24 for males) or 6 µg/µL (n = 24 for females and n = 24 for males). Infusions were administered 15 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire
a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Data are presented as mean + SEM. *p < 0.05, **p < 0.01, ***p < 0.001, in comparison to saline infused control animals.

The reduction in female total activity was also reflected by the significant increase in female non-social non-locomotor behaviors \[\chi^2(4) = 46.154, p < .001; \text{Figure 39A}\] by SCH23390 at all doses [1 µg/µL: \(U = 500, z = 4.371, p < .001; 2 \mu g/µL: U = 538, z = 5.155, p < .001; 4 \mu g/µL: U = 543, z = 5.258, p < .001; 6 \mu g/µL: U = 521, z = 4.804, p < .001\]. This can be accounted for by the significantly increase in female solitary inactivity \[\chi^2(4) = 55.742, p < .001; \text{Figure 39C}\] by drug treatment at all doses tested [1 µg/µL: \(U = 522, z = 4.825, p < .001; 2 \mu g/µL: U = 555, z = 5.505, p < .001; 4 \mu g/µL: U = 558, z = 5.567, p < .001; 6 \mu g/µL: U = 548, z = 5.361, p < .001\]. In addition, female self-grooming was significantly reduced \[F(4, 115) = 3.451, p = .011; \text{Figure 39E}\] by SCH23390 at the three highest doses [2 µg/µL: \(t(46) = 3.057, p = .004; 4 \mu g/µL: t(46) = 2.724, p = .009; 6 \mu g/µL: t(46) = 3.138, p = .003\], while the lowest dose [1 µg/µL: \(t(46) = 1.962, p = .056\)] only trended towards a decrease.

Similarly, male non-social non-locomotor behaviors were significantly increased \[\chi^2(4) = 60.719, p < .001; \text{Figure 39B}\] by SCH23390 at all doses [1 µg/µL: \(U = 557, z = 5.547, p < .001; 2 \mu g/µL: U = 568, z = 5.774, p < .001; 4 \mu g/µL: U = 574, z = 5.897, p < .001; 6 \mu g/µL: U = 559, z = 5.588, p < .001\]. This was accounted for by the significant increase in male solitary inactivity \[\chi^2(4) = 66.929, p < .001; \text{Figure 39D}\] by drug treatment at all doses [1 µg/µL: \(U = 562, z = 5.650, p < .001; 2 \mu g/µL: U = 576, z = 5.938, p < .001; 4 \mu g/µL: U = 576, z = 5.938, p < .001; 6 \mu g/µL: U = 572, z = 5.856, p < .001\].
Additionally, male self-grooming was significantly reduced \([F(4, 115) = 11.488, p < .001; \text{Figure 39F}]\) by SCH23390 at the three highest doses \([2 \mu g/\mu L: t(46) = 3.586, p = .001; 4 \mu g/\mu L: t(46) = 5.643, p < .001; 6 \mu g/\mu L: t(46) = 5.949, p < .001]\).

**Figure 39.** Non-social non-active behaviors (in sec) for observer (OBS) female [A, C, E] and male [B, D, F] mice that received a single intra-nucleus accumbens microinfusion of either saline vehicle \((n = 24\) for females and \(n = 24\) for males), or the dopamine (DA) D1-type receptor antagonist SCH23390 at 1 \(\mu g/\mu L\) \((n = 24\) for females and \(n = 24\) for males), 2 \(\mu g/\mu L\) \((n = 24\) for females and \(n = 24\) for males), 4 \(\mu g/\mu L\) \((n = 24\) for females and \(n = 24\) for males) or 6 \(\mu g/\mu L\) \((n = 24\) for females and \(n = 24\) for males). Infusions were administered 15 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1\% ground cinnamon (CIN) or 2\% ground cocoa (COC) flavored diet. Intra-nucleus accumbens SCH23390 effects on female [A] and male [B] non-social non-locomotor behaviors, female [C] and male [D] solitary inactivity, and female [E]
and male [F] self-grooming are shown. Data are presented as mean ± SEM. **p < 0.01, ***p < 0.001, T = .05 < p < 0.1, in comparison to saline infused control animals.

No other significances were found. In particular, intra-NAc SCH23390 did not produce any abnormal stereotypic behaviors for either sex.

There were no main effects of the estrous cycle, or interactions with intra-NAc SCH23390 for any single or grouped behaviors, which was likely due to the small number of mice at each phase, per treatment group (Table 7).

**Table 7.** Sample sizes (n’s) for each estrous phase, per treatment group, for the social transmission of food preferences (STFP) intra-NAc SCH23390 experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proestrus</th>
<th>Estrus</th>
<th>Diestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>n = 6</td>
<td>n = 7</td>
<td>n = 11</td>
</tr>
<tr>
<td>SCH23390 at 1 µg/µL</td>
<td>n = 5</td>
<td>n = 9</td>
<td>n = 10</td>
</tr>
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<td>SCH23390 at 2 µg/µL</td>
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<td>n = 4</td>
<td>n = 16</td>
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<td>SCH23390 at 4 µg/µL</td>
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<td>n = 7</td>
</tr>
<tr>
<td>SCH23390 at 6 µg/µL</td>
<td>n = 5</td>
<td>n = 8</td>
<td>n = 11</td>
</tr>
</tbody>
</table>
Table 8. Summarized intra-NAC SCH23390 effects on grouped behaviors during the 30 min social interactions for the STFP experiment.

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
<th>Saline treated sex difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Activity</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
</tr>
<tr>
<td>Total Social Behavior</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
</tr>
<tr>
<td>Agonistic Behavior Delivered</td>
<td>↓</td>
<td>↓</td>
<td>↑ [F] Females &lt; Males</td>
</tr>
<tr>
<td>Agonistic Behavior Received</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Total Agonistic Behaviors</td>
<td>↓</td>
<td>↓</td>
<td>↑ [F] Females &lt; Males</td>
</tr>
<tr>
<td>Dominance Score</td>
<td>↓</td>
<td>↓</td>
<td>↑ [F] Females &lt; Males</td>
</tr>
<tr>
<td>Social Investigation</td>
<td>↓</td>
<td>↓</td>
<td>↑ [F][T] Females &gt; Males</td>
</tr>
<tr>
<td>Non-social Behaviors</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Non-social Locomotor Behaviors</td>
<td>↓</td>
<td>↓</td>
<td>↑ [T] Females &lt; Males</td>
</tr>
<tr>
<td>Non-social Non-locomotor Behaviors</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
</tbody>
</table>

— no significant drug effect; ↓ significant decrease; ↑ significant increase; “T” indicates statistical trend (.05 < p < 0.1). Unless otherwise specified with an “F” (which indicates frequency effect), all effects are on duration.
Table 9. Summarized intra-NAC SCH23390 effects on single behaviors during the 30min social interactions for the STFP experiment.

<table>
<thead>
<tr>
<th>Social Behaviors</th>
<th>Females</th>
<th>Males</th>
<th>Saline treated sex difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Following the DEM</td>
<td>↓</td>
<td>↓</td>
<td>[T] Females &gt; Males</td>
</tr>
<tr>
<td>Dominant Behavior</td>
<td>↓</td>
<td>↓</td>
<td>[T] Females &gt; Males</td>
</tr>
<tr>
<td>Attacks Delivered</td>
<td>—</td>
<td>[F]↓</td>
<td>[F] Females &lt; Males</td>
</tr>
<tr>
<td>Boxing</td>
<td>—</td>
<td>↓</td>
<td>Females &lt; Males</td>
</tr>
<tr>
<td>Open Aggression</td>
<td>—</td>
<td>↓</td>
<td>Females &lt; Males</td>
</tr>
<tr>
<td>Avoidance of DEM</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Submissive Behavior</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Attacks Received</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Defensive Upright Posturing</td>
<td>—</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Social Inactivity</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Oronasal Investigation</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
</tr>
<tr>
<td>Body Investigation</td>
<td>↓</td>
<td>↓</td>
<td>[T] Females &gt; Males</td>
</tr>
<tr>
<td>Anogenital investigation</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
</tr>
<tr>
<td>Stretched Approach</td>
<td>[F]↑</td>
<td>[F]↑</td>
<td>—</td>
</tr>
<tr>
<td>Approaching and/or Attending to the DEM</td>
<td>↑</td>
<td>↑</td>
<td>Females &gt; Males</td>
</tr>
<tr>
<td>Non-social Behaviors</td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Horizontal Exploration</td>
<td>↓</td>
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<td>—</td>
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<tr>
<td>Vertical Exploration</td>
<td>↓</td>
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<td>Females &lt; Males</td>
</tr>
<tr>
<td>Digging</td>
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<td>↑↓</td>
<td>—</td>
</tr>
<tr>
<td>Abnormal Stereotypies</td>
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<td>—</td>
</tr>
<tr>
<td>Solitary Inactivity</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Self-Grooming</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
</tr>
</tbody>
</table>

— no significant drug effect; ↓ significant decrease; ↑ significant increase; “T” indicates statistical trend (.05 < p < 0.1). Unless otherwise specified with an “F” (which indicates frequency effect), all effects are on duration.
Baseline Sex Differences Among Saline Infused Mice During the Social Interactions in the STFP

Male mice were generally more agonistic (e.g., more boxing and open aggression) and engaged in more non-social active behaviors (i.e., vertical exploration) than females, who on the other hand, engaged in more social investigatory-type behaviors (e.g., body investigation) than males (Figure 40; Table 8 and 9).

Independent samples t-tests conducted on saline-infused males versus saline infused females revealed a trend towards males engaging in non-social locomotor behaviors longer than females \( t(46) = -1.841, p = .072 \). This may be explained by the finding that males engaged in vertical exploration for significantly longer than females \( U = 391, z = 2.124, p = .034 \); Figure 40A]. Hence, males treated with saline engaged in non-social active behaviors more than saline treated females.

The frequency of total agonistic behaviors \( U = 464, z = 3.629, p < .001 \); Figure 40B] and dominance score frequency \( t(46) = -3.673, p = .001 \); Figure 40B] was significantly higher for males. This may be explained by the finding that the frequency of agonistic behaviors delivered was significantly greater for males than females \( U = 432, z = 2.970, p = .003 \); Figure 40B]. Specifically, the frequency of attacks delivered was significantly greater for males \( t(46) = -5.302, p < .001 \); Figure 40B]. In addition, boxing \( U = 503.5, z = 4.750, p < .001 \); Figure 40A] and open aggression \( U = 508.5, z = 4.957, p < .001 \); Figure 40A] was significantly greater for males. Interestingly, there was a trend towards more dominant behavior for females \( t(46) = 1.961, p = .056 \]. Hence, male mice treated with saline displayed more overt agonistic behaviors than females treated with saline.
Approaching and/or attending to the DEM was significantly greater for females than males \([t(46) = 2.215, p = .032; \text{Figure } 40A]\). Furthermore, the social investigation frequency trended towards being greater for females \([t(46) = 1.712, p = .094]\). This may be partly explained by the finding that body investigation trended towards being greater for females \([t(46) = 1.969, p = .055]\). In addition, there was a trend towards females following the DEM longer than males \([t(46) = 1.842, p = .072]\). Hence, females treated with saline engaged in social investigatory-type behaviors more than males treated with saline.
Figure 40. Baseline sex differences for observer (OBS) mice that received a single intra-nucleus accumbens microinfusion of saline vehicle ($n = 24$ for females [black bars] and $n = 24$ for males [white bars]). Infusions were administered 15 minutes prior to a 30 minute social interaction where OBS mice had the opportunity to acquire a food preference from a same-sex demonstrator (DEM) mouse that recently consumed either a 1% ground cinnamon (CIN) or 2% ground cocoa (COC) flavored diet. Sex differences are shown for various grouped and single behaviors for duration [A] and frequency [B] data. “Non-Soc Loco” stands for non-social locomotor behaviors; “Follow” stands for following the DEM; “Dominant” stands for dominant behavior; “Open Aggr” stands for open aggression; “Body Inves” stands for body investigation; “Appr Attn” stands for approaching and/or attending to the DEM; “Vertical” stands for vertical exploration; “Ago Del” stands for agonistic behavior delivered; “Tot Ago” stands for total agonistic behaviors; “Dom Score” stands for dominance score; “Soc Inves” stands for social investigation; “Attacks Del” stands for attacks delivered. Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, $T = 0.05 < p < 0.1$, saline infused females versus saline infused males.
DISCUSSION

Effects of Intra-Nucleus Accumbens SCH23390 on Social Learning and Food Intake.

This study found that SCH23390 infused into the NAc shell did not affect social learning (Figure 30 and 31) or food intake (Figure 32) in either male or female mice. However, drug treatment regulated many social and non-social behaviors during the 30 minute social interactions in both sexes (summarized in Table 8 and 9). There were no effects of the estrous cycle.

Analyses on the CIN preference scores (Figure 30) revealed while females infused with SCH23390 at 4 µg/µL (Figure 30G) and males infused with SCH23390 at 1 µg/µL (Figure 30D) displayed no food preference at 2, 6 and 8 hours, this was not a full block of social learning, since these mice still displayed a socially acquired food preference at 4 hours. Paradoxically, analyses on the DEM preference scores (Figure 31) revealed that OBS females treated with SCH23390 at 1 µg/µL (Figure 31C) had a higher food preference than saline control animals at 4 hours into the choice test, however it should be noted that this was likely simply a result of the unusually low DEM preference score for the control mice at this time point. Indeed, usually vehicle treated mice maintain a high value DEM preference score up to 4 (or even 6) hours into the test (as seen in Matta et al., 2017). Analyses on the DEM preference scores further showed that while OBS females infused with SCH23390 at 4 µg/µL (Figure 31G) and males infused with SCH23390 at 1 µg/µL (Figure 31D) had lower food preferences than control animals, both of these groups treated with SCH23390 still showed food preferences higher than chance levels. Hence, the results on the DEM preference scores were in parallel with the
results of the CIN preference scores. Taken together, these results suggest that intra-NAc SCH23390 may have weakened the socially transmitted food preference, but did not fully block it in either males nor females. Thus, DA D1-type receptors in the NAc either play a small role, or no role in social learning in the STFP.

NAc DA plays a prominent role in processing incentive salience associated with motivationally relevant behaviors (Berridge, 2007; Floresco, 2015; Kelley, 2004; Salamone & Correa, 2012). It is therefore surprising that we found no effects of intra-NAc SCH23390 on social learning in this study. However, these results are consistent with a study conducted by Malkusz et al., 2012, which found that SCH23390 (or even Raclopride) infused into the shell of the NAc did not impair the acquisition of fructose-conditioned flavor preferences (Malkusz et al., 2012). Hence, NAc DA D1-type receptors are not involved in either socially acquired food preferences, nor individually acquired food preferences for certain palatable foods, which suggests that in the two types of learning, NAc DA D1-type receptors are similarly not involved.

Our findings that social learning was not affected by DA D1-type receptor antagonism indicates that the NAc is not one of the brain regions of action underlying the results of Choleris et al., 2011 with systemic treatments. It could be argued that had we used another dose range of SCH23390, or employed a different delay period we would have seen a social learning impairment. Such assumptions warrant further research into the dopaminergic mediation of social learning.

Intra-NAc SCH23390 did not influence male or female total food intakes (Figure 32). Such findings may be generalizable since other studies also find that blocking NAc shell DA D1-type receptors does not affect total food intake on standard laboratory
rodent food in hungry rats (Baldo et al., 2002). Moreover, these results are consistent with our previous studies with systemic (Choleris et al., 2011) and hippocampal administrations (Matta et al., 2017) showing no effects of DA D1-type receptor antagonism on food intake in the STFP. Hence, NAc DA D1-type receptors are not involved in feeding behavior in mice within the context of socially learned food preferences.

It is interesting to note that while oronasal investigation was reduced by the highest dose of SCH23390 for both sexes (Figure 36C and D), social learning was unaffected by this dose. As shown by Clipperton et al., 2008, there is no correlation between oronasal investigation and the strength of a socially learned food preference. As discussed by Choleris et al., 2011, acquiring a food preference in the STFP may only require a certain amount of exposure to the socially carried food odor.

There were no effects of the estrous cycle or interactions with intra-NAc SCH23390 on either social learning or total food intake. These results are surprising given that DA release in the NAc fluctuates over the rat estrous cycle (Thompson & Moss, 1997), and mouse STFP and feeding is regulated by the estrous cycle (Choleris et al., 2011). This can be attributed to the low number of females for each phase, per dose of SCH23390 (see Table 7). Different than the current study, the experiment conducted by Choleris et al., 2011 using systemic treatments (but not the Matta et al., 2017 study using hippocampal administrations) found an interaction between SCH23390 and the estrous cycle on the STFP, although further mean comparisons did not reveal any significances, which was (again) likely due to the small number of females for each phase, per dose.
Clearly, future research on the role of female gonadal hormones and their interactions with mesolimbic DA in the mediation of the STFP is required.

Collectively, this study finds that DA D1-type receptors in the NAc do not regulate social learning or food intake in male and female mice. It is possible that hippocampal DA and its receptors play a fairly strong role in the mediation of social learning in the STFP in mice, and other brain regions (including the NAc) may not be involved.

**Effects of Intra-Nucleus Accumbens SCH23390 on Behavior during the Social Interactions.**

Intra-NAc SCH23390 reduced both total activity (Figure 33A and B) and total social behaviors (Figure 33C and D) for males and females. The reduced total activity was paralleled by an increase in non-social non-locomotor behaviors displayed, including solitary inactivity (Figure 39). While SCH23390 effects on agonistic behaviors were more pronounced for male mice (Figure 35), drug treatment decreased agonistic behaviors, increased submissive behaviors, and led to a negative dominance score for both sexes (Figure 35B and Figure 37B). Social investigation, including oronasal, body and anogenital investigation was reduced by drug treatment (Figure 36) for both OBS males and females towards a familiar DEM (summarized in Table 8 and 9).

There were effects of drug treatment on social behaviors for both males and females, whereby total social behavior was reduced for both sexes (Figure 33C and D).
With regards to male social behaviors, we found that male total agonistic behaviors (Figure 35A) were reduced by intra-NAc SCH23390. This effect was partially accounted for by the reduced agonistic behaviors delivered (Figure 35C), including dominant behavior (Figure 35G), attacks delivered (Figure 35J), boxing (Figure 35E), and open aggression (Figure 35I). In direct parallel with these effects, there was an increase in male agonistic behaviors received (Figure 35D), including submissive behavior (Figure 35H) and defensive upright posturing (Figure 35F). Collectively, this reduction in agonistic behaviors and increased submissive behaviors for males led to a negative dominance score (Figure 35B). Certain non-agonistic social behaviors were also affected by intra-NAc SCH23390 for males. Specifically, male social inactivity, approaching and/or attending to the DEM, and stretched approaches were increased. In addition, there were effects of drug treatment on many social investigatory-related behaviors for males. That is, male social investigation was reduced (Figure 36B), which was reflected by the decrease in body (Figure 36F) and anogenital investigation (Figure 36H), as well as following the DEM (Figure 36J). Interestingly, as mentioned, male oronasal investigation (Figure 36D) was reduced by SCH23390 at 6 µg/µL, however, this dose did not affect social learning. Thus, NAc DA D1-type receptors mediate many social behaviors in interactions between adult males that were familiar with each other, including agonistic and social investigatory-type behaviors.

Various non-agonistic social behaviors were affected by intra-NAc SCH23390 in females. Specifically, social inactivity, approaching and/or attending to the DEM, and stretched approaches were increased. Additionally, there was a decrease in female social investigation (Figure 36A), including a reduction in body (Figure 36E) and anogenital
investigation (Figure 36G), as well as following the DEM (Figure 36I). Notably, oronasal investigation (Figure 36C) was reduced by SCH23390 at 6 µg/µL, although social learning was unaffected by drug treatment. Some agonistic behaviors for females were also affected by intra-NAc SCH23390. Specifically, female total agonistic behaviors (Figure 37A) were reduced, which could be partly explained by the reduced agonistic behaviors delivered (Figure 37C), including dominant behavior (Figure 37E). In parallel with these effects, there was an increase in agonistic behaviors received (Figure 37D), including submissive behavior (Figure 37F). This reduction in agonistic behaviors and increased submissive behaviors displayed collectively resulted in a negative dominance score (Figure 37B) for female mice. Hence, similar to males, NAc DA D1-type receptors regulate agonistic and social investigatory behaviors in adult female mice towards a familiar same-sex conspecific.

NAc DA D1-type receptors have been shown to be involved in mediating many complex social behaviors. For instance, SCH23390 infused into the NAc shell regulates the prosocial optogenetic effects of adult female social interactions towards a novel (same-sex) conspecific (Gunaydin et al., 2014). Additionally, social play behavior is reduced by infusing SCH23390 into the NAc in juvenile male rats that were highly motivated to play with a familiar same-sex animal as a result of being socially isolated beforehand (Manduca et al., 2016). Furthermore, intra-NAc SCH23390 increases social approach behavior in adult female mice exposed to consecutive social defeat by a familiar same-sex aggressor (Campi et al., 2014). The results of this study on social behaviors between adult familiar same-sex animals are highly consistent with those
above, and add social interactions in the STFP as another social situation that is regulated by NAc DA D1-type receptors.

Drug treatment resulted in negative dominance scores for OBS males (Figure 35B) and females (Figure 37B), which indicates that they were the subordinate animals of the pair. These effects on agonistic behaviors are in line with those of other studies, which find that infusing SCH23390 into the NAc eliminates monogamy-related aggression in male prairie voles towards unfamiliar females (Aragona et al., 2006) and reduces aggression in a resident-intruder task in male mice (Couppis & Kennedy, 2008). On the other hand, infusing a DA D1-type receptor agonist into the NAc facilitates social dominance in male rats (Kooij et al., 2018). In parallel with these results, we have now shown an involvement of NAc DA D1-type receptors in dominant and subordinate behaviors towards a familiar same-sex animal within the context of the STFP.

The overall decrease in total activity for both sexes (Figure 33A and B) could be partially explained by the reduction in non-social locomotor behaviors, including vertical and horizontal exploration (Figure 34). Interestingly, while female (Figure 34G) and male (Figure 34H) digging was increased by SCH23390 at the highest dose, digging for males was actually decreased by the three lowest doses. The reduction in total activity was also reflected by the increase in non-social non-locomotor behaviors, including solitary inactivity, while self-grooming was reduced for both sexes (Figure 39). Additionally, there was an increase in non-social behaviors (Figure 38) for males and females. The reduced total activity levels found here are not surprising, given that NAc DA D1-type receptors have been shown to regulate locomotor activity (Dreher & Jackson, 1989; Essman et al., 1993; Smith-Roe & Kelley, 2000). It should be noted that in the current
study, intra-NAc SCH23390 infusions did not produce any abnormal stereotypic behaviors, which has been reported at higher doses (Cooper & Al-Naser, 2006). The fact that this study found no effects of drug treatment on social learning (Figure 30 and 31) or feeding behavior (Figure 32), but found numerous effects of SCH23390 on the social interactions highlights the advantages of performing a thorough ethological analysis, as this allowed us to delineate between effects on socially learned food preferences versus effects on locomotion as well as specific social and non-social behaviors. Collectively, these effects suggest that the doses of SCH23390 used in the current study were not simply too low for the STFP, but rather these effects may be specific to social interactions, and the STFP is truly not dependent upon NAc DA D1-type receptors.

Few studies have examined sex differences in NAc DA signaling (Trainor, 2011). Andersen and Teicher, 2000 found that male rats show more pronounced DA D1 receptor binding in the NAc than female rats, however, this effect is not always found, as Ferris et al., 2007 found no sex differences in NAc DA D1 receptor binding in adult rat brains. This study found no sex differences in SCH23390 treated mice during the social interactions in the STFP other than the finding that open aggression, boxing, and attacks delivered were reduced, while defensive upright posturing was increased for males (Figure 35), while none of these behaviors were affected by drug treatment in females. This may be simply explained by a floor effect for these overt agonistic behaviors in females (see results on baseline sex differences, Figure 40), given that M. musculus female aggression levels are low to begin with (Jacobson-Pick et al., 2013). The results of the current study are in line with those of Campi et al., 2014, which found no sex differences in DA D1 receptor expression in the NAc in association with social defeat in
mice. The findings of the present study are different than our previous dorsal hippocampal work that finds clear sex differences during the social interactions in response to DA D1- and D2-type receptor antagonists, whereby males show a greater reduction in agonistic-type behaviors, while females show a greater reduction in social investigatory-type behaviors (Matta et al., 2017, see also Chapter 2 in this thesis). DA D1- and D2-type receptor antagonism in the dorsal hippocampus therefore reduces the behaviors that each sex was already largely engaged in to begin with. These results suggest a hippocampal dependent pattern of sex-dependent effects on the social interactions in the STFP. The NAc may therefore not be involved in the regulation of such sex differences, and highlights the selective importance of hippocampal DA receptors in the mediation of sexually dimorphic social behaviors in the STFP in mice.

To the best of our knowledge, this is the first study to implicate a role for the NAc in social interactions within the context of the STFP in familiar male and female OBS mice. This study emphasizes the importance of performing a comprehensive ethological analysis, as we have shown that NAc DA D1-type receptors mediate social and non-social behaviors, but not social learning or feeding behavior in males and females. This study also highlights the importance of brain region comparisons (e.g., dorsal hippocampus versus NAc) in the specificity of dopaminergic effects on social behaviors.
CHAPTER 5: General Discussion
Summary of Key Findings.

This thesis has shown, for the first time (to the best of our knowledge), that antagonizing DA D2-type receptors in the dorsal hippocampus blocks female, but not male social learning in the STFP (see Chapter 2). Blocking hippocampal DA D2-type receptors also sex-dependently mediated the social interactions, whereby males had a greater reduction in agonistic-type behaviors, while females had a greater reduction in social investigatory-type behaviors. The sex-dependent social learning impairment could not be accounted for by changes in total food intake, or reduced exposure to the socially transmitted diet odor found on the breath of the DEM. An olfaction control study also revealed that the social learning blockade could not be explained by changes in olfactory discrimination between the two foods used during the choice test. It is therefore likely that the effects of hippocampal DA D2-type receptor blockade were on learning and memory processes, within the context of social learning.

Using in vivo microdialysis, Chapter 3 showed that dorsal hippocampal DA release mainly changed in accordance to novelty in a sexually dimorphic manner: DA levels increased in males, and declined in females. Furthermore, this sex difference in hippocampal DA release found within the context of the STFP could not be accounted for by differences in exposures to the socially transmitted diet transferred by the DEM, since there were no differences in oronasal investigations between males and females. I propose that this sex difference is mediated by differences in DA transmission, whereby female mice may rely more on tonic firing of DA neurons from the VTA to the dorsal hippocampus, whereas male mice may rely more on phasic firing (discussed below).
Chapter 4 showed that infusing a DA D1-type receptor antagonist (SCH23390) into the shell of the NAc did not affect social learning or food intake in the STFP in male and female mice. However, there were many effects of drug treatment on the social interactions, including reductions in social behaviors such as social investigation and agonistic behaviors, and increases in submissive behaviors in both sexes. The NAc is therefore not a site of action underlying the findings of Choleris et al., 2011 using systemic treatments.

Based on this body of work and a model developed by Lisman and Grace, 2005, I propose a socially relevant novel odor detection mechanism, and sexually dimorphic actions through both tonic and phasic neurotransmission by which mesolimbic DA may be mediating social learning in male and female mice.

*Dorsal Hippocampal Dopamine and Socially Relevant Novel Odor Detection.*

The VTA is the main source of dorsal hippocampal DA release (Edelmann & Lessmann, 2018; Frey et al., 1990; Lisman & Grace, 2005; McNamara et al., 2014; Wise, 2004). A model by Lisman and Grace, 2005 proposes that the VTA-hippocampal dopaminergic loop regulates novelty detection associated with salient stimuli (Lisman and Grace, 2005). For this to occur, the hippocampus first processes a novel stimulus encountered in the environment (Brankack et al., 1996; Floresco et al., 2003). Through interactions with intermediate structures (including the NAc and ventral pallidum), the signal eventually arrives to the VTA, which is finally able to mediate hippocampal dependent engram formation through changes in hippocampal DA release via direct projections (Hansen and Manahan-Vaughan, 2014; Lisman and Grace, 2005).
This thesis adds to the original model developed by Lisman and Grace, 2005, where we have now shown that dorsal hippocampal DA may also process a novel food odor emitted by a familiar DEM animal during a social interaction. It is well established that the VTA-hippocampal DA loop regulates reward related memory functions through DA D1-type receptor activity in the dorsal hippocampus (reviewed in Hansen and Manahan-Vaughan, 2014). After showing a role for dorsal hippocampal DA D1-type receptors (Matta et al., 2017), we have shown here (Chapter 2) that there is also an involvement of dorsal hippocampal DA D2-type receptors in the STFP in females. I propose that the dopaminergic loop that processes novelty detection may also be involved in detecting a novel diet odor transferred by a DEM, and that both DA D1-type and D2-type receptors in the dorsal hippocampus may play a distinct role in the formation of this memory trace in males and females. It is possible that via LTP (Kulla & Manahan-Vaughan, 2003) and LTD (Chen et al., 1996) mechanisms (reviewed in Hansen & Manahan-Vaughan, 2014) in the current studies, the hippocampus was able to add meaning to the DA releasing stimuli (novel food odor emitted by the familiar DEM), and integrated this information into the engram by acting through DA D1-type receptors in males and females (Matta et al., 2017), and DA D2-type receptors in females only (Chapter 2). Given the results of Chapter 3, I further showed the memory trace associated with social learning involved increases in dorsal hippocampal DA in males, and decreases in females (discussed below).

It can be hypothesized that the sex differences in hippocampal DA release in association with novelty detection could be partly explained by sex differences in DA receptor distributions in the dorsal hippocampus. Indeed, such hypotheses would be in
agreement with our intrahippocampal pharmacological work on social learning in the STFP showing that males are more sensitive to DA D1-type receptor antagonism than females (Matta et al., 2017), and that females (but not males) are affected by DA D2-type receptor antagonism (see Chapter 2 in this thesis). All five DA receptors are expressed in the rodent hippocampus (reviewed in Edelmann & Lessmann, 2018). A recent study conducted by Wei et al., 2018 using male and female mice with DA D1 and D2 promotor-driven green fluorescent protein (GFP) expression found no sex differences in either DA D1 or D2 receptor expression in the dorsal (or ventral) hippocampus (Wei et al., 2018). However, it is possible that there are sex differences in receptor expression for other individual DA receptor subtypes (D3, D4, D5) in the hippocampus of mice. A thorough anatomical quantification of these other DA receptors in the hippocampus of both male and female mice would therefore be valuable. Given the DA projections to the hippocampus, it is possible that hippocampal DA actions through these other individual DA receptor subtypes were a critical step in the sex-dependent novelty detection signal. Such hypotheses warrant further studies examining novelty processing involving social stimuli and potential sex differences in DA circuitry.

Few studies have examined whether motivation affects the hippocampal-VTA DA loop (Otmakhova et al., 2013). The reciprocal crosstalk between the hippocampus and the VTA also involves actions through the NAc (together with the ventral pallidum), the so-called “downward arm” of the VTA-hippocampal DA loop (Lisman & Grace, 2005; Otmakhova et al., 2013). It is possible that the NAc may be a structure where motivational signals arriving from other brain regions (such as the PFC) may integrate with novelty signals originating from the hippocampus (Otmakhova et al., 2013). Indeed,
the NAc mediates goal directed behaviors (reviewed in Salamone & Correa, 2012), plays a role in motivationally relevant hippocampal depend forms of learning (Ito & Hayen, 2011), and gating information (Goto & Grace 2008; Grace et al., 2007; Yin et al., 2008). Hence, motivation may stimulate the NAc and this may therefore be a key component in the novelty signal underlying memory consolidation (Otmakhova et al., 2013). While this aspect of the loop has remained largely unexplored, a study by Wolosin et al., (2009) found that subjects who were motivated by a high reward to do well on a memory test had greater VTA and hippocampal activation than subjects anticipating only a modest reward. However, the results of Chapter 4 show that NAc DA D1-type receptors may not be involved in the integration of novel information signaling associated with social learning. I therefore propose that NAc DA D1-type receptors play a lesser role (or no role) in the processing of the socially relevant DA dependent engram.

The functional implications of the novelty detection system driven by DA are unknown (Otmakhova et al., 2013). However, an exploration bonus hypothesis has been offered by Kakade and Dayan (2002), which proposes that novel events have the potential to be rewarding, whereas previously encountered unrewarded familiar stimuli have lost this feature. Thus, dopaminergic actions associated with novelty may drive learning by biasing approach/exploratory-related motivated behaviors (Kakade & Dayan, 2002; Otmakhova et al., 2013).
Phasic and tonic modes of dopaminergic transmission have been proposed to underlie different postsynaptic mechanisms, and mediate distinct behaviors in male rodents (Bromberg-Martin et al., 2010; Cohen et al., 2002; Floresco et al., 2003; Grace et al., 2007). That is, phasic signaling is activated by primary or conditioned rewards (Schultz et al., 1997), and is hypothesized to operate as a neural teaching signal in males (Schultz, 1998). On the other hand, tonic DA levels are more involved in affecting the direction of behavior when having to pick between varying reward types (Niv, 2007; St. Onge et al., 2012), and may be more so mediated by the absolute number of DA neurons activated in males (Floresco et al., 2003; Howland et al., 2002). However, more recent research conducted on male rats suggests the two modes of transmission are actually complementary, whereby tonic and phasic DA activity may work in tandem in plasticity mechanisms that involve persistent motivational processes to regulate memory consolidation (Lohani et al., 2018).

As discussed in Chapter 2 and 3, tonic DA activity produces low (but stable) DA levels that only activate D2-type receptors because these are high affinity DA receptors (Edelmann & Lessmann, 2018; van Wieringen et al., 2013). Instead, phasic DA activity induces rapid rising (but brief) high DA levels (Schultz, 1999; Lisman and Grace, 2005) that activate even D1-type receptors, which are low affinity DA receptors (Edelmann & Lessmann, 2018; Hsu, 1996). The results of Chapter 2 show that dorsal hippocampal DA D2-type receptors mediate social learning in females. These effects may be mediated by tonic DA activity. Indeed, tonic DA signaling is functionally significant, where after
bouts of phasic activity, sustained DA that slowly accumulates in the extracellular space may be involved in maintaining and consolidating networks triggered during learning and memory in males (Durstewitz et al., 2000). The encoding of memories may even be modulated by tonic DA activity by lowering the threshold for LTP in synapses (Shohamy & Adcock, 2010).

Most DA dependent types of learning (which includes the STFP) rely on both tonic and phasic modes of neurotransmission (Salamone & Correa, 2012). Indeed, both fast and slow states may work on a continuum to mediate similar processes in males (Lohani et al., 2018). For example, tonically activated DA levels may be enhanced by secondary actions triggered by phasic DA transmission in male rats (Lohani et al., 2018). Our body of work suggests that both modes of signaling may be important for the dopaminergic mediation of social learning in the STFP in male and female mice. The results of Chapter 3 showed sex-dependent changes in dorsal hippocampal DA release in association with social learning. I propose that the increases in hippocampal DA release for males may be explained by phasic actions of DA neurons, whereas the decreases in hippocampal DA release in females may be explained by tonic DA actions. Unfortunately, few studies have investigated sex differences in tonic and phasic DA transmission. A study conducted by Anstrom et al., 2009 found that aggressive encounters in socially defeated male rats are associated with increased phasic DA activity in the VTA to NAc DA projection. Unfortunately, Anstrom et al., 2009 only tested male (but not female) rats. On the other hand, a study conducted by Calipari et al., 2017 investigated sex differences in the VTA to NAc DA projection in mice. Specifically, Calipari et al., 2017 used in vivo electrophysiology and fast-scan cyclic voltammetry
and found that female mice in estrus have greater basal VTA DA neuron activity, higher tonic release of DA, and are more responsive to phasic stimulations than are male mice and diestrus females (Calipari et al., 2017). While Calipari et al., 2017 did not examine the VTA to hippocampal DA projection, it is possible that if they had applied the same procedures in VTA to hippocampal DA cells during social learning in the STFP, they would have found similar sex differences in DA transmission in association with social learning. Further research examining sex differences in tonic and phasic DA activity is therefore warranted.

Some of these hypotheses in terms of sex differences in tonic and phasic DA signaling can be addressed with further investigations employing both microdialysis and FSCV methods. The detection limit of microdialysis is 2-3 orders of magnitude greater than the detection limit of FSCV (Moghaddam et al., 1990; Westerink & Justice, 1991), which makes microdialysis a more useful technique at measuring low (tonic) DA levels (Lohani et al., 2018). Nevertheless, it has been suggested that microdialysis can measure “slow phasic” DA levels since it can detect changes (e.g., increases followed by decreases) that occur over a slow time scale (several minutes). On the other hand, FSCV is the most suitable approach for measuring “fast phasic” rapid changes in DA activity that occur on a seconds-based time scale (Hauber, 2010; Salamone & Correa, 2012; Segovia et al., 2011; Westerink & Justice, 1991). Thus, further studies employing in vivo microdialysis and FSCV in the investigation of sex differences in tonic and phasic DA activity in association with social learning would be useful.

The NAc receives glutamatergic afferents from the ventral subiculum of the hippocampus, and while this projection may even have excitatory actions on VTA DA...
neuron activity (Floresco et al., 2001), in view of my results I propose that the interaction between the NAc and the VTA-hippocampal DA loop involving NAc DA D1-type receptor signaling (likely through phasic DA activity) either plays a small role, or no role in the regulation of social learning in the STFP in either sex.

The functional impact of dorsal hippocampal DA release that occurs in response to phasic versus tonic activity of DA neurons is unclear (Edelmann & Lessmann, 2018). Given the results of Matta et al., 2017, as well as the results of Chapter 2 and 3, I collectively propose that phasic firing of DA neurons activates hippocampal DA D1-type receptors and subsequently mediates male STFP to a greater extent than female STFP, while tonic firing of DA neurons activates only hippocampal DA D2-type receptors and subsequently mediates only female STFP. It can therefore be speculated that males and female process socially acquired information about foods from other animals in distinct ways. For social learning to occur in males, there may need to be changes in the so-called “signal-to-noise” or “synaptic chatter” (i.e., spontaneous firing of neurons; Destexhe et al., 2003), which may be achieved by enhancing postsynaptic dorsal hippocampal DA D1-type receptor transmission. Instead, for female social learning to occur, there is a reduction in DA release to possibly lower the “synaptic chatter” and focus attention through enhanced dorsal hippocampal presynaptic auto-inhibitory DA D2-type receptor activity. Taken together, sexual dimorphisms in DA transmission may underlie different ways in which males and females process information related to reward, including the motivation to reproduce and drive to attain food (Schultz, 2006).
Conclusions and Significance of Studying Mesolimbic Dopamine.

Our knowledge of the neurobiology of social learning is still evolving (Matta et al., 2016). This body of work has shown that mesolimbic DA and its receptors are involved in the mediation of social learning. Understanding the mesolimbic DA system can provide insight into reward related signals that may affect memory formation and behavior. Investigations into the role of DA in social learning may lead to procedures that can be used to improve social learning and teaching methods in humans (Matta et al., 2016).

This line of research has implications for the understanding of neurological social disorders involving the DA system. For example, symptoms including hallucinations and delusions found in schizophrenic patients are usually treated with DA D2-type receptor antagonists such as haloperidol (Seeman et al., 1993; Seeman & Seeman, 2014). Moreover, attention deficit hyperactivity disorder (ADHD) is most often treated with stimulants that increase brain DA (Biederman et al., 2006). Furthermore, symptoms of autism spectrum disorder (ASD) are currently being treated with drugs that lower DA levels (Lai et al., 2014; Sun et al., 2008).

Little is known about the potential sex differences in the dopaminergic circuits that may regulate social psychiatric disorders. Differences in mesolimbic DA functioning between females and males may underlie the sex-bias in DA dependent social disorders including depression, ASD, ADHD and schizophrenia, where such sex differences may be partly mediated by an interplay between gonadal hormones and the DA system (reviewed in Loke et al., 2015). For example, schizophrenia is slightly more common among males (7 males to every 5 females), however, females have a greater likelihood of
being diagnosed later in life during the peri-menopausal period (Hafner, 2003; Lindamer et al., 1997). Moreover, ASD is more prevalent in males (4 males for every female diagnosed; Fombonne, 2003), ADHD is three times more common among males (Balint et al., 2009), while depression is twice as common among females (Marcus et al., 2005; Nolen-Hoeksema, 1987; Weissman et al., 1996). Hence, a better understanding of the mechanisms underlying sex differences in mesolimbic DA and its receptors would be beneficial for designing novel therapeutic agents that would have more suitable effects in each sex. Thus, using both males and females, as well as investigating possible actions of the estrous cycle in social neuroscience is crucial in understanding such disorders (Choleris et al., 2018). Ultimately, this line of research may provide new insights into understanding sex differences in the incidence of psychiatric disorders with a dysfunctional mesolimbic DA system.
LIMITATIONS

There are some limitations in the studies conducted. For instance, for the intrahippocampal Raclopride experiments (Chapter 2), when implanting intracerebral guide cannulae into the dorsal hippocampus, holes were punctured into the membrane surrounding the hippocampus (alveus). This meant that the drug used (Raclopride) might have ended up elsewhere in the brain. Similarly, for the intra-NAc experiment (Chapter 4), it is possible that SCH23390 may have diffused into other brain regions (e.g., ventricles). However, the dye infusions conducted suggest that the microinfusions were mostly localized to the dorsal hippocampus (Chapter 2) or NAc (Chapter 4). Nevertheless, it is unlikely that Chicago blue dye and the drugs used (Raclopride and SCH23390) have the same diffusion characteristics.

Another potential limitation is the order of the exposures for the in vivo microdialysis experiment (Chapter 3). That is, the exposures were not counterbalanced throughout testing. Since sample collection occurred over a period of several hours, we conducted each trial in the same sequence so that the time between learning in the STFP and testing during the choice test was always constant. This allowed us to control for potential confounds in terms of learning versus memory processes (short delay versus long delay); however, this may have resulted in some carryover/order of presentation effects. Indeed, (as discussed in Chapter 3) it is possible that the OBS mice spent more time investigating the DEM diet odor over the NON-DEM diet odor simply because they were more hungry at the later time point. Similar order of presentation effects may have occurred for other conditions.
CHAPTER 6: References


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