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THE EFFECTS OF APOMORPHINE ON SEXUAL BEHAVIOR AND AGGRESSION IN MALE GOLDEN HAMSTERS

by

Molly M. Hyer

A Thesis

Presented to the Faculty of
Bucknell University
In Partial Fulfillment of the Requirements for the Degree of
Master of Science in Psychology

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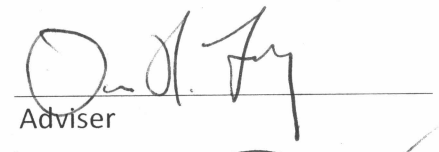

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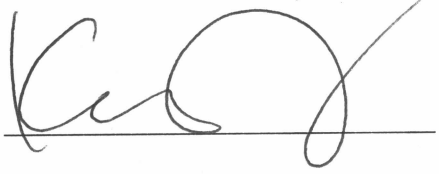

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Abstract

The general dopamine agonist apomorphine has been shown to have mostly facilitative effects on sexual behavior in rodents (Domingues & Hull, 2005; Bitran & Hull, 1987). A study looking at the effects of apomorphine on sexual behavior in male golden hamsters observed that after systemic injections of apomorphine the males became aggressive towards the estrous females (Floody, unpublished). Studies on aggressive behavior have shown that apomorphine has facilitative effects on aggression in rodents (Nelson & Trainor, 2007; van Erp & Miczek, 2000; Ferrari, van Erp, Tornatzky, & Miczek, 2003). The studies presented here attempt to unravel the effects that apomorphine has on sexual and aggressive behavior in male golden hamsters. Studies 1, 2, 3, and 4 focused on the effects of apomorphine on aggression and Study 5 focused on the effects of apomorphine on sexual behavior. It was important for the purposes of this study to have separate, specific measures of aggression and sexual behavior that did not involve a social context that would involve multiple behaviors and motivations. The measure used to assess aggression was flank marking behavior. The measure used to assess sexual behavior was the number of vocalizations in response to sexual stimuli. The results from Studies 1, 2, and 3 suggested that apomorphine increased aggressive motivation in a dose-dependent manner. In Studies 1 and 2 there was a high occurrence of stereotyped cheek pouching that interfered with the flank marking behavior. In Study 3 the procedure was modified to prevent cheek pouching and flank marking was observed uninhibited. Study 5 suggested a decrease in vocalizations after apomorphine treatment. However, this decrease may have been a result of the increase in stereotyped licking behavior. Results suggested that systemic apomorphine treatments increase aggressive motivation in hamsters. The increase in aggressive motivation may confuse the perception of the sensory signals that the males receive from the estrous females. They may have perceived the estrous female as a nonestrous female which they would normally associate with an aggressive interaction (Lehman, Powers, & Winans, 1983).

The neurotransmitter dopamine (DA) has been linked to the regulation of many behaviors in animals (Nelson & Trainor, 2007; Dominguez & Hull, 2005; Bitran & Hull, 1987). The role that DA plays in the regulation of sexual behavior has been studied extensively. DA appears to play a facilitative role in several aspects of copulatory behavior in rodents. Male rodents follow a specific behavioral pattern during a sexual encounter; locomotor pursuits, mounting, thrusting, penile erection and insertion, ejaculation, post-ejaculation grooming, and quiescence (Hull, Muschamp, & Sato, 2004). Microinjections into the medial preoptic area (MPOA), the brain area most closely associated with male sexual behavior, of DA agonists, such as apomorphine, a largely unselective DA agonist, facilitate the efficiency of copulation and genital reflexes (reducing the latency of achieving erection or ejaculation), whereas microinjections into the MPOA of DA antagonists, such as haloperidol, inhibit these behaviors (Dominguez & Hull, 2005; 2006). Subcutaneous administration of apomorphine decreases ejaculation thresholds, reducing the time to reach ejaculation as well as increasing the percentage of animals that reach ejaculation (Dominguez & Hull, 2005; Paglietti, Quarantotti, Mereu, & Gessa, 1977). Microdialysis used to measure samples taken from the MPOA during copulation in rats showed an increase in extracellular DA (Sato et al., 1995). Extracellular DA in the MPOA increases once a male rat detects an estrous female and remains high throughout copulation. The DA release caused by the detection of a female positively correlates with copulation ability (Hull & Dominguez, 2006). Castrated males that have a deficit in extracellular DA in the MPOA, as well as a deficit in behavior, show a partial restoration of sexual activity as a result of treatment with apomorphine (Szczypka, Zhou, & Palmiter, 1998).

Studies examining sexual motivation, as opposed to copulation, have shown that increased levels of DA induce sexual motivation. This increase in DA-induced sexual motivation also facilitates copulatory behaviors (Bitran & Hull, 1987; Hull & Dominguez, 2007). The increase in DA levels after the detection of a female reported in the study above suggests that increased DA levels play a role in the anticipation of copulation as well as actual copulatory behavior (Hull & Dominguez, 2006). Exposure to an actual female, as opposed to just detection of the female through odors and sounds, stimulates DA release in males, further indicating DA's role in anticipation of a sexual encounter (Szczypka, Zhou, & Palmiter, 1998).

Levels of DA in the nucleus accumbens (NA) increase when rats are exposed to the chamber where they had previous sexual encounters with a female (Meisal, Camp, & Robinson, 1993). The dopamine agonist SKF-38393 increased the time spent in a goal compartment with an estrous female indicating increased sexual motivation (Dominguez and Hull, 2005; Hull, Muschamp, & Sato, 2004). Haloperidol, on the other hand, increases the latency to approach an estrous female but not to approach an empty goal compartment. Here, haloperidol decreased sexual motivation but did not affect motivation for an empty goal compartment (a sexually unrelated goal) (Hull & Dominguez, 2007; Hull, Muschamp, & Sato, 2004). Microinjections of the DA antagonist cis-flupenthixol into the MPOA reduce the number of times a male will approach a goal box containing a receptive female as compared to empty goal boxes (Dominguez and Hull, 2005). The findings presented here suggest that DA antagonists will reduce sexual motivation while DA agonists will increase sexual motivation. Based on the above studies, it appears that DA facilitates both sexual motivation and copulatory behaviors.

Studies have also implicated DA in the regulation of aggression (Nelson & Trainor, 2007; van Erp & Miczek, 2000; Ferrari, van Erp, Tornatzky, & Miczek, 1998). The evidence from these studies suggests that DA may play a facilitative role in aggression as well as sexual behavior. In fact, the DA antagonist haloperidol has been clinically approved to treat aggression in psychotic human patients (Couppis & Kennedy, 2008). In rodents, the role that DA plays in aggression has been studied extensively. Accumbal DA in rats increases during positively reinforcing behaviors such as sexual behavior, as well as stressful events such as foot shocks or social defeat (Van Erp and Miczek, 2000). Van Erp and Miczek (2000) proposed that this DA pattern would also occur in aggressive encounters due to the combination of the stress, motoric demand, and reward components involved in such encounters. Tidey and Miczek (1996) proposed that stress-related changes in dopaminergic activity in the mesocorticolimbic reward system are potentially related to arousal or anticipatory fear. Van Erp and Miczek (2000) used microdialysis to measure samples taken from Long-Evans rats during the resident-intruder task to assess changes in neurotransmitters, specifically DA and serotonin. They found that DA levels in the nucleus accumbens and the prefrontal cortex increased significantly by 30-40% immediately following the aggressive encounter.

Similarly, Ferrari, van Erp, Tornatzky, and Miczek (2003) found that male Long-Evans rats that have had aggressive encounters at the same time each day will show physiological changes just prior to the scheduled encounter suggesting that they are anticipating the event. Some of the physiological changes include increased heart rate and body temperature. In the same study, male Long-Evans rats had scheduled aggressive encounters for 10 straight days at the beginning of their dark cycle. Levels of DA in the NA were measured by microdialysis before and during the encounters to determine the neurological changes surrounding an aggressive encounter. Results showed that rats that had experienced repeated aggressive encounters had a 60-70% increase in DA levels twenty minutes prior to the time of the aggressive encounter (the beginning of the dark cycle) and these DA levels remained elevated at 50-60% more than baseline throughout the period of the expected aggressive encounter (Ferrari et al., 2003).

DA is taken up from the synapse through dopamine transporters (DAT). In DAT knockout mice extracellular levels of DA are increased. These same mice show significantly higher levels of aggression compared to wild type controls (Rodriguiz, Chu, Caron, & Wetsel, 2004). Skrebuhhova-Malmros et al. (2000) found that repeated small doses of apomorphine induced behavioral aggression in rodents. Following the apomorphine induced aggression, treatments with DA antagonists subsequently prevented further aggressive behavior (Kask & Harro, 2000; Rudissaar et al., 1999; Matto, Allikmets, & Skrebuhhova, 1998). A study by Couppis and Kennedy (2008) used an operant contingency paradigm to assess DA and the rewarding effects of aggression. In this paradigm, male mice were trained to nose-poke for access to an intruder male. The increase in correct nose pokes indicated that these mice were experiencing rewarding effects with access to aggression. After a combined treatment with a D_1 antagonist and a D_2 antagonist, two different types of DA receptors, to the nucleus accumbens, nose poking for aggression was significantly reduced. This paradigm also eliminated the complex social encounters that are normally used to study aggressive behavior. In this study only the relationship between DA and the motivation for aggression was examined (Couppis & Kennedy, 2008). These data suggest that DA may play a facilitative role in initiating an aggressive response in rodents through increasing aggressive motivation.

Based on the research presented above it appears that DA plays a role in facilitating sexual behavior and aggressive behavior in at least some rodents. In golden hamsters (*Mesocricetus auratus*) these two behaviors can be closely linked. By nature, hamsters are a solitary species and when confronted with another individual can be extremely aggressive (delBarco-Trillo, McPhee, & Johnston, 2009; David, Cervantes, Trosky, Salinas, & Delville, 2004). Aggression even occurs between males and females if the female is not in estrous (Gutzler, Karom, Erwin & Albers, 2010; delBarco-Trillo, LaVenture, & Johnston, 2009). A study examining the effects of apomorphine on sexual behavior found an increase in aggression of the males towards the females and a decrease in the efficiency of sexual behaviors (Floody, unpublished data). This suggested that DA may not play a facilitative role in sexual behavior in hamsters, which is inconsistent with its pivotal role for sexual behavior in other rodents. However, other studies on the effects of apomorphine in rodent sexual behavior have shown it to mostly have facilitory effects. One study found that apomorphine treatments of 0.025 mg/kg reduced ejaculatory latencies and the postejaculatory interval in male golden hamsters suggesting an excitation of sexual motivation and behavior in this species (Arteaga, Motte-Lara, & Velazquez-Moctezuma, 2002). Butcher, Butcher, and Larsson (1969) reported that apomorphine administered systemically decreased the number of intromissions and the latency to ejaculation in male rats suggesting a facilitory role of apomorphine on sexual behavior. Paglietti, Quarantotti, Mereu, and Gessa, (1977) also found that subcutaneous administration of apomorphine reduced the number of intromissions necessary to reach ejaculation. Apomorphine also decreased the latency to intromission and the latency to ejaculation. A study looking at naturally, sexually sluggish male rats found that 0.5 mg/kg of apomorphine significantly increased the expression of sexual behaviors in these sexually sluggish males (Tagliamonte, Fratta, Del Fiacco, & Gessa, 1974). A study by Mas (1995) found that apomorphine would induce sexual behavior in male rats that had been allowed to copulate unrestricted until they had reached satiety.

Other studies examining the role of apomorphine in sexual behavior have found apomorphine to have general facilitory effects but they have also found some cases of apomorphine-induced inhibition. In a study comparing acute and chronic administration of apomorphine the authors reported that acute

treatments resulted in decreased numbers of mounts and intromission prior to ejaculation as well as an increase in ejaculation frequency. However, the chronic administration of apomorphine sustained the decreased mount and intromissions but not the increase in ejaculation frequency. Apomorphine treated animals also showed an elongated post-ejaculatory interval (Olivier, et al., 2007). Agmo and Fernandez (1988) found that systemic apomorphine treatments (0.05-0.15 mg/kg) did not induce copulatory behavior in castrated male Wistar rats. Hull et al. (1986) reported that low doses of apomorphine infused into the ventricles of male rats reduced the number of ejaculations, slowed the rate of intromitting, and decreased the percentage of mounts that resulted in vaginal intromissions indicating an inhibition of sexual behavior. Mas, Fumero, Fernandez-Vera, and Gonzalez-Mora (1995) found that in vivo microdialysis from the MPOA of male Sprague-Dawley rats showed that during sexual refractoriness, the period after unrestricted copulation when a male rat is sexually inactive towards an estrous female, levels of the DA metabolite dihydroxyphenylacetic acid (DOPAC) were significantly elevated compared to the copulation period. Prior to when the male rat would resume sexual activity these levels were significantly reduced. This indicated that DOPAC levels were increased with reduced sexual behaviors and decreased with expression of sexual activity. Clark and Smith (1985) compared the effects of low and high doses of apomorphine on young and older male rats. High doses of apomorphine (0.4-3.0 mg/kg) reduced sexual behavior in the young and old male rats. Lower doses (0.05-0.8 mg/kg) reduced the ejaculatory threshold in the younger males by reducing latency and frequency of intromissions before ejaculation. In tests six minutes after the treatment there was an overall reduction in the number of males mating after the high (0.4-0.8 mg/kg) doses of apomorphine. They, along with Agmo and Fernandez (1988), proposed that low doses of apomorphine may be stimulating DA autoreceptors while high doses of apomorphine may be activating postsynaptic receptors and triggering inhibitory neuronal pathways. The results from these studies suggest that the role DA plays in sexual motivation and behavior is unclear. DA could be inducing sexual excitation, inhibiting sexual behavior, or increasing aggressive motivation. It also appears that the role DA plays may depend on the method of administration as well as the amount of activation.

A study by Lehman, Powers, and Winans (1983) examining the role of the stria terminalis in copulatory behavior saw a similar change in male hamster behavior towards the estrous females. In their study, they found that lesions to the stria terminalis reduced copulatory behavior in male hamsters and increased their aggression towards estrous females. To explain this reduction in copulatory behavior they examined the efferent basolateral and central amygdala (associated with escape and defense in hamsters) pathways to the stria terminalis. In encounters when the female was not sexually responsive she displayed intense aggression towards the male. In this species, the female is larger and dominant over the male and these cues may make the males hesitant to approach a female (delBarco-Trillo, LaVenture, & Johnston, 2009; Petrulis, Widener, & Johnston, 2004). The efferent pathways to the stria terminalis may convey complex sensory information about the female that was previously associated with aggressive encounters. Lehman, Powers, and Winans (1983) proposed that this information would inhibit the male's approach to the female. However, when a female is sexually responsive her scent and ultrasonic vocalization cues are enough to suppress any wariness the male may have about approaching her. Stria terminalis lesions may disrupt processing of these cues.

The results observed by Floody (unpublished) and by Lehman, Powers, and Winans (1983) suggest that disruption of neural processing through lesioning or DA manipulations may confuse the cues that a male hamster must process to effectively copulate with a female rather than engage in an agonistic conflict with a female. These studies raise the question of whether the increased DA levels independently affect sexual behavior and aggression, if the increase in DA increases aggression which in turn disrupts sexual behavior, or if DA affects sexual behavior and indirectly affecting aggressive behavior. To further examine this change in typical behavior, the role that DA plays in sexual and aggressive motivation in male golden hamsters must be explained in more specific detail. However, it is important that the measures used focus on only one motivational state at a time so that the role of DA can be more clearly defined for each specific motivational state and then applied to sexual and aggressive behaviors. This is particularly important in male golden hamsters since aggression plays such a major role in their social behaviors. To look at these two motivational states a design is needed that does not involve social encounters.

Floody and Pfaff (1981; 1977a; 1977b; 1977c) proposed that ultrasonic calling in both male and female hamsters is a form of sexual advertisement. In hamsters, vocalizations play an important role in sexual behavior. Hamsters are solitary and highly aggressive to other individuals (Petrulis, Widener, & Johnston, 2004), making vocalizations necessary to advertise willingness to mate (Floody & Pfaff, 1977a; 1977c; Johnston, 1980; delBarco-Trillo, LaVenture, & Johnston, 2009). In more social rodents, such as rats, vocalizations play an important role in aggressive encounters as well as in sexual encounters. Through posturing and ultrasonic vocalizations the losing animal will signal his submission, terminating the attacks and making group living possible. Ultrasonic calls are not seen in aggressive encounters between hamsters. The dominant male will always attack the subordinate male since they are not social animals (Sales, 1972). In hamsters, female reproductive calls inform potential mates of her reproductive state. The frequency of vocalizations is highest when the female is in estrus. Males call more in the presence of an estrous female to facilitate lordosis (the female's mating posture) and following her removal (Floody & Pfaff, 1977; Cherry, 1989). This increase in male ultrasonic vocalizations in response to an absent female suggests that one purpose of these calls is to achieve male female contact (Floody & Pfaff, 1977b). In a Y-maze task it was found that both males and females are attracted to natural and synthetic ultrasounds. These synthetic ultrasounds will also elicit vocalizations in both males and females (Floody & Pfaff, 1977c). Playbacks of a male's ultrasounds following the removal of a female from the male's presence will elicit lordosis in females, indicating the importance that these vocalizations have for copulatory behavior in hamsters (Cherry, 1989).

Hamsters will produce ultrasonic vocalizations not only in response to the calls of conspecifics but to their odors as well. Male hamsters will exhibit increased ultrasound rates in response to female odors (Floody & Pfaff, 1977c). Johnston and Kwan (1984) reported that vaginal discharge from female hamsters stimulates ultrasonic vocalizations. They proposed that vaginal scent marks are integral for mate location. The scent marks that a male will encounter elicit ultrasonic vocalizations that the female will hear and respond to with her own vocalizations. Males will investigate vaginal scent marks from estrous females significantly longer than scent marks from non-estrous females (delBarco-Trillo, LaVenture, & Johnston,

2009). In a study by Johnston (1980) investigating male hamsters' responses to vaginal secretions from females at different points in the estrous cycle, he found that males showed a preference for the scent marked bedding from the home cages of estrous females compared to non-estrous females. The males did not show a preference for the scent marked bedding from cages of vaginectomized estrous females compared to the vaginectomized non-estrous females suggesting that the vaginal scent marks are a critical part of the female's advertisement to mate. The stimulus was critical for the male to determine if a female is in the estrous state (Johnston, 1980). The combination of ultrasonic vocalizations and female vaginal discharge odors help to locate a mate and facilitate copulation (Floody & Pfaff, 1977c; Johnston, 1980). The studies presented here suggest that ultrasonic vocalizations in response to vaginal scent markings are relatively specific to sexual motivation in hamsters.

A behavioral measure that has been used to show aggression in hamsters is flank marking (delBarco-Trillo, McPhee, & Johnston, 2009; Petrulis, Widener, & Johnston, 2004; Hayden-Hixson & Ferris, 1991; Ferris & Potegal, 1988; Albers, Axelson, Ferris, & Shinto, 1987). Hamsters have sebaceous glands on the dorsal portion of each flank called flank glands that they use to mark their territory. The number of flank marks increases when a test animal is introduced to the cage of another animal, male or female (Johnston, 1975a). Ferris and Potegal (1988) used flank marking as a measure of aggressive behavior when examining the effects of vasopressin and serotonin on aggressive displays. Potegal et al. (1996) indicated that flank marking is a species typical behavior of hamsters that is shown most often in agonistic encounters. Their research focused on the corticomedial amygdala (CMA), which has been implicated in offensive aggression. The study showed that electrical CMA stimulation significantly increased flank-marking behavior to a level twice that of baseline (Potegal et al., 1996). Hayden-Hixson and Ferris (1991) found that, after an increase in gonadal steroids, male hamsters exhibited an increase in flank marking. Studies on the relationship between dominant and subordinate male hamsters have also used flank marking as an indicator of aggression. Subordinate males will flank mark less than dominant males indicating reduced agonistic motivation (Petrulis, Widener, & Johnston, 2004; Albers, Axelson, Ferris, & Shinto, 1987).

When introduced into the cage of a female, a male expresses a cyclic pattern of flank marking. Males will mark more when a female is not in estrous and less when she is sexually receptive. Johnston (1980) proposed that the vaginal scent marks from the estrous female advertise her willingness to mate and reduce agonistic tendencies in the male. This cyclic pattern of marking suggests that increased flank marks are mainly a reflection of agonistic social tendencies but can be affected by an increase in sexual motivation (Johnston, 1975a). This conclusion is supported by Johnston's (1984) research showing that flank marking increases after an aggressive encounter. Overall, male hamsters flank mark at the highest frequencies following encounters with other males or non-estrous females, both of which would involve aggression. The strongest elicitor of flank marking from one male is the flank marks from another male (Johnston, 1975b). These studies combined with the data presented previously suggest that flank marking is a sign of aggressive motivation in hamsters.

The previous studies show that the neurotransmitter dopamine plays a role in facilitating both sexual behavior and aggressive behavior in some rodents. However, it appears that DA's role in sexual behavior and aggressive behavior in hamsters may be different. To tease apart the role that DA has on measures of both sexual behavior and aggressive behavior, these types of motivation need to be examined separately. Research by Floody and Pfaff (1977a; 1977b; 1977c) suggests that ultrasonic vocalizations of male hamsters in response to a combination of female vaginal secretions (delBarco-Trillo, LaVenture, & Johnston, 2009; Johnston; 1980) and synthetic vocalizations could measure sexual motivation with greater specificity than levels of sexual behavior during exposure to a stimulus animal. The research on flank marking and aggression, presented previously, suggests that flank marking by male hamsters in response to odors from other male hamsters measures aggressive motivation, again, with greater specificity than in more complex social situations such as aggressive encounters or the resident-intruder paradigm. The following series of studies examines the role that DA plays in aggression and sexual motivation through observations of flank marking behavior in response to a conspecific male's odors in the conspecific's home cage as a measure of aggression and ultrasound production in response to synthetic vocalizations and vaginal secretions from an estrous female as a measure of sexual motivation. Treatments with the

nonselective DA agonist apomorphine were administered immediately prior to an observation of the expression of flank marking behaviors (Studies 1, 2, & 3) and immediately prior to an observation of the exhibition of ultrasonic vocalizations (Study 5). Study 4 examined the effects of apomorphine on aggression as measured by the latency of a male hamster to attack another male hamster in an aggressive encounter, which is a more traditional measure of aggression. The results from these studies should suggest how an increase in DA is affecting male hamster behavior. It was hypothesized that DA affects sexual and aggressive motivation independently, DA affects aggression which then disrupts sexual behavior, or DA affects sexual behavior which indirectly increases aggression. An increase in flank marking behavior after apomorphine treatment would indicate an increase in aggressive motivation. This potential increase in aggression may be suppressing the sexual behavior that a male normally expresses towards an estrous female. A reduction in ultrasonic vocalizations following apomorphine treatment would suggest that an increase in DA reduces sexual motivation and thus indirectly increases aggression since, with the reduction in sexual motivation, the natural aggressive tendencies of the male hamsters are being expressed in their agonistic behavior towards the females. These potential results would account for the behavior seen in Floody (unpublished) and Lehman, Powers, and Winans (1983) as well as improve our understanding of how DA affects both sexual and aggressive motivation. If the results suggest that DA facilitates sexual motivation then this conclusion will indicate the role DA plays in hamsters. This conclusion would be similar to what has been shown in previous research on other rodents. This will also indicate that further studies need to be conducted to understand the behaviors seen in Floody (unpublished) and Lehman, Powers, and Winans (1983). Perhaps a closer examination of the different DA receptor subtypes or brain areas that may play a role in both sexual and aggressive motivations would be appropriate.

Study 1

The purpose of Study 1 was to determine the effects of the general dopamine agonist apomorphine (R-(-)-Apomorphine hydrochloride hemihydrate) on aggression as measured by flank marking behavior in male golden hamsters. Previous studies have shown that dopamine is involved in many behaviors including aggression (Nelson & Trainor, 2007; Bitran & Hull, 1987; van Erp & Miczek, 2000). Van Erp and Miczek (2000) proposed that dopamine levels would increase during aggressive encounters from the combined activation of the stress, motoric, and reward pathways that all play a role in aggressive behavior. Ferrari, van Erp, Tornatzky, and Miczek (1998) showed that rats that had consistent aggressive encounters experienced an increase in dopamine levels prior to and during the scheduled encounter. Repeated doses of apomorphine have been shown to induce aggressive behaviors in rodents (Skrebuhhova-Malmros et al., 2000). These studies suggest that dopamine may play a facilitative role in aggressive behavior.

To understand the role that dopamine plays in aggression it is important to tease apart the complex social interactions and focus on the aggressive behavior. It is possible to isolate the aggression in male hamsters by observing flank marking behavior as opposed to an aggressive encounter. Male hamsters have sebaceous glands on their flanks that they use to mark their environment (Johnston, 1975). Previous studies have suggested that flank marking increases as a result of increased aggressive motivation (Johnston, 1975; Ferris, 1997; Potegal et al., 1996). When a male hamster is introduced to the home environment of another male he will begin flank marking. Flank marking is highest after an aggressive encounter between two males (Johnston, 1984; 1975). Males will flank mark significantly less in the cage of an estrous female indicating a reduction in aggressive motivation in response to an increase in sexual motivation (Johnston, 1975a). These studies indicate that flank marking can be a measure of aggression without the added complexity of a social encounter. In this study, aggression was measured through the latency and frequency of flank marking. Varying doses of apomorphine were administered prior to the observation to determine if apomorphine had an effect on aggression as measured by flank marking.

Subjects

Male golden hamsters (N=16) aged six months to one year and bred in the laboratory were individually housed in cages measuring 34.5 x 20.5 x 17.5 cm with wire fronts and bottoms and a solid back and walls. Paper towels and paper plates served as bedding material and food and water were available ad libitum. All hamsters were housed in one of two colony rooms on a reversed light cycle (10 hr dark, 14 hr light). The hamsters were most active during the dark period when all tests were conducted.

Behavioral Measures

Data were collected through observation. The behaviors that were recorded were frequency of flank marking, latency to flank mark, and incidence of cheek pouching. Flank marking was defined by an arched back, erect tail and ears, and rubbing of the flank along a surface. The behavior can vary widely across animals so it is necessary to have a specific operational definition. Whenever a hamster rubbed its flank along a surface it was counted as one flank mark. If the animal paused during rubbing without breaking his posture and then continued the rubbing it was scored as two flank marks. When an animal would mark and then, without breaking posture, turn and mark with the other side two flank marks were scored. If an animal assumed the flank marking posture and made the rubbing action without contacting a surface one flank mark was scored, although this rarely occurs (Johnston, 1975). An instance of cheek pouching was scored when an animal filled both his cheek pouches with food or bedding. If only one cheek pouch was filled the animal would still flank mark using the scent gland on the unpacked side of his body. If the animal had cheek pouched during the habituation period it was scored.

Drugs

Apomorphine (R-(-)-Apomorphine hydrochloride hemihydrate purchased from Sigma-Aldrich) treatments were administered through intraperitoneal injection fifteen minutes before observation. Injections were given using a 27 G needle attached to a 1.0ml syringe. Four doses of apomorphine (0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg, and a saline control) were given to each animal in a counterbalanced order

with 48 hours separating injections. Each animal received two rounds of each dose. Each dose was mixed and coded by a third party so the observers were blind to the treatment each animal was receiving.

Procedure

Testing began within two to two and a half hours following the onset of the dark period. The study included 16 males assigned to 8 test pairs that were maintained throughout the study. Test pairs were maintained to prevent the fluctuation of stimulus scent quality to which each animal was exposed during testing in the home cage of his partner. Each animal was weighed on the day of testing to determine the appropriate injection volume to be administered (weight/1000 ml). The first pair of animals received their injections and was placed back into their respective home cages. The home cages were placed on an observation table in a room separate from the colony for a fifteen minute habituation period that was necessary for the drug to take effect. The tops of the cages were covered with plexiglas pieces (55 x 40 x 0.5 cm) to prevent the animals from climbing out while maintaining visibility for the observer. At the twelve minute mark after the first injection the second pair of animals was injected then placed in habituation. This created a continuous schedule of observing one pair during the subsequent pair's habituation period.

Once the two test animals had spent fifteen minutes habituating in their respective home cages, each animal was lifted out of his cage then placed into the, now unoccupied, home cage of his test partner and the stop watch was started. Both animals were observed at the same time by one observer. The latency to flank mark, the number of flank marks, and incidence of cheek pouching were recorded. The observation lasted for ten minutes. At the end of the observation, the animals were returned to their home cages and then returned to the colony room.

Results

The purpose of Study 1 was to determine if different levels of apomorphine had an effect on flank marking behaviors in male hamsters. Frequency of flank marking for each animal after each treatment

(saline, 0.1 mg/kg, 0.25 mg/kg, and 0.50 mg/kg of apomorphine) was analyzed using a repeated measures analysis of variance (ANOVA). Each animal received each dose twice and the two measures of flank marking frequency were averaged. There were no significant effects of apomorphine treatment on the frequency of flank marking. A second analysis including only tests when flank marking was observed (N=9) showed no significant effects of the drug treatment.

The latency of each animal to flank mark after each treatment was also recorded. Previous studies have shown that an increase or decrease in latency is an indicator of motivational state for that specific behavior (Gutzler, Karom, Erwin, & Albers, 2010; Caldwell & Albers, 2004; Dominguez, 2005; & Hull, 2004). Only tests when flank marking occurred were included in the analysis (N=9). The latency to flank mark was analyzed using a repeated measures ANOVA. However, the analysis showed no significant effect of apomorphine treatment on latency to flank mark.

There was a high occurrence of cheek pouching behavior that may have played a role in the latency to flank mark and the frequency of flank marking. Of the 54 observations when cheek pouching occurred, 37 of these observations occurred without flank marking being observed at any point during that same observation (69%). When cheek pouching did not occur throughout the duration of an observation (74 observations), there were only 18 out of these 74 observations when flank marking also did not occur (24%). This suggests that cheek pouching may have interrupted flank marking behavior. Incidences of cheek pouching were analyzed using the Cochran's Q statistic. The Cochran's Q statistic is a nonparametric test used to analyze whether three or more related sets of frequencies differ from each other. The analysis showed a significant difference in incidence of cheek pouching across the four apomorphine doses ($Q(3) = 11.92$, $p \leq 0.05$). In the saline condition 44% of the animals cheek pouched one or more times, after the 0.1 mg/kg of apomorphine 81% cheek pouched, 94% cheek pouched after the 0.25 mg/kg dose, and 63% cheek pouched after the 0.50 mg/kg dose (Fig. 1). The significant Cochran's Q indicated that animals cheek pouched more frequently after receiving apomorphine treatment compared to the saline control. A repeated measures ANOVA of flank marking frequency excluding tests on which an animal cheek pouched (N=10) showed no significant effect of apomorphine treatment on flank mark frequency.

A disparity in frequency of flank marking for some of the animals suggests that the overall group of subjects could be split into high marking and low marking animals. There was a clear distinction of frequency of flank marking between the animals that were high markers and the animals that were low markers. This separation attempted to take into account the high frequency of cheek pouching in some of the animals. When the 16 animals were separated into high flank markers that had an average flank marking frequency of greater than five within the 10 minute observation period across all treatments (N=8) and low flank markers that had an average flank marking frequency of less than five within the 10 minute observation period (N=8) there was a significant dose x group interaction effect, $F(3, 42)=3.55$, $p=0.02$, of apomorphine dose on frequency of flank marking between the high marking and the low marking groups (Fig. 2). Analyses of the high marking group approached a significant main effect of dose, $F(3, 21) =2.906$, $p=0.059$, and had a significant linear contrast, $F(1, 7) =22.393$; $p=0.002$. The significant linear contrast indicated that there was a significant decrease in flank marking frequency as a result of increased apomorphine doses in the high marking group. However, an analysis of flank marking in the high group excluding days when an animal cheek pouched (N=6) resulted in no significant differences.

Discussion

These results indicate that flank marking was significantly decreased with apomorphine treatments in high flank marking animals. This suggests that in high marking animals activation of DA receptors by apomorphine reduces aggressive motivation. Apomorphine treatment also dose-dependently increased cheek pouching behavior. The observed increase in cheek pouching behavior disrupted displays of flank marking behavior.

Study Two

In Study 1, male golden hamsters were given different doses of apomorphine treatments and then observed for flank marking behavior as a measure of aggression. During the habituation and observation

periods of Study 1 the hamsters exhibited a high frequency of cheek pouching behavior. When a hamster had both cheek pouches filled with either food or bedding flank marking behavior may have been disrupted. This potential confound made it difficult to determine the effect that apomorphine had on flank marking behavior in Study 1. Subsequent pilot testing indicated that, if the hamsters were left in the colony room for the 15 minutes it took for the apomorphine to take effect, as opposed to being placed on the observation bench, cheek pouching prior to the observation did not occur in any of the tests. This led to a modification of the procedure from Study 1. In Study 2, the hamsters were habituated to the apomorphine in the colony room and taken to the observation bench once the 15 minute habituation period had passed. The purpose of this change was to reduce the instances of cheek pouching during the habituation period that may have been disrupting the flank marking behavior. The goal of Study 2 was to determine the effect of apomorphine on flank marking behavior while limiting the instances of cheek pouching. Male hamsters were given different doses of the apomorphine treatment, habituated in the colony room, and then observed for flank marking behavior.

Subjects

Male golden hamsters (N=18) bred in the laboratory were used in this study. The ages ranged from six months to two years. Seven of the males had been used in the first study but they were paired with naïve animals in Study 2 and no behavioral differences were observed between the experienced and the naïve animals. Housing was identical to Study 1. The animals were most active in the dark period when the testing was conducted.

Behavioral Measures

Behaviors were again recorded through observation. The frequency of flank marking, the latency to flank mark, and incidence of cheek pouching were recorded for each animal after each drug treatment. Operational definitions were the same as in Study 1.

Drugs

Drug (R-(-)-Apomorphine hydrochloride hemihydrate purchased from Sigma-Aldrich) treatments were administered as in Study 1. In Study 2, three doses of apomorphine (0.25 mg/kg, 0.5 mg/kg, and a saline control) were administered to each animal in a counterbalanced order with 48 hours separating injections. The 0.1 mg/kg dose that was used in Study 1 was eliminated in this study due to extremely low differences from the saline condition. Each animal received two rounds of each dose. Each dose was mixed and coded by a third party so the observers were blind to the treatment each animal received.

Procedure

Testing began within 2 to 2.5 hours after the onset of the dark period. 18 males were assigned to 9 test pairs that were maintained throughout the study. A procedure similar to Study 1 was followed. Each animal was weighed on the day of testing to determine the volume of the injection given (weight/1000 ml). The first pair of animals each received their respective injections and was returned to their respective home cages for the 15 minute habituation period. Pilot testing following the first study indicated that cheek pouching occurred more frequently if the animals habituated outside of the colony room. Therefore, unlike Study 1, in which the animals spent 15 minutes habituating on the observation table, the animals were returned to the colony room following their injections for the habituation period. The purpose of this change was to reduce the incidence of cheek pouching that was occurring during the habituation period on the observation table. At the 12 minute mark after the first injection, the second pair of animals was injected then placed in habituation. This created a continuous schedule of observing one pair during the subsequent pair's habituation period.

Following the 15 minute habituation period, the two home cages of the first test pair were placed on the observation table. Each animal was lifted out of his cage then placed into the, now unoccupied, home cage of his test partner and the stop watch was started. The tops of the cages were covered with plexiglas pieces (55 x 40 x 0.5 cm) to prevent the animals from climbing out while maintaining visibility for the observer. Both animals were observed at the same time by one observer. The latency to flank mark,

the number of flank marks, and incidence of cheek pouching were recorded. The observation lasted for ten minutes. At the end of the observation the animals were returned to their home cages and then returned to the colony room.

Results

The purpose of Study 2 was to determine if apomorphine had an effect on flank marking behavior while the likelihood of cheek pouching behavior was reduced. The procedure was modified from Study 1 so that the animals habituated in the colony room instead of on the observation table to reduce the incidence of cheek pouching. Frequency of flank marking after each apomorphine treatment was recorded and analyzed using repeated measures ANOVA. The analysis showed no significant effects of apomorphine treatment on frequency of flank marking in the second study (N=18).

The latency to flank mark after each apomorphine treatment was also recorded. A repeated measures ANOVA was used to analyze the effects of apomorphine treatment on the latency to flank mark. Where there was a sphericity problem that violated the assumptions underlying the analysis, the raw data were transformed using a log transformation. Excluding tests where an animal did not exhibit any flank marking (N=15), the analysis showed a significant main effect of apomorphine dose on latency to flank mark, $F(2, 28)=19.16$, $p<0.00$. A pairwise comparison of the main effect of dose indicated that the latency to flank mark after the 0.25 mg/kg (M=31.23s, SEM=0.07, $p<0.00$) and 0.5 mg/kg (M=45.73s, SEM=0.11, $p<0.00$) doses was significantly shorter than after the saline control (M=123.83s, SEM=0.12) (Fig. 3).

Despite the change in procedure to reduce the incidence of cheek pouching the behavior still occurred at similar levels to Study 1 (42.2% in Study 1 and 43.5% in Study 2). However, the procedure was successful in that cheek pouching occurred later in the observation period, as compared to during the habituation period in study one, so flank marking behavior was observed. As opposed to Study 1 in which 69% of the cases when an animal cheek pouched he did not flank mark, in Study 2 there were only 12 out of 47 (26%) observations when an animal cheek pouched that flank marking was not observed. A Cochran's Q statistic was again used to analyze the incidences of cheek pouching. There was a significant

effect of apomorphine dose on cheek pouching ($Q(2) = 9.80$, $p \leq 0.05$). 33% of the saline control animals cheek pouched, 78% cheek pouched after the 0.25 mg/kg dose, and 61% of the animals cheek pouched after the 0.5 mg/kg dose (Fig. 4). Similar to Study 1, the animals' incidence of cheek pouching increased with apomorphine treatment compared to the saline control.

A repeated measures analysis of the frequency of flank marking excluding tests on which an animal cheek pouched (N=8) showed that apomorphine dose did have a significant main effect on frequency of flank marking, $F(2, 14)=5.582$, $p=0.016$. Pairwise comparisons of the main effect of dose showed that frequency of flank marking was significantly higher after the 0.25 mg/kg (M=16.31, SEM=2.73, $p=0.007$) and 0.5 mg/kg (M=13.25, SEM=4.32, $p=0.035$) doses of apomorphine compared to the saline control (M=6.94, SEM=2.44) (Fig. 5). There was a significant linear contrast indicating that there was an increase of flank marking with increasing apomorphine dose, $F(1, 7)=6.84$, $p=0.04$.

Discussion

The results from Study 2 indicate that apomorphine decreased the latency to flank mark after apomorphine treatment. Cheek pouching behavior occurred later in the observation than in Study 1 so flank marking measures were able to be observed in more tests. However, apomorphine treatment increased cheek pouching behavior similarly to the effects observed in Study 1. Once tests when cheek pouching occurred were excluded from the analysis, apomorphine treatment dose-dependently increased flank marking behavior. These results contradict the findings from Study 1 that apomorphine decreased flank marking in high flank marking animals. The results from Study 2 suggest that DA receptor activation by apomorphine increases aggressive motivation as measured by flank marking in male hamsters.

Study Three

Studies 1 and 2 examined the effects of varying levels of apomorphine on flank marking as a measure of aggression in male golden hamsters. In Study 1 there was a high occurrence of cheek pouching

that disrupted flank marking behavior. Study 2 attempted to reduce cheek pouching by habituating the animals in the colony room so that flank marking behavior could be observed without disruption. Unfortunately, in Study 2 cheek pouching behavior was not decreased overall. However, when cheek pouching occurred in Study 2 it did occur later in the observation as opposed to occurring during the habituation period as in Study 1. The later occurrence of cheek pouching increased the initial amount of flank marking that occurred since the animals would flank mark for the at least first part of the observation before they cheek pouched. However, the continued high occurrence of cheek pouching overall in Study 2 may still have disrupted flank marking behavior. The purpose of Study 3 was to completely eliminate the possibility of cheek pouching behavior by removing all food and bedding from the cages before the observation and therefore observe the effects of apomorphine on flank marking behavior in an uninterrupted manner. I also expected to replicate the significant decrease in latency to flank mark as a result of increasing apomorphine dose as seen in Study 2. Finally, I expected to show a significant increase in flank marking as a result of increased apomorphine dose as was shown in the analysis from Study 2 when cheek pouching observations were excluded.

Subjects

Male golden hamsters (N=12) approximately 1 year old and bred in the laboratory were used in this study. Seven of the animals had been used in Study 2 but they were paired with naïve animals and no behavioral differences were observed. Housing was identical to Study 1 and Study 2. All observations were conducted during the dark period when the animals were most active.

Behavioral Measures

Behavioral measures were recorded through observation. Latency to flank mark and frequency of flank marking were recorded. Operational definitions were the same as in Study 1 and Study 2.

Drugs

Drug (R-(-)-Apomorphine hydrochloride hemihydrate purchased from Sigma-Aldrich) treatments were administered were administered the same as in Studies 1 and 2. One dose of apomorphine (0.5 mg/kg) and a saline control were given to each animal in a counterbalanced order with 48 hours separating injections. The lower doses of apomorphine used in Studies 1 and 2 (0.1 mg/kg and 0.25 mg/kg) were eliminated in this study since a significant drug effect was seen with the highest dose in the previous studies. Each animal received two rounds of each dose. Each dose was mixed and coded by a third party so the observers were blind to the treatment each animal was receiving.

Procedure

Testing began within two to two and a half hours after the onset of the dark period. 12 males were assigned to 6 test pairs that were maintained throughout the study. A procedure similar to Studies 1 and 2 was followed. Each animal was weighed on the day of testing to determine the volume of the injection given (weight/1000 ml). The first pair of animals each received their respective injections and was returned to their respective home cages for the 15 minute habituation period. Similar to Study 2, the animals were habituated in the colony room. At the 12 minute mark after the first injection, the second pair of animals was injected then placed in habituation. This created a continuous schedule of observing one pair during the subsequent pair's habituation period.

At 15 minutes the two test animals were brought into the testing room. Both cages were placed on the observation bench. Both male hamsters were taken out of their respective home cages and placed to the side. All of the bedding and food was removed from both of the cages so that they were empty for the observation period. The purpose of this was so that the animals did not have any material with which to cheek pouch. The food and bedding was kept separated so that it could be returned to the appropriate cage at the end of the observation. Once the cages were emptied the two test animals were placed into their partner's cage. A plexiglas cover (55 x 40 x 0.5 cm) was placed over-top of the cages to prevent the animals from climbing out. The stop watch was started. The observation was broken down into five, two

minute blocks. The first block ranged from 0-2:00 minute, the second block ranged from 2:00-4:00 minutes, and so on. The purpose of this breakdown was to observe any changes in flank marking behavior over the course of the observation. The latency to flank mark was recorded and each flank mark was recorded within the appropriate time block. The observation lasted for a total of ten minutes. At the end of ten minutes each animal was replaced in his home cage, his bedding and food were returned, and both animals were returned to the colony room.

Results

The purpose of Study 3 was to examine the effects of apomorphine on flank marking behavior as a measure of aggression while completely eliminating the possibility of cheek pouching behavior. The change in procedure in Study 3 (removing all food and bedding from the test cages) was successful in eliminating cheek pouching behavior from every observation. Without the cheek pouching behavior flank marking was able to be observed uninterrupted. Each animal received two rounds of each treatment. The measures of frequency of flank marking from both rounds were averaged. A repeated measures analysis of variance (ANOVA) of the effect of apomorphine treatment on latency to flank mark (N=12) showed a significant difference, $F(1, 11)=10.77$, $p=0.007$ (Fig. 6). This analysis indicated that latency to flank mark was significantly longer after the saline control treatment (M=44.3 s, SEM=11.8) compared to the 0.5 mg/kg apomorphine treatment (M=20.5 s, SEM=6.7).

The observation of flank marking was conducted over a ten minute period. Flank marking occurrences were broken down into five, two minute blocks. Block 1 lasted from 0-2:00 minutes, Block 2 lasted from 2:00-4:00 minutes, Block 3 lasted from 4:00-6:00 minutes, Block 4 lasted from 6:00-8:00 minutes, and Block 5 lasted from 8:00-10:00 minutes. A two factor repeated measures analysis with one factor being treatment (two levels) and one factor being time block (five levels) indicated that there was a significant main effect of treatment on number of flank marks ($F(1,11) =4.80$, $p=0.05$), a significant main effect of time block on number of flank marks ($F(4, 44) =32.99$, $p<0.00$), and no significant interaction effect.

A repeated measures analysis on frequency of flank marking indicated that there was a significant effect of apomorphine treatment on frequency of flank marking; $F(1, 11)=4.77$, $p=0.05$ (Fig. 7). Hamsters flank marked significantly more after they had been given the high apomorphine treatment (M=20.33, SEM=3.7) compared to when they had been given the saline treatment (M=12.41, SEM=2.6).

The main effect of time block and a significant linear contrast of time block ($F(1, 11) =45.17$, $p=0.00$) indicated that the male hamsters marked significantly more at the beginning of the observation than towards the end (Fig. 8). In time block 1 they marked an average of 7.85±1.14 times, in time block 2 they marked an average of 3.98 ±0.83 times, in time block 3 they marked an average of 1.79±0.31 times, in time block 4 they marked an average of 1.31±0.33 times, and in time block 5 they marked an average of 1.46±0.38 times. There was a significant difference between block 1 and blocks 2 ($p<0.00$), 3 ($p<0.00$), 4 ($p<0.00$), and 5 ($p<0.00$). There was a significant difference between block 2 and blocks 3 ($p=0.006$), 4 ($p=0.003$), and 5 ($p=0.002$). There was a significant difference between block 3 and block 4 ($p=0.05$) but not block 5. There was no significant difference between blocks 4 and 5.

Discussion

The data from this study indicated that activation of DA receptors by apomorphine increased aggressive motivation in male hamsters when the possibility for cheek pouching was eliminated. The significant reduction in latency to flank mark after apomorphine treatment was successfully replicated from Study 2. The difference in the latencies measures between Studies 2 and 3 (overall longer latencies in Study 2) may have been a result of the removal of the bedding and food from the cages in Study 3. In Study 2 the animals may have spent more time at the beginning of the observation investigating the food and bedding that was in their partner male's cage making their overall latencies longer. It does not appear to be a drug effect since both the saline and the drug treated animals in Study 3 reduced their overall latencies suggesting that they spent less time in the beginning of the observation investigating the cage.

The significant difference in flank marking indicated that apomorphine treatment increases aggressive motivation as measured by flank marking in male hamsters. The significant difference in flank

marking within each time block indicated that male hamsters express more aggressive motivation earlier in the observation period compared to later. The lack of a significant interaction between treatment and time block suggests that treatment did not affect the changes across blocks but it did affect the rate within each block.

Study 4

The purpose of Study 4 was to determine if apomorphine had an effect on aggression within the context of a social encounter. Many previous studies of aggressive behavior in rodents have examined aggression in a social context such as an aggressive encounter between two males. These studies have shown that dopamine may have a facilitative role in aggression (Van Erp & Miczek, 2000; Ferrari, van Erp, Tornatzky, & Miczek, 1998). Studies 1 and 2 looked at the role of dopamine in aggressive motivation without the added complexity of a social context. Study 4 examined the effects of apomorphine on an aggressive encounter between two male golden hamsters which, although it includes the social context, is a more traditional measure of aggression.

Procedure

Encounters were staged between two male hamsters to measure apomorphine's effects on attack latency. Each male was tested with the same male he was paired with previously in Study 2 (N=18). On both test days each animal was weighed to determine the appropriate injection volume (weight/1000 ml). Each member of a pair of animals was injected with the same dose of either apomorphine (0.5 mg/kg) or saline so they were treated equally for the encounter. To determine treatment order the nine pairs were ordered using counterbalancing with the restriction that the two treatment orders occurred at the same frequency. Ten minutes following the injection each animal in a pair was placed into the test arena (55 x 53.5 x 28 cm) on opposite sides of a plexiglas barrier. The tests were videotaped starting with the habituation period and lasting throughout the encounter. The animals had five minutes to habituate to the

test arena. At five minutes the barrier was lifted. The stop watch was started when the animals came within 2 cm of each other. The latency of the first bite attack was measured. An attack was counted when one or both of the males bit the other and they engaged in a roll. As soon as the animals engaged in an attack they were separated and returned to their home cages. The test arena was wiped down with a wet paper towel before the next pair of animals was tested.

Results

A repeated measures ANOVA was used to analyze the latency of the first bite attack between the two males in the test arena initiating an aggressive encounter. The latency till the first bite attack after the saline treatment was 19.8 s (SEM=9.5). The latency until the first bite attack after the high apomorphine treatment was 11.3 s (SEM=3.2). The analysis showed no significant effect of apomorphine dose on the latency to bite (N=9). The latencies in both conditions were so short that a floor effect occurred and a drug effect was not detectable.

Study Five

The purpose of Study 5 was to examine the effects of apomorphine on male golden hamster ultrasonic vocalizations in response to female sexual stimuli. Ultrasonic vocalizations in hamsters have previously been used as an indicator of sexual motivation (Floody & Pfaff; 1977). Male hamsters will increase their calls in the presence of an estrous female to induce lordosis and when the female is removed in an attempt to locate her. It is believed that the males increase calling in the absence of the female to initiate male-female contact (Floody & Pfaff, 1977b). Other, more social rodents use vocalizations to signal their submission during an aggressive encounter to make social living possible. Golden hamsters are a solitary, highly aggressive species that do not exhibit vocalizations during aggressive encounters. An aggressive encounter between two hamsters always ends with the dominant male attacking the subordinate (Sales, 1972).

These studies indicate that male hamster ultrasonic vocalizations are a result of sexual motivation not aggressive motivation. It is important in this study to use a measure of sexual motivation that does not involve a social encounter so that the effects on sexual motivation alone can be observed without the added complexity of a social situation. In Study 5, males were presented with vaginal secretions from estrous females and ultrasonic vocalizations that mimic calling from an estrous female. Previous studies have shown that male hamsters will respond to these two stimuli with ultrasonic vocalizations (Johnston, 1984; Johnston, 1979; Floody & Pfaff, 1977c). These two stimuli presented together were intended to induce sexual motivation in the male hamster that would result in ultrasonic vocalizations. These vocalizations could be recorded and compared after varying apomorphine treatments to determine the effects of apomorphine on sexual motivation in male hamsters as measured by ultrasonic vocalizations.

Subjects

Male (N=14) and female (N=8) golden hamsters aged 8 to 18 months and bred in the laboratory were individually housed in cages measuring 34.5 x 20.5 x 17.5 cm with wire fronts and bottoms and a solid back and walls. Paper towels and paper plates served as bedding material and food and water were available ad libitum. All male hamsters were housed in one of two colony rooms and the females were housed in the other. The rooms were on a reversed light cycle (10 hr dark, 14 hr light). The hamsters were most active during the dark period when all tests were conducted.

Behavioral Measures

Data were collected through observation and recorded on paper. Two observers were present. One observer played the synthetic vocalizations while the second observer recorded the vocalizations and other behaviors made by the male hamster. Behaviors that were recorded included vocalizations, expression of interest in the scent mark, expression of excessive licking behavior, and flank marking. Vocalizations were defined as any vocal sound made by the male hamster that could not be attributed to another behavior (i.e. licking or scratching). Interest in the scent mark was defined as the male spending any time sniffing and/or licking the middle of the plexiglas piece containing the female sample. Excessive

substrate licking behavior was defined as a male licking the floor or walls of the aquarium greater than 30 times and in a continuous manner. Many of the males would lick the floor and walls of the aquarium two or three times during their investigation of the environment but then cease the behavior. When obsessive substrate licking was observed the male hamster would begin licking the floor or walls of the aquarium very rapidly and continue the behavior for minutes at a time. Flank marking behavior was defined as in Studies 1, 2, and 3.

Drugs

Drug (R-(-)-Apomorphine hydrochloride hemihydrate purchased from Sigma-Aldrich) treatments were administered the same as in Studies 1, 2, 3, and 4. Three doses of apomorphine (0.25 mg/kg, 0.5 mg/kg, and a saline control) were given to each animal in a counterbalanced order with the restriction that each order occurred at the same frequency. There were 48 hours separating each injection given to an animal. Each animal received two rounds of each dose. The doses were mixed and coded by a third party so the observers were blind to the treatment each animal was receiving.

Apparatus

Vocalizations were observed using a wide-band high-frequency microphone attached to a QMC ultrasonic receiver tuned to 35 kH to make audition of the ultrasonic vocalizations possible for the observers. Synthetic ultrasonic vocalizations were presented using a Krohn-Hite Corporation function generator and high frequency capacitance speaker. The ultrasonic calls were consistent 100 ms, 35 kHz tones mimicking natural hamster calls (Floody & Pfaff, 1977). A glass aquarium, measuring 21 x 26.5 x 41 cm, was placed on the observation bench. The high-frequency speaker was positioned at a downward facing angle over one end of the aquarium. The high frequency microphone was positioned so it extended out over the middle of the aquarium, facing downward, and was 26 cm above the floor of the aquarium. The frequency of vocalizations made by the male hamster were tabulated on paper.

Procedure

Estrous cycles were established for each of the females. The testing schedule was designed so that there were two females exhibiting lordosis on each day of testing. Vaginal secretion samples were taken from each of the two females using cotton-tipped applicators (Puritan Sterile Cotton-Tipped Applicators). A sample from one female was used to mark four plexiglas pieces. Each sample consisted of a mark (approximately 4 x 1 cm) made using about 4 swipes of the cotton tipped applicator across the center of each plexiglas piece (7.5 x 10 cm). Samples taken from two estrous females and weighed indicated that the average amount of the vaginal secretion applied to each piece of plexiglas was 0.54 mg ± 0.09 mg. Each piece of plexiglas was used for only one observation and then cleaned.

On testing days, each male received his injection and was returned to his cage in the colony room for the 15 minute habituation period needed for the drug to take effect. At 15 minutes the hamster was brought into the quiet, dimly lit test room and placed into the aquarium and the stop watch was started. The hamster was observed for a two minute habituation period. At two minutes the scented plexiglas piece was placed into the aquarium. Following the introduction of the plexiglas, five synthetic ultrasonic vocalizations were played every thirty seconds. All vocalizations made by the male hamster were tabulated on paper and a record was kept of when each vocalization occurred throughout the observation (i.e. pre stimuli, first minute, second minute, etc.). It was necessary to distinguish the pre stimuli vocalizations from the post stimuli vocalizations since hamsters will often vocalize when first introduced to a new environment. The two minute habituation period in the test environment reduced the chances that vocalizations sounded after the stimuli had been presented were in response to the novel environment.

Vocalizations, expression of interest in the scent mark, excessive substrate licking, and flank marking behaviors were recorded. The observation lasted for a total of seven minutes. It consisted of a two minute habituation period and five minutes with the scent and vocal stimuli present. One observer timed the test and played the synthetic vocalizations while a second observer listened and recorded the

vocalizations and behaviors made by the male hamster. At the end of each observation the aquarium was wiped down with a wet paper towel and allowed to dry before the next observation.

Results

The purpose of Study 5 was to determine if different levels of apomorphine had an effect on male hamster vocalization frequency in response to estrous female vaginal secretions and synthetic vocalizations that represented sexual cues from an estrous female. Vocalizations were recorded prior to the presentation of the sexual stimuli and during the presentation of the sexual stimuli. The purpose of this separation was to determine if the hamsters' vocalizations changed in response to the stimulus as well as the varying apomorphine treatments. A two factor repeated ANOVA was used to analyze the results with one factor being the presentation of the stimulus (two levels, pre and during) and the second factor being the apomorphine treatment (three levels). On the last day of testing one of the male hamsters exhibited abnormal behavior during testing and died later that day. He was excluded from all of the analysis to reduce any variability he may have introduced. The analysis (N=13) showed no main effects of stimulus presentation or apomorphine treatments. There was a significant interaction between the presentation of the stimulus and the apomorphine treatment, $F(2, 24) = 3.60$, $p=0.04$ (Fig. 9). There was a significant linear contrast of treatment, $F(1, 12) = 7.43$, $p=0.02$, suggesting that vocalizations decreased significantly after each treatment.

A closer examination of the interaction effect was conducted. Paired samples t-tests were conducted at each level of the apomorphine treatment between the two levels of presentation of the stimuli (pre and during) to determine if there was an effect of presentation of the stimuli. There was a significant difference in number of vocalizations after the saline treatment prior to the presentation of the stimuli than during presentation of the stimuli, $t(12) = -2.95$, $p=0.01$ (Fig. 9). The number of vocalizations after the saline treatment prior to exposure to the stimuli (M=1.15, SEM=0.57) was significantly lower than the number of vocalizations during presentation of the stimuli (M=4.35, SEM=1.06). There was no significant

difference in vocalization frequency prior to or during the presentation of the stimuli after either the 0.25 mg/kg or the 0.5 mg/kg apomorphine treatments (Fig. 9).

Two, separate repeated measures ANOVAs were conducted to examine more closely the effect of apomorphine treatment on vocalization frequency. The first analysis was conducted examining the difference between the three treatment doses on the number of vocalizations prior to the presentation of the stimuli. There was no significant difference in vocalizations prior to the presentation of the stimuli after any of the treatments. The second analysis was conducted examining the difference of vocalization frequency during the presentation of the stimuli after each treatment. There was a significant effect of treatment on vocalizations during stimuli presentation, $F(2, 24) = 4.75$, $p=0.02$ (Fig. 9). The males vocalized significantly less after the 0.5 mg/kg apomorphine treatment (M=1.04, SEM=0.28) than after the saline treatment (M=4.35, SEM=1.05). There was no significant difference after the 0.25 mg/kg apomorphine treatment (M=2.04, SEM=0.63). However, there was a significant linear contrast, $F(1, 12) = 7.33$, $p=0.02$.

During the observation periods three notable behaviors were recorded. The first behavior was whether or not a male hamster exhibited interest in the vaginal scent mark. Interest was operationally defined as if the male hamster spent any time sniffing and/or licking the middle of the plexiglas piece with the female sample on it. A Cochran's Q nonparametric test was run on the expression of interest to determine if there was a drug effect on whether or not a hamster showed any interest in the scent mark. The analysis showed a significant difference in incidence of expression of interest after apomorphine treatment, (N=14) Q (2) =7.00, p=0.03 (Fig. 10). After the saline treatment 100% of the animals showed interest in the scent. After the low apomorphine treatment 72% of the animals showed interest. After the high apomorphine treatment 64% showed interest in the scent. A Sign test can be used to determine if two related samples are different. A Sign test was used to determine the difference in expression of interest between each of the apomorphine treatments. The analysis approached significance between the saline and the 0.5 mg/kg apomorphine treatment (p=0.06). The analysis showed no significant differences between the saline and the 0.25 mg/kg apomorphine treatment. This analysis suggests that there was significantly

less interest shown in the scent mark after the 0.25 mg/kg apomorphine treatment than after the saline treatment.

The second notable behavior that was observed was an excessive licking stereotypy exhibited by the males. This excessive substrate licking behavior was operationally defined as a male licking the floor or walls of the aquarium greater than 5 times and in a continuous manner. A Cochrane's Q test was used to analyze the incidence of expression of licking behavior after each treatment. The analysis showed that there was a significant difference in incidence of licking behavior between the treatments, (N=14) Q (2) =15.17, p=0.001 (Fig. 11). After the saline treatment 0% of the animals showed the licking stereotypy. After the 0.25 mg/kg apomorphine treatment 43% of the animals showed the licking stereotypy one or more times. After the 0.5 mg/kg apomorphine treatment 79% of the animals expressed the licking stereotypy one or more times. A Sign test was used to determine the differences in expression of excessive licking of the substrate after each of the apomorphine treatments. There was a significant difference between the saline treatment and the 0.25 mg/kg apomorphine treatment (p=0.03). There was a significant difference between the saline and the 0.5 mg/kg apomorphine treatment (p=0.002). This indicated that there was a significant increase in excessive substrate licking behavior after the 0.5 mg/kg apomorphine dose.

Only two incidences of flank marking behavior occurred during the observations of vocalizations. Both cases of flank marking occurred after an animal had received the apomorphine treatment. In one of the two cases the animals marked only one time. In the second of the two cases the animal marked six times. Two cases of flank marking were not enough for statistical analysis.

Discussion

The results from Study 5 indicated that the male hamsters vocalized significantly more in response to the stimuli after the saline treatment than prior to the stimuli. This indicated that with no apomorphine treatment male hamsters will respond to the sexual stimuli with increased vocalizations. The significant difference in vocalizations during the presentation of the stimuli after the saline and the high

apomorphine treatments combined with the significant linear contrast suggests that apomorphine treatment significantly reduced vocalizations in response to the stimuli. The expression of interest in the vaginal scent mark approached a significant difference between the saline and the 0.5 mg/kg apomorphine treatments suggesting that with increased apomorphine treatments interest expressed in the scent mark decreased. The opposite effect occurred in expression of excessive substrate licking. There was a significant increase in this stereotypy following the 0.25 mg/kg apomorphine treatment and the 0.5 mg/kg apomorphine treatment in a linear fashion. This suggests that with increasing apomorphine treatments excessive substrate licking is expressed more frequently.

There are two possible conclusions from this data. The first is that the hamsters did not notice the stimuli after they had received the apomorphine treatments and therefore did not experience an increase in vocalizations. This could have occurred through a flaw in the procedure, such as possibly an ineffective presentation of the scent mark, which is unlikely since the same procedure produced an effect after the saline treatment. The second, and more likely conclusion, suggests that the hamsters did take note of the stimuli but the apomorphine suppressed the expression of sexual behavior as observed through vocalizations.

There were some limitations to Study 5 that were observed after completion of the study. The two minute habituation period before presentation of the stimuli was necessary to determine if there was a difference in vocalization frequency after presentation of the stimuli. This is important so that it can be determined if the hamsters are affected by the stimuli regardless of drug effects. In Study 5 a difference was observed indicating that the males responded to the presence of the stimuli. However, golden hamsters will often vocalize in response to being disrupted in their habitats. Moving the hamsters from the colony room to the observation room could have led to an increase in their vocalization frequency upon immediate placement into the test chamber. A more appropriate procedure would have been to give the animals a one or two minute habituation period to the test chamber and then a two minute observation period without the presence of the stimuli. This would reduce any chances that the vocalizations recorded prior to the presentation of the stimuli were only in response to moving the males rather than in response to the test

arena without the stimuli. However, in Study 5 there was still a low enough frequency of vocalizations in the two minutes prior to the presentation of the stimuli that an effect was still observed.

The observations of expression of interest in the scent mark and the licking stereotypy could have been more specific. With the current operational definition of expression of interest there is no real way to differentiate between levels of interest expressed. As it stands now, the only analysis that can be conducted is on either any expression of interest or total lack of expression of interest. Recording the amount of time an animal spent in contact with the scent mark would be a more appropriate measure of the amount of interest the male was expressing in the scent mark. A more comprehensive observation of the excessive licking behavior would also be more appropriate. Timing the expression of the behavior would give a clearer indication of differences in the behavior following different drug treatments.

General Discussion

The results from these studies suggested a role for apomorphine in aggressive and sexual motivation in male golden hamsters. Previous studies have suggested that apomorphine treatments largely have a facilitative effect on sexual behavior (Arteaga, Motte-Lara, & Velazquez-Moctezuma, 2002; Bitran & Hull, 1987; Paglietti, Quarantotti, Mereu, & Gessa, 1977; Butcher, Butcher, & Larsson, 1969). Other studies have suggested that the amount and the route of administration are important factors to determine if apomorphine will act in a facilitative role or as an inhibitor of sexual behavior (Olivier, et al., 2007; Agmo & Fernandez, 1988; Hull et al., 1986). On the other hand, studies looking at aggressive behavior have indicated that increases in DA are associated with increased aggressive motivation and behavior (Nelson & Trainor, 2007; van Erp & Miczek, 2000; Ferrari, van Erp, Tornatzky, & Miczek, 2003). This suggests that apomorphine, as a DA agonist, may lead to increased aggression. This relationship has been shown in studies looking directly at the effects of apomorphine on aggression (Skrebuhhova-Malmros et al., 2000; Kask & Harro, 2000; Rudissaar et al., 1999; Matto, Allikmets, & Skrebuhhova, 1998). However, the effects that apomorphine has on aggressive and sexual behavior in male golden hamsters have been

previously shown to be inconclusive (Floody, unpublished). The results from these studies suggest that apomorphine has a facilitative effect on aggressive motivation as measured by flank marking behavior which has previously been associated with aggressive motivation in golden hamsters (delBarco-Trillo, McPhee, & Johnston, 2009; Petrulis, Widener, & Johnston, 2004; Hayden-Hixson & Ferris, 1991; Ferris & Potegal, 1988; Albers, Axelson, Ferris, & Shinto, 1987). These results also suggest that apomorphine had an inhibitory effect on ultrasonic vocalizations in response to sexual stimuli.

Studies 1, 2, and 3 examined how apomorphine affected aggression through flank marking behavior. The results from Study 1 suggested that, in high flank markers, flank marking behavior was decreased by apomorphine treatments. The results from Studies 2 and 3 differed from Study 1 in that they suggested that the latency to flank mark decreased and the frequency of flank marking was increased with apomorphine treatments. The difference in the results from these studies could have been due to the occurrence of cheek pouching behavior in Studies 1 and 2. Previous studies looking at apomorphine have observed an expression of stereotyped behavior in the animals receiving the apomorphine treatments (Tieppo, Nasello, & Felicio, 1997; Schnur & Martinez, 1989; Tagliamonte, Fratta, Del Fiacco, & Gessa, 1974, Butcher, Butcher, & Larrson, 1969). In Studies 1 and 2 this stereotypy was expressed by the male hamsters through cheek pouching behavior. The behavior was so frequent in Study 1 that it limited the number of observations of flank marking behavior since an animal that had his cheek pouches full of food and/or bedding would not flank mark. This caused a large variability in the expression of flank marking behavior by each male hamster and led to the discussion to divide the males into a high or a low marking group. In the high marking group, flank marking decreased significantly with apomorphine treatments. These results suggest that aggressive motivation was decreased with apomorphine treatment in the high flank marking animals. However, these data are inconsistent with previous studies (Skrebuhhova-Malmros et al., 2000; Kask & Harro, 2000; Rudissaar et al., 1999; Matto, Allikmets, & Skrebuhhova, 1998). The high occurrence of cheek pouching behavior may have skewed the data by interfering with the expression of flank marking since flank marking behavior was never observed in conjunction with cheek pouching. Studies on apomorphine-induced stereotyped behaviors have suggested that this stereotypy will disrupt

other behaviors (Tieppo, Nasello, & Felicio, 1997; Schnur & Martinez, 1989; Tagliamonte, Fratta, Del Fiacco, & Gessa, 1974, Butcher, Butcher, & Larrson, 1969). An analysis of the high marking group excluding observations when cheek pouching occurred showed no significant differences between treatments.

Studies 2 and 3 were conducted with the intent of examining the effects of apomorphine treatment on flank marking behavior without cheek pouching behavior. In Study 2 the procedural modification reduced the occurrence of cheek pouching behavior during the habituation period, thus more instances of flank marking were observed in all of the animals. The analysis with a larger sample size of observations showed a significant reduction in latency to flank mark after apomorphine treatment suggesting an increase in aggressive motivation. An analysis conducted on observations of flank marking that excluded the observations in Study 2 when an animal cheek pouched showed a significant increase in frequency of flank marking after apomorphine treatment suggesting an increase in aggressive motivation. These results contradict the findings from Study 1. However, the reduction of cheek pouching behavior in Study 2 possibly led to a more appropriate analysis of uninterrupted flank marking behavior.

Study 3 was conducted to further examine the effects of apomorphine on flank marking behavior while completely eliminating the possibility of cheek pouching behavior so that the conflicting results from Studies 1 and 2 could be clarified. To control for this, all of the food and bedding was removed from the cages of the test animals, thus cheek pouching was not observed in any of the tests. With regard to cheek pouching, the analysis from Study 3 was more accurate than the analyses from Studies 1 and 2. However, Study 2 also showed a positive effect of apomorphine on aggression through the reduction in the latency to flank mark while using multiple doses of apomorphine. This linear trend strongly supports the conclusion that apomorphine increases aggressive motivation. Combined with the results from Study 3 that showed a significant decrease in latency to flank mark and a significant increase in frequency of flank marking after the apomorphine treatment, these results suggest that apomorphine increases aggressive motivation in male golden hamsters.

The data from Study 5 suggested that apomorphine treatment reduced the male hamsters' vocalizations in response to sexual stimuli. The observations of expression of interest indicated that expression of interest in the vaginal scent mark was nearly significantly lower after the 0.5 mg/kg apomorphine treatment. It appears that this is a drug effect suggesting that with the 0.5 mg/kg apomorphine treatment the hamsters showed less interest in the scent. One possible conclusion is that the reduction in expression of interest after the 0.5 mg/kg apomorphine treatment is related to the expression of the licking stereotypy. In Study 5 it was noted that after the 0.25 mg/kg and 0.5 mg/kg apomorphine treatments the male hamsters exhibited an excessive licking of the substrate. The linear trend suggests that this behavior increased with increasing apomorphine dosage. Similar to the cheek pouching behavior in Studies 1 and 2, as well as consistent with previous literature (Tieppo, Nasello, & Felicio, 1997; Schnur & Martinez, 1989; Tagliamonte, Fratta, Del Fiacco, & Gessa, 1974, Butcher, Butcher, & Larrson, 1969), it appears that apomorphine treatment causes an increase in stereotyped behaviors. It seems that the increase in excessive licking of the substrate may have led to the decreased expression of interest and the reduced number of vocalizations after the 0.5 mg/kg apomorphine treatment. After the 0.5 mg/kg apomorphine treatment the animals may have been spending a majority of their time excessively licking the substrate rather than expressing interest in the scent mark or vocalizing in response to the sexual stimuli.

The data after the saline treatment in Study 5 showed that the animals did notice the sexual stimuli and they responded to it with increased vocalizations. After the 0.25 mg/kg apomorphine treatment, these same animals did not show a change in vocalization rate following the presentation of the stimuli, as observed in the t test analysis of change in vocalization rate before and during presentation of the stimuli. After the 0.25 mg/kg apomorphine treatment they still expressed interest in the scent mark in 54% of the observations suggesting that they did notice it despite an increase in obsessive substrate licking. Their lack of an increase in vocalizations suggests that the apomorphine treatment may have been directly suppressing expression of this behavior. On the other hand, the increase in excessive substrate licking may have contributed to the suppression of vocalizations. After the 0.5 mg/kg apomorphine treatment the animals again did not show a change in vocalization rate following the presentation of the stimuli. In this group,

interest in the scent may have been reduced as a result of the obsessive substrate licking as well. Overall the results from this study suggest that with no apomorphine treatment male hamsters will increase their vocalizations in response to sexual stimuli that have been previously associated with sexual behavior in hamsters (Floody & Pfaff, 1977a; 1977b; 1977c; Johnston, 1980; delBarco-Trillo, LaVenture, & Johnston, 2009).

After apomorphine treatments in Study 5 vocalizations in response to these same stimuli appear to be suppressed. It is highly likely that the suppression of the vocalizations is a result of the increase in the licking stereotypy. The literature on apomorphine-induced stereotyped behaviors suggests that the stereotypy does disrupt other behaviors (Tieppo, Nasello, & Felicio, 1997; Tagliamonte, Fratta, Del Fiacco, & Gessa, 1974, Butcher, Butcher, & Larrson, 1969). Schnur and Martinez (1989) conducted a study focusing on the expression of stereotyped behaviors induced by apomorphine treatments. They found that in female hamsters expression of stereotyped gnawing behavior increased in a dose-related fashion. Stereotyped licking increased after a 1.0 mg/kg dose of apomorphine. Tieppo, Nasello, and Felicio (1997) found that, in rats, 0.6 mg/kg of apomorphine induced continual expression of licking or gnawing of the test cage. The apomorphine dosage used in Study 5 that induced the highest amount of stereotyped licking behavior (0.5mg/kg) is similar to the amounts that caused high levels of stereotypy in these studies. This suggests that the licking stereotypy may have disrupted the expression of vocalizations. This conclusion is supported by the nature of the stereotyped behaviors that suggests that other behaviors, such as vocalizations, may be suppressed by the overwhelming tendency of the animals to express the stereotyped behaviors. This conclusion is consistent with the conclusions from Schnur and Martinez (1988) about female hamsters. Interestingly, a study on the effects of various drugs on ultrasonic vocalizations in gerbils indicated that apomorphine decreased vocalizations in these animals (Thiessan & Upchurch, 1981). A high dose of apomorphine was used (5.0 mg/kg) but no observations of stereotyped behaviors were reported. The authors draw no firm conclusions but this suggests that vocalizations may be reduced by apomorphine in gerbils.

If the disruption of the vocalizations in response to the sexual stimuli is a result of the increases in apomorphine-induced licking stereotypy then it may be that other DA agonists would result in an increase in vocalizations in response to the sexual stimuli. This increase in sexual behavior as measured by increased vocalizations would be consistent with the literature on the effects of DA agonists on sexual behavior in rodents (Dominguez & Hull, 2005; Bitran & Hull, 1987). Many of the studies that have reported sexual facilitation by apomorphine were using social contexts to assess sexual behavior and motivation such as mounts, intromissions, and ejaculations that require the presence of a male and a female to be observed (Arteaga, Motte-Lara, & Velazquez-Moctezuma, 2002; Bitran & Hull, 1987; Paglietti, Quarantotti, Mereu, & Gessa, 1977; Butcher, Butcher, & Larsson, 1969). The intent in this study was to not use a social context to measure sexual behavior. The measure of vocalizations in response to sexual stimuli used in Study 5 was intended to give more a specific account of how apomorphine affected sexual behavior while not including other behaviors and motivations that would be seen in a more social measure. It was fairly clear in Study 5 that the licking stereotypy was disrupting expression of vocalizations. The same could be true for the cheek pouching stereotypy and flank marking in Studies 1 and 2. This measure of sexual behavior in hamsters is still beneficial to assess sexual behavior in a nonsocial context. However, it could be that a DA agonist other than apomorphine, such as the dopamine precursor L-DOPA, SK-38393, or SDN 919 (Dominguez & Hull, 2005; Bitran & Hull, 1987), would show facilitation of the vocalizations in response to the sexual stimuli suggesting that the role DA is playing in male hamster sexual behavior is consistent with the literature.

These results explain the findings observed by Floody (unpublished) and elaborate on the findings observed by Lehman, Powers, and Winan (1983). Lehman, Powers, and Winan (1983) suggested that male hamsters that have had their sensory processing disrupted may confuse the signals that an estrous female is giving. As a result of their strong association with the female's overly aggressive behavior when she is not in estrus the males that have disrupted processing may be attributing these characteristics to estrous females and will respond to the estrous female in an aggressive manner. The observations made by Floody (unpublished) see a similar pattern of behavior. Male hamsters that received apomorphine treatments and

were subsequently presented with an estrous female expressed aggression towards the estrous female. The highest apomorphine treatment resulted in the most aggression displayed. This is consistent with the results seen in Studies 2 and 3. It appears that with these doses of apomorphine aggressive motivation is increased in male hamsters. This increase in aggression is consistent with previous studies on apomorphine's effects (Nelson & Trainor, 2007; van Erp & Miczek, 2000; Ferrari, van Erp, Tornatzky, & Miczek, 1998). With this increase in aggressive motivation, the male's perception of the cues presented by the estrous female becomes confused and the male responds to the female in an aggressive manner. The results from the present study combined with the literature on apomorphine-induced aggression and the study by Lehman, Powers, and Winans (1983) suggest that males receiving apomorphine treatments experienced a disruption of processing as a result of the apomorphine administration and were responding to the estrous female in an aggressive manner that is normally reserved for nonestrous females.

Further studies are needed to examine the relationship between DA, aggression, and sexual behavior in hamsters. This study only focused on systemic administrations of the general DA agonist apomorphine. It was important to focus on this drug and method of administration to explain the results that were observed by Floody (unpublished) when the same method was used. However, the literature suggests that apomorphine, and DA in general, is mainly a facilitator of sexual behavior in rodents (Arteaga, Motte-Lara, & Velazquez-Moctezuma, 2002; Bitran & Hull, 1987; Paglietti, Quarantotti, Mereu, & Gessa, 1977; Butcher, Butcher, & Larsson, 1969). This literature, however, primarily focuses on rats. The high levels of aggression observed between male and nonestrous female hamsters that Lehman, Powers, and Winan (1983) suggest account for the behavioral response of the male hamsters with disrupted cue processing may explain the aggression expressed by the males to the estrous females observed in Floody (unpublished). Studies on sexual behavior in rats, however, do not report obvious increases in aggression as a result of apomorphine treatment (Dominguez & Hull 2005; Bitran & Hull, 1987). However, in non-copulatory models an apomorphine-induced increase in aggression has been observed in rats (Skrebuhhova-Malmros et al., 2000; Kask & Harro, 2000; Rudissaar et al., 1999; Matto, Allikmets, & Skrebuhhova, 1998). It appears that the difference in aggression between these species accounts for the

differences observed in their sexual and aggressive reactions to apomorphine treatment. The highly aggressive nature of hamsters may contribute to why the increase in aggression towards estrous females is seen so obviously following apomorphine treatment. The results from the studies presented here suggest an explanation for the inconsistencies in the effects of apomorphine on copulatory behavior in rats presented in the literature (Bitran & Hull, 1987; Hull & Dominguez, 2005). The induction of aggression in hamsters by apomorphine appears to de disrupting sexual behavior in these males. This could also be the case in rats. The apomorphine treatments that have been reported to inhibit or have no effect on sexual behavior may in fact be increasing aggression in rats. However, since they are not as overly aggressive as hamsters the aggression may not be as obvious. This increase in apomorphine-induced aggression in rats may be disrupting sexual behavior, leading to the inconsistencies seen in the literature.

The literature on sexual behavior in rats suggests that other DA agonists, as well as apomorphine, facilitate sexual behavior (Bitran & Hull, 1987). This is important to consider for future work on sexual behavior in hamsters. Other DA agonists that are known sexual behavior facilitators, L-DOPA, SK-38393, or SDN 919 (Dominguez & Hull, 2005; Bitran & Hull, 1987), may be more effective in this capacity on hamsters than apomorphine if they do not induce the aggressive behaviors. However, the literature on apomorphine's effects on sexual behavior has shown some variability which suggests that it should not be completely ruled out as a facilitator of sexual behavior in hamsters. As reported in Bitran and Hull (1987), systemic treatments of apomorphine decrease the number of intromissions and the latency to ejaculation. Butcher, Butcher, and Larsson (1969) observed that a dose of 0.8 mg/kg of apomorphine significantly reduced intromission frequency despite observations of stereotyped gnawing behavior. Tagliamonte, Fratta, Del Fiacco, and Gessa, (1974) used a 0.5 mg/kg dose of apomorphine that significantly increased the expression of sexual behaviors in sexually sluggish males. These studies all suggest that apomorphine can facilitate aspects of sexual behavior. Agmo and Fernandez (1988) saw that in castrated male Wistar rats, apomorphine had no effect on sexual behavior from 0.05 mg/kg to the 0.15 mg/kg range. Higher doses resulted in stereotyped behavior expression. Butcher, Butcher, and Larsson (1969) observed that a systemic treatment of 0.8 mg/kg of apomorphine decreased the intromission frequency before ejaculation

and increased the post-ejaculatory interval. They also observed an increase in stereotyped gnawing behavior. These studies suggest that at higher doses, apomorphine acts as a facilitator of sexual behavior. However, at lower doses apomorphine appears to have no effect in castrated males. This literature has interesting implications for the present study. The doses that were used in the present study ranged from 0.01 mg/kg to 0.5 mg/kg and were administered systemically. Based on the conclusions from the literature any facilitative effects on sexual behavior in male hamsters should have been observed after the 0.25 or the 0.5 mg/kg doses of systemic apomorphine. However, the stereotypy interrupted any observations that could have been made. Perhaps a lower dose that would not induce the stereotypy and was administered into the MPOA as reported by Hull, Bitran, Pehek, Warner, Band, and Holmes (1986), would facilitate sexual behavior in male hamsters.

Other modes of administration should be explored to determine the effects that DA has on aggressive and sexual behaviors in hamsters. The medial preoptic area (MPOA) has been associated with DA, copulatory behaviors (Dominguez & Hull, 2005; 2006) and aggressive behaviors (Floody, 2009). This may be an area on which to focus to further examine the relationship between copulation, aggression, and DA. Apomorphine is also a non-selective DA agonist. A more specific agonist or antagonist could indicate a common DA receptor for aggression and sexual behavior. Couppis and Kennedy (2008) reported that the D_2 agonist N-propylnorapomorphine has been shown to increase aggression under predatory, stress, and isolation induced aggressive paradigms. D_1 agonists have also been shown to increase aggression but motor effects and an inability to replicate the data across species has limited interpretations. The results from the present study suggest that there is a relationship in male hamsters between apomorphine activated DA receptors, aggression, and sexual behavior. However, the exact nature of that relationship is still undetermined.

Figures

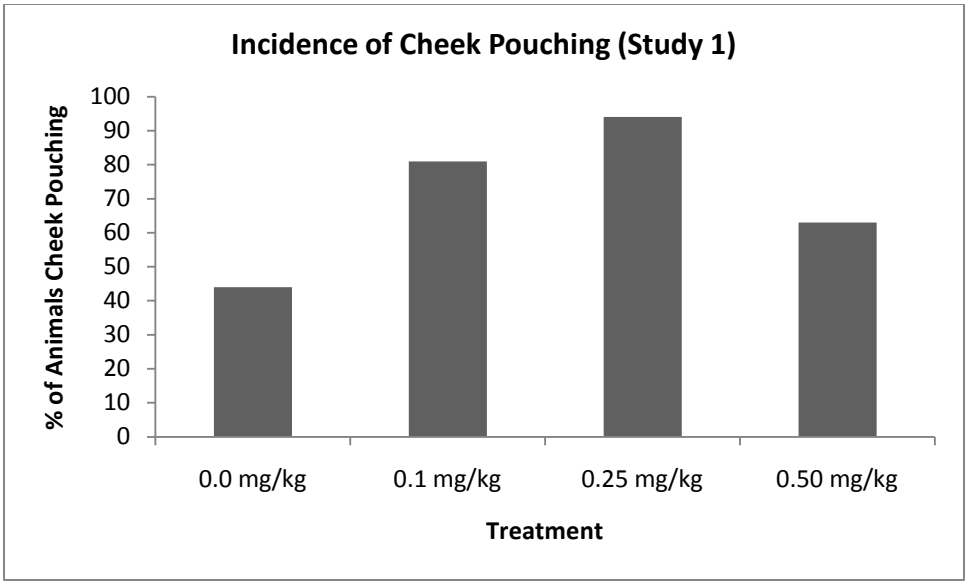

Figure 1: Apomorphine treatment significantly increased incidence of cheek pouching in Study 1.

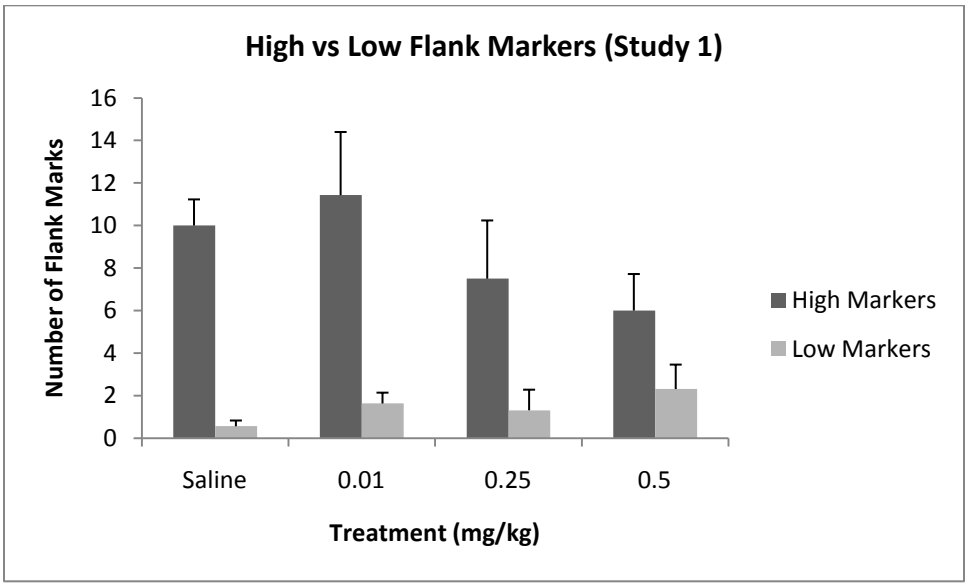

Figure 2: A significant dose X group interaction on frequency of flank marking. The group of high flank markers showed a significant linear contrast indicating that apomorphine treatment decreased frequency of flank marking in Study 1.

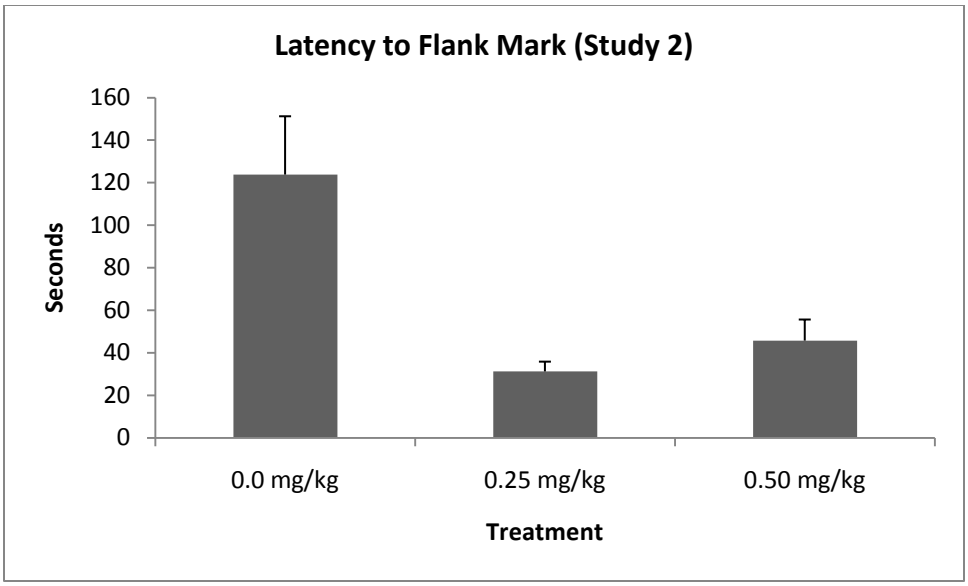

Figure 3: Apomorphine treatment significantly reduced the latency to flank mark in Study 2. The data presented here are untransformed.

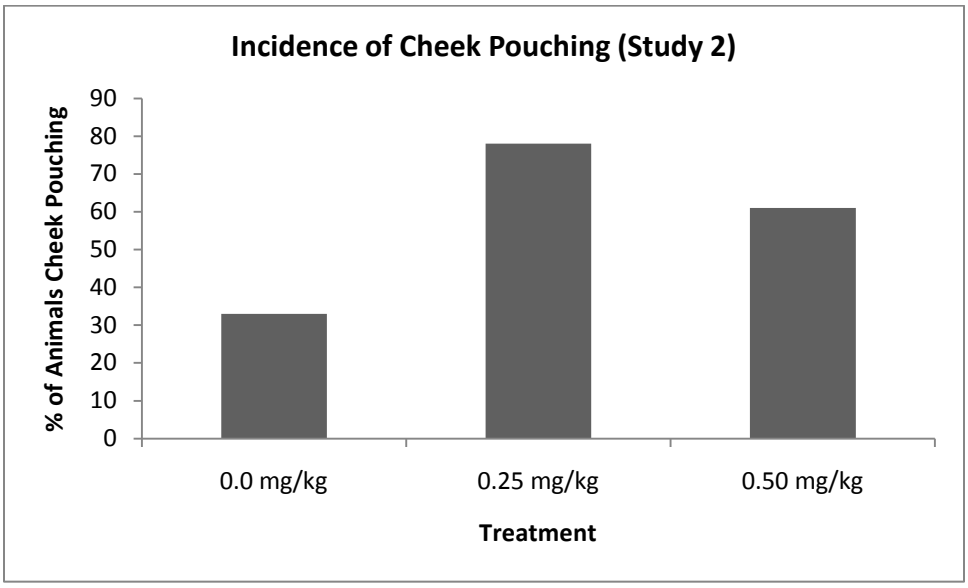

Figure 4: Apomorphine significantly increased the incidence of cheek pouching in Study 2.

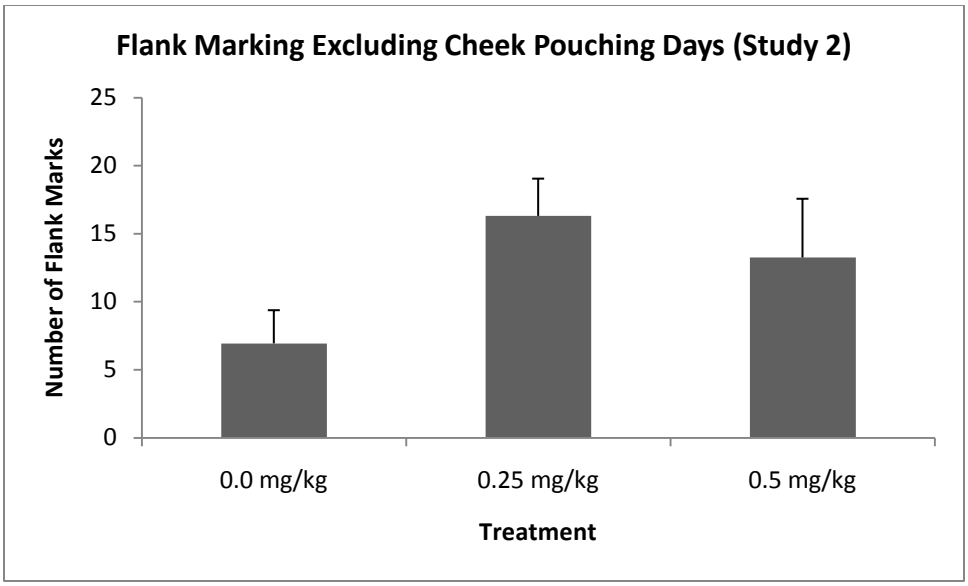

Figure 5: Excluding the days were an animal cheek pouched, apomorphine significantly increased frequency of flank marking in Study 2.

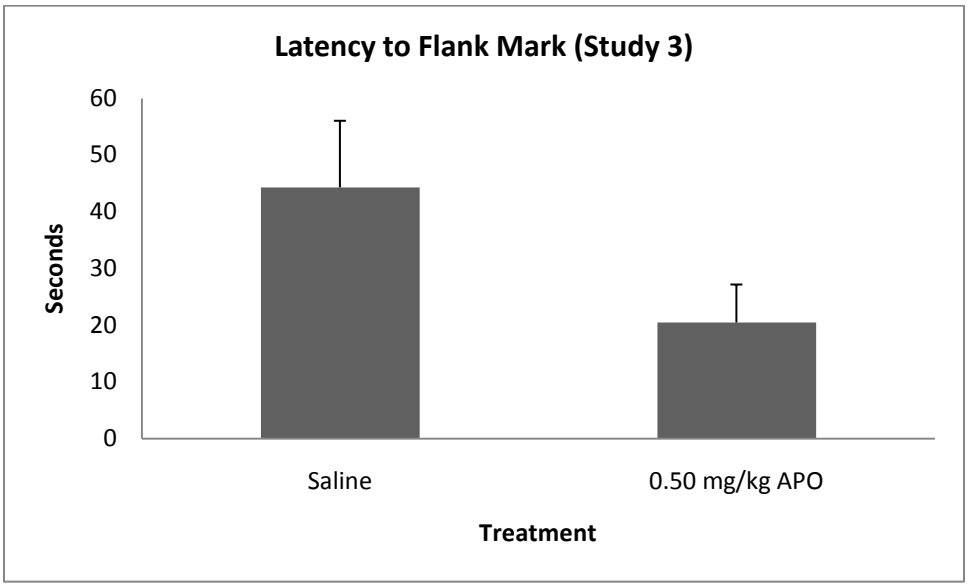

Figure 6: Apomorphine treatment significantly reduced the latency to flank mark in Study 3.

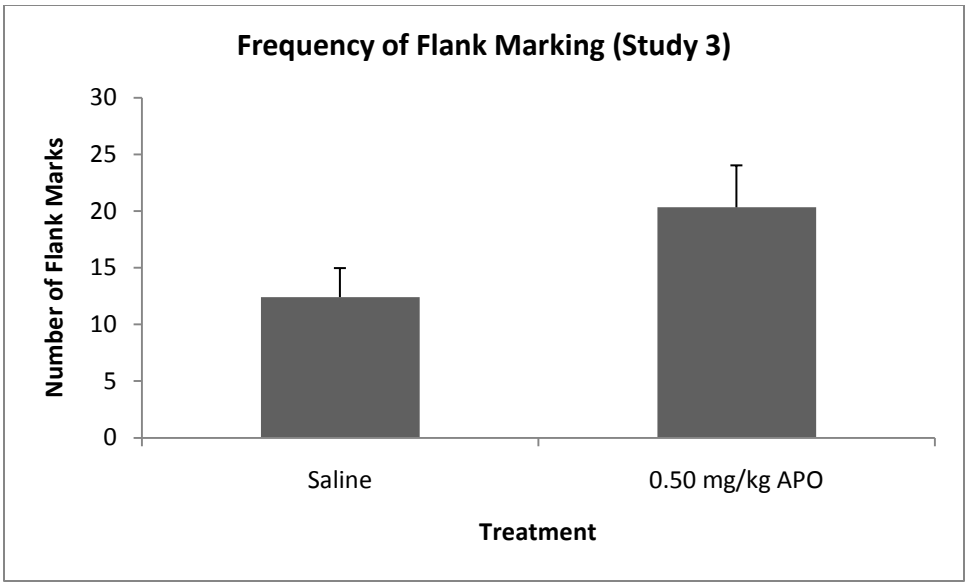

Figure 7: Apomorphine treatment significantly increased frequency of flank marking in Study 3.

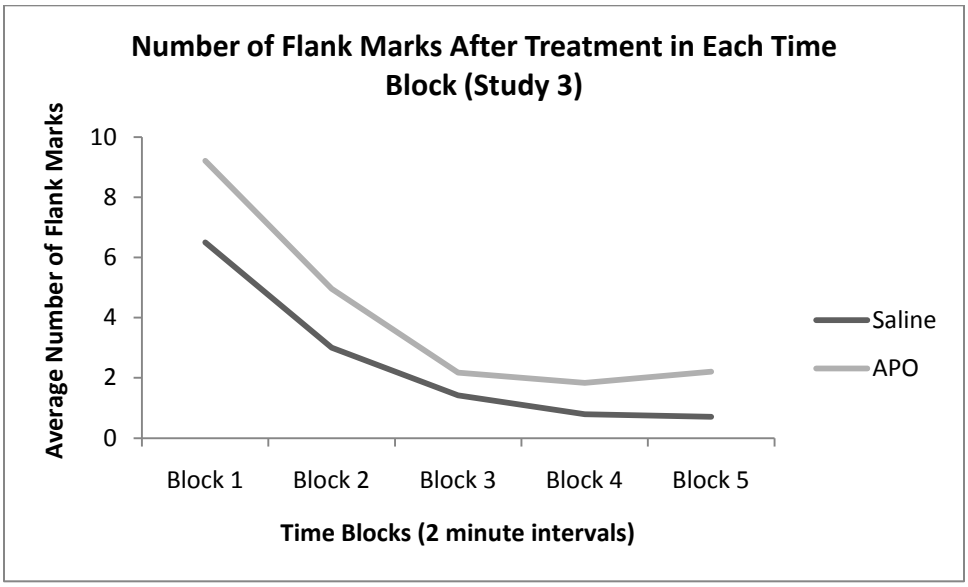

Figure 8: Flank marking frequency was significantly greater earlier in the observation period than later for both the saline and the apomorphine treatments in Study 3.

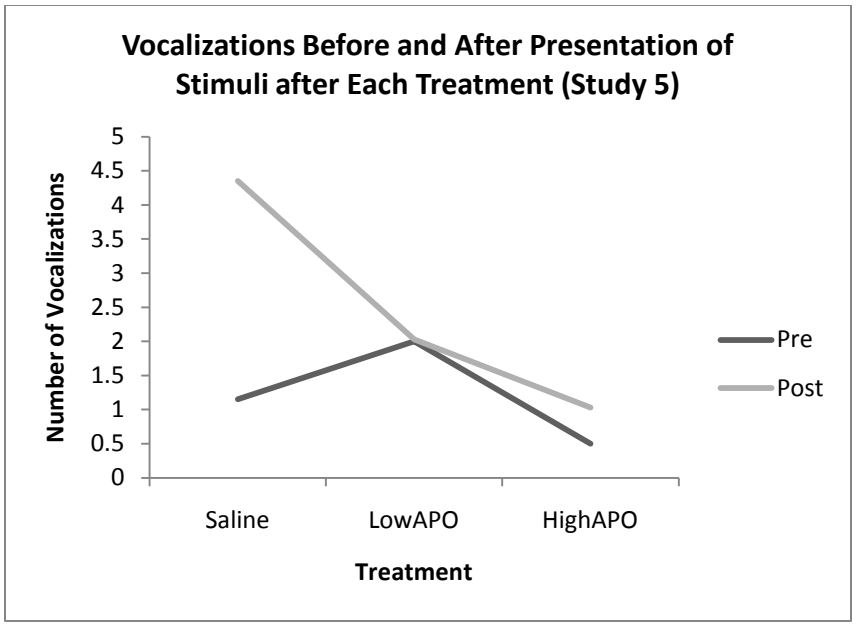

Figure 9: After the saline treatment the males vocalized significantly more after presentation of the stimuli. Apomorphine treatment significantly suppressed vocalization frequency after presentation of the stimuli in Study 5.

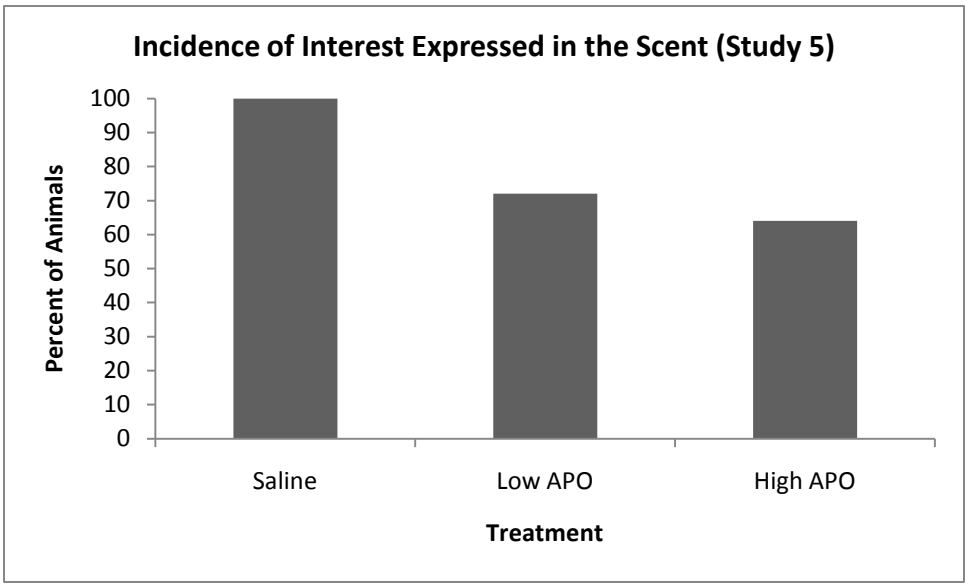

Figure 10: Expression of interest in the scent mark was significantly decreased by the apomorphine treatment in Study 5.

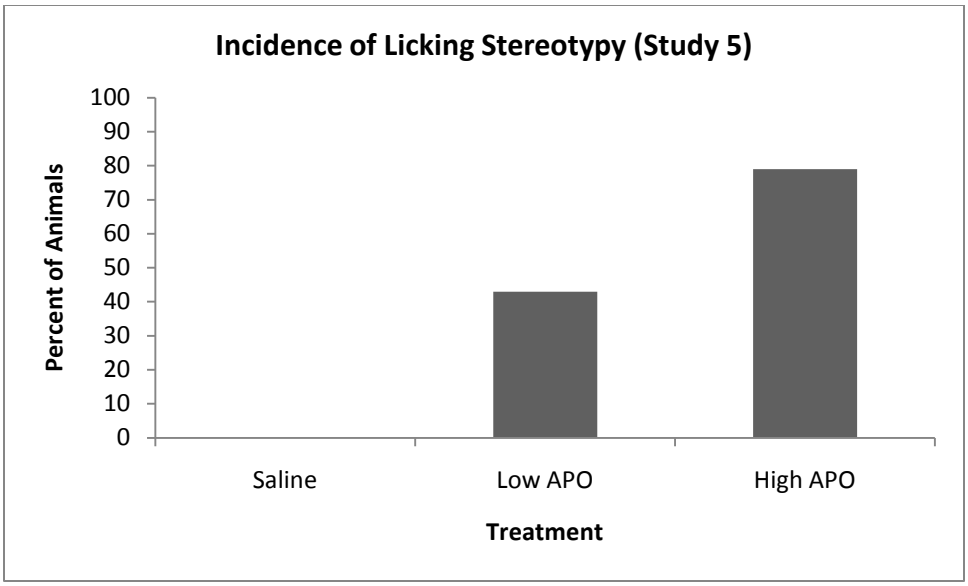

Figure 11: Expression of obsessive licking behavior was significantly increased with apomorphine treatment in Study 5.

References

Agmo, A. & Fernandez, H. (1988). Dopamine and sexual behavior in the male rat: a reevaluation. *Journal of Neural Transmission, 77*, 21-37.

Albers, H., Axelson, J., Ferris, C., & Shinto, L. (1987). Scent marking and the maintenance of dominant/subordinate status in male golden hamsters. *Physiology and Behavior, 40*(5), 661-664.

Arteaga, M., Motte-Lara, J., & Velazquez-Moctezuma, J. (2002). Effects of yohimbine and apomorphine on the male sexual behaviour pattern of the golden hamster (Mesocricetus auratus). *European Neuropsychopharmacology, 12*, 39-45.

Bitran, D., & Hull, E. (1987). Pharmacological analysis of male rat sexual behavior. *Neuroscience and Behavioral Reviews, 11*, 365-389.

Butcher, L., Butcher, S., & Larsson, K. (1969). Effects of apomorphine, amphetamine, and nialamide on tetrabenazine-induced suppression of sexual behavior in the male rat. *European Journal of Pharmacology, 7*, 283-288.

Caldwell, H. & Albers, H. (2004). Effect of photoperiod on vasopressin-induced aggression in Syrian hamsters. *Hormones and Behavior, 46*, 444-449.

Cherry, J. (1989). Ultrasonic vocalizations by male hamsters: parameters of calling and effects of playback on female behaviour. *Animal Behaviour, 38*(1), 138-153.

Clark, J. & Smith, E. (1985). Effects of apomorphine on sexual behavior in young and middle aged rats. *Neurobiology of Aging, 8*, 153-157.

Couppis, M. & Kennedy, C. (2008). The rewarding effect of aggression is reduced by nucleus accumbens dopamine receptor antagonism in mice. *Psychopharmacology, 197*, 449-456.

David, J., Cervantes, M., Trosky, K., Salinas, J., & Delville, Y. (2004). A neural network underlying individual differences in emotion and aggression in male golden hamsters. *Neuroscience, 126*, 567-578.

delBarco-Trillo, J., LaVenture, A., & Johnston, R. (2009). Male hamsters discriminate estrous state from vaginal secretions and individuals from flank marks. *Behavioural Processes, 82*, 18-24.

delBarco-Trillo, J., McPhee, M., & Johnston, R. (2009). Nonagonistic familiarity decreases aggression in male Turkish hamsters, *Mesocricetus brandti. Animal Behavior*, *77*, 389-393.

Dominguez, J., & Hull, E. (2005). Dopamine, the medial preoptic area, and male sexual behavior. *Physiology and Behavior*, *86*, 356-368.

Ferrari, P., van Erp, A., Tornatzky, W., & Miczek, K. (2003). Accumbal dopamine and serotonin in anticipation of the next aggressive episode in rats. *European Journal of Neuroscience*, *17*, 371-378.

Ferris, C. & Potegal, M. (1988). Vasopressin receptor blockade in the anterior hypothalamus suppresses aggression in hamsters. *Physiology and Behavior*, *44*(2), 235-239.

Floody, O. (2009). Effects on hamster vocalization and aggression of carbachol injections into the MPOA/AH. *Physiology and Behavior*, *96*, 294-299.

Floody, O. (1979). Behavioral and physiological analyses of ultrasound production by female hamsters (Mesocricetus auratus). *American Zoology*, *19*, 443-455.

Floody, O. (1981). The hormonal control of ultrasonic communication in rodents. *American Zoology*, *21*, 129-142.

Floody, O., & Pfaff, D. (1977). Communication among hamsters by high-frequency acoustic signals: I. Physical characteristics of hamster calls. *Journal of Comparative and Physiological Psychology*, *91*(4), 794-806.

Floody, O., Pfaff, D., & Lewis, C. (1977). Communication among hamsters by high-frequency acoustic signals: II. Determinants of calling by females and males. *Journal of Comparative and Physiological Psychology*, *91*(4), 807-819.

Floody, O., & Pfaff, D. (1977). Communication among hamsters by high-frequency acoustic signals: III. Responses evoked by natural and synthetic ultrasounds. *Journal of Comparative and Physiological Psychology*, *91*(4), 820-829.

Gutzler, S., Karom, M., Erwin, W., & Albers, H. (2010). Arginine-vasopressin and the regulation of aggression in female Syrian hamsters. *European Journal of Neuroscience*, *31*, 1655-1663.

Hayden-Hixson, D. & Ferris, C. (1991). Steroid-specific regulation of agonistic responding in the anterior hypothalamus of male hamsters. *Physiology and Behavior, 50*(4), 793-799.

Hull, E., Bitran, D., Pehek, E., Warner, R., Band, L., & Holmes, G. (1986). Dopaminergic control of male sex behavior in rats: Effects of an intra-cerebrally infused agonist. *Brain Research, 370*, 73-81.

Hull, E., Du, J., Lorrain, D., & Matuszewich, L. (1997). Testosterone, preoptic dopamine, and copulation in male rats. *Brain Research Bulletin, 44*(4), 327-333.

Hull, E., Muschamp, J. W., & Sato, S. (2004). Dopamine and serotonin: influences on male sexual behavior. *Physiology and Behavior, 83*, 291-307.

Hull, E., & Dominguez, J. (2006). Getting his act together: Roles of glutamate, nitric oxide, and dopamine in the medial preoptic area. *Brain Research, 1126*, 66-75.

Hull, E., & Dominguez, J. M. (2007). Sexual behavior in male rodents. *Hormones and Behavior, 52*, 45-55.

Johnston, R. (1980). Responses of male hamsters to odors of females in different reproductive states. *Journal of Comparative and Physiological Psychology, 94*(5), 894-904.

Johnston, R. (1975). Scent marking by male golden hamsters (Mesocricetus auratus): I. Effects of odor and social encounters. *Zeitschrift fur Tierpsychologie, 37*, 75-98.

Johnston, R. (1975). Scent marking by male golden hamsters (Mesocricetus auratus): II. The role of the flank gland scent in the causation of marking. *Zeitschrift fur Tierpsychologie, 37*, 138-144.

Johnston, R., & Kwan, M. (1984). Vaginal scent marking: effects on ultrasonic calling and attraction of male golden hamsters. *Behavioral and Neural Biology, 42*, 158-168.

Kask, A., & Harro, J. (2000). Inhibition of amphetamine and apomorphine induced behavioral effects by neuropeptide Y Y1 receptor antagonist BIBO 3304. *Neuropharmacology, 39*, 1292-1302.

Kollack-Walker, S. & Newman, S. (1995). Mating and agonistic behavior produce different patterns of FOS immunolabelling in the male Syrian hamster brain. *Neuroscience, 66*(3), 721-736.

Lehman, M., Powers, J., & Winans, S. (1983). Stria terminalis lesions alter the temporal pattern of copulatory behavior in the male golden hamsters. *Behavioural Brain Research, 8*, 109-128.

Mas, M. (1995). Induction of mating behavior by apomorphine in sexually sated rats. *European Journal of Pharmacology, 280*(3), 331-334.

Mas, M., Fumero, B., Fernandez-Vera, J., & Gonzalez-Mora, J. (1995). Neurochemical correlates of sexual exhaustion and recovery as assessed by in vivo Microdialysis. *Brain Research, 675*, 13-19.

Matto, V., Allikmets, L., & Skrebuhova, T. (1998). Apomorphine induced aggressiveness and [3H] citalopram binding after antidepressant treatment in rats. *Pharmacology, Biochemistry, and Behavior, 59*(3), 747-752.

Meisel, R., Camp, D., & Robinson, T. (1993). A microdialysis study of ventral striatal dopamine during sexual behavior in female Syrian hamsters. *Behavioral Brain Research, 55*, 151-157.

Nelson, R. & Trainor, B. (2007). Neural mechanisms of aggression. *Nature Reviews: Neuroscience*, 8, 536-546.

Olivier, J. et al. (2007). Effects of acute and chronic apomorphine on sex behavior and copulation-induced neural activation in the male rat. *European Journal of Pharmacology, 576*, 61-76.

Paglietti, E., Quarantotti, B., Mereu, G., & Gessa, G. (1977). Apomorphine and L-DOPA lower ejaculation threshold in the male rat. *Physiology and Behavior, 20*, 559-562.

Petrulis, A., Weidner, M., & Johnston, R. (2004). Recognition of competitors by male golden hamsters. *Physiology and Behavior, 81*, 629-638.

Potegal, M., Hebert, M., Decostar, M., & Meyerhoff, J. (1996). Brief, high-frequency stimulation of the corticomedial amygdala induces a delayed and prolonged increase of aggressiveness in male syrian golden hamsters. *Behavioral Neuroscience, 110*(2), 401-412.

Rodriguiz, R., Chu, R., Caron, M., & Wetsel, W. (2004). Abberrant responses on social interaction of dopamine transporter knockout mice. *Behavioral Brain Research, 148*, 185-198.

Rudissar, R., Pruus, K., Skrebuhova, T., Allikmets, L., & Matto, V. (1999). Modulatory role of 5-HT3 receptors in mediation of apomorphine-induced aggressive behavior in male rats. *Behavioral Brain Research, 106*, 91-96.

Sales, G.D. (1972). Ultrasound and aggressive behaviour in rats and other small mammals. *Animal Behaviour, 20*, 88-100.

Sato, Y. et al. (1995). Dopamine release in the medial preoptic area during male copulatory behavior in male rats. *Brain Research, 692*(1-2), 66-70.

Schnur, P., & Martinez, R. (1989). Independent effects of morphine and apomorphine on stereotyped gnawing in the hamster. *Pharmacology, Biochemistry, and Behavior, 32*(3), 589-594.

Skrebuhhova-Malmros, T., Pruus, K., Rudissaar, R., Allikmets, L., & Matto, V. (2000). The serotonin 5-HT2A receptor subtype does not mediate apomorphine induced aggressive behavior in male Wistar rats. *Pharmacology, Biochemistry, and Behavior, 67*, 339-343.

Szczypka, M., Zhou, Q., & Palmiter, R. (1998). Dopamine-stimulated sexual behavior is testosterone dependent in mice. *Behavioral Neuroscience, 115*(5), 1229-1235.

Tagliamonte, A., Fratta, W., Del Fiacco, M., & Gessa, G. (1974). Possible stimulatory role of brain dopamine in the copulatory behavior of male rats. *Pharmacology, Biochemistry, and Behavior, 2*, 257-260.

Thiessen, D., & Upchurch, M. (1981). Haloperidol and clonidine increase, and apomorphine decreases ultrasonic vocalizations by gerbils. *Psychopharmacology, 75*(3), 287-290.

Tidey, J. & Miczek, K. (1996). Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. *Brain Research*, 721, 140-149.

Tieppo, C., Nasello, A.,& Felicio, L. (1997). Modulation of apomorphine-induced stereotyped behavior by cholecystokinin. *Program of Neuro-pharmacology and Biological Psychiatry, 21*, 683-695.

van Erp, A. M., & Miczek, K. A. (2000). Aggressive behavior, increased accumbal dopamine, and decreased coritical serotonin in rats. *The Journal of Neuroscience, 20*, 9320-9325.