Dopaminergic Regulation of Pair Bonding in the Socially Monogamous Prairie Vole

by

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Dedication

This dissertation is dedicated to my mother, María Esther Leal, and to the memory of my grandfather Juan Tirado and undergraduate mentor Dr. Maria Teresa Velez (MTV). Your self-sacrifice is the reason myself, and so many others, have been able to pursue the American Dream.

Esta tesis esta dedicada a mi madre, María Esther Leal, y memoria de mi abuelo Juan Tirado y mi mentora la Dra. María Teresa Vélez (MTV). Sus sacrificios son la razón porque yo, y muchos otros, hemos podido conseguir el sueño Americano.

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Abstract

The development and maintenance of pair bonds in the socially monogamous prairie vole is regulated in part by the mesolimbic dopamine system. My dissertation work investigates dopamine signaling mechanisms involved in the development of these selective social attachments, the adaptations of this system in the maintenance of such bonds, and finally, how oxytocin may interact with dopamine in these processes.

I first replicate findings showing that the mixed D2/D3 receptor agonist, quinpirole when infused into the nucleus accumbens shell, induces robust partner preferences. I subsequently sought to determine if selective activation of D2 or D3 receptors within the this region, via infusions of the specific D2R agonist 5,6,7,8-Tetrahydro-6-(2-propenyl)-4H-thiazolo[4,5-d]azepine-2-amine dihydrochloride (B-HT 920) or the specific D3R agonist R(+)-2-Dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (7-OH-DPAT), influenced partner preference formation. Partner preference testing showed that selective activation of D3 receptors is sufficient to induce partner preferences while D2 receptor activation is not.

In a second set of experiments I utilized these same drugs in combination with fast scan cyclic voltammetry to measure DA release inhibition via autoreceptors in the striatum. B-HT 920 had highest effect in the dorsal striatum whereas quinpirole was more effective in the nucleus accumbens shell at inhibiting dopamine release than either the selective D2 or D3 agonist. Surprisingly, 7-OH-DPAT, showed similar effects in both regions examined. This result was unanticipated as inhibition of dopamine release by agonists is thought to reflect receptor distribution and reports in other species show greater density D3 receptors in the nucleus accumbens shell compared with dorsal striatum. Our results suggest that the prairie vole shows a unique response to quinpirole compared to other species or that the distribution of D3 receptors in the prairie vole may not follow the same pattern as other species.

In a final set of experiments, I utilized fast scan cyclic voltammetry to measure

dopamine release dynamics in the nucleus accumbens shell of sexually naïve and 28-day pair bonded prairie voles. We demonstrate that pregnancy status is reflected in dopamine release in males after 28 days of pairing such that males from pairs that became pregnant quickly after pairing show the largest increases. Additionally, we test the effects of the dopamine D2/D3 receptor agonist, quinpirole, on electrically stimulated dopamine release in the dorsal striatum and nucleus accumbens as a measure of dopamine autoreceptor functionality. Interestingly, dose response curves to treatment with quinpirole showed that pair bonded prairie voles show subsensitive dopamine autoreceptor activity. In a final experiment, we show that increased oxytocin tone can mimic this effect of pair bonding at the dopamine autoreceptor in sexually naïve animals.

Together, these data provide support for an essential role for the DA D3 receptor subtype in regulating the development of social attachments in male prairie voles. Additionally, these data show a long-term adaptation in DA signaling in pair bonded prairie voles, which is both sex-specific and fecundity-dependent. Finally, I show that oxytocin facilitates this neuroadaptation in prairie voles that have not yet been pair bonded. These data represent an important advance for the field since these signaling mechanisms may be valid therapeutic targets to treat social deficits in humans. Taken together, these research findings have provided valuable insight into the role of the DA and oxytocin systems in affiliative behavior and also have broad implications for our understanding of social bonding as a motivated behavior.

Chapter 1
Introduction

General Introduction

Animals are drawn to seek natural rewards such as food, shelter, and sex. Dopamine (DA) signaling within the mesolimbic system is known to encode the learning and motivational properties of these rewards[1–4]. Social bonding utilizes this same circuitry for the positive reinforcement of social interactions including the motivated approach of other sex conspecifics and maintenance of relationships with mating partners and offspring[5]. Specifically, activation of this system underlies the development of selective social attachments whereas neuroplasticity of this circuitry underlies their maintenance. In certain highly prosocial species, such as prairie voles, oxytocin (OT) signaling functions within this "reward" network to regulate social behavior[6–9]. Specifically, it has been suggested that the coordinated action of OT and DA within striatal circuitry functions to attribute salience to social stimuli in a context-dependent fashion[8,10]. Additionally, it is theorized that DA may work in conjunction with endogenous opioids to mediate the hedonic properties of social interactions and may motivate approach of familiar partners and rejection of novel conspecifics during the pair bond maintenance phase[11–15]. Thus, the DA system is actively engaged in motivating social behavior and is uniquely

positioned within striatal reward circuitry to influence the learning and hedonic properties of social incentives via its interaction with the OT and opioid systems.

Here, I review evidence for the function of mesolimbic DA in the regulation of social attachments (i.e., "pair bonds") in the socially monogamous prairie vole. I will first discuss the prairie vole's natural history and how their social structure poses benefits for the study of social attachment as well as how these behaviors are studied in the laboratory. I will then discuss how DA regulates affiliative behaviors in the early development of pair bonds, caretaking behaviors directed toward pups, and the aggressive behaviors characteristic of later stages of bonding. Finally, I will discuss how DA may function in mesolimbic circuits to attribute salience to stimuli that have either positive or negative valence, via its interaction with the OT and opioid systems. I briefly discuss some evolutionary considerations that must be kept in mind in our interpretation of these findings and consider how the study of the neurochemical convergence between DA and OT may facilitate our understanding of motivated behavior of a social nature.

The Prairie Vole as an Animal Model of Social Attachment

The socially monogamous prairie vole (*Microtus ochrogaster*) is a strong model for the study of social bonding due to its highly prosocial mating strategy[16–21]. Prairie voles form lifelong pair bonds that are characterized by a preference for a familiar partner, rejection of novel conspecifics, and equal distribution of caretaking responsibilities such as nest guarding, food gathering, and offspring care[7]. In particular, their offspring caretaking strategy is unique in that the male and female equally share tasks such as huddling, brooding, and retrieving pups which is not common in males of other species[22].

Importantly, prairie voles show a readily observable behavioral transition from the highly affiliative pair bond "development" phase to the aggressive and territorial pair bond "maintenance" phase. Affiliative displays such as allogrooming and huddling are characteristic of the development phase while aggressive displays that include biting, chasing, and lunging at novel conspecifics are characteristic of the pair bond maintenance phase[23]. Importantly, the data gathered using this model have shown that the biological systems regulating social attachment in voles also underlie the expression of social relationships in humans[24]. These characteristics make the prairie vole an optimal translational model to study the neuroscience of social attachment[25–31].

Prairie Vole Behavior

Prairie voles are burrowing rodents native to the Midwestern United States and Canada[32]. They are a philopatric species, meaning that offspring will often remain in the parental nest into adulthood and help rear subsequent litters[22,33–37]. Female prairie voles do not ovulate spontaneously and instead require prolonged exposure to the pheromones present in male urine to induce sexual receptivity (i.e., behavioral estrus), and subsequent mating to achieve ovulation[38]. Importantly, while in the nest, the presence of the mother inhibits the younger females' sexual maturation even when they are in the presence of the appropriate stimulus[39–41]. Thus, sexually naïve adult female prairie voles remain functionally prepubescent until prolonged exposure to a non-related male outside of the parental nest induces their first ovulatory cycle[42]. This social structure results in multigenerational nests that consist of one breeding pair, multiple litters, and often, additional non-reproductive adults[22,36].

Pair Bond Development and Affiliative Contact

Upon encountering a novel conspecific of the other sex, prairie voles will often approach and engage in olfactory investigation, grooming, and ultimately settle into their unique side-by-side "huddling" behavior[18]. Huddling is considered a central characteristic of prairie vole pair bonds[43–45]. Indeed, non-monogamous vole species, such as the montane vole, do not show this contact proneness and instead choose to stay away from novel conspecifics even when in enclosed habitats[16,23]. Importantly, neuroanatomical differences in the expression of OT receptors within striatal circuitry among prairie and montane voles reflect their different social organization, suggesting a role for this neuropeptide in the regulation of pair bonding[46,47].

The high level of contact present during early interactions among prairie voles is theorized to maximize the amount of pheromone transfer and the induction of female sexual receptivity[48]. This unique feature of the prairie vole ovulatory cycle provides further support for the importance of social contact in monogamous species. It has been observed that while regular estrous cycles are absent in most *Microtus* prior to exposure to male stimuli, non-monogamous species do not rely on extended affiliative contact to effectively induce estrus[49]. In fact, time to induced estrus and time in affiliative contact vary in a predictable stepwise function so that monogamous species require longer exposure to male stimuli to achieve behavioral estrus[50–54]. This suggests, then, that prolonged motivation to remain in close contact to the female is imperative for male prairie voles.

Upon interaction with a non-related male outside of the natal nest, female prairie voles will achieve behavioral estrus within 24 but up to 72 hours (variation that has meaningful significance and is discussed below) and will mate for a period of approximately 30 hours[42,48,50,55,56]. The pair remains together throughout gestation and the female will give birth to a litter at 21-23 days. In the presence of a male, female prairie voles go into post-partum estrus and will typically achieve pregnancy within 48 hours of parturition[55,57]. In fact, after the first copulation, the majority of subsequent pregnancies will result from copulations during subsequent post-partum estrus periods. Thus, the initial interactions that induce behavioral estrus and ovulation are crucial for setting in place, both behaviorally and neurohormonally, the long-term success of the bond. This male-female association (i.e., pair bond), will continue throughout multiple breeding seasons until a member of the pair dies[58].

Pair Bond Maintenance and Aggressive Rejection of Novel Conspecifics

Once prairie voles have mated and achieved pregnancy they will remain together throughout their lifetimes. That is, they will continue to engage in social monogamy with the same partner throughout multiple mating seasons until a member of the pair dies. During this time, prairie voles are known to actively defend their home territory against novel conspecifics via aggressive displays that include chasing, lunging, and biting[23]. These aggressive displays are directed at both sexes which suggests that the behavior serves both to protect the current bond and prevent the establishment of new bonds[17,49,59–63]. Based on these observations, it is theorized that at this stage in pair bonding, voles remain highly sensitive to social stimuli, however new conspecifics are

now perceived as aversive and motivated behaviors are directed toward aggressive rejection or avoidance rather than the previous investigation and close contact that are associated with the development phase[15].

Pair Bonding and Biparental Offspring Care

Prairie voles give birth to altricial young that depend on both parents for nourishment, thermoregulation, and shelter. Among most mammals male parental care is sparse or completely absent[64]. From an evolutionary perspective, for the male, caring for young is most advantageous when there is a guarantee that the male is caring for his own offspring. In monogamous mating pairs, most pups are generally exclusively sired by the same two partners, and so remaining in the nest to ensure survival of the young can present a direct fitness benefit for that male[65].

Prairie voles engage in biparental care of offspring and both the male and female have been seen to engage in caretaking behaviors, including nest maintenance and construction, caching food, runway construction, care of the young, grooming, retrieval, and brooding (Figure 1.1)[22]. Interestingly, male voles have been seen to have higher survivorship when living alone than when living in pairs, and they have also been observed to lose weight during the trajectory of their pair bonds showing that the male takes on substantial energetic costs in exchange for the opportunity to rear offspring[66,67]. Importantly, for the male, the increased survivorship of offspring reared byparentally outweighs the costs of long term monogamy.

Some parenting behaviors such as nursing can solely be expressed by new mothers and represent a sequence of adaptive action patterns that include licking,

grooming, nursing, and huddling which are reliably triggered by specific cues in the environment such as pup sounds, smells, and tastes[68]. For example, ultrasonic vocalizations reliably trigger pup retrieval and exposure to pup scent can induce caretaking behaviors[69–71]. It has been hypothesized that the maternal prairie vole brain experiences similar neuroplasticity as has been observed in the rat such as sensitized sensory processing of infant cues such as pup odor and ultrasonic vocalizations (USVs) making the mother more likely to effectively determine her pups' needs and protect them from harm [72,73]. New mothers vary in the amount of affiliative interaction they display with their pups, and individual differences in the manifestation of these behaviors (i.e., licking, grooming, and feeding) displayed by mothers can have lifelong and even multi-generational effects on offspring[74].

Measuring Pair Bonding Behavior in the Laboratory

As discussed above, pair bond development is characterized by affiliative social interactions that include huddling, mating, and grooming and can be assayed via the partner preference test[75]. The pair bond maintenance phase is characterized by aggressive rejection of novel conspecifics and can be measured via the resident intruder test[50,75–78]. The measurement of partner preferences and aggressive displays toward a home "intruder" have been established as reliable assays for the measure of the existence of a pair bond as well as pair bond strength, respectively, and when combined with the breadth of tools at a neuroscientists' and behaviorists' disposal, these serve as powerful tools to study the neurobiology of social attachment[30,31,77]. Figure 1.2 illustrates these two behavioral assays, which are explained in detail below.

Partner Preference Test to Measure Pair Bond Development

The "partner preference" is considered to be the first observable measure of pair bond development[75,79]. This test borrows from traditional reward learning paradigms like conditioned place preference (CPP) to test the rewarding properties of an unconditioned stimulus[80,81]. In the CPP paradigm, each chamber of a two chambered apparatus is designed to have different contextual cues and is subsequently paired with an either rewarding or neutral unconditioned stimulus. During conditioning sessions the rewarding stimulus will be continually paired with one chamber, while the neutral stimulus will be paired with the other chamber. After repeated pairings, the environment paired with the rewarding stimulus is imbued with secondary rewarding value and itself can act as a conditioned stimulus to elicit approach behavior. Whereas the chamber paired with the neutral stimulus will not elicit such behavior. On test day, the test animal is allowed to roam both chambers freely and time spent in the reward paired or neutral chamber are recorded[82]. From these measurements it is inferred that if the unconditioned stimulus was potentially rewarding, the animal will choose to spend time in the reward-paired chamber and this is termed a "conditioned place preference" (CPP).

A similar paradigm is used to measure pair bonding in prairie voles. The "partner preference" test consists of placing a test subject (of either sex) in a three-chamber apparatus in which two of the chambers house tethered stimulus animals. One chamber contains the "partner" (animal with whom the test subject has been paired; i.e., a conspecific that is familiar to the subject) another chamber contains a novel "stranger" (with which the subject is not familiar), and a third chamber that remains empty during the

test. For three hours the test animal is allowed to roam freely among all the chambers and cage entries, time spent in cage, and time in side-by-side contact (scored in minutes for a total of 180 total maximum time in contact) are quantified. When time spent in side-by-side contact with the partner exceeds time spent in contact with the novel animal, an animal is said to have developed a "partner preference"[83–86]. Importantly, under these test conditions, most animals, as has been observed with several species of birds and chimpanzees, would choose the novel animal, having already mated with one animal, they explore alternative "pairs" to increase their odds of reproduction[87–89]. Monogamous species, such as the prairie vole, however, show a reliable preference for their familiar partner during this test which is displayed via huddling behavior[75]. Importantly, two limitations should be kept in mind when interpreting data from this paradigm. First, without any adaptations, this paradigm does not isolate aspects of the individual stimuli, for example, it cannot rule out that an animal may just show a preference for a familiar partner based on scent (e.g., animal "smells" like home). Second, it should be kept in mind that while this assay is reliably able to determine if a long-term bond has been developed, or has the potential to be developed (in some variations of the paradigm), it is unlikely to decipher more nuanced variations of bonding behavior and also limits our interpretation of the interaction to the point of view of the test subject. That is, this assay provides little information on the behavior of the stimulus animals and how that impacts the subject animal's choice. Future incorporation ultrasonic vocalization recordings during the partner preference test and deeper behavioral analysis of the interactions between each of the members of the chamber, not only measurements of side by side huddling, will provide us with a richer dataset on how the reciprocal interactions between the stimulus animal

and test subject impact an animal's decision to remain with their partner or explore other options.

Resident-Intruder Test: Aggression as an Index of Pair Bond Strength

Sexually naïve prairie voles show high levels of affiliation. However, if they have cohabitated for as little as 24 hours, both males and females will begin to display aggressive behavior toward novel conspecifics[9,90]. These displays are referred to as "selective aggression" and are measured via the "resident-intruder test" and the data gathered are used as an index of pair bond strength[77,79,91,92]. In the laboratory, animals will be paired for two weeks of cohabitation and mating in which they will naturally establish this home cage as their "territory". The resident-intruder test consists of measuring aggressive interactions for a short period of time (typically 10 minutes) between the subject animal "resident" and an unfamiliar "intruder" that is introduced into the home cage[62]. Intruders and residents can be of any sex as both sexes display selective aggression behavior[93]. Aggressive behaviors such as lunges, attacks, offensive rearing, biting, and chasing are recorded[23,59,90].

Dopamine Signaling and Pair Bonding

As discussed above, prairie voles show an impressive behavioral plasticity in the transition from sexually naïve to pair bonded. The incentive to first develop a monogamous bond and remain in contact with a mating partner and offspring as well as the drive to aggressively reject novel partners requires strongly motivated behaviors that are a result of activation of the mesolimbic DA system[94–96]. Studies on the prairie vole

have shown that activation of this system and subsequent neuroplasticity of this circuitry supports the trajectory from sexually naïve to fully pair bonded status in prairie vole pairs[59,97]. It is important to note however, as I will review below, that while DA itself is broadly involved in motivated behaviors it is through its interaction with other systems (i.e., OT, opioids) that it can effectively focus motivational action on specific facets of social reward such as that associated with social exploration, mating, and parental/maternal care.

DA Signaling Mechanisms

The mesolimbic DA system is central to motivational processing and learning[1,2,98]. DA is a slow acting neuromodulator (i.e., binding results in activation of intracellular cascades via second messengers rather than engaging direct effects on opening of ion channels) that can bind to one of 5 known receptor subtypes which are classified as either D1-like or D2-like based on their activation or inhibition of adenylate cyclase, respectively[99,100]. The D1 class of receptors includes the D1 and D5 receptor subtypes while the D2 class of receptors includes D2, D3, and D4 subtypes. Both the D1 and D2 class of receptors are expressed on striatal medium spiny neurons (MSNs)[100–103]. MSNs that express D1 receptors project directly to the substantia nigra pars reticulate from the direct pathway while D2 expressing MSNs project to the external part of the globus pallidus via the indirect pathway[101,102]. Comparatively, the D1 receptor has a lower affinity for DA than the D2 receptor. Thus, D1 receptors require higher levels of DA to be released for them to become activated, such as those levels observed during burst firing. The D1 receptor is expressed on medium spiny neurons that contain dynorphin (the endogenous

ligand for kappa-opioid receptors) and activation of these receptors increases the release of dynorphin[104–107]. Comparatively, the D2 class of receptors has a higher affinity for DA meaning that it can be activated by lower levels of the ligand such as those released during tonic firing. Additionally, D2-like receptors are expressed on neurons that contain enkephalin (endogenous ligand for mu-opioid receptors) that mediate positive hedonics generally, and in particular, positive associations to a mating experience in the prairie vole[11,12,108–111]. Uniquely, despite their opposing intracellular effects and expression, these receptors often work together to facilitate the expression of diverse behaviors relating to motor behavior, motivation, and learning, and as we review below, complex social behaviors such as pair bonding in the socially monogamous prairie vole[112].

Role of DA Signaling in Pair Bond Formation

In female prairie voles, mating during estrus results in an increase of extracellular DA in the NAc of up to 51%[113]. Importantly, only a minor increase in DA release is observed when females are not sexually receptive and exposed to a male. Yet, it must be noted that DA increases during mating are also observed among other non-monogamous species such as the rat and the Syrian hamster suggesting that although DA is necessary for the formation of a pair bond, it is not sufficient[114,115]. Additionally, nonspecific blockade of DA receptors inhibits the development of partner preferences even in the presence of mating[116–118]. Thus, a combination of female receptivity and DA activation during mating are key components in the development of partner preferences.

Experimentally targeting of specific DA receptor classes has shown that activation of D2-like receptors and subsequent inhibition of cAMP signaling, in the rostral NAc shell,

but not the dorsal shell or core, induces partner preferences even in the absence of mating, whereas blockade of these receptors in this same region blocks partner preferences even after 24 hours of mating (Figure 1.3)[59,117,119]. Additionally, blockade of D2-like receptors, but not D1 receptors, inhibits the development of partner preferences in both sexes, while activation of D1 receptors prevents the development of pair bonds[116,117]. Considered in light of the knowledge of receptor affinities in each class, these data suggest that low levels of DA that preferentially activate D2 like receptors are important for the development of pair bonds.

Role of DA Signaling in Pair Bond Maintenance

As mentioned above, the pair bond maintenance phase is characterized by high levels of aggression toward novel conspecifics and this aggression is also considered a motivated behavior[15]. This behavior is regulated by the activation of DA D1 type receptors within the NAc shell. Importantly, blockade of these receptors has shown to block selective aggression and activation of these receptors in sexually naïve animals blocks the development of partner preferences[59]. An upregulation of D1 receptors is observed in the NAc shell and not other regions of the striatum in males that are have cohabitated for two weeks and successfully achieved pregnancy. And this upregulation is correlated with the amount of selective aggression exhibited by the males of the pair, suggesting that this upregulation in receptor expression prevents affiliative social contact with novel animals thereby protecting the established bond[15,59]. That is, due to the higher expression of D1 receptors (which mediate aggressive behaviors toward novel conspecifics) the activation of these receptors prevents the development of new pair bonds. Because DA

D1 type receptors are the low affinity type that require high levels of DA to be activated, this suggests that burst firing in the presence of novel social stimuli, can result in the activation of D1 neurons and subsequent activation of kappa-opioid receptors via the release of dynorphin, resulting in aggressive rejection of novel conspecifics[63].

Additionally, another form of neuroplasticity has been observed in pair bonded prairie voles. Specifically, stimulated DA release is enhanced within the NAc shell of pair bonded male prairie voles. However, these increases are correlated with pregnancy onset in the female such that males from pairs that became pregnant sooner, displayed the largest DA increases[97]. In light of the findings discussed above, the increased DA release that is induced by pair bonding may serve to preferentially activate DA D1 receptors and lead to aggressive rejection of novel mating partners.

Role of DA in Mother-Infant Attachment

It is theorized mother-infant bonding could be an evolutionary precursor to pair bonding and that the neurocircuitry for familial attachments has adapted to accommodate the development of attachments with mating partners[120]. Importantly, maternal behaviors, much like pair bonding, are highly motivated actions that come at a high energetic cost for the mother. As such, the DA system has been shown to be recruited in the invigoration of caretaking behaviors as well as the neural plasticity that accompanies motherhood. Thus, the study of DA in maternal attachments can provide insights into how DA regulates pair bonding.

Research on this topic has established that DAergic circuitry within the ventral tegmental area (VTA) and nucleus accumbens (NAc) are key to the expression of

maternal caregiving. During licking and grooming EEG shows increased activity in the VTA[121]. Microdialysis has shown increases in DA release in the NAc during pup interactions[122]. DA release within the NAc has been correlated with "quality of maternal behavior" in the rat in particular with pre-weaning contact between the mother and pups[123–125]. Additionally, *in vivo* voltammetric measurement of dopamine release during maternal behavior shows that DA release is highest in those mothers that are considered "high licking and grooming"[126]. Blocking of DA signaling by damage to DA cell bodies via 6-hydroxydopamine lesions or radiofrequency lesions within the VTA block the expression of maternal behaviors demonstrating that DA originating from the VTA projections onto the NAc is required for the expression of maternal behavior[127–129].

It is believed that the VTA is invigorating maternal behavior via its interactions with the OT system especially within the MPOA[130]. Direct infusion of OT into the VTA increases DA release in the NAc suggesting a mechanism by which social interaction can impact DA signaling[131]. Additionally, high licking and grooming mothers show increased OT expression in the mPOA and the paraventricular nucleus of the hypothalamus and increased projections of OT cells from both of these regions into the VTA[131]. D1 receptor agonist treatment into the MPOA or NAc can stimulate the onset of maternal behavior. Further, maternal behavior can be triggered by pup scent and this form of "maternal memory" for pup cues has been shown to be mediated by olfactory discrimination processes mediated by DA D2 like receptors[132].

Role of DA in Male Parental Behavior

Because male parental behavior is rare in mammals, its study has been limited to the few species which display this behavior and only until recently has DA regulation of this behavior in prairie voles been investigated. Sexually naïve and pair bonded prairie voles will both display pup caretaking behaviors. Pair-bonded prairie voles, however, display greater pup caretaking[133]. Pair bonded males show similar c-Fos expression as females after experience with a pup suggesting that the same regions that regulate maternal behavior may also regulate paternal caregiving in this species as well[134]. Pair bonding also increases the expression of a specific dopamine receptor mRNA (D3) in the NAc but not in the VTA, which is the source of NAc DA[133]. Non-selective blockade of DA receptors results in decreased pup licking and grooming in males, increased duration of huddling behaviors and decreased latency to approach pups[135].

DA Interactions with Other Systems

Opioid Systems in Pair Bonding

As a whole, the aforementioned data suggest that the DA system may work to invigorate motivation for the seeking of a mate, maintenance and defense of a bond and offspring, however we cannot account for valence attributions that are made toward these social stimuli. DA signaling on its own can only account for the motivational and learning properties of these social rewards and as I will discuss below, it is only when DA acts in conjunction with the opioid system that hedonic valences are applied to these stimuli[24,63,136,137]. The interaction of DA with opioid systems is important for the main behavioral transitions discussed above, that is, positive hedonics associated with social contact and mating with a partner will reinforce the maintenance of this social proximity

with the partner while negative hedonics associated with new social conspecifics prevents from the establishment of new bonds[138–140].

Mu-opioid receptor and the attribution of positive hedonics to the mating partner

Opioid signaling within the striatum is shown to contribute to the hedonic processing of rewards[110,136,137,141]. In particular, positive rewards are mediated via the activation of μ-opioid receptors (MORs) within the ventral pallidum and the rostro-dorsomedial NAc[142]. In regards to social rewards, the activation of MORs within DA circuitry mediates the positive hedonic aspects of mating and affiliative social interactions[143–145].

In the prairie vole, the development of pair bonds relies on the attribution of positive hedonics to experiences with the mating partner. Blockade of MORs within the dorsal striatum, but not within the NAc shell prevent partner preference formation[12]. Peripheral activation of these receptors results in an increase in affiliation in sexually naïve prairie voles while blockade of these receptors decreases the number of mating bouts in newly paired voles and inhibits the development of pair bonds[12,146]. At the time these were published, these data suggested that the NAc shell was not necessary for the attribution of positive hedonics to the mating partner. However, upon further examination, Resendez (2013) and colleagues found that the involvement of MORs was segregated to the specific regions of the NAc known to be involved in the attribution of positive hedonics to social stimuli (i.e., rostro-dorsomedial NAc)[11].

k-opioid Regulation of Aggressive Rejection

Once a pair bond has been established, prairie voles will perceive novel conspecifics entering the home territory as “intruders” and will aggressively reject and avoid interaction[18,147,148]. Importantly, kappa-opioid receptor (KOR) activation in the NAc shell is necessary for selective aggression behavior in prairie voles[13,15]. Importantly, activation of KORs is associated with negative affect, aversion, and attenuated value of rewards[149–156]. Aversive stimuli will activate these receptors within the NAc, and their activation via site specific pharmacology results in conditioned place aversions[151]. Importantly, this behavioral transition in which a previously rewarding stimulus is now perceived as aversive is similar to that which prairie voles experience from their transition from sexually naïve to pair bonded (transition from acceptance of novel social interactions to aggressive rejection)[15].

Oxytocin Systems in Pair Bonding

Oxytocin (OT) is synthesized primarily in magnocellular neurons of the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus, and is transported to the posterior pituitary where it is released into the periphery[157,158]. Secretory vesicles containing OT are present within PVN and SON neurons and are capable of somatodendritic release into the extracellular space. This property allows for the diffusion of OT to distant targets via volume transmission[157,159]. Additionally, a smaller subset of parvocellular OT neurons in the PVN send direct projections to the amygdala, hippocampus, NAc, ventral tegmental area (VTA), and brainstem[157,160]. Critically, it has been demonstrated that the distribution of OT receptors in the brain is both species

dependent and correlated with within species differences in social behaviors such as pair bonding[46,161].

OT, DA, and Social Salience

As mentioned above, the interaction of DA and OT systems within the NAc shell is necessary for the development of pair bonds in female prairie voles, and blockade of one with activation of the other is not conducive of a pair bond[162]. OT has been shown to impact a variety of social behaviors of both positive and negative valence depending on the context in which these are presented[163]. Thus, while oxytocin is often regarded for its role in mother infant bonding, empathy, trust, and in-group preference, it has also been shown to play a role in aggression, punishment, and envy[163]. One parsimonious account of how OT can accomplish such varied roles is via its coordinated action with DA which allows it to modulate attentional control toward a social stimulus and contextual cues. Indeed, OT and DA circuitry overlap in regions relevant to the processing of rewards and threats (Figure 1.4) supporting the idea that OT may be "tuning" social salience.

Evolutionary Considerations

Monogamous mating strategies influence reproductive success across the lifespan and thus have been influenced by the same evolutionary pressures for reproduction that regulate polygyny. Thus, it is important to consider the interplay between evolutionary constraints and benefits to understand how monogamy is an adaptive strategic option weighed against promiscuity (i.e. one potential answer to an evolutionary problem of survival and reproduction).

For the female prairie vole, male presence is not only important for raising and protecting offspring, it is also beneficial during pregnancy; a female prairie vole is less likely to carry a pregnancy to term if separated from her partner[50]. For this reason, it is advantageous for the male to remain with the female not only for some time after mating (to ensure paternity and to ward off potentially infanticidal males) but also to protect and feed the female throughout gestation to ensure successful delivery[164] and successful post-partum care of pups. Increased survival of genetically-related offspring is a likely evolutionarily-maintained benefit for species in which males remain with the female throughout pregnancy. Additionally, during the postpartum estrus period, familiar pairs are more likely to successfully impregnate the female[57].

Additionally, behaviors characteristic of the vole social structure such as the presence of juveniles in the nest appears to have several positive effects on the health of the nest as a whole, such as higher rates of survival and earlier eye opening for new pups, increased opportunities for parents to leave the nest and forage (and thus more food resources for the new litter), and more opportunities for social contact during early development[165].

Thus, a strategy in which a high-quality mate is attained can afford both members of the bond stable mating opportunities, greater assurance of paternity, increased likelihood of raising healthy offspring, and security of food and territory. There is an important caveat, however: the male's individual commitment to the bond is impacted by the pairs' reproductive potential. It has been recorded that male pair-bonded prairie voles that fail to efficiently impregnate their partner are more likely to leave a current bond to form another and it has been observed that plasticity in the dopamine system associated

with pair bonding is also correlated to the timely pregnancy of the female[166]. That is to say, a successful pair bond that has a likelihood of persisting and induces plasticity of the DA system (and perhaps motivation to remain in the nest) is defined by both mating and pregnancy, and by the timing of these events as well (which suggests reproductive compatibility). Importantly, despite these observations, we know very little about how the individual neurobiological processes such as the variations in a male's ability to induce and a female's ability to achieve estrous as well as the processes that contribute to the expression of associated neuro and behavioral plasticity and their timing impacts male fidelity.

Introduction to the Experiments in the Dissertation

DA plays a role in the development and maintenance of pair bonds, and its interaction OT and opioid systems are well known to influence learning and hedonic aspects of rewards. The neural circuitry implicated in pair bonding and affiliative behavior in the prairie vole shares many similarities with that of reward and addiction[24]. The mesolimbic DA system has been proposed to have evolved to mediate incentive behaviors associated natural rewards[167]. It has been shown that drugs of abuse exert their effects on this same system, essentially "hijacking" adaptive circuitry to produce a slew of maladaptive behaviors [30]. As discussed herein, pair bonds are regulated by activation of the DA system and interactions between drug reward and social reward have been observed in prairie voles.

Male prairie voles pretreated with amphetamine fail to show mating induced partner preferences and conversely, the presence of social bonds reduces the rewarding properties of amphetamine[168,169]. Importantly, this illustrates the bidirectional relationship

through which healthy social attachment can attenuate the rewarding properties of drugs of abuse and addiction can reduce the rewarding properties of social attachments.

The studies in this dissertation utilize the prairie vole to investigate the DA signaling mechanisms and adaptations involved in the development of selective social attachments. Insight into the role of the DA system in this context has implications for our understanding of social bonding as a motivated behavior. Future work on this model will undoubtedly provide a unique perspective on comorbidities between drug addiction and social deficits, as well as an understanding of how healthy social attachment can decrease vulnerability to drug use.

Overview of this Dissertation

Evolutionary selection pressures have shaped monogamous mating strategies in a variety of species. Mammals are unique in that only an estimated 3%-9% of mammal species adopt a monogamous mating structure in which individuals mate with one partner over an extended period of time and both partners actively care for offspring. The development of this behavior across evolutionary time would have shaped biological systems that energized seeking of and remaining in prolonged contact with a mating partner and offspring. Indeed, monogamous species such as the socially monogamous prairie vole, express neurochemical systems that process social interaction as highly rewarding. Of particular interest is the regulation of social behavior by the neurotransmitter dopamine (DA). As discussed above a*ctivation* of DA receptors and *neuroplasticity* in mesolimbic DAergic circuitry underlies the development and maintenance of the prairie voles' monogamous attachments, termed "pair bonds".

My dissertation studies utilize this animal model to: (1) investigate the DA signaling mechanisms involved in the development of selective social attachments; (2) determine the adaptations of this system after the development of such bonds; (3) characterize the interaction of the traditional bonding neuropeptide, OT, on DA signaling within the NAc. Together, these research findings provide valuable insight into the role of the DA system in this context and have broad implications for our understanding of social bonding as a motivated behavior.

The goal of my dissertation work was to increase our understanding of the DAergic mechanisms that regulate pair bonding in particular in relation to the activation of the DA system necessary for the development of pair bonds, and the plasticity associated with pair bond maintenance in the socially monogamous prairie vole. Because previous data suggest that low levels of DA that preferentially activate the D2 class of receptors are necessary for the development of pair bonds, and the DA D3 receptor has the highest affinity for DA within the D2 class, is highly localized to the NAc shell (region important for pair bonding and association of positive hedonics with a mating partner), and is shown to be involved in social recognition processes, we hypothesize that the DA D3 receptor mediates pair bond development in the socially monogamous prairie vole. Additionally, previous data show that DA release within the NAc of pair bonded voles is increased after 2 weeks of pairing. We hypothesize that this adaptation will persist after 28 days of pairing (at which point prairie voles are caring for first litter) and that this adaptation may underlie the sustained motivation required for the maintenance of a long-term bond. Further, while increases in DA release have been measured, the mechanism responsible for this is not known. By incorporating data gathered from the field of drug addiction which addresses

the various DA adaptations that can arise from drug abuse, we hypothesize that pair bonding increases DA release via an alteration in DA autoreceptor function. Finally, we investigate a possible role for OT in this neuroplasticity.

Summary of the following chapters:

Chapter 2: This chapter shows a key role for the DA D3 receptor subtype in the regulation of pair bond formation in male prairie voles. I utilize site-specific pharmacology to demonstrate that selective DA D3 receptor activation can induce partner preferences in sexually naïve male prairie voles. Importantly, subtype specific activation of D2 receptors did not have an effect on pair bonding behavior.

Chapter 3: Chapter 2 utilized a variety of selective DA agonists to induce partner preference behavior. Here, I utilize fast-scan cyclic voltammetry to characterize the functional activity and selectivity of these drugs in the prairie vole brain. The data collected show that these drugs have high efficacy in the striatum. However, unexpectedly, we did not replicate findings that the putative DA D3 receptor agonist is most effective in the NAc shell than the DS (as shown elsewhere[170]). This discrepancy may be a result of the following: (1) the agonist is not as selective for the D3 receptor subtype or not selective at the doses used in this study, or (2) the DA D3 receptor is not as dense in this region in the prairie vole as has been measured in mice and rats. These findings should be interpreted cautiously in relation to the behavioral data presented in Chapter 1 due to the fact that FSCV experiments in slices can only determine terminal regulation of DA release. Thus, these findings do not invalidate behavioral data that have been collected,

but instead suggest that perhaps post synaptic mechanisms may be important for the regulation of this behavior.

Chapter 4: Here I present data that support findings that prairie voles show pair bonding dependent increases in DA release in the rostral nucleus accumbens shell (Resendez et al., 2016). Importantly, while the previous work had demonstrated this adaptation after 2 weeks of pairing, I show that this adaptation is maintained beyond the birth of the first litter (and into the active biparental caretaking phase). Additionally, I characterize adaptations in autoreceptor function as the neuroplasticity that underlies the observed DA increase. I then combine these novel findings of DA system adaptations in pair bonding with the broad database of research that has shown a role for the oxytocin system in the regulation of prairie vole pair bonding by demonstrating that increased oxytocin tone can mimic the effect of pair bonding at the DA terminal.

Chapter 5: Here I discuss the broader implications and limitations of this collection of work. I will propose future directions to continue the study of DA regulation of pair bonding, especially in regards to the characterization of the neuroplasticity associated with this behavior.

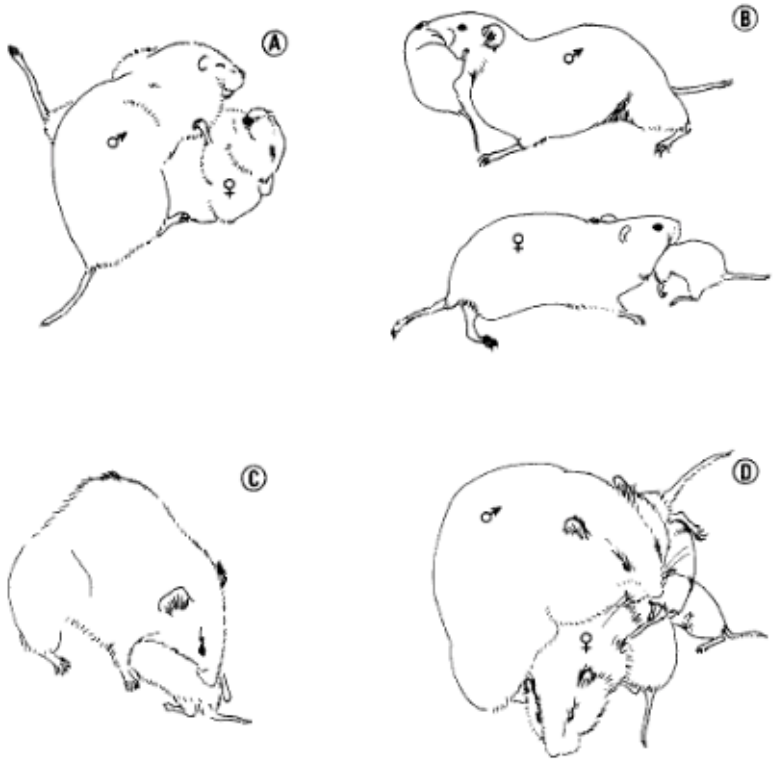

Figure 1.1. Parental behaviors in prairie voles. Prairie voles engage in biparental care of offspring and both the male and female have been seen to engage in caretaking behaviors, including nest maintenance and construction, caching food, runway construction, care of the young, grooming, retrieval, and brooding. **(A)** Sleeping posture exhibited only by mated pairs **(B)** Male or female retrieving pup **(C)** Male or female grooming a pup **(D)** Mated pair brooding a litter.

Illustration from Thomas, J. A. & Birney, E. C. Parental care and mating system of the prairie vole, Microtus ochrogaster. *Behav. Ecol. Sociobiol.* **5,** 171–186 (1979).

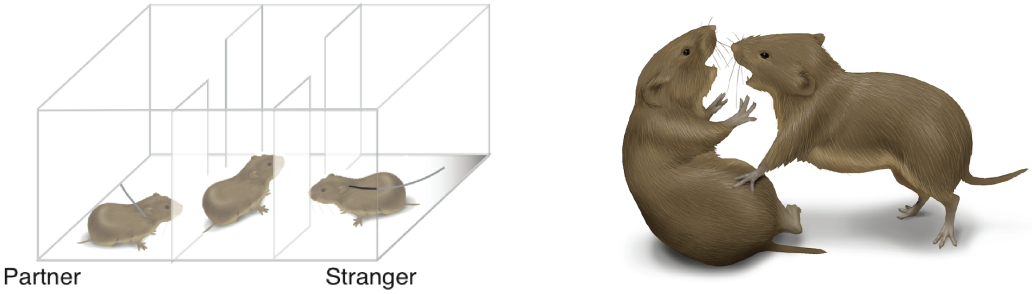

Figure 1.2. Measuring Pair Bonding. (A) Partner Preference Test. The test consists of placing a test subject (of either sex) in a three-chamber apparatus in which two of the chambers house tethered stimulus animals. One chamber contains the “partner” (animal with whom the test subject has been paired; i.e., a conspecific that is familiar to the subject) another chamber contains a novel “stranger” (with which the subject is not familiar), and a third chamber that remains empty during the test. For three hours the test animal is allowed to roam freely among all the chambers and cage entries, time spent in cage, and time in side-by-side contact are quantified. If time spent in side-by-side contact (in minutes; for a maximum total of 180 minutes) with the partner exceeds time spent in contact with the novel animal, an animal is said to have developed a “partner preference”. **(B) Resident Intruder Test.** Pair bonded prairie voles show high levels of aggression toward novel conspecifics from which we can infer pair bond strength. After two weeks of pairing, the resident-intruder test is conducted and will consist of measuring aggressive interactions for a short period of time (typically 10 minutes) between the subject animal “resident” and an unfamiliar “intruder” that is introduced into the home cage. Aggressive behaviors such as lunges, attacks, offensive rearing, biting, and chasing are recorded.

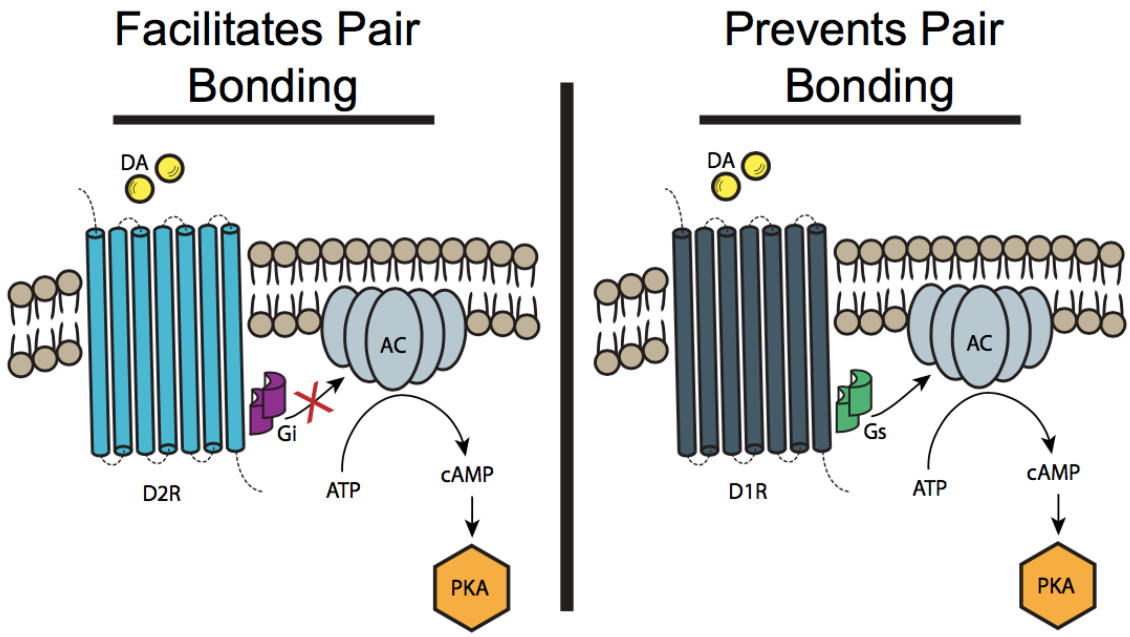

Figure 1.3 Dopamine signaling and pair bond formation. Pair bond formation is facilitated by decreased activity of the cAMP system (left panel Gi -> AC prevented), whereas increased activity of this system (right panel) prevents this behavior. Because D2-like activation and subsequent decreases in cAMP activity and facilitate pair bond formation. Abbreviations: DA, dopamine; D2R, D2 Receptor; D1R, D1 receptor; Gi, inhibitory g protein; Gs, stimulatory g protein; AC, adenylate cyclase; ATP, adenosine triphosphate ;cAMP, cyclic adenosine monophosphate ; PKA, protein kinase A

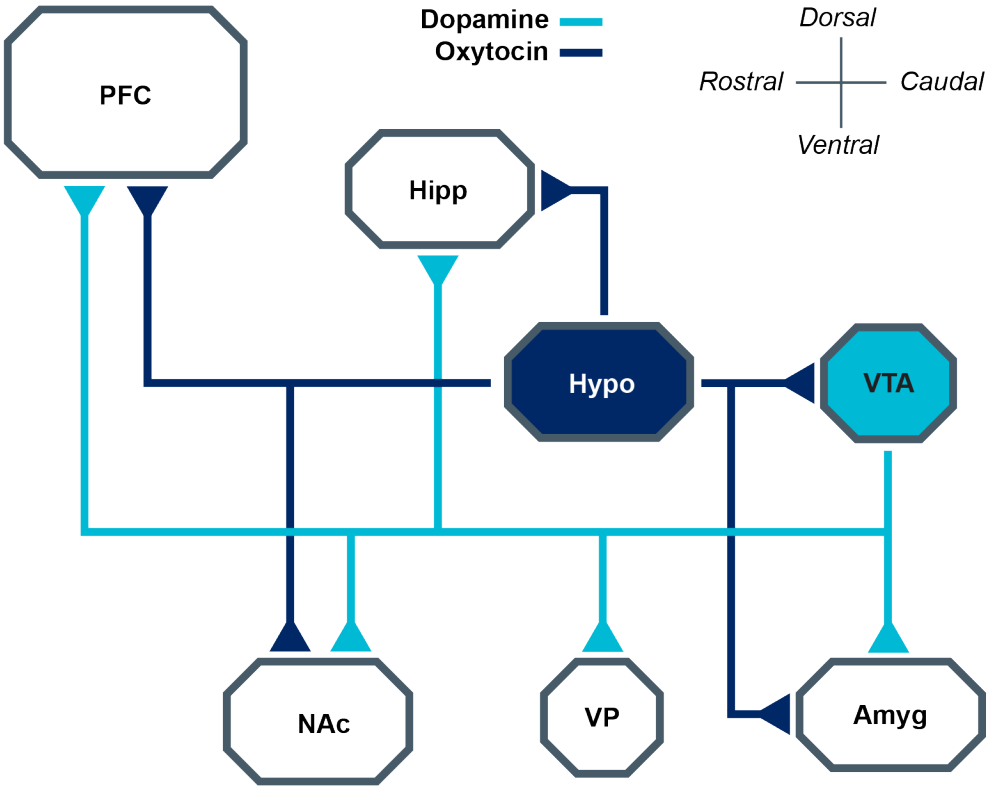

Figure 1.4. Oxytocin and DA Signaling in Pair Bonding/Motivation. Simplified illustration of DA and OT signaling. The regions depicted here are thought to participate in the processing of motivation and social stimuli. DAergic fibers within the mesocorticolimbic pathway (light blue) project from the ventral tegmental area VTA are key regulators of motivation. These DAergic projections onto the VP are the major output of this pathway which then connects to motor regions to invigorate behavior. Activity within this pathway can be modulated by OT (dark blue). Abbreviations: Amyg, amygdala; Hipp, hippocampus; Hypo, hypothalamus; NAc, nucleus accumbens; PFC, prefrontal cortex; VP, ventral pallidum; VTA, ventral tegmental area.

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Chapter 2

Dopamine D3 Receptors Within the Nucleus Accumbens Shell Mediate Pair Bond Formation

Abstract

In the socially monogamous prairie vole, activation of D2-like dopamine receptors within the rostral nucleus accumbens shell promotes pair bond formation. However, the specific dopamine receptor (i.e., D2 or D3) responsible for this remains unknown. Here, we first replicate findings showing that a mixed D2/D3 receptor agonist, quinpirole (.04 ng), when infused into the nucleus accumbens shell, induces robust partner preferences. We subsequently sought to determine if selective activation of D2 or D3 receptors within the rostral nucleus accumbens shell, via infusions of the specific D2R agonist 5,6,7,8-Tetrahydro-6-(2-propenyl)-4H-thiazolo[4,5-d]azepine-2-amine dihydrochloride (BHT-920; 3 ng) or the specific D3R agonist R(+)-2-Dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (7-OH-DPAT; 3 ng), influences partner preference formation. Indeed, selective activation of D3 receptors is sufficient to induce partner preferences while selective D2 receptor activation is not. Importantly, activation of D3 receptors increased general affiliative contact during the cohabitation period. Together, these findings show a sufficient role for the D3 receptor in the development of partner

preferences in male prairie voles and have broad implications for our understanding of how dopamine mediates an adaptive motivated behavior.

Introduction

The socially monogamous prairie vole (*Microtus ochrogaster*) serves as an ethologically sound model for the study of social bonding[1,2]. Unlike most mammals, prairie voles establish life-long pair bonds in which both male and female show a stable preference for each other, reject novel mating partners, and equally share in offspring and nest caretaking[2–6]. Development and maintenance of these bonds relies on activation of the mesolimbic dopamine (DA) system, which is centrally involved in reward processing and the generation of motivated behavior[7–10]. Specifically, activation of D2-like DA receptors within the rostral nucleus accumbens (NAc) shell promotes partner preference formation in sexually naïve prairie voles[7,11]. However, the D2-like class of receptors includes D2, D3, and D4 subtypes, and the specific receptor(s) responsible for promoting partner preference formation are not yet known.

Determining the individual contributions of these receptors poses unique challenges due to their similar binding affinity and overlapping expression[12,13]. Both D2 and D3 receptors are expressed in the striatum and share a high degree of homology (75%) in their binding sites[14]. However, D3 receptors are strong candidates for mediating the rewarding properties of social interactions due to their more concentrated expression within the NAc shell (the specific region of the striatum known to be important for pair bonding behavior), compared to D2 expression, which is dense throughout the whole of the striatum (i.e., dorsal and ventral striatum)[15–19]. Additionally, it has been suggested that

low levels of DA which preferentially act on D2-like receptors may be responsible for the development of pair bonds. Within the D2 class, D3 receptors show the highest affinity for DA, making them a likely target of the low levels of DA theorized to be released during partner preference formation. Further, D3 receptors are known to influence motivated behaviors including social recognition, male parental behavior, and addiction[20–25]. These qualities make the D3 receptor a strong candidate for mediating the development of pair bonds in the prairie vole.

In the current study we examined the role of D2 and D3 receptors in the regulation of partner preference behavior via site specific administration of DA subtype specific agonists. We first replicated findings showing that the administration of a mixed D2/D3 receptor agonist (quinpirole; .04 ng) into the rostral NAc shell induces robust partner preferences (huddling, rejection of novel mating partners, pup care)[7]. In a separate set of sexually naïve animals, we subsequently administered D2 and D3 selective agonists (7-OH-DPAT and BHT 920; 3 ng) to target these receptor subtypes and found that activation of DA D3 receptors was sufficient to induce partner preferences in sexually naïve animals in the absence of mating while activation of D2 receptors was not. These data are the first to show a role for the DA D3 receptor subtype in development of partner preferences in male prairie voles and perhaps a role for coactivation of D2 and D3 receptors in these processes.

Materials and Methods

Subjects. Subjects were sexually naïve adult male prairie voles (60-150 days old) bred at the University of Michigan (UM), weaned at 21 days and housed in single-sex sibling

pairs. Test subjects were randomly assigned to a test condition. Animals were on a 14:10h light-dark cycle and all experiments were conducted between 0800h and 1800h. All procedures were conducted in accordance with the UM animal care guidelines and approved by the UM Institutional Animal Care and Use Committee.

Stereotaxic Cannulation. For all surgeries, subjects were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine mixture at 0.1% body weight. Animals received stereotaxic surgery and were implanted with a 26-gauge bilateral guide cannula (Plastics One, Roanoke, VA) aimed at the NAc shell (+1.7 mm A/P; ±1 mm M/L; dorsomedial: −4.2 mm D/V; ventral: −4.5 mm D/V) as described in Resendez, et al., 2012. 10 mg/kg ketoprofen analgesic was provided preemptively, after surgery, and 24 hours after the first dose was administered. Animals were given 3-5 days to recover in their home cage with their cage mate. Additional pain relief was provided as needed.

Microinfusion. On test day, a 33-gauge stainless steel injector was used to infuse 200 nl/side at a rate of 200 nl/min of either vehicle or vehicle + drug mixture. For partner preference induction experiments animals received infusions of either aCSF (n=7) or aCSF containing .04 ng of quinpirole (n=4), 3 ng BHT-920 (n=6), or 3 ng 7-OH-DPAT (n=6) into the rostral NAc shell. Injectors were left in place for one minute after injection to ensure diffusion into the desired region (See “Verification of Cannula Placements”).

Cohabitation. Upon site-specific infusions of drug or vehicle, male subjects were paired with a novel ovariectomized female (thus, not sexually receptive) and allowed to cohabit

for 6 hours in the absence of mating. It has been determined that this length of pairing, in the absence of mating, does not induce a partner preference[8,26]. Thus, when used in combination with targeted pharmacological manipulations, this paradigm provides a reliable method to examine the neurobiological mechanisms that underlie partner preference formation. The first 10 minutes of every hour during the initial 6-hour pairing period were scored for affiliative behavior (olfactory investigation, friendly follow, anogenital sniffing, active contact, allogrooming, huddling) and locomotion (cage crosses). Additionally, the full 6-hour cohabitation period was scored for mating behavior and no animals were observed to mate during this time[27–29].

Partner Preference Testing. After the 6-hour cohabitation period, partner preference tests were conducted using a partner preference apparatus that consists of three chambers separated by 2 inch partitions (Figure 2.1). These partitions separate chambers that house on either side a tethered stimulus female (i.e., partner or stranger), and an empty center chamber. The subject (sexually naïve prairie vole) is then allowed to roam freely among the three chambers for a total of 3 hours (for further detail see [30]). "Partners" were females with which the test subjects had been paired for the 6-hour period, and "strangers" were age and weight matched ovariectomized females that had not previously encountered the subject. Subject animals were placed in the center compartment and were allowed to roam all three chambers for 3 hours (180 min)[31,32]. Video recordings were scored for huddling behavior in Behavior Tracker 1.0 Software (www.behaviortracker.com) at 4x speed by experimenters blind to treatment group assignment and inter-observer reliability was confirmed at 95%. Total time in side-by-side

contact with partner and stranger were compared and partner preferences were determined by statistical comparison of mean time spent with partner with mean time spent with stranger (see "Statistics and Data Analysis")[32–34].

Verification of Cannula Placements: Following the partner preference test in which huddling behavior with a partner and stranger animal is scored, animals received infusions of 200 nl/min/side of 20% Chicago Sky Blue 6B solution (Sigma Aldrich St. Louis, MO) in 0.9% saline for verification of cannula placements post mortem. Subjects were killed via rapid decapitation and brains were snap frozen in powdered dry ice and stored at -80°C until sectioning. Cannula placements were determined by an experimenter blind to treatment condition and only subjects whose cannula placements were in the rostral region of the NAc were included in analyses.

Statistics and Data Analysis. All statistical analyses were carried out using GraphPad Prism (Version 7, GraphPad Software, Inc., San Diego, CA) and the Statistical Package for the Social Sciences (SPSS version 21; IBM SPSS Inc., Chicago, Illinois). Data from partner preference experiments were analyzed using a two-way ANOVA in which stimulus animal (partner or stranger) and treatment were used as factors. In addition, within treatment analysis to compare time in contact with the partner and stranger was performed with a paired t-test. In order to determine if drug treatment affected affiliative or locomotor activity during the cohabitation period, a two-way ANOVA (treatment x time) followed by Tukey's post hoc test was used[27,31]. Additionally, a two-way ANOVA (treatment x chamber) followed by Tukey's post hoc test was also used to determine if

drug treatment affected total amount of time spent in each individual chamber during the partner preference test. Total contact time during partner preference testing and during cohabitation period were compared via one way-ANOVA. In all cases, statistical significance was set at $p < 0.05$.

Results

D2/D3 Receptor Induced Partner Preferences

Previous work utilizing a mixed D2/D3 receptor agonist to induce partner preferences showed a key role for D2-like receptors in the development of pair bonds[7]. Analysis of the overall data set using a 2-way ANOVA revealed a significant main effect of stimulus animal F (1,38) = 35.78, p = 0.0001, and an interaction effect between treatment and stimulus animal F (3, 38) = 6.62, p = 0.0010. In order to determine which groups preferentially spent time in contact with partners over strangers, Student's t-tests were performed. We first replicated these data showing that animals that received injections of .04 ng of the mixed D2/D3 receptor agonist, quinpirole, into the rostral NAc shell formed robust partner preferences in the absence of mating t(4)=6.703, p = 0.0068 (Figure 2.2). Animals treated with quinpirole did not differ significantly in locomotor behavior during the 6 hour cohabitation period F(5, 60) = 0.27, p = 0.9268 (Table 2.2), nor did they differ from controls in their total amount of affiliation during the 6 hour cohabitation period F(1, 48) = 2.76, p = 0.1030 (Table 2.1). Animals treated with quinpirole, did vary significantly in their total contact time (i.e., contact with "partner" and "stranger") during the partner preference test F(1, 5) = 13.83, p = 0.0137. These results are logical as this is mostly a reflection of the high amount of contact with the partner

during the partner preference test. Importantly, while they showed high levels of contact with their partner, they did not differ significantly in the amount of time spent in each chamber of the apparatus $F(2, 24) = 4.12$, $p = 0.0291$ (Figure 2.2), showing that they investigated the additional options.

Our replication of D2/D3 induced partner preferences in the rostral NAc shell emphasizes the importance of this region in the expression of this behavior; however, concluding that the responsible receptor subtype or subtypes can be identified based on these findings is not possible due to the use of a non-selective D2-like agonist. In fact, while quinpirole is regarded as a mixed agonist, its affinity for D3 receptors has been shown to be higher, at times, than that of D2[14,35]. Thus, in order to determine the receptor subtype(s) (D2R or D3R) responsible for pair bonding, the selective D2 receptor agonist BHT-920 (3 ng) and the selective D3 agonist 7-OH-DPAT (3 ng) were administered into the rostral NAc shell in the subsequent experiment.

D2 receptor activation does not induce partner preferences

In order to determine whether D2 receptor activation is responsible for pair bonding, the selective D2 receptor agonist BHT-920 was administered into the rostral NAc shell in a second cohort of sexually naïve male prairie voles. This agonist has been shown to be selective for the D2 subtype via fast scan cyclic voltammetry (FSCV) in mouse striatal slices[36]. Unlike treatment with quinpirole, males treated with the selective D2 agonist were not significantly different in their partner preference than those treated with vehicle $t(6) = 0.740$, $p = 0.4922$ (Figure 2.2). Treatment with the D2 agonist did not impact total contact time $F(1, 5) = 0.41$, $p = 0.5500$ or total time spent in each chamber

of the partner preference apparatus $F(2, 30) = 0.03$, $p = 0.9731$. Treatment with B-HT 920 showed a trend toward increasing affiliative behaviors during the 6 hour cohabitation period $F(1, 48) = 2.93$, $p = 0.0934$. Treatment with the D2 receptor agonist did, however, decrease locomotion during the 6 hour cohabitation period (F (1, 60) = 6.35, $p = 0.0144$ (Table 2.2). This is not surprising as D2 receptors are highly involved in motor behavior and the use of other DA agonists in other species has been shown to impact locomotion via its actions at the Nac[37,38]. Importantly, this demonstrates that even at a physiologically relevant dose, partner preference behavior was not affected. These findings may provide some additional insight in that there are two known isoforms of the DA D2 receptor which are expressed mainly at pre (D2 short; D2S) or post synaptic sites (D2 long; D2L) and drug effects on locomotion are mediated via D2L receptors, suggesting that B-HT 920 may selectively activate post synaptic (i.e., D2L) receptors in the prairie vole[39].

D3 receptor activation induces partner preferences

In order to asses the contribution of the D3 receptor to pair bond formation we used the selective D3 agonist 7-OH-DPAT and infused it into the rostral NAc shell in a third set of sexually naïve male prairie voles. Males treated with the selective D3 agonist in the rostral shell showed significant partner preferences $t(6) = 4.275$, $p = 0.0079$ (Figure 2.2). Animals treated with 7-OH-DPAT did not differ significantly in their total contact time (i.e., contact with either animal) $F(1, 4) = 0.06$, $p = 0.8137$ during partner preference test or locomotion as assessed by cage crosses during the cohabitation period $F(5, 60) = 1.80$, $p = 0.1270$ (Table 2.2), although they did show a slight trend for exploring the partner chamber of the apparatus $F(2, 27) = 2.74$, $p = 0.08$ (Figure 2.2). Importantly, this

experimental group was the only group to show a significant increase in affiliative behavior during the 6 hour cohabitation period $F(1, 54) = 4.34$, $p = 0.0420$ (Figure 2.3). Additionally, this affiliative behavior showed an increase throughout the 6 hours of pairing $F(5, 54) = 5.53$, $p = 0.0004$. The results allow us to conclude that the ability of D3 receptor agonist treatment to induce partner preferences may be via increased social investigation during the cohabitation period. Importantly, the fact that the animals showed only a trend for the partner chamber of the apparatus demonstrates that despite developing a pair bond, the animals investigated the novel options.

Discussion

DA in Partner Preference Formation

The development of monogamous pair bonds in the prairie vole is a motivated behavior important for reproduction. Thus, it is not surprising that the DA system, which regulates motivation to seek natural rewards such as food and shelter, also mediates behaviors relevant to the pursuit, development, and maintenance of pair bonds[7,8,11,27,28,40–43]. The present findings expand on previous work from Aragona (2006) to show for the first time that within the D2-like class of DA receptors, it is the NAc DA D3 receptors that are critically involved in the development of partner preferences in the socially monogamous prairie vole.

Here, we first replicate findings showing that the mixed D2/D3 receptor agonist, quinpirole, when injected into the rostral NAc shell, can induce partner preferences under short cohabitation periods in the absence of mating. We then follow up by showing that a

selective D3 receptor agonist (7-OH-DPAT) injected into this same region can induce partner preferences but a selective D2 receptor agonist (B-HT 920) cannot. These findings suggest that the mixed D2/D3 agonist, quinpirole may be inducing its effects either by binding to D3 receptors, or that it may be activating both D2 and D3 receptors concurrently. Indeed, it is known that D2/D3 receptors can form dimers (i.e., receptor complexes) and that these have higher affinity for DA than either receptor on its own[44]. This hypothesis can be tested via brain collection after the cohabitation period subsequent assaying of heterodimers within the NAc via immunoprecipitation for D2 and D3 receptor complexes. An alternative explanation for the efficacy of the mixed D2/D3 agonist is the observation that transient activation of D3 receptors can increase agonism efficacy at D2 receptors[44,45]. That is, the transient activation of D3 receptors may "prime" D2 receptors to more efficiently activate intracellular signaling after binding of DA.

DA Activation and Affiliative Behavior

Animals treated with the selective D3 receptor agonist, 7-OH-DPAT, showed increased affiliative behaviors such as olfactory investigation, friendly following, anogenital sniffing, active contact, allogrooming, and huddling. Because pairing was ended at 6 hours, we cannot know how long this effect on affiliation persisted, and future research should investigate these parameters. Interestingly, while D2 receptor activation did not induce partner preferences, there was a slight trend for increased affiliation in animals treated with B-HT 920 (D2 agonist). The fact that both D2 and D3 receptors increased affiliative behaviors, yet only D3 receptor activation was able to induce pair bonds, suggests that D3 receptors may be working with other systems within this circuitry,

or that they are unique in other properties that contribute to social behaviors. Oxytocin and opioid circuitry are known to interact with DA in this region and are theorized to contribute to the attribution of hedonic value to salient stimuli, such as meeting a potential mating partner[46,47]. Future work should determine the interactions of D3 receptors with hedonic processing systems present within the NAc[48,49].

DA D3 Receptors in Motivated Behavior

The NAc

Autoradiography is the most common way to measure receptor expression. While it has been shown that the D3 receptor is highly expressed in the more limbic regions of the NAc in rats, mice, and humans, we do not have measures of these receptors in prairie voles[50]. If these receptors are similarly expressed in prairie voles, then this would support the assumption that DA is working in concert with other systems to mediate pair bonding behavior. In fact, DA receptor expression and signaling measurements in prairie voles have shown that they are consistent with that of other rodents, whereas the expression of OT differs significantly among monogamous and non-monogamous species[7,51,52]. Based on these observations, it may be possible to assume that it is the action of DA in relation to the restricted expression of OT receptors in this region that underlies the development of pair bonds. Future work on this model should characterize the specific mechanisms of such interactions by determining the existence of receptor complexes and coactivation of these receptors during the cohabitation period.

Drug Taking

In recent studies, the D3 receptor has being targeted for the treatment of drug addiction as well as other psychiatric disorders such as Parkinson's disease[53,54]. In particular, the D3 receptor has been implicated in the learning and salience attribution processes present during the binging and intoxication stages of addiction[55,56]. Additionally, D3 receptors are expressed within circuitry that mediates drug-, cue-, and stress-induced reinstatement of drug seeking[57–59]. Further, Individuals that have died from cocaine overdose show heightened D3 receptor expression in the striatum , and rats treated with D3 receptor antagonists will stop self-administering cocaine[60,61]. Antagonist treatment abolishes conditioned place preference (CPP) for cocaine and morphine[62]. Further, blockade of D3 receptors blocks cue-induced reinstatement of cocaine seeking, and deletion of the D3 receptor gene results in attenuation of cocaine-induced place preferences in rats[63–68]. It has been established that social bonding processes share many neurobiological mechanisms with other motivated processes like addiction, and the D3 receptor may underlie aspects of this connection.

Conclusion

In the prairie vole, the development of pair bonds is an adaptive and highly motivated behavior regulated by mesolimbic DA circuitry[69]. The present study demonstrates a role for the DA D3 receptor in the development of a preference for a familiar partner. These receptors are highly expressed within the NAc which is the region of the striatum that regulates pair bonding behavior and can be activated by low levels of DA which are hypothesized to be released during the highly affiliative stages that are characteristic of the early development of pair bonds[12,50]. Interestingly, receptors in this

region have been implicated in salience attribution processes in drug addiction, and thus, determining the mechanism by which D3 receptors may mediate salience attributions for an adaptive behavior such as pair bonding in the prairie vole, can help inform our understanding of these processes in maladaptive conditions.

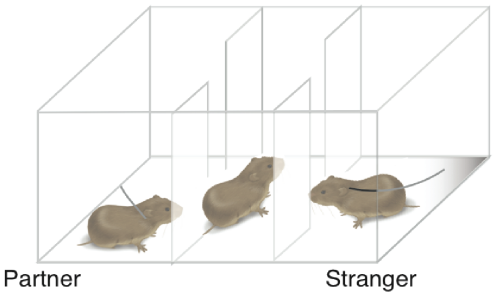

Figure 2.1. Illustration of Partner Preference Test. The test consists of placing a test subject (male) in a three-chamber apparatus in which two of the chambers house tethered stimulus animals (ovariectomized females). The partner is that animal with whom the test subject has been paired and the stranger is a novel conspecific. For three hours the test animal is allowed to roam freely among all the chambers and cage entries, time spent in cage, and time in side-by-side contact are scored in minutes for a total maximum of 180 minutes. If time spent in side-by-side contact with the partner exceeds time spent in contact with the novel animal, an animal is said to have developed a “partner preference”.

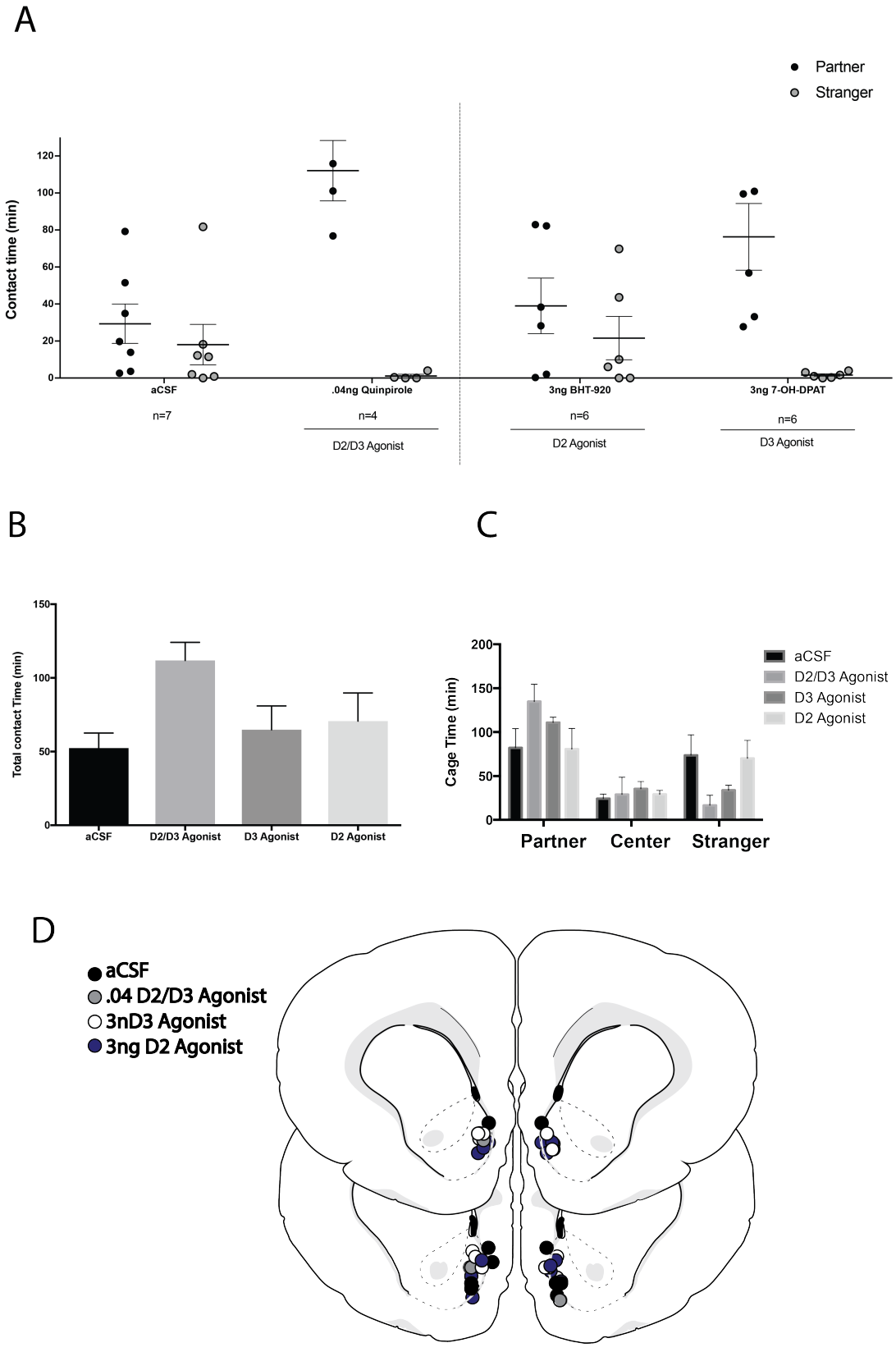
A
Partner
Stranger
Contact time (min)
aCSF
.04ng Quinpirole
3ng BHT-920
3ng 7-OH-DPAT
n=7
n=4
n=6
n=6
D2/D3 Agonist
D2 Agonist
D3 Agonist
B
Total contact Time (min)
aCSF
D2/D3 Agonist
D3 Agonist
D2 Agonist
C
Cage Time (min)
aCSF
D2/D3 Agonist
D3 Agonist
D2 Agonist
Partner
Center
Stranger
D
aCSF
.04 D2/D3 Agonist
3nD3 Agonist
3ng D2 Agonist

Figure 2.2. Activation of DA D3 receptors in the NAc shell induces partner preferences in sexually naïve males. (A) Animals that received injections of .04 ng of the mixed D2/D3 receptor agonist, quinpirole, into the rostral NAc shell formed robust partner preferences in the absence of mating $t(4)=6.703$, $p = 0.0068$. Animals treated with B-HT 920 (D2 Agonist). Unlike treatment with quinpirole, males treated with the selective D2 agonist were not significantly different in their partner preference than those treated with vehicle $t(6) = 0.740$, $p = 0.4922$. Males treated with the selective D3 agonist in the rostral shell showed significant partner preferences $t(6) = 4.275$, $p = 0.0079$. **(B)** Animals treated with quinpirole, did vary significantly in their total contact time (i.e., contact with "partner" and "stranger") during the partner preference test $F(1, 5) = 13.83$, $p = 0.0137$. Animals treated with B-HT 920 (D2 Agonist) did not differ in their total contact time $F(1, 5) = 0.41$, $p = 0.5500$. Animals treated with 7-OH-DPAT did not differ significantly in their total contact time (i.e., contact with either animal) $F(1, 4) = 0.06$, $p = 0.8137$ during partner preference test. **(C)** Animals treated with quinpirole did not differ significantly in the amount of time spent in each chamber of the apparatus $F(2, 24) = 4.12$, $p = 0.0219$, showing that they investigated the additional options. Animals treated with B-HT 920 (D2 Agonist) did not differ in their time spent in each chamber of the partner preference apparatus $F(2, 30) = 0.03$, $p = 0.9731$. Animals treated with 7-OH-DPAT showed a slight trend for exploring the partner chamber of the apparatus $F(2, 27) = 2.74$, $p = 0.0824$**.** In all cases, statistical significance was set at $p < 0.05$.

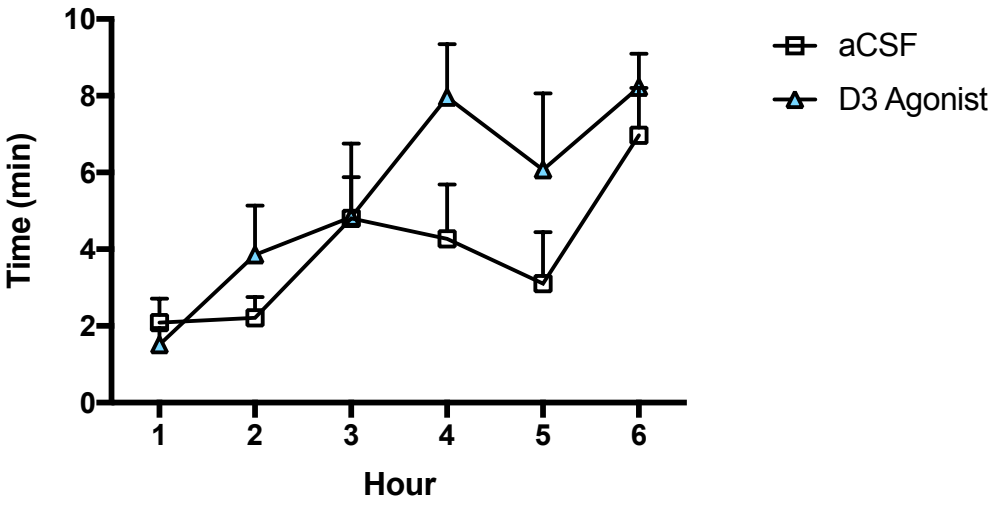

Figure 2.3. Affiliative behavior during the cohabitation period. Animals treated with 7-OH-DPAT (D3 Agonist) showed a significant increase in affiliative behavior during the 6 hour cohabitation period $F(1, 54) = 4.34$, $p = 0.0420$ (Figure 2.3). Additionally, this affiliative behavior showed an increase throughout the 6 hours of pairing $F(5, 54) = 5.53$, $p = 0.0004$. The results allow us to conclude that the ability of D3 receptor agonist treatment to induce partner preferences may be via increased social investigation during the cohabitation period. Only the first 10 minutes of every hour are scored for affiliation, thus the maximal score for each hour is 10. Affiliative behaviors scored included olfactory investigation, friendly follow, anogenital sniffing, active contact, allogrooming, and huddling. In all cases, statistical significance was set at $p < 0.05$.

Locomotion						
	Hour					
Group	1	2	3	4	5	6
aCSF	21±3.92	12.67±2.80	5.83±1.47	5±2.22	3.33±1.50	1.33±0.95
.04ng D2/D3 Agonist	16±3.19	9.25±2.72	3.5±3.5	4.25±3.28	3.5±2.18	1.75±1.18
3ng D3 Agonist	28±14.94	9.8±7.24	4.4±2.38	3.8±3.10	2.2±0.8	1.2±0.8
3ng D2 Agonist	9.75±1.50	6±0.88	3.25±0.20	0.75±0.61	2.75±0.841	3±1.05

Table 2.1. Locomotor behavior during 6 hour cohabitation period. Animals treated with quinpirole did not differ significantly in locomotor behavior during the 6 hour cohabitation period $F(5, 60) = 0.27$, $p = 0.9268$. Treatment with the D2 receptor agonist did, however, decrease locomotion during the 6-hour cohabitation period ($F(1, 60) = 6.35$, $p = 0.0144$. This is not surprising as D2 receptors are highly involved in motor behavior and the use of other DA agonists in other species has been shown to impact locomotion via its actions at the Nac[37,38]. Importantly, this demonstrates that even at a physiologically relevant dose, partner preference behavior was not affected. Animals treated with 7-OH-DPAT did not differ significantly in their locomotion as assessed by cage crosses during the cohabitation period $F(5, 60) = 1.80$, $p = 0.1270$. All data are presented as mean $\pm$ standard error of the mean (SEM). In all cases, statistical significance was set at $p < 0.05$.

Affiliative Behavior

Group	Hour					
	1	2	3	4	5	6
aCSF	2.09 ± 0.63	2.21 ± 0.54	4.8 ± 1.08	4.27 ± 1.42	3.11 ± 1.35	6.97 ± 1.24
.04ng Quin	2.05 ± 0.80	2.78 ± 1.04	7.9 ± 0.79	5.61 ± 1.99	4.53 ± 1.67	7.85 ± 1.88
3ng BHT-920	2.79 ± 0.66	4.34 ± 1.04	4.56 ± 1.63	7.42 ± 1.45	6.06 ± 1.09	6.17 ± 1.78
3ng 7-OH-DPAT	1.52 ± 0.44	3.85 ± 1.29	4.84 ± 1.92	7.96 ± 1.39	6.07 ± 1.99	8.22 ± 0.87

Table 2.2. Affiliative Behaviors during 6 hour cohabitation period. Animals treated with quinpirole did not vary significantly in the amount of affiliation during the 6 hour cohabitation period $F(1, 48) = 2.76$, $p = 0.1030$. Treatment with B-HT 920 showed a trend toward increasing affiliative behaviors during the 6 hour cohabitation period $F(1, 48) = 2.93$, $p = 0.0934$. Animals treated with 7-OH-DPAT (D3 Agonist) showed a significant increase in affiliative behavior during the 6 hour cohabitation period $F(1, 54) = 4.34$, $p = 0.0420$ (Figure 2.3). Additionally, this affiliative behavior showed an increase throughout the 6 hours of pairing $F(5, 54) = 5.53$, $p = 0.0004$. The results allow us to conclude that the ability of D3 receptor agonist treatment to induce partner preferences may be via increased social investigation during the cohabitation period. Only the first 10 minutes of every hour are scored for affiliation, thus the maximal score for each hour is 10. All data are presented as mean ± standard error of the mean (SEM). In all cases, statistical significance was set at $p < 0.05$.

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Chapter 3

Functional Fast Scan Cyclic Voltammetry Assay to Characterize Dopamine D2 and D3 Autoreceptors in the Prairie Vole Striatum

Abstract

Presynaptic dopamine D2 and D3 receptors can control the synthesis and release of dopamine via their function as autoreceptors. In mice and rats these receptors have been shown to exhibit a restricted expression that differs in a meaningful way— the D2 receptor is dense throughout the striatum while the expression of the D3 receptor is highly localized to the nucleus accumbens shell. With the use of fast scan cyclic voltammetry in striatal slices, we can take advantage of these unique signaling and expression properties to characterize the selectivity and functional activity of putative D2 and D3 selective agonists in the prairie vole brain based on their ability to quickly and completely inhibit dopamine release in these regions. Here, we measured the efficacies of the D2-selective agonist 5,6,7,8-Tetrahydro-6-(2-propenyl)-4H-thiazolo[4,5-d]azepine-2-amine dihydrochloride (B-HT 920), the D3 selective agonist R(+)-2-Dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (7-OH-DPAT), and the mixed D2/D3 receptor agonist, quinpirole. B-HT 920 had highest efficacy in the dorsal striatum whereas quinpirole was more effective in the nucleus accumbens shell at inhibiting dopamine

release than either the selective D2 or D3 agonist. Surprisingly, the D3 agonist, 7-OH-DPAT, showed similar effects in both regions examined. This result was unanticipated as inhibition of dopamine release by agonists is thought to reflect receptor distribution and reports in other species show greater density D3 receptors in the nucleus accumbens shell compared with dorsal striatum. Our results suggest that the prairie vole shows a unique response to quinpirole compared to other species and that the distribution of D3 receptors in the prairie vole may not follow the same pattern as in other species.

Introduction

Unlike most mammals, the socially monogamous prairie vole (*Microtus ochrogaster*) forms a lifelong bond characterized by sexual exclusivity, aggressive rejection of novel mating partners, and biparental care of offspring[1–6]. Motivation to seek out and engage with a potential mating partner and further remain in the nest to care for offspring is necessary for the development and maintenance of these bonds and so, it is not surprising that dopamine (DA) D2-like receptor signaling within the mesocorticolimbic system, which regulates the expression of motivated behavior, mediates the expression of the prairie vole's unique sociality as well[7–12].

The use of D2-like receptor agonists to manipulate pair bonding behavior have elucidated many aspects of prairie vole pair bonding, however, the functional properties of these agonists in the prairie vole brain have not been tested. Importantly, receptors within the D2 class are highly homologous, thus, determining their functional activity is imperative for the analysis of behavioral data. Here, we characterize the effects of drugs that bind the 2 DA receptor subtypes and provide surprising and useful insights for the interpretation of behavioral studies that have used these pharmacological agents.

DA Signaling

DA signaling within the nucleus accumbens (NAc) regulates the development and maintenance of pair bonds in this species[7,13]. Neuroanatomical studies of this region in

other species have determined that this is a heterogeneous region composed of individual components known to regulate a variety of behaviors relating to motivation and hedonic processing[10,14]. In particular, the striatum can be subdivided into the dorsal striatum (DS), and the ventral striatum (VS) or NAc, which itself is comprised of distinct shell and core regions. The DA cell bodies innervating the DS and NAc regions originate from two distinct components of the midbrain, the Substantia nigra (SN) innervates the DS while the ventral tegmental area (VTA) innervates the NAc[15]. For the prairie vole, it is the VTA projection onto the rostral NAc, but not the caudal NAc that regulates the development and maintenance of pair bonds in this species[7].

Additionally, these regions display different dopamine signaling characteristics. The DS expresses high levels of extracellular DA and higher rates of DA uptake than the NAc[16–18]. The individual regions of the NAc also display distinct DA signaling. Specifically, the core has been shown to have higher levels of extracellular DA and higher levels of electrically evoked DA release and uptake than the shell region[19–21].

DA binds to any of 5 receptors which are categorized as D1-like or D2-like based on their activation or inhibition of adenylate cyclase, respectively. The D1 class of receptors includes the D1 and D5 receptors which are low affinity receptors activated by high levels of dopamine release[22]. The D2 class of receptors includes D2, D3, and D4 receptors which are high affinity receptors, that is they are sensitive to low levels of DA release such as that present during tonic signaling[22]. It is theorized that low levels of DA release, such as those levels present during tonic firing, are important for the development of pair bonds in the prairie vole, as pharmacological manipulations that selectively target these receptors within the NAc can induce partner preferences in

sexually naïve prairie voles of both sexes[13,23–25]. The ability of slow DA signaling to produce dramatic and long lasting effects on behavior such as pair bonding, highlight the many ways in which DA can impact behavior via variations in release and uptake mechanisms that are further fine-tuned within the regions and receptors on which it is acting.

FSCV for the study of drug selectivity and autoreceptor functionality

When expressed at the presynaptic DA terminal, the D2 and D3 receptor subtypes function as autoreceptors[26–28]. Autoreceptors are homeostatic regulators of dopamine release which modulate extracellular levels of DA through a negative feedback loop. Increasing extracellular concentrations of DA result in a reduction of further DA release via the autoreceptors. Measurements in rats, mice, and humans have shown that the D2 autoreceptor is dense throughout the DS and NAc while the D3 autoreceptor is most dense in the NAc[29–34]. This unique property allows for the study of the functional properties, and in particular, the selectivity of drugs that target these receptors by measuring the ability of these drugs to inhibit DA release in discrete regions of the striatum known to exhibit different densities of D2 and D3 receptor expression, like the DS and NAc

In slice preparations, bath application of selective agonists activates D2 and D3 autoreceptors, subsequently reducing the amount of stimulated DA release[26]. More effective agonists induce full DA inhibition more quickly, and at lower doses. Thus, bath application of agonists followed by FSCV measures in discrete brain areas known to express different receptor subtypes provides a means by which to characterize

pharmacological agents as either D2 or D3 preferring[35,36]. If the prairie vole brain is assumed to have similar distribution of D2 and D3 receptors as other species such as mice and rats[31,35,37–39], then D2 preferring agonists that bind to autoreceptors should show similar efficacy in the DS and NAc while D3 preferring agonists should show higher efficacy within the NAc shell.

Here, we test the effects of DA D2 and D3 agonists that have been previously used in behavioral studies of prairie vole pair bonding via FSCV measures of electrically stimulated DA release in the prairie DS and NAc. We measured the efficacies of the DA D2-selective agonist, B-HT 920, the selective D3 agonist, 7-OH-DPAT, and the mixed D2/D3 receptor agonist, quinpirole at inhibiting DA release within the DS and NAc. B-HT 920 showed the highest efficacy in the DS, whereas quinpirole was the most effective drug tested in the NAc shell, inhibiting DA release to a greater extent than either the selective D2 or selective D3 agonists alone. These results suggest that quinpirole, is particularly well suited because of its high efficacy for dopamine receptors in the nucleus accumbens shell of the prairie vole which would support findings showing that this is a highly effective manipulation in prairie vole behavioral studies.

Materials and Methods

Subjects. Subjects were sexually naïve adult male prairie voles (60-150 days old) bred at the University of Michigan (UM), weaned at 21 days and housed in single-sex sibling pairs. Test subjects were randomly assigned to a drug condition. Animals were on a 14:10h light-dark cycle and all experiments were conducted between 0800h and 1800h.

All procedures were conducted in accordance with the UM animal care guidelines and approved by the UM Institutional Animal Care and Use Committee.

Slice preparation. Animals were killed via rapid decapitation and one hemisphere of the brain was used for FSCV experiments[40]. Left/right hemispheres used for FSCV were counter balanced. FSCV hemisphere was place in pre-cooled preoxygenated (95% O_2/5% CO_2) high sucrose-aCSF buffer for 10 min. High sucrose aCSF consisted of 180 mM sucrose, 30 mM NaCl, 4.5 mM KCl, 1 mM $MgCl_2$, 26 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, and 10 mM d-glucose (aCSF followed same recipe without addition of sucrose; both solutions were made in house and stored for use within 2 weeks)[41]. 400 µm-thick coronal striatal slices that included sections of the DS and VS were taken with a vibrating tissue slicer (Leica, Buffalo Grove, IL). Slices were maintained in oxygenated aCSF at room temperature for 1 h. After 1 hour a slice was transferred to a custom-made submersion recording chamber (Custom Scientific, Denver, CO) for recording and perfusion with selective agonists.

FSCV. In order to determine the functional activity of selective D2 and D3 receptor agonists, we conducted FSCV as previously described[35,36]. During experiments, a bipolar stimulating electrode and carbon fiber recording electrode (produced in house) and calibrated to a known DA concentration as described previously[42,43] were placed in the DS or rostral Nacc shell. Using software written in LABVIEW (National Instruments, Austin, TX; Tarheel CV University of North Carolina, Chapel Hill) a triangular ramp sweeping from -0.4V to +1.2V versus a Ag/AgCl reference was applied to the carbon-

fiber electrode at a rate of 10Hz. Once DA release was stabilized, the slice was bathed in increasing concentrations of quinpirole, B-HT 920, or 7-OH-DPAT (0.001, 0.01, 0.03, 0.1, 0.3, 1, 3, 10μM). Fifteen second recordings consisting of a 1 pulse 350μA stimulation at the 5 second mark were made at regular 5 minute intervals for a total of 6 recordings per drug dose (30 minutes per dose).

Statistics and Data Analysis. Data analysis and quantification of stimulated DA release was conducted as described previously[35,44]. Current versus time plots and electrically stimulated DA release rates were determined via use of Michaelis-Menten equations as described in[19]. All statistical analyses were carried out using GraphPad Prism (Version 7, GraphPad Software, Inc., San Diego, CA) and the Statistical Package for the Social Sciences (SPSS version 21; SPSS Inc., Chicago, IL). Data are presented as the mean ± standard error of the mean (SEM). For dose response experiments, the change in DA peak was compared to pre-drug values for each individual animal producing percent change in stimulated DA release. Percent change was then plotted against concentration (M). Data were assessed with a one-way ANOVA comparing drugs within each region (NAc shell or DS) followed by a Bonferroni post hoc test. In all cases, statistical significance was set at $P < 0.05$.

Chemicals. B-HT 920, 7-OH-DPAT were purchased from Tocris Bioscience (Minneapolis, MN), Quinpirole hydrochloride was purchased from Sigma Aldrich (St. Louis, MO). All drugs were made the morning of the experiment and dissolved in aCSF

Results

Bath-applying agonists to slice preparations results in a reduction in stimulated DA release. More effective agonists induce full DA inhibition more quickly, and at lower doses. Thus, bath application of agonists followed by FSCV measures in discrete brain areas known to express distinct receptor subtypes provides a means by which to characterize pharmacological agents as either D2 or D3 preferring[35]. As an example, representative release traces in the absence and presence of three doses of quinpirole (0 μM, 0.03 μM, 1 μM) are shown in Figure 3.1a. We hypothesized that D3 preferring agonists would show highest efficacy (i.e., strongest inhibition of DA release stimulated by a single electrical pulse) in the NAc shell region where D3 receptor expression has been found to be high in mice and rats[30,31], while D2 preferring agents would show similar inhibition in both regions as it is expressed densely throughout the dorsal and ventral striatum.

Maximal Response Compared at 1μM

To make a direct comparison between drugs within these regions, the concentration of 1μM was chosen as treatment with this concentration produced effects in all slices. Table 3.1 summarizes the maximal DA release at this concentration for each of the three drugs on both brain regions. Efficacy values are expressed as percent of drug effect in relation to pre-drug value defined as 100%. Because these are expressed as a percentage of pre-drug value, lower percentages (i.e., lower amount of DA release compared to baseline) reflect greater effect of the agonist within the specified region.

The mixed D2/D3 receptor agonist, quinpirole, exhibited the greatest DA release inhibition at 1 μM in the NAc shell with 18.77% release compared to baseline, although it also showed relatively greater inhibitory efficacy in the DS at 20.90% release compared to baseline. The inhibition effect of 1μM of the D3 receptor agonist was not significant in the NAc or DS with 49.67% and 46.06% release from baseline, respectively (although larger variation was observed in DS responses). Interestingly, the DA inhibition effect of the selective D3 agonist, 7-OH-DPAT, observed here was similar to those recorded in the DS of mice[35]. Finally, B-HT 920 DA inhibition (D2 receptor agonist) was comparable to that of the D3 receptor agonist in the shell with 42.56%, however treatment with B-HT 920 within the DS showed the maximum inhibition effect for this region with 12.21%.

FSCV Assay of D2/D3 Agonists in the Prairie Vole Striatum

Agonist efficacy was evaluated by measuring its effects on stimulated DA release within the NAc and the DS. All drugs inhibited DA release in a concentration dependent fashion that could be fitted to a monophasic curve (Figure 3.1, 3.2). Based on previous autoradiography and FSCV studies, we expected B-HT 920 to be equally effective at inhibiting DA release within the DS and NAc shell, as the expression of the D2 receptors within these regions has been observed to be relatively homogenous[31,35,45–48]. Per region analysis via a two-way ANOVA revealed a significant main effect of drug treatment F (2, 135) = 31.34, p = 0.0001 and drug dose F (8, 135) = 84.19, p = 0.0001 in the NAc shell. Post hoc analysis indicated that quinpirole was more effective at inhibiting DA release in the NAc shell at all doses administered compared to either of the drugs that bind to the D2 or D3 receptors wih a higher affinity. In the DS, analysis via a two-way ANOVA

revealed a significant main effect of drug treatment F (2, 71) = 11.55, p = 0.0001 and drug dose F (8, 71) = 34.50, p = 0.0001. Post hoc analysis indicated that B-HT920 was more effective at inhibiting DA release in the DS from treatment with .03uM and higher. These results were surprising as previous measurements of B-HT920 inhibition effects in the mouse brain showed comparable results in these regions.

Discussion

Here, we test the effects of DA D2 and D3 agonists that have been previously used in behavioral studies of prairie vole pair bonding via FSCV measures of electrically stimulated DA release in the prairie vole DS and NAc. We measured the efficacies of the D2-selective agonist, B-HT 920, the selective D3 agonist, 7-OH-DPAT, and the mixed D2/D3 receptor agonist, quinpirole, at inhibiting DA release within the DS and NAc shell. B-HT 920 had highest efficacy in the DS. We report here that quinpirole was more effective at inhibiting DA release within the NAc shell than either of the subtype selective agonists supporting the idea that this drug is particularly effective in the prairie vole NAc. These results suggest that quinpirole, is particularly well suited because of its high efficacy for dopamine receptors in the nucleus accumbens shell of the prairie vole.

The D3 agonist, 7-OH-DPAT, showed similar effects in both brain regions examined, the NAc shell and DS. This result was unanticipated as reports on other species show a higher density of the D3 DA receptor in the NAc shell[49]. This discrepancy may be a result of the following: (1) the agonist is not selective for the D3 receptor subtype or not selective at the doses used in this study in the prairie vole, or (2) the DA D3 receptor is not as dense or functionally active in this region in the prairie vole as it is in other

species. It should be noted, that these findings would not invalidate behavioral data acquired from the use of these drugs. Instead, these data should be interpreted with a sensitivity to the fact that FSCV experiments in slices investigate only one part of this system, that is, FSCV can only determine terminal regulation of DA release and cannot account for activity of these receptors in behavioral experiments on which it acts on an intact system. Additionally, FSCV can only infer drug selectivity and receptor functionality and not receptor density, thus, it is imperative to measure D3 receptor expression in the prairie vole striatum to determine if the limited effects of D3 activation are a result of low receptor density.

Additionally, the effect of B-HT 920 in the DS was also unexpected as previous measurements of B-HT 920 in the mouse brain showed comparable inhibition with this drug in the DS and NAc. Behavioral observations (see Chapter 2) have shown that use of this drug in prairie voles impacts locomotion. Locomotor behavior at D2 receptors in the NAc is mainly mediated by activation of post-synaptic D2L receptors. FSCV is limited in that it can only determine the effects of this drug on terminal release via activation of autoreceptors. However, the data included in Chapter 2 suggest that in the prairie vole, this drug may have a greater affinity for the post-synaptic receptors, rather than pre-synaptic receptors, which do not directly regulate DA release. Additionally, we demonstrate that the selective D2 agonist is most effective at inhibiting DA release within the DS and this may be either a result of varied expression or functionality of these receptors within the NAc and DS of prairie voles.

The mixed D2/D3 agonist, quinpirole, showed the greatest effect at the NAc, compared to the selective D2 and D3 agonists tested. Importantly, this region mediates

pair bonding behavior as well as the attribution of positive hedonics to a mating partner[50,51]. Because quinpirole is a mixed agonist, and neither agonist alone could produce as strong responses in the NAc, this suggests that quinpirole is particularly well formulated to exploit meaningful functional differences within this region (perhaps by concurrent activation of both D2 and D3 receptors). These findings are consistent with the robust partner preferences that are induced via quinpirole treatment in prairie voles[7]. Future work can determine if this is the case by utilizing quinpirole in the presence of selective D2 and D3 antagonists in slice preparations.

Conclusions

These data provide further evidence that the unique signaling properties of the striatum can be used in combination with techniques such as FSCV to measure the selectivity of drugs for the D2 and D3 autoreceptor. Two interesting findings suggest that DA D2-like receptor expression and/or functionality is unique in prairie voles compared to other rodent species. Indeed, future work on this model should characterize the expression of these receptors in the prairie vole brain, and determine whether concurrent activation of D2 and D3 receptors results in meaningful functional differences.

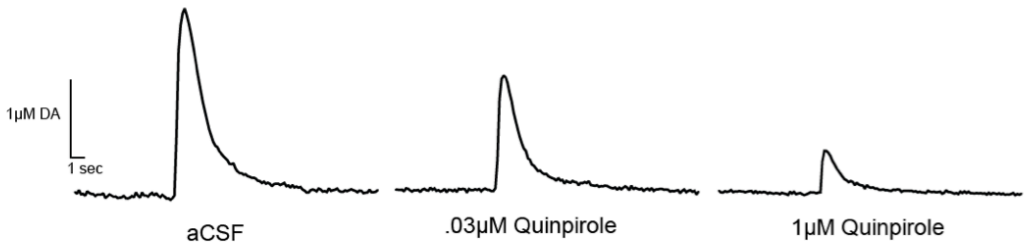

Figure 3.1. Effect of increasing concentrations of quinpirole on maximal evoked dopamine release in the DS

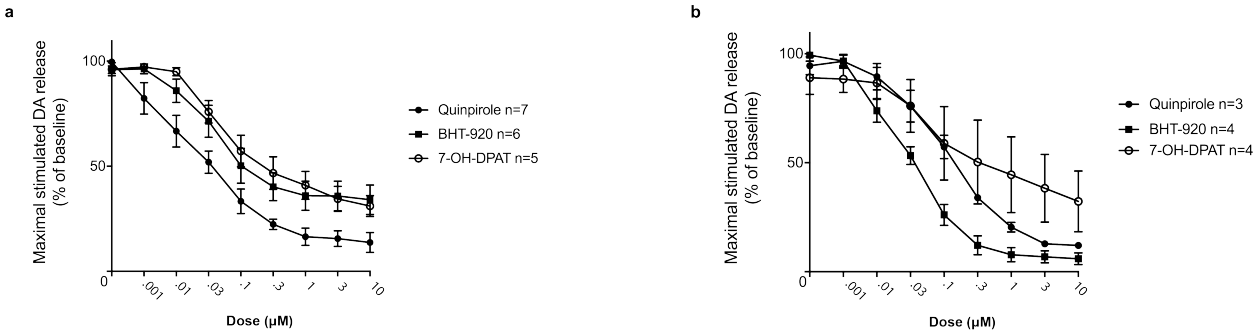

Figure 3.2. Effect of increasing concentrations of Quinpirole, B-HT 920 and 7-OH-DPAT on maximal evoked dopamine release in the striatum. NAc shell (a) and DS (b) the log concentration of drug is graphed on the horizontal axis versus the amount of dopamine released expressed as a percentage of the baseline (prior to agonist exposure).

	Region	
Drug	**Shell**	**DS**
	mean±SEM effect (%)	**mean±SEM effect (%)**
Quinpirole	18.77±2.20	20.90±0.96
7-OH-DPAT	49.67±5.41	46.06±13.69
B-HT 920	42.56±3.78	12.21±2.90

Table 3.1. Maximal Inhibition Effect at 1uM doses of Dopamine D2 and D3 Agonists Direct comparisons of DA release inhibition were made at 1uM doses of Quinpirole, 7-OH-DPAT, and B-HT 920. Percentages represent percent decrease in DA response from baseline.

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Chapter 4

Dopamine Autoreceptor Subsensitivity Underlies Pair Bonding Induced Increases in Stimulated Dopamine Release in the Prairie Vole

Abstract

The expression of monogamous pair bonds in the prairie vole is regulated in part via dopamine signaling within the nucleus accumbens shell region of the striatum. We have previously shown that a 14-day pairing period results in increased stimulated dopamine release within this region in a manner that reflects reproductive compatibility of the pair. Here, we utilize fast scan cyclic voltammetry to measure dopamine release dynamics in the nucleus accumbens shell of sexually naïve and 28-day pair bonded prairie voles. We demonstrate that similar to the 14-day pair bonding period point, pregnancy status is reflected in dopamine release in males after 28 days of pairing such that males from pairs that became pregnant quickly after pairing show the largest increases. Additionally, we test the effects of the dopamine D2/D3 receptor agonist, quinpirole, on electrically stimulated dopamine release in the dorsal striatum and nucleus accumbens as a measure of dopamine autoreceptor functionality. Interestingly, pair bonded prairie voles show subsensitive dopamine autoreceptor activity, as demonstrated by a quinpirole dose effect curve analysis. Together, our findings show that pair bonding increases stimulated dopamine release in regions relevant to reward processing and motivation in male prairie

voles in a fecundity dependent fashion and suggests that alterations in dopamine autoregulation either via reduced dopamine autoreceptor expression or functionality may underlie these increases. In a final experiment, we show that increased oxytocin tone can mimic this effect of pair bonding at the dopamine autoreceptor in sexually naïve animals. Together, these data provide valuable information on how the dopamine system can be reorganized based on experience.

Introduction

The development and maintenance of social bonds is a critical component of human well-being, however, we do not have a comprehensive understanding of the neurobiological mechanisms that underlie these processes[1]. The socially monogamous prairie vole is a useful research model for the study of social bonding because it develops lifelong pair bonds in which both male and female contribute equally in pup caretaking and aggressively reject novel conspecifics[2]. Furthermore, while a variety of species express monogamous strategies only through breeding seasons or while offspring are in the nest, few mammals form lifelong pair bonds like the prairie vole[3–8]. The mechanisms that underlie the long term nature of prairie vole pair bonding are not fully characterized however, the mesolimbic dopamine (DA) system has been found to play a central role in these processes[9–14]. Specifically, *plasticity* within this circuitry has been implicated in the behavioral transition during which prairie voles will stop engaging in affiliative investigation of novel social stimuli as occurs during the pair bond development phase and begin to express the highly aggressive behavior typical of the maintenance phase during which prairie voles reject novel conspecifics[11,12]. This behavioral transition reliably reflects the strength of a given pair bond and is correlated with that prairie vole pairs' reproductive success[12]. That is, males from pairs that became pregnant quickly after pairing show higher levels of aggression and a higher likelihood to remain with that mate than those that become pregnant after a delay[11,15].

In particular, it has been shown that pair bonding increases stimulated DA release within the nucleus accumbens (NAc) in prairie voles that have cohabitated for two weeks[13]. Because D1 receptors are the low affinity type that require high levels of DA to be activated, this adaptation is hypothesized to bias DA signaling to activate D1 type receptors[16–18]. Activation of D1 receptors subsequently activates kappa opioid receptors (KORs) via the release of dynorphin[11,12]. KORs are known to be involved in aversive processing[19–26], and thus, it is hypothesized the DAergic adaptations thought to bias this network toward the activation of aversive hedonic processing underlies the transition from affiliation to aggressive rejection observed in prairie voles[12]. Because this behavioral transition is reflective of pair bond strength and predicts the long-term maintenance of the bond, this suggests that the DAergic neuroplasticity that underlies these processes may be the mechanism that results in the enduring nature of prairie vole pair bonds.

Determining a Mechanism

The mechanism by which DA release is increased in the prairie vole is not known, however, plentiful research within the field of drug addiction has measured alterations of DA signaling after drug experience, and these provide a theoretical scaffolding on which to make predictions about the mechanisms regulating the observed increase in DA release in pair bonded prairie voles.

Drug Use Induced Neuroplasticity

All drugs that are abused by humans alter DAergic signaling and repeated use of these substances will result in adaptations of this circuitry[27–30]. Specifically, drug reward

is regulated by activity within the mesolimbic DA projections originating in the ventral tegmental area (VTA) and synapsing onto the NAc[31]. Importantly, these neural adaptations also underlie the behavioral transition from which a drug can go from casual use and the induction of positive hedonic states to the habitual use of these substances despite negative consequences[30]. One reported mechanism by which drugs of abuse can alter DA signaling (and perhaps long-term drug reinforcement) is via their alteration of DA autoreceptor function[32–34].

DA Autoreceptors

D2 and D3 DA autoreceptors are located presynaptically on nerve terminals within the NAc and dorsal striatum (DS) as well as in somatodendritic regions within the VTA itself[35]. DA D2 and D3 DA autoreceptors are a key homeostatic mechanism within this circuitry[36–38]. These receptors operate in a negative feedback manner to inhibit DA release, DA synthesis, and DA neuronal firing[35,37,38]. Extended access to amphetamine (AMPH) has been shown to reduce the ability of DA autoreceptors to inhibit DA release in rats[39]. Importantly, in the extended access paradigm used in this study, animals can regulate their own intake and it was observed that escalation of intake paralleled the reduced function of D2/D3 receptors[39]. Additionally, priming with AMPH or repeated systemic treatment with AMPH has been shown to increase VTA neuron firing via the reduced function of D2 autoreceptors[40–44]. Because AMPH experience results in a similar adaptation as pair bonding (i.e., increased DA release) and this effect is mediated at the level of the DA autoreceptor, we hypothesized that these same adaptations underlie the observed increase in DA release in pair bonded prairie voles.

Measuring Autoreceptor Function via Fast Scan Cyclic Voltammetry (FSCV) in Striatal Slices

In slice preparations, application of selective agonists activates presynaptic D2 and D3 DA autoreceptors, which regulate extracellular levels of DA through a negative feedback loop (i.e., increased levels of extracellular DA inhibit further release via the DA autoreceptors)[38]. Bath-applying agonists to slice preparations results in a reduction in stimulated DA release that reflects agonist potency. That is, more effective agonists induce full DA inhibition. Therefore, application of DA agonists known to reliably activate these receptors followed by FSCV measures provides a means by which to characterize DA autoreceptor function[45,46].

Previous work from our lab has shown that plasticity within the DA system of male prairie voles underlies behavioral transitions that are necessary for the long-term maintenance of pair bonds, and that these are correlated with the timing of pregnancy in the female. Based on these data, we propose to investigate whether this adaptation is maintained at 28 days post-pairing. Additionally, because AMPH use results in a similar adaptation as pair bonding (i.e., increased DA release in the NAc) and this is mediated via alterations in DA autoreceptor function, we hypothesized that these same adaptations underlie the observed increase in DA release in pair bonded prairie voles.

Materials and Methods

Subjects. Subjects were sexually naïve adult prairie voles (60-150 days old) bred at the University of Michigan (UM), weaned at 21 days and housed in single-sex sibling pairs.

Test subjects were randomly assigned to a drug condition. Animals were on a 14:10h light-dark cycle and all experiments were conducted between 0800h and 1800h. All procedures were conducted in accordance with the UM animal care guidelines and approved by the UM Institutional Animal Care and Use Committee.

Determining Pregnancy: After 28 days of pairing males with adult females, and prior to FSCV recordings, we determined pair fecundity and grouped pairs into the following categories: not pregnant, sub-optimally pregnant, or optimally pregnant. We determined pregnancy status based on the average neonatal weight of offspring as described previously[11,15]. Briefly, our lab has determined that weights of 0.3g correspond to lengths of 10mm and 10 days of pregnancy (optimal pregnancy), 0.165g correspond to lengths of 5mm and 3-5 days of pregnancy (suboptimal pregnancy). Thus, animals were categorized as either optimally pregnant (with neonatal weight of 0.3g or above or suboptimally pregnant from 0-0.3g. For animals that had already had one litter, we used the weight of the second pregnancy for group assignment. To do this we used the day of the first litter's birth as day 0 in pairing. It should be noted that all animals that had successfully had one litter became optimally pregnant immediately after parturition.

Slice preparation. Animals were killed via rapid decapitation and one hemisphere of the brain was used for FSCV experiments[11]. Right/left hemispheres used for FSCV were counter balanced. FSCV hemisphere was place in pre cooled preoxygenated (95% O_2/5% CO_2) high sucrose-aCSF buffer for 10 min. High sucrose aCSF consisted of 180 mM sucrose, 30 mM NaCl, 4.5 mM KCl, 1 mM $MgCl_2$, 26 mM $NaHCO_3$, 1.2 mM NaH_2PO_4,

and 10 mM d-glucose[47]. 400 µm-thick coronal striatal slices that included sections of the DS and VS were taken with a vibrating tissue slicer (Leica, Buffalo Grove, IL). Slices were maintained in oxygenated aCSF at room temperature for 1 h. After 1 hour at room temperature the slice was transferred to a custom-made submersion recording chamber (Custom Scientific, Denver, CO)

FSCV. During experiments, a bipolar stimulating electrode and carbon fiber recording electrode (produced in house and calibrated to a known DA concentration as in[48,49] were placed in the dorsal striatum (DS) or rostral Nacc shell. Using software written in LABVIEW (National Instruments, Austin, TX; Tarheel CV University of North Carolina, Chapel Hill) a triangular ramp sweeping from -0.4V to +1.2V versus a Ag/AgCl reference was applied to the carbon-fiber electrode at a rate of 10Hz. Once DA release was stabilized, the slice was bathed in increasing concentrations of quinpirole (0.001, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 20, 30, 300µM). Fifteen second recordings consisting of a 1 pulse 350µA stimulation at the 5 second mark were made at regular 5 minute intervals for a total of 6 recordings per drug dose (30 minutes per dose). For animals receiving OT treatment, baseline DA recordings were made for 30m prior to the addition of a .1uM dose of OT to the bath. After addition of oxytocin these slices underwent a quinpirole dose response as described above.

Statistics and Data Analysis. Data analysis and quantification of stimulated DA release was conducted as in[45,50]. Current versus time plots and electrically stimulated DA release rates were determined via use of Michaelis-Menten equations as previously described [51]. All statistical analyses were carried out using GraphPad Prism (Version 7, GraphPad

Software, Inc., San Diego, CA) and the Statistical Package for the Social Sciences (SPSS version 21; SPSS Inc., Chicago, IL). Data are presented as the mean ± standard error of the mean (SEM). For dose response experiments, the change in DA peak was compared to predrug values for each individual animal producing percent change in stimulated DA release. Percent change was then plotted as dose-response curve in log concentration (M). Data were fitted using nonlinear regression curve fit to determine log EC50 values. Data for pairing status (sexually naïve, pair bonded), and quinpirole dose-response curve were analyzed by a two-way analysis of variance (ANOVA) with Bonferroni *post hoc* test to compare group differences. Differences in baseline DA release were assessed with t-test. In all cases, statistical significance was set at $P < 0.05$.

Chemicals. All drugs were made the morning of the experiment and dissolved in aCSF. Quinpirole hydrochloride was purchased from Sigma Aldrich (St. Louis, MO).

Results

Increased DA Release at 28 days post-pairing

In order to measure whether increased DA release within the NAc shell was maintained at 28 days post-pairing, we completed real time DA measures via FSCV in brain slices. As hypothesized, following 28 days of cohabitation and mating, pair bonded males F (3, 15) = 5.089, p = 0.0011 showed increases in DA release with post hoc analysis showing that successful breeders showed higher levels of DA release in the NAc shell than both sibling housed controls and paired animals that had not become pregnant (p = 0.0178; p= 0.0424). Females did not show this same effect F (3, 17) = 1.717, p = 0.2013 Previous data had shown that both males and females showed increases in DA

after two weeks of pairing, but increases in females were much lower than those observed in males. Our data suggest that the minimal increase in females at 14 days may not be sustained at 28 days post pairing, further emphasizing that this neurosplasticity is important for the maintenance of pair bonds in males.

DA Autoreceptor Function

To determine the functional activity of D2 and D3 DA autoreceptors to inhibit stimulated DA release, we bathed striatal slices from sexually naïve and pair bonded prairie voles in the D2/D3 DA receptor agonist quinpirole to establish a dose response curve. Pre-drug values were defined as 100% and subsequent measures were expressed as percent of drug effect in relation to pre-drug value. A two-way ANOVA revealed a significant main effect of quinpirole dose $F(7, 84) = 83.7$ $p = 0.00$ for pair-bonded males (Figure 4.2). Additionally, there was also an interaction (dose x pairing status) effect $F(7, 84) = 3.10$ $p = 0.00$. *Post hoc* Bonferroni analysis indicated that quinpirole was less effective at inhibiting DA release at 1, 3, 10, 20, 30, 300 μM doses in pair bonded animals.

OT Treatment Impairs Autoreceptor Function in Sexually Naïve Males

In the above section we determined that pair bonding altered autoreceptor function in male prairie voles. In order to determine if this effect could be mediated by OT receptors, we bathed slices from sexually naïve animals in 0. 1 μM dose of OT in aCSF during the entire dose response experiment. Pre-drug (OT and quinpirole) were defined as 100% and subsequent measures were expressed as percent of drug effect in relation to pre-drug value. A two-way ANOVA revealed a significant interaction effect (quinpirole

dose x oxytocin treatment) $F(8, 88) = 13.69$ $p = 0.00$ and *post hoc* Bonferroni analysis indicated that quinpirole was less effective at inhibiting DA release at doses higher than 0.03 μM in slices that contained a .1 μM dose of OT in the bath (Figure 4.3).

Discussion

A marked behavioral plasticity has been shown to be a hallmark in prairie vole pair bonding. Sexually naïve prairie voles that first encounter a potential mating partner will investigate the novel animal and perceive this social stimulus as rewarding. After animals have pair bonded, however, they transition to perceiving novel social stimuli as aversive and aggressively reject or avoid new potential partners. Increases in DA release that preferentially activate D1 receptors, and subsequently KORs via dynorphin release, are thought to underlie this behavioral transition. This transition is necessary for the maintenance of long term pair bonds[12]. Here, we characterize a potential mechanism by which these increases are mediated: a subsensitive DA autoreceptor dose-response curve that results in enhanced increases in extracellular DA during neuronal firing in pair bonded males. Furthermore, since OT mimics this effect, the results taken together suggest that OT plays a role in mediating this process.

Motivational Significance of 28 Days

Unlike most mammals, prairie voles engage in biparental care of offspring which includes behaviors like nest maintenance and construction, foraging and feeding, grooming, and brooding. Investigations into prairie vole parental behavior have shown that males take on significant costs in exchange for caring for offspring such as weight

loss and increased mortality[52,53]. The costs accrued by the male suggest that the drive to remain in the nest despite negative consequences must be a highly motivated behavior with the ultimate motive to increase pup survivorship and thus produce a net benefit of reproduction for the male.

The female prairie vole gives birth 21-23 days after becoming pregnant. In the presence of a male (which is most often the case), female prairie voles go into post-partum estrus and will typically achieve pregnancy within 48 hours of parturition[54,55]. Thus, at 28 days, a successful pair will most often have one litter in the nest that is completely dependent on the parents, and is also expecting a second litter within 3 weeks. This cycle will continue throughout the breeding seasons and across the prairie vole's lifetime.

Oxytocin Signaling in Pair Bonding

Autoradiographical studies comparing monogamous species of vole with non-monogamous montane voles[56] found stark differences in the expression of oxytocin receptors, setting the stage for the extensive research that has discovered a key role for this neuropeptide in the regulation of pair bonding behavior. Diffuse OT receptor blockade via antagonist treatment in female prairie voles blocks mating induced partner preference formation, while administration of OT facilitates the formation of partner preferences in the absence of mating[57]. Blockade of OT receptors via antagonist treatment prevents partner preferences induced by D2-like receptor agonists, while administration of a D2-llike antagonist prevents the formation of OT induced partner preferences. These findings suggest a reciprocal relationship exists between the OT and DA systems in pair bonding, however, the mechanisms of their interaction in relation to this behavior are not clear.

Observations that direct infusion of OT into the VTA of rats can modulate DA release in the NAc demonstrated a direct role for OT to modulate mesocorticolimbic projections[58]. Recent reports have shown receptor complexes between D2-Like DA receptors and OT receptors in the striatum after social experience in rats[59]. Additionally, upon binding OT, the affinity of DA for the D2 receptor was observed to increase intracellular signaling via the CREB signaling pathway (illustrated in Figure 4.4 adapted from[59]). As both of these receptors are g-protein coupled receptors binding inhibitory g proteins, they may work synergistically upon co activation by altering cAMP singling, inhibition of which induces partner preferences in the absence of mating[60,61].

Conclusion

The data presented here suggest a potential mechanism for the increased DA release seen in pair bonded prairie vole males, and we suggest that this may underlie the sustained motivation required to remain in a lifelong pair bond. Specifically, we demonstrate that increased DA release in pair bonded males is maintained at 28 days post-pairing in a manner that reflects female pregnancy status. Additionally, we characterize subsensitive DA autoreceptor function in pair bonded males, suggesting this as a possible mechanism regulating the observed increased DA release that may be mediated by OT.

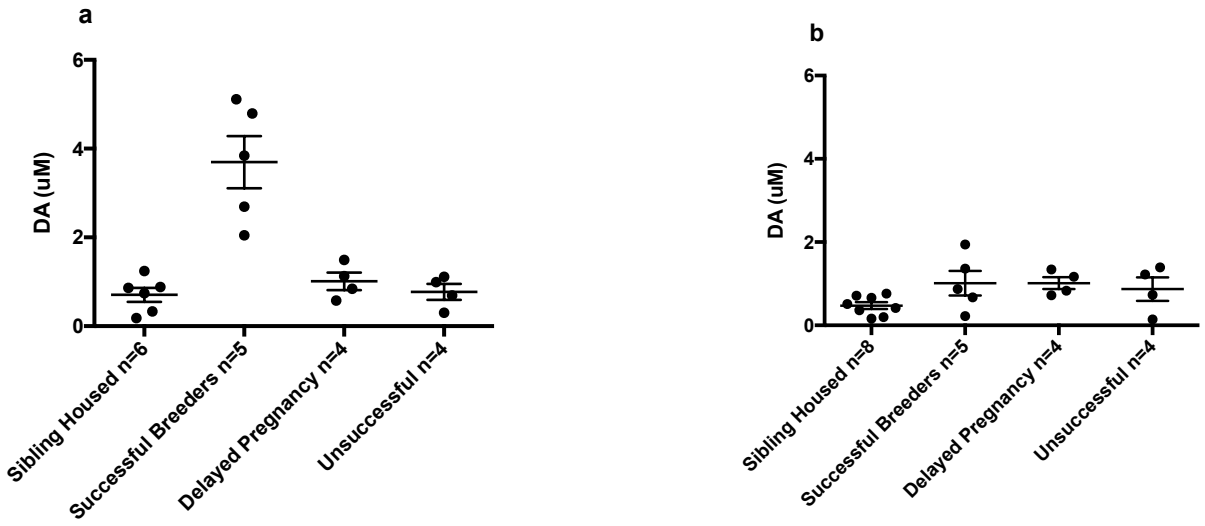

Figure 4.1 Increased baseline DA release in males 28 days post-pairing. Following 28 days of cohabitation and mating, pair bonded males (a) showed increases in DA release F (3, 15) = 5.089, p = 0.0011 with post hoc analysis showing that successful breeders showed higher levels of DA release in the NAc shell than both sibling housed controls and paired animals that had not become pregnant (p = 0.0178; p= 0.0424). Females (b) did not show this same effect F (3, 17) = 1.717, p = 0.2013.

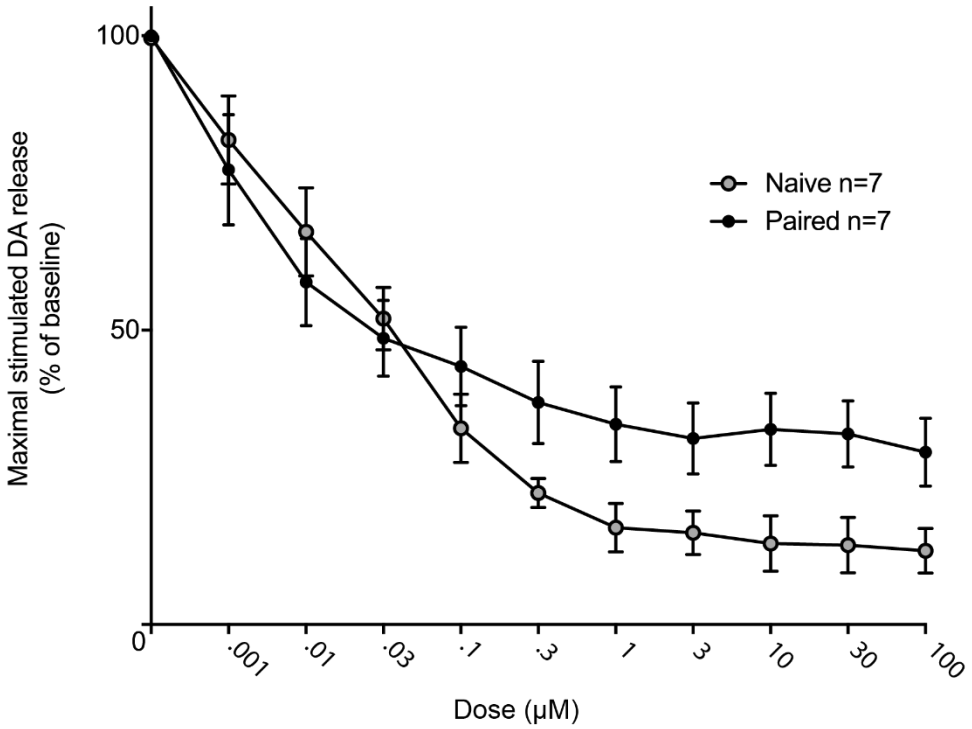

Figure 4.2 Altered DA autoreceptor function in pair bonded males. To determine the functional activity of D2 and D3 DA autoreceptors to inhibit stimulated DA release, we bathed striatal slices from sexually naïve and pair bonded prairie voles in the D2/D3 DA receptor agonist, quinpirole to establish a dose response curve. Pre-drug values were defined as 100% and subsequent measures were expressed as percent of drug effect in relation to pre-drug value. A two-way ANOVA revealed a significant interaction (dose x pairing status) effect $F(7, 84) = 3.10$ $p = 0.00$. *Post hoc* Bonferroni analysis indicated that quinpirole was less effective at inhibiting DA release at 1, 3, 10, 20, 30, 300 μM doses in pair bonded animals suggesting subsensitivity of autoreceptor function. Data are expressed as mean ± SEM.

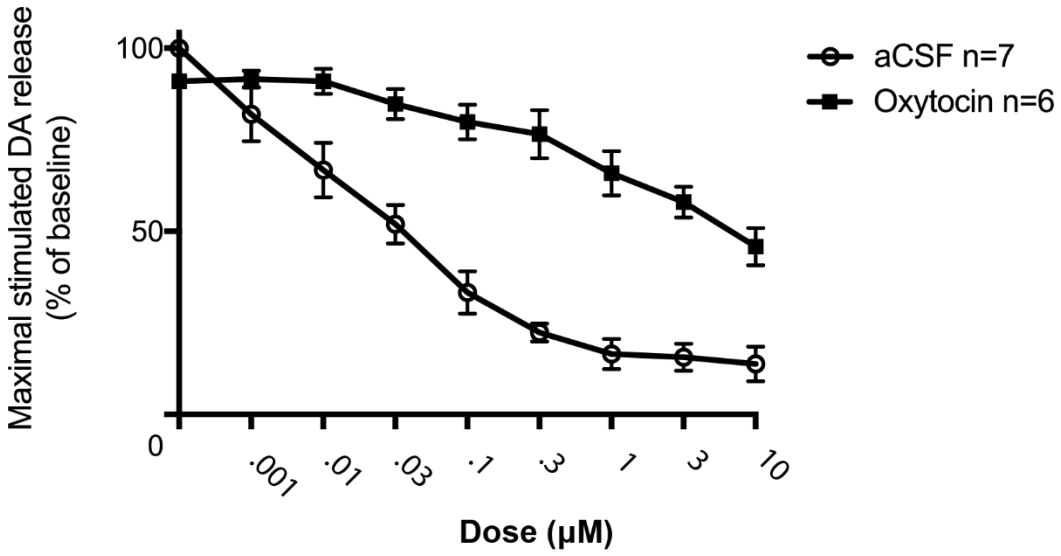

Figure 4.3 OT Treatment impairs DA autoreceptor function in sexually naïve males.
Slices from sexually naïve males were bathed in 0.1 μM dose of OT in aCSF during the entire quinpirole dose response experiment. Pre-drug (0 μM OT and quinpirole) were defined as 100% and subsequent measures were expressed as percent of drug effect in relation to pre-drug value. A two-way ANOVA revealed a significant interaction effect (quinpirole dose x oxytocin treatment) $F(8, 88) = 13.69$ $p = 0.00$ and *post hoc* Bonferroni analysis indicated that quinpirole was less effective at inhibiting DA release at doses higher than 0.03 μM in slices that contained a 0.1 μM dose of OT in the bath suggesting that the presence of OT can alter DA autoreceptor function in sexually naïve prairie voles in a manner similar to that of pair bonded animals. Data are expressed as mean ± SEM.

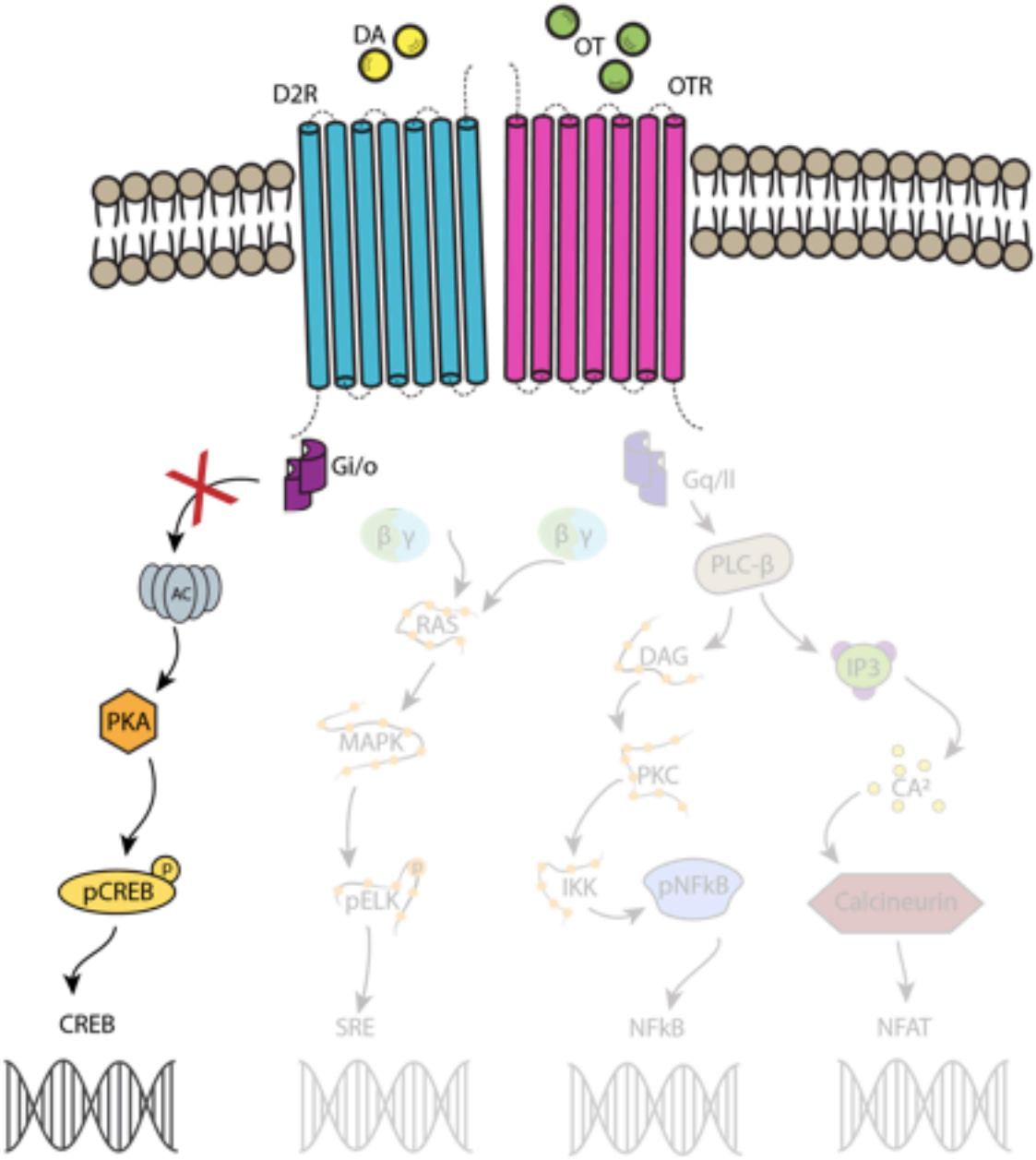

Figure 4.4. OT/DAD2 Heterodimers increase intracellular signaling via CREB
Heterodimer complexes have enhanced activation of the Gi/o mediated, inhibition of the AC-PKA-pCREB

Illustration adapted from Romero-Fernandez, W., Borroto-Escuela, D. O., Agnati, L. F. & Fuxe, K. Evidence for the existence of dopamine D2-oxytocin receptor heteromers in the ventral and dorsal striatum with facilitatory receptor-receptor interactions. *Mol Psychiatry* **18,** 849–850 (2013).

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Chapter 5

Discussion

General Summary

The development of social bonds is an evolutionarily adaptive behavior that is thought to have developed because it afforded humans higher likelihood of offspring survival and group living afforded resources in the form of defense, food, offspring care[1–4]. In modern humans, the development and stability of these bonds is correlated with resilience in mental health and shown to reduce the rewarding properties of drugs of abuse [5–9]. Alternatively, a physical or *perceived* lack of such bonds, referred to as 'loneliness', increases perceptions of stress, impairs immune and cognitive function, and is correlated with risk-taking behavior and a variety of psychiatric diagnoses such as drug abuse[10–13]. Additionally, disorders such as scizhophrenia and autism have marked social deficits that impact daily functioning. For these reasons, it is of great public health relevance to better understand the neural mechanisms underlying the development and maintenance of social attachments. The study of the neurobiology underlying social bonding requires an animal model that displays selective social attachments.

The socially monogamous prairie vole has emerged as a useful model system to study social attachments as it demonstrates selective social bonds in its life history strategy[14,15]. Prairie voles form lifelong pair bonds that are characterized by the sharing of a home territory, biparental care of offspring, and mate guarding[14,16,17]. DA signaling within the NAc has been discovered to be important for the regulation of these motivated

behaviors[18–20]. In particular, selective *activation* of high affinity receptors via tonic DA signaling underlies the pair bond maintenance phase, while *plasticity* within this system that increases DA release to bias the system to activate low affinity D1 receptors[18,20,21].

This system is centrally involved in reward processing and the generation of motivated behavior and thus, it is not surprising that it is also critical for the processing of social reward [22–25] In this system, DA containing neurons project from the ventral tegmental area (VTA) in the midbrain onto D1-like or D2-like expressing medium spiny neurons (MSNs) in the NAcc[26]. D1 receptors are postsynaptic, excitatory, low affinity type receptors that require high levels of DA to be activated, such as during fast "phasic" burst firing [24,25,27–30]. In contrast, DA D2-like receptors are found both postsynaptically on target cells and presynaptically on dopaminergic neurons, they are inhibitory and have a high affinity for DA, allowing them to respond to low "tonic" DA concentrations[25,27].

DA in the NAc signals reward

In particular, the NAc region of the striatum is involved in the processing of natural rewards such as mating or the consumption of a palatable food and these reliably trigger DA release within this region, whereas the blockade of NAc DA receptors disrupts reward seeking[31–34]. Not surprisingly, this region is also involved in the rewarding processing of other rewards such as drugs of abuse[35,36]. The activity of DA within this region for reinforcing natural and artificially concentrated rewards such as drugs of abuse may underlie the protective nature of social attachments against the development of addictions. Additionally, the processes that underlie salience attributions to cues that predict a natural reward (such as the presence of a mating partner or other social contact),

or a drug reward, can all be dysregulated in conditions such as addiction, schizophrenia, and autism spectrum disorders, demonstrating the value of the study of these processes in the prairie vole to characterize effective treatments for these conditions.

Contribution of This Dissertation

The goal of my dissertation work was to increase our understanding of the role of DA system activation and neuroplasticity in the development and maintenance of pair bonds in the socially monogamous prairie vole. Previous work on this model system has demonstrated that activation of D2 like receptors is necessary for the development of pair bonds while activation of D1 like receptors is necessary for their maintenance. Based on the affinity of DA for different DA receptors (D2, high; D1, low,), we can infer that low levels of DA that activate D2 receptors are necessary for the development phase while high levels of DA which selectively activate D1 receptors are important regulators of the maintenance phase. This suggests that the preferential activation of D1-like or D2-like receptors may reflect dynamic DA signaling across the early and late stages of pair bonding —suggesting a model in which tonic DA signaling facilitates the development of the bond while phasic DA signaling facilitates pair bond maintenance. In order to begin to parse out the activational and neuroplastic processes involved in pair bonding we conducted the following studies:

Study 1: DA D3 Receptor in Partner Preference Formation

As mentioned above, low levels of DA that preferentially activate the D2 class of receptors are thought to mediate pair bonding behavior. The DA D3 receptor subtype has the highest affinity for DA within the D2 class, is highly localized to the NAc shell (region

important for pair bonding and association of positive hedonics with a mating partner), and is shown to be involved in social recognition processes[37,38]. Thus, we hypothesized that the DA D3 receptor mediates pair bond development in the socially monogamous prairie vole.

In the lab, pair bonding is operationally defined as preferential side by side contact with an opposite sex conspecific[39,40]. Preferential contact is referred to as a 'partner preference'. Partner preferences are induced in the lab via extended cohabitation periods and tested via a 'partner preference test'. Alternatively, experimental manipulations can allow for induction of a partner preference under conditions that normally would not induce pair bonding such as short cohabitation periods in the absence of mating[39].

Animals treated with a mixed D2/D3 receptor agonist (quinpirole) or a D3 agonist (7-OH-DPAT) alone into the NAc shell showed development of partner preferences in the absence of mating and under short cohabitation periods. Importantly, selective activation of D2 receptors via site specific injections of B-HT 920 were not able to induce partner preferences in sexually naïve prairie voles.

Two main points can be gathered from these data. First, these findings suggest that the mixed D2/D3 agonist, quinpirole may be inducing its effects either by binding to D3 receptors, or that it may be activating both D2 and D3 receptors concurrently. Indeed, it is known that D2/D3 receptors can form dimers (i.e., receptor complexes) and that these have higher affinity for DA than either receptor on its own[41]. This hypothesis can be tested via brain collection after the cohabitation period and subsequent assaying of heterodimers within the NAc via immunoprecipitation for D2 and D3 receptor complexes. An alternative

explanation for the efficacy of the mixed D2/D3 agonist is the observation that transient activation of D3 receptors can increase agonism efficacy at D2 receptors[41,42]. That is, the transient activation of D3 receptors may "prime" D2 receptors to more efficiently activate intracellular signaling after binding of DA.

A second point to be made from these data are that the animal's social behavior varied based on drug treatment. Animals treated with the selective D3 receptor agonist showed increased affiliative behaviors such as olfactory investigation, friendly following, anogenital sniffing, active contact, allogrooming, and huddling. Interestingly, while D2 receptor activation did not induce partner preferences, there was a trend for increased affiliation in animals treated with B-HT 920 (D2 agonist). The fact that both D2 and D3 receptors had the capacity to increase general affiliation, yet only D3 receptor activation was able to induce pair bonds, suggests that D3 receptors may be working with other systems within this circuitry, or that they are unique in other properties that contribute to social behaviors. Oxytocin and opioid circuitry are known to interact with DA in this region and are theorized to contribute to the attribution of hedonic value to salient stimuli, such as meeting a potential mating partner[43,44]. Future work should determine the interactions of D3 receptors with hedonic processing systems present within the NAc[45,46].

Autoradiography is the most common way to measure receptor expression. While it has been shown that the D3 receptor is highly expressed in the more limbic regions of the NAc in rats, mice, and humans, we do not have measures of these receptors in prairie voles[37]. If these receptors are similarly expressed in prairie voles, then this would support the assumption that DA is working in concert with other systems to mediate pair bonding behavior. In fact, DA receptor expression and signaling measurements in prairie voles

have shown that they are consistent with that of other rodents, whereas the expression of OT differs significantly among monogamous and non-monogamous species[18,47,48]. Based on these observations, it may be possible to assume that it is the action of DA in relation to the restricted expression of OT receptors in this region that underlies the development of pair bonds. Future work on this model should characterize the specific mechanisms of such interactions by determining the existence of receptor complexes and coactivation of these receptors during the cohabitation period.

Schizophrenia and the attribution of salience

It is believed that phasic DA release mediates behaviorally relevant responses, whereas tonic release mediates the amplitude of these responses[49]. DA functions via volume transmission across long distances and in the process of reaching its distal targets it can be diluted[50]. D3 receptors can be activated by these low levels of DA, such as those present during tonic firing, due to their high affinity for the neurotransmitter[51]. Thus, it is suggested that D3 receptor sensitivity may underlie excessive attributions of salience to certain stimuli. In the case of schizophrenia, it is theorized that heightened D3 receptor sensitivity results in a state of “aberrant salience” [52]. Specifically, it is believed that when the DA system is hyper responsive, an individual cannot differentiate relevant from irrelevant stimuli, and thus will assign high salience to stimuli which should be ignored[52,53]. This is unique in that in the prairie vole, it is believed that the mating partner is imbued with high salience, however, in the case of the vole this salience attribution is highly adaptive. Likewise, high salience attributions to drug related cues are only aberrant when these guide behavior so that an individual pursues drugs despite negative

consequences. Based on these arguments, future research should investigate whether there is plasticity in the signaling of D3 receptors in pair bonded prairie voles. Additionally, studies of mate associated cues can be combined with direct D3 manipulations to determine the ability of the D3 receptor to direct salience attributions toward these stimuli.

Study 2: FSCV Assay of DA D2/D3 Selective Drugs in the Prairie Vole Striatum

Here, we test the effects of DA D2 and D3 agonists that have been previously used in behavioral studies of prairie vole pair bonding via FSCV measures of electrically stimulated DA release in the prairie vole DS and NAc. We measured the efficacies of the D2-selective agonist, B-HT 920, the selective D3 agonist, 7-OH-DPAT, and the mixed D2/D3 receptor agonist, quinpirole, at inhibiting DA release within the DS and NAc. B-HT 920 had highest efficacy in the DS, whereas quinpirole was most effective at inhibiting DA release within the NAc shell than either the subtype selective agonists. These results were surprising as the expression of DA D3 receptors is dense in the NAc shell of other species, thus we expected to see increased efficacy of 7-OH-DPAT within this region. Two surprising findings suggest that DA D2-like receptor expression or functionality is unique in prairie voles compared to other rodent species. Indeed, future work on this model should characterize the expression of these receptors in the prairie vole brain, and determine whether concurrent activation of D2 and D3 receptors results in meaningful functional differences.

The D3 agonist, 7-OH-DPAT, showed similar effects in both regions examined. This result was unanticipated as reports on other species show a high density of this receptor in the NAc shell[37]. This discrepancy may be a result of the following: (1) the

agonist is not as selective for the D3 receptor subtype or not selective at the doses used in this study, or (2) the DA D3 receptor is not as dense or functionally active in this region in the prairie vole as it is in other species. It should be noted, that these findings should not invalidate behavioral data acquired from the use of these drugs. Instead, the data here should be interpreted with a sensitivity to the fact that FSCV experiments in slices investigate only one part of this system, that is, FSCV can only determine terminal regulation of DA release and cannot account for activity of these receptors in behavioral experiments on which it acts on an intact system. Additionally, FSCV can only infer drug selectivity and receptor functionality and not receptor density, thus, it is imperative to measure D3 receptor expression in the prairie vole striatum to determine if the limited effects of D3 activation are a result of low receptor density.

Additionally, the effect of B-HT 920 in the DS was also unexpected as previous measurements of B-HT 920 in the mouse brain showed similar inhibition effects for the DS and NAc suggesting that the expression of these receptors was homogenous throughout the whole of the striatum. Behavioral observations (see Chapter 2) have shown that use of this drug in prairie voles impacts locomotion. Locomotor behavior at D2 receptors in the NAc is mainly mediated by activation of post-synaptic D2L receptors. FSCV is limited in that it can only determine the effects of this drug on terminal release via activation of autoreceptors. However, the data included in Chapter 2 suggest that in the prairie vole, this drug may have highest affinity for the post synaptic receptors which do not directly regulate DA release.

The mixed D2/D3 agonist, quinpirole, showed the highest effect at the NAc compared to selective D2 and D3 agonists. Importantly, this region mediates pair bonding

behavior as well as the attribution of positive hedonics to a mating partner[48,54]. Because quinpirole is a mixed agonist, and neither agonist alone could produce as strong responses in the NAc, we believe quinpirole may have a unique profile of activity, exploiting meaningful functional differences within this region (perhaps by concurrent activation of both D2 and D3 receptors). These findings are consistent with the robust partner preferences that are induced via quinpirole treatment in prairie voles[18]. Future work can determine if this is the case by utilizing quinpirole in the presence of selective D2 and D3 antagonists in slice preparations.

Study 3: Partner Preferences Lead to Subsensitivity of DA D2/D3 Autoreceptors

Here I present data that support findings that prairie voles show pair bonding dependent increases in DA release in the rostral nucleus accumbens shell (Resendez et al., 2016). Importantly, while the previous work had demonstrated this adaptation after 2 weeks of pairing, I show that this adaptation is maintained beyond the birth of the first litter (and into the active biparental caretaking phase). Additionally, I characterize a subsensitivity in autoreceptor function as the possible neuroplasticity that underlies the observed DA increase. I then combine these novel findings of DA system adaptations in pair bonding with the broad database of research that has shown a role for the oxytocin system in the regulation of prairie vole pair bonding by demonstrating that increased oxytocin tone can mimic the effect of pair bonding at the DA terminal. The observations of neuroplasticity in this system have high translational value. In the vole, this neuroplasticity underlies salience attributions that mediate the long term motivation to remain with one partner. However, in other conditions, such as drug addiction, these

salience attributions can contribute to persistent maladaptive behaviors such as drug seeking despite negative consequences. Thus, the characterization of these changes can give insight into how drugs of abuse can impact motivation and additionally, how social bonding can help buffer the rewarding effects of drugs of abuse.

DA in Partner Preference Formation

The development of monogamous pair bonds in the prairie vole is a motivated behavior important for reproduction. Thus, it is not surprising that the DA system, which regulates motivation to seek natural rewards such as food and shelter, also mediates behaviors relevant to the pursuit, development, and maintenance of pair bonds[7,18,20,55–60]. The present findings expand on previous work from the Aragona[18] laboratory to show for the first time that within the D2-like class of DA receptors, the NAc DA D3 receptors are critically involved in the development of partner preferences in the socially monogamous prairie vole.

Social Attachments and Drug Reward

The neural circuitry implicated in pair bonding and affiliative behavior in the prairie vole shares many similarities with that of reward and addiction [5,7,61]. The mesolimbic DA system has been proposed to have evolved to mediate incentive behaviors associated natural rewards and reproduction[62]. It has been shown that drugs of abuse exert their effects on this same system, essentially “hijacking” adaptive circuitry to produce a slew of maladaptive behaviors[6]. As surveyed here, pair bonds are regulated by activation of several structures in this system, demonstrating significant overlap with the effects of

drugs of abuse on these systems. This is further demonstrated by evidence showing that male prairie voles pretreated with amphetamine fail to show mating induced partner preferences and conversely, the presence of social bonds reduces the rewarding properties of amphetamine[63,64]. Specifically, the neuroplasticity associated with pair bonds (but not other social experiences such as a short term exposure) underlie a protective effect by which psychostimulant effects of amphetamine are attenuated. In particular, the increases in DA release after pair bonding selectively activate DA D1 receptors resulting in the activation of KOR via dynorphin and the alterations in this system also buffer against the rewarding properties of AMPH. Importantly, this illustrates the bidirectional relationship through which healthy social attachment can attenuate the rewarding properties of drugs of abuse and addiction can reduce the rewarding properties of social attachment. Future work on this model will undoubtedly provide a unique perspective on comorbidities between drug addiction and social deficits, as well as an understanding of how healthy social attachment can decrease vulnerability to drug use.

Integration with knowledge of D1 and D2 like receptor function

It must be addressed that the data presented in this dissertation seem to demonstrate opposite roles for D1 and D2 receptors than the traditional conceptualization of striatal D1 and D2 function[65,66]. When activated optogenetically, striatal neurons expressing D1 receptors have been shown to enhance reward learning whereas activation of D2 like receptors in this region are shown to reduce reward learning. From these observations, a general dogma has been established that D1 and D2 receptors play opposing roles in reward learning[67]. The data presented here however, show that

DA D2-like receptors, namely, DA D3 receptors, mediate the learning of a social reward. Thus, we must address how these seemingly opposing results can be incorporated into the current knowledge base. First, we must consider that it is possible that DA receptor function may differ in the prairie vole. However, this is unlikely as DA release dynamics and receptor expression (thus far only D1 like and D2 like receptor expression has been measured) are consistent to that expressed in mice and rats[18,48]. A more likely explanation could be that other systems which are known to mediate aspects of social behavior in the prairie vole may impact DA processing to facilitate more nuanced behavioral outputs. Indeed, recent work has found that coordinated action of oxytocin, serotonin, and dopamine mediate social learning in the mouse[68]. In agreement, the data presented in this dissertation suggest that interactions with OT may be able to mediate the learning of a socially rewarding stimulus via the D2 DA receptor in the prairie vole. The future of the field should expand toward a systems approach to better determine how DA can produce novel effects on behavior based on region of release, amount of release, temporal pattern, receptor binding, and other systems with which it interacts. This field of research has been limited as targeting these systems in a cell specific manner has proven to be very technically difficult. However, recent data from Calipari and colleagues have begun to test critical aspects of the established model[69]. Lemos and colleagues have also found that behavioral experience such as stress can produce interactions between corticotropin releasing factor (CRF) and dopamine systems to switch CRF action in the nucleus accubens from appeitive to aversive, further demonstrating how the DA system can adapt after experience[70].

It is believed that DA acts as a single "neural currency" for the learning of motivationally relevant stimuli including that of a social nature[71,72]. It is logical that such a diversely used neurotransmitter should be fine-tuned via interactions with other systems as well as via neuroplasticity based on experience so as to allow a single neural modulator to produce nuanced behavioral effects with the ultimate goals of increasing evolutionary fitness. Thus, while at first glance, our data could be interpreted as contradicting the current dogma, a more likely narrative is that as the field expands our use of a variety of behavioral paradigms (of which social bonding represents only a small portion of research in the field of DA) we will find that it is the various interactions of DAergic, neurohormonal, and opioid signaling that can produce these nuanced effects on behavior.

Future Directions

Several experiments should explore the possibility of DA and OT receptor complexes and their expression across the development of a pair bond. Ideally, brain collection should be completed soon after meeting and later on in pairing (e.g., 3 hour, 6 hour, 12 hour, 1 week, 2 week post pairing), in order to gain an understanding of whether these receptor complexes are being formed and whether the timeline of their expression may impact the strength of a bond. Additionally, as we have observed here, the strength of a bond is correlated to the pregnancy status of the female. Thus, it follows that we should determine whether the expression of these receptor complexes is correlated to the pregnancy status of the female and if so, what neurochemical, hormonal, or sensory signals trigger the expression of these receptors? Finally, along this same vein, as female pregnancy status is correlated to increased DA release in male prairie voles and this

neuroplasticity is believed to underlie the maintenance of these relationships, it is imperative to determine how the male of the pair becomes aware of the reproductive success of the pair. One possible mechanism to determine this sequence of events it to have sexually receptive females (estrogen primed) that are physiologically incapable of becoming pregnant. After pairing, mating bouts and male DA release could be correlated to determine if this DA release is correlated instead to the male's perception of successful copulation, rather than fertilization. Indeed, these data will increase our understanding of social bonding in a monogamous rodent and have implications for our understanding of human relationships.

Conclusion

Social bonding utilizes reward circuitry in combination with valence processing to direct behavior in adaptive ways toward social stimuli that are rewarding, and away from stimuli that may pose a threat. As discussed above, the neural circuitry for the processing of natural rewards such as the development of pair bonds can be hijacked by drugs of abuse. It is interesting that pair bonding induces adaptations at the autoreceptor similar to those induced by treatment with AMPH, and that pair bonding has been seen to attenuate drug reward[7,64]. Many paradigms used to test the reinforcing properties of drugs of abuse do not adequately model the type of drug use that exists in humans. When addressing this question, Calipari and colleagues found some interesting results: the temporal pattern of cocaine intake can produce biphasic effects at the DA transporter (DAT)[73]. While continuous intake, and presumed sustained high levels of drug, results in tolerance, intermittent short access results in sensitization. While it is clear that pair

bonding, much like drug addiction, results in long lasting changes in DA neurotransmission, little is known of the effects of pair bonding on the DAT and how this contributes to the enduring nature of these ties. In an effort to better understand the neuroplasticity associated with this adaptive behavior, as well as understand how these systems are also altered in disease states, future work should seek to determine whether pair bond induced alterations in DAT function are characteristic of sensitized or tolerant DAT systems.

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