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Effects of dopamine receptor antagonism with haloperidol on nurturing behavior in the biparental prairie vole

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Abstract

Dopamine (DA) receptor activity in lactating rats is critical for retrieval and licking of pups, whereas its inactivity facilitates quiescent nursing. The role of DA in the maternal behavior of other species and its role in paternal behavior are unknown. This experiment examined the effects of the DA antagonist haloperidol (HAL) on parental behavior in the biparental prairie vole (*Microtus ochrogaster*). Three days after birth of pups, parental behavior of male and female voles was observed for 30 min beginning 1 h after intraperitoneal injection of 0.1, 0.5, or 2.5 mg/kg of HAL. Controls received the propylene glycol vehicle. Control males were slower to contact pups, licked them more, and quiescently huddled/nursed less than control females. Even at the lowest dose of HAL that had no effect on general activity, pup licking was decreased in both sexes and the latency to contact pups increased in males. The latency to contact pups was most increased in females by the highest HAL dose. Retrieval of pups was not often displayed by any group. HAL dose-dependently decreased the latency and increased the duration of huddling/nursing in both sexes, but did not affect litter weight gains. These data indicate some subtle species differences in the dopaminergic regulation of parenting, as well as sex differences in the sensitivity of some vole parental behaviors to HAL.

Keywords: Antipsychotics; Dopamine; Lactation; Maternal behavior; Neuroleptic; Nursing; Paternal behavior; Sex differences; Voles

1. Introduction

Similar to many goal-directed behaviors in animals (Robbins and Everitt, 1996), there is extensive evidence indicating that dopamine (DA) is a neurotransmitter critical for the motivation (Stern and Keer, 1999), reward value (Fleming et al., 1994), and motoric display of some maternal behaviors in lactating rats (for reviews, see Bridges, 1996; Stern, 1996; Stern and Lonstein, 2001). After reductions in dopaminergic activity via systemic (Giordano et al., 1990; Silva et al., 2001; Stern, 1991; Stern and Keer, 1999; Stern and Taylor, 1991) or central (Hansen et al., 1991a,b, 1993; Keer and Stern, 1999; Numan and Nagle, 1983) manipulations, ongoing active maternal behaviors in lactating rats such as retrieving pups to the nest and licking them are severely impaired. In contrast, quiescent nursing behavior and milk letdown are not impaired after such reductions in dopaminergic activity, but rather, are facilitated if a sufficient number of pups gain access to the nipples and suckle (Stern and Taylor, 1991). Not only are ongoing active maternal behaviors in lactating rats affected by dopaminergic manipulations, but recent indirect evidence suggests that fluctuations in DA neurotransmission within areas of the female rat brain, such as the medial preoptic area and striatum, may also be necessary for the periparturitional onset of these behaviors (Lonstein et al., 2001; Olazabal et al., 2001).

Considering the wealth of information that exists in rats, it is surprising that with the exception of a few studies in mice (Aston-Mills et al., 1999; Spielewoy et al., 2000; Wegener et al., 1988) and one study in sheep (Kendrick et al., 1992), nothing is known about the relationship between DA and maternal care in other species. Furthermore, there has been no examination of DA's function in the control of paternal care (i.e., caregiving behaviors displayed by males) in any species. To address these questions, this experiment examined the effects of the mixed D1/D2 DA receptor antagonist haloperidol (HAL) on parental care in prairie

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voles (*Microtus ochrogaster*). Prairie voles are a unique rodent model to study social behavior because they show many indices of monogamy, including pairbonding after copulation and biparental care after pups are born (Carter et al., 1995). Even though male prairie voles do not lactate, sires and dams show an identical repertoire of parental behaviors that differ only in their patterning and duration (Lonstein and De Vries, 1999a; Solomon, 1993).

Relatively little is known about the neurochemical control of parental care in prairie voles. Chronic treatment with estradiol promotes parental responding in the typically infanticidal virgin female (Lonstein and De Vries, 1999b), and a single infusion of the neuropeptide arginine–vasopressin into the lateral septum increases parental responsiveness in groups of virgin males that are low-responders (Wang et al., 1994). Chronic treatment with the serotonin reuptake inhibitor fluoxetine produces a small, but statistically significant, reduction in parental responsiveness in sexually and parentally experienced males, but not in lactating females (Villalba et al., 1997).

Examining HAL's effects on parental behavior in prairie voles makes it possible not only to provide valuable comparative information about dopaminergic effects on parental behavior in lactating female rodents, but also information about DA's influence on nurturant behaviors in males, as well as possible sex differences in its influence. It is parsimonious to suggest that many of the neurochemical mechanisms regulating parental care, including a role for DA, are conserved across rodent species and between the sexes.

2. Methods

2.1. Subjects

Subjects are male and female F4 generation prairie voles (*M. ochrogaster*) born and raised in our colony, which was established in 1996 at the University of Massachusetts, Amherst, from breeding stock originating from offspring of voles captured in 1994 from Urbana, IL provided by Dr. Betty McGuire (Smith College, Northampton, MA) and Dr. Zuoxin Wang (Florida State University, Tallahassee, FL). To produce breeding pairs used as subjects, adult virgin female and male prairie voles were socially isolated for 3 days after which the females were placed in the cage of an unfamiliar male; behavioral estrus, copulation, and ovulation ensue within 24 h in this species (e.g., Carter et al., 1989).

2.2. Housing

Animals were housed in plastic cages (48×28×16 cm) containing wood chips, wood shavings, and substantial hay covering. Animals were maintained on a 1410 h light/dark cycle (lights on at 0800 h) with an ambient temperature of 21 °C. Water and a food mixture containing cracked corn, whole oats, sunflower seeds, and Purina rabbit chow (ratio of 1:1:2:2) were available ad libitum. Subjects had been weaned at 20 days of age and housed with their same-sex littermates in groups of two to four animals per cage prior to mating. After mating, subjects remained with their mates until behavioral testing.

2.3. Drugs

The mixed DA D1/D2 receptor antagonist HAL (Sigma, USA) was dissolved in propylene glycol at 10 mg/ml and then diluted with additional propylene glycol to the appropriate dose (0.1, 0.5, or 2.5 mg/kg). HAL was readily soluble in propylene glycol but was soluble in saline only at a very acidic pH. Although HAL is also readily soluble in ethanol, there was concern about ethanol's effects on the behaviors being examined in this study. Subjects were weighed to the nearest 0.1 g and received a single intraperitoneal injection of HAL (0.1, 0.5, or 2.5 mg/kg) or propylene glycol ($n=8$ subjects/dose/sex). Solutions were diluted such that the subjects, whose average weight was ~40 g, received 20 μl of solution per 10 g of body weight. Doses were chosen by the effects of similar doses of HAL to influence maternal behavior in lactating rats (Giordano et al., 1990; Silva et al., 2001; Stern, 1991; Stern and Keer, 1999; Stern and Taylor, 1991).

2.4. Behavioral observation

Subjects were undisturbed after pairing and the presence of pups was checked each morning beginning 21 days after pairing. The day of birth was designated as Day 1 postpartum. Litters were adjusted to contain five pups within 24 h after birth, which is a typical size for litters of prairie voles. On the morning of Day 3 postpartum (1000–1230 h), both the sire and dam were removed from their home cage and were individually placed in clean, clear polypropylene cages (48×28×16 cm) containing wood chips for bedding, a small amount of hay, food, and water. One hour later, subjects were weighed, received an injection of one dose of HAL or propylene glycol, and were returned to the test cage. Behavioral testing began 1 h later, the time when similar doses of HAL have their maximal effects on maternal behavior in lactating rats (Silva et al., 2001). Sires and dams were simultaneously tested in separate observation cages because we wanted to test dams and sire simultaneously, but not in a way that a subject's activities would influence their mate's. Furthermore, behavioral observation in the home cage is difficult in our laboratory because the substantial amount of hay and bedding that the voles are provided with in their home cage obscures many details of their behavior.

Sires and dams received litters during testing that contained a combination of their own pups and foster pups of approximately the same age (±1 day) obtained from lactating prairie voles in our colony. There is no evidence that prairie voles can discriminate between their own and foster young, and similar to rats, will readily be parental toward both. Pups

were placed under a warm lamp during the 2-h separation from their parents and were expressed of feces and urine immediately prior to behavioral testing. Behavioral testing began by weighing the pups to the nearest 0.1 g and scattering them in the test cage opposite to where the subject was sitting. Behavior of the subject was then continuously recorded for 30 min with a custom-made computerized data acquisition system designed to provide data on latency, frequency, and duration of numerous behaviors (Lonstein and De Vries, 1999a,b). Pup-oriented active behaviors included sniffing and licking them. Carrying pups from one position to another is very rare in parental prairie voles (e.g., Lonstein and De Vries, 1999a,b) but was measured in the few cases that it occurred; in most cases, once the parent moved across the cage to contact some of the pups, any stray pups in close proximity crawled to the parent. Two mutually exclusive measures of huddling behaviors included actively hovering over the pups while performing other activities, as well as being quiescently positioned over the litter in a nursing posture which typically follows periods of active hovering. Non-pup-oriented activities recorded were self-grooming, exploration away from the pups, nesting or burrowing in the shaved wood bedding, and eating or drinking. Three observers blind to subject condition were involved in data collection; interobserver reliability was consistently >95% for the behavioral variables reported. If a subject did not make contact with a pup within 5 min after the beginning of the test, the test was briefly paused while the pups were placed approximately 1 in. in front of the subject. After moving the pups closer to the nonresponding subjects, all subjects made contact with the pups independently, so it was not necessary to place subjects directly on top of the litter (see Stern and Taylor, 1991). In no case did a subject attack the pups. After the 30-min observation, pups were removed from the test cage and weighed. Subjects and pups were then returned to their home cages. The procedures used in this experiment were approved by the University of Massachusetts Committee on the Care and Use of Animals in Research and are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.5. Statistical analyses

To examine for sex differences in behavior, data from control subjects that received the zero dose of HAL were compared using paired *t* tests. Data were also analyzed with a two-way analysis of variance (ANOVA) using sex (male and female) and dose of HAL (0, 0.1, 0.5, 2.5 mg/kg) as factors. These were followed by Fisher's Least Significant Difference post hoc analyses. Correlations between behaviors were analyzed with Pearson's correlation coefficient. Total time spent with pups was calculated by the sum of the time spent hovering over the litter while active plus the time spent quiescently huddling/nursing. Total activity was the summed duration of all active parental and nonparental behaviors displayed (i.e., sniffing, licking, exploring, self-grooming, carrying pups, feeding, drinking, and nesting). The latency to begin huddling/nursing was determined as the beginning of the first bout of huddling/nursing that lasted longer than 30 s. The latency to contact pups for initially unresponsive subjects that had the litter moved closer to them by the observer was 300 s (the time that pups were moved) plus the time thereafter taken by the subject to contact the pups. Statistical significance was indicated by $P \leq .05$.

3. Results

3.1. Sex differences in parental behavior of controls

There were a few significant sex differences in the behavior of control subjects. Similar to data reported previously (Lonstein and De Vries, 1999a,b), males were slower to make initial contact with pups than females ($t_7 = 2.62$, $P \leq .04$) and took more time from the start of testing to actively hover over them ($t_7 = 2.53$, $P \leq .04$). However, if the latency to actively hover over the pups is calculated from the moment that subjects first contacted them, males' latency was similar to females ($t_7 = 0.06$, $P \geq .96$). Males licked pups

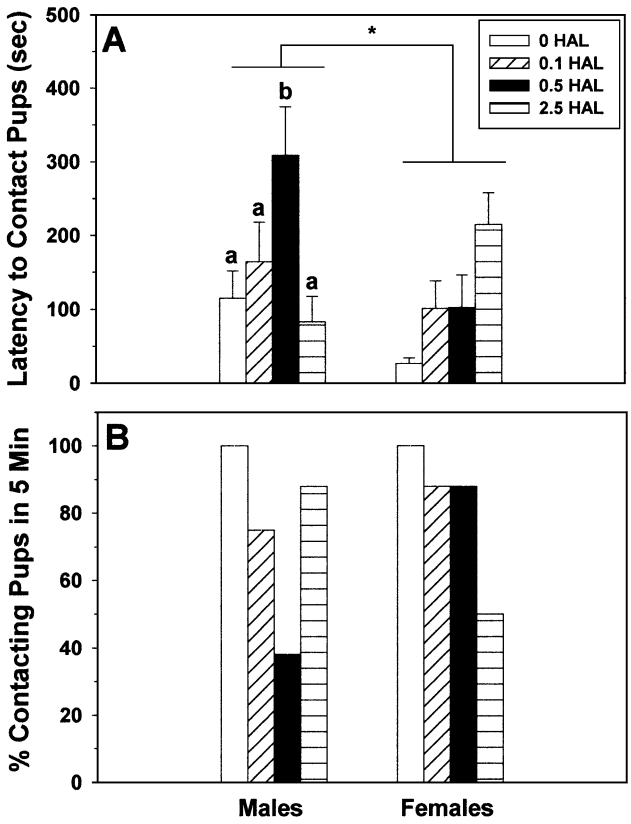

Fig. 1. (A) Latency (mean±S.E.M.) for male and female prairie voles treated with vehicle or HAL to contact pups. (B) Percentage of subjects contacting pups within 5 min after beginning of testing. *Indicates presence of significant Sex×Dose interaction. Main effects of dose (collapsed across sex) indicated by letters above males' bars, with different letters indicating significant differences between doses, $P \leq .05$.

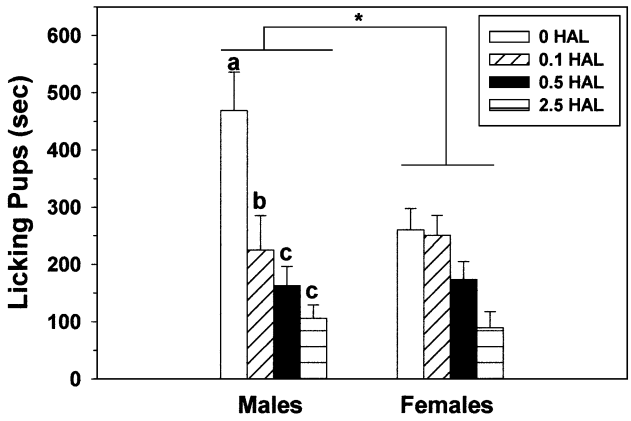

Fig. 2. Duration of time (mean±S.E.M.) spent licking the pups during a 30-min observation by male and female prairie voles treated with vehicle or HAL. *Indicates presence of significant Sex × Dose interaction. Main effects of dose (collapsed across sex) indicated by letters above males' bars, with different letters indicating significant differences between doses, $P \leq .05$.

significantly more than females ($t_7 = 3.03$, $P \leq .02$) but spent less time in physical contact with them ($t_7 = 2.62$, $P \leq .04$). This was not because males actively hovered over the litter less than females ($t_7 = 1.78$, $P \geq .12$), but rather because they spent less time in the quiescent huddling/nursing postures ($t_7 = 2.59$, $P \leq .04$).

Similar sex differences were found when main effects for sex were obtained using a two-way ANOVA [latency to contact pups: $F(1,56) = 3.4$, $P \leq .07$; latency to hover over pups: $F(1,56) = 3.5$, $P \leq .07$; total duration in contact with pups: $F(1,56) = 7.8$, $P \leq .01$; duration of quiescent huddling/nursing: $F(1,56) = 9.4$, $P \leq 0.005$]. There was a Sex×Dose interaction for the duration of time spent licking the pups [$F(3,56) = 3.3$, $P \leq .05$]. Carrying the pups (retrieval or mouthing) and nest building were not displayed by most subjects and were of very short duration in the few cases when observed. These variables were, therefore, not subjected to statistical analyses.

3.2. Effects of HAL on parental behaviors

3.2.1. Active behaviors

HAL affected most active parental and non-parental behaviors during the 30-min observation. Increasing dose of HAL produced increasingly longer latencies for females to contact pups after reunion. However, a different effect of HAL was found on this measure in males, and it increased their latency to contact the pups only at the low doses. In fact, at the highest dose of HAL, the latency to contact the pups was completely normal in males [dose: $F(3,56) = 3.3$, $P \leq 0.05$; sex by dose: $F(3,56) = 5.3$, $P \leq .005$; Fig. 1A]. The percentage of subjects initiating contact pups within 5 min after the beginning of testing was affected in a similar pattern by HAL (males: $\chi^2 = 9.3$, $df = 3$, $P \leq .03$; females: $\chi^2 = 7.4$, $P = .061$; Fig. 1B). Duration of time spent licking the pups was decreased by HAL in both sexes, with all doses

Table 1
Parental and nonparental behaviors in male and female prairie voles after treatment with 0, 0.1, 0.5, or 2.5 mg/kg HAL

Dose	0	0.1	0.5	2.5	Significant effects
Latency (s)					
Hover over pups:					Dose, Sex×Dose
from start of test					
Males	119± 37[a]	168 ± 56[ab]	314 ± 67[ab]	87 ± 34[bc]	
Females	30 ± 7	103 ± 37	105 ± 43	218 ± 43	
from first contact					–
Males	4 ± 1	5 ± 2	6 ± 3	4 ± 1	
Females	4 ± 1	2 ± 1	2 ± 1	3 ± 1	
Huddling/nursing:					Sex×Dose
from start of test					
Males	353± 51	328 ± 45	408 ± 68	169 ± 35	
Females	223± 44	256 ± 45	232 ± 72	270 ± 44	
Duration (s)					
Self-groom					Dose
Males	20 ± 6[a]	62 ± 22[b]	36 ± 9[a]	20 ± 10[a]	
Females	34 ± 12	54 ± 24	29 ± 16	4 ± 1	
Explore					–
Males	22 ± 10	22 ± 13	27 ± 8	13 ± 4	
Females	7 ± 2	16 ± 6	16 ± 10	8 ± 2	
Total activity					Dose
Males	563±66[a]	435 ± 43[a]	265 ± 35[b]	182 ± 25[c]	
Females	457±47	445 ± 53	318 ± 30	132 ± 35	
Time in contact with pups					Sex, Dose, Sex×Dose
Males	1645 ± 45[a]	1602 ± 53[a]	1402 ± 56[b]	1659 ± 61[a]	
Females	1758 ± 9	1656 ± 9	1676 ± 43	1577 ± 43	

Main effects of dose (collapsed across sex) indicated by superscript letters, with columns with all different letters indicating significant differences between doses, $P \leq .05$.

being significantly different from each other, with the exception of the 0.5-mg/kg vs. the 2.5-mg/kg doses [$F(3,56)=14.6$, $P\leq.0001$; Fig. 2]. In contrast to the decrease in licking the pups, self-grooming was increased in duration by the 0.1-mg/kg dose but not at the other doses [$F(3,56)=3.3$, $P\leq.03$; Table 1]. Duration of time spent exploring the test cage was not affected by HAL [$F(3,56)=0.8$, $P\geq 0.5$]. Compared to controls, total activity was less in subjects treated with the 0.5-mg/kg and 2.5-mg/kg doses HAL [$F(3,56)=26.3$, $P\leq 0.0001$; Table 1].

3.2.2. Quiescent huddling/nursing behaviors

Similar to the latency to contact the pups after reunion, HAL increased the latency to hover over the pups differently in males and females, with females being affected at all doses but males only affected at lower doses (Table 1). The latency from the beginning of testing to begin quiescently huddling/nursing the pups showed a Sex×Dose interaction [$F(3,56)=2.8$, $P\leq.05$], such that the latency in females was virtually unaffected by any dose of HAL but was shortest in males treated with the highest dose of HAL. However, when the latency to begin huddling/nursing the pups was measured from the time of the subjects' first contact with pups, no significant Sex×Dose interaction was found, but HAL dose-dependently decreased the latency for both sexes (Fig. 3). Duration of hovering over the pups was reduced by all doses of HAL in both sexes [$F(3,56)=20.9$, $P\leq.0001$; Fig. 4A], whereas the duration of time spent huddling/nursing was increased by the 0.5- and 2.5-mg/kg doses [$F(3,56)=12.2$, $P\leq.0001$; Fig. 4B]. Collapsed across sex, total time spent in physical contact with pups was significantly decreased by the 0.5-mg/kg dose of HAL [$F(3,56)=4.3$, $P\leq.05$; Table 1], and there was also a Sex×Dose interaction on this measure [$F(3,56)=5.26$, $P\leq.005$].

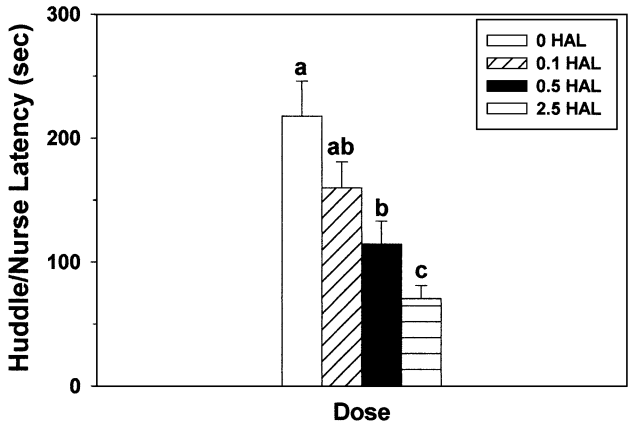

Fig. 3. Latency (mean±S.E.M.) for prairie voles (collapsed across sex) treated with vehicle or HAL to begin huddling/nursing the litter after making initial contact with them. Differences between doses indicated by different letters above bars, $P\leq.05$.

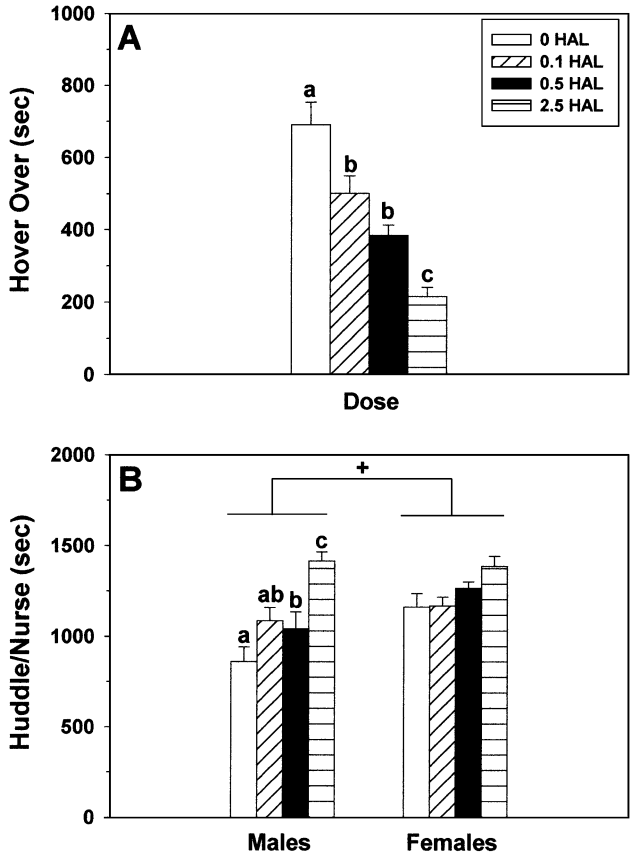

Fig. 4. Duration of time (mean±S.E.M.) spent by prairie voles treated with vehicle or HAL (A) actively hovering over the litter and (B) quiescently huddling/nursing. $^{+}$Indicates significant main effect of sex. Different letters above bars indicate significant main effects of dose, collapsed across sex in both panels, $P\leq.05$.

3.3. Correlations between active and inactive behaviors

Consistent with the main effects of HAL, when collapsed across sex and treatment, subjects that licked pups less had longer latencies to contact them ($r=-.35$, $P\leq.005$), spent less time actively hovering over the litter ($r=+.87$, $P\leq.0001$), but more time quiescently nursing/huddling ($r=-.41$, $P\leq.0001$). Subjects that spent more time quiescently nursing/huddling spent less time actively hovering over the litter ($r=-.80$, $P\leq.0001$). Duration of licking the pups was positively correlated with total activity ($r=+.84$, $P\leq.0001$), while the latency to contact the pups was negatively correlated with total activity ($r=.33$, $P\leq.01$).

3.4. Subject and litter weights

Female subjects weighed more than males [43±1 vs. 38±1 g; $F(1,56)=6.0$, $P\leq.02$] and the weights of the litters provided to males and females were similar prior to testing [167±3 vs. 163±3 mg; $F(1,56)=1.1$, $P\geq.3$]. By the end of the 30-min observation, however, litters interacting with

females gained a small amount of weight (2.9 ± 1.7 mg) during the 30-min observation, whereas litters interacting with males lost a small amount of weight (1.3 ± 0.7 mg) [$F(1,56) = 4.5$, $P \leq .05$]. There were no effects of parental HAL treatment on weight gains or losses in the pups [$F(3,56) = 0.1$, $P \geq .9$].

4. Discussion

4.1. Similarities and differences between species in the effects of HAL on parental behaviors

Doses of HAL similar to those used in the present study have previously been demonstrated in lactating rats to dose-dependently reduce active maternal behaviors such as retrieval of pups, licking the pups, and nest building (Giordano et al., 1990; Silva et al., 2001; Stern and Taylor, 1991). These effects are due to central DA receptor antagonism because peripherally acting DA antagonists do not produce these effects (Stern and Taylor, 1991), and the effects can be reversed with the DA agonist apomorphine (Giordano et al., 1990). The effects of HAL on parental behavior in prairie voles are, in part, similar to those found in rats. Indeed, the duration of time that prairie voles spent licking the pups was dose-dependently decreased by HAL. Prairie voles of neither sex readily retrieve pups (see Lonstein and De Vries, 1999a,b), possibly because the precocial prairie vole pup is born with teeth (Salo et al., 1994) and their very strong and persistent grip on the nipples virtually eliminates the likelihood that it would be accidentally separated from the dam under most circumstances. Considering this, it was very unlikely to observe an effect of HAL on this behavior in voles. As will be discussed in detail below, HAL also delayed the speed at which voles of both sexes initiated contact with pups.

Opposite to its effects on active maternal behaviors in lactating rats, HAL increases their propensity to quiescently nurse the pups, not only reducing the time taken to adopt a nursing posture over the pups, but also increasing the total duration of nursing (Stern, 1991; Stern and Taylor, 1991). Almost identical results were found in HAL-treated voles of both sexes, with the latency to begin quiescently huddling over/nursing the pups after contacting them reduced by almost 75% and its duration increased over 25% by the 2.5-mg/kg dose. These results exemplify the fact that maternal behavior is not a unitary process that will be globally facilitated or impaired by a given experimental manipulation. Rather, it consists of individual behaviors that in some cases can be influenced in the opposite direction of others. HAL likely facilitates quiescent huddling/nursing both by reducing the display of some active behaviors, which obviously need to be suppressed before quiescence can be observed, but also by increasing the effectiveness of ventral somatosensory inputs provided by pups that are necessary for the sire or dam's quiescence and huddling/nursing behavior (Stern, 1991; Stern and Johnson, 1990; Stern et al., 1992).

The motor inhibition and postural alterations seen in male rats during HAL-induced catalepsy are quite similar to what occurs during quiescent nursing behavior in lactating females and the DA mechanisms underlying these processes may be similar (De Ryk et al., 1980). In fact, male rats can be induced to show female-like nursing postures when treated with HAL and provided ventral stimulation by pups (Stern, 1991). Dopaminergic pathways in the brain that regulate these processes in lactating rats include both the nigrostriatal (Numan and Nagle, 1983) and mesolimbic systems (Keer and Stern, 1999; Hansen et al., 1993). Similar systems may be necessary for these processes in parental prairie voles of both sexes, and site-specific infusions of DA antagonists into the striatum and other sites would clarify this question.

It is possible that the impairment in active parental behaviors and enhancement of inactive nursing/huddling behaviors in HAL-treated rats and voles is due to a general disruption in motor activity resulting from the cataleptic effects of HAL (Silva et al., 2001). This may very well be true at relatively high doses of HAL, but probably not at lower doses. Doses of HAL less than approximately 0.5 mg/kg produce relatively little or no significant degree of catalepsy in rats (Cambell et al., 1988; Sanberg et al., 2001; Stern and Keer, 1999; Stern and Taylor, 1991; Wolgin, 1985), and accordingly, little change in dopaminergic activity within the striatum (e.g., Honma and Fukushima, 1976; Patterson and Schenk, 1991). The effects of these low doses of HAL are more specific to the dam's motivation to be maternal and lactating rats treated with low doses of HAL are impaired in their ability to retrieve and lick the pups, but are not cataleptic and will readily make contact with pups and sniff them (Giordano et al., 1990; Stern and Keer, 1999), as well as leave the nest for food (Giordano et al., 1990). Similarly, it was found herein that even the lowest dose of HAL (0.1 mg/kg) significantly reduced licking of the pups and actively hovering over the litter, but not total activity. It is more likely that low doses of HAL disrupt dopaminergic activity necessary for the motivation to perform active parental behaviors, influences the reinforcing value of the pups, and/or affects perception of the sensory cues they emit (De Vry et al., 1989; Lopez and Ettenberg, 2001, 2002; Mobini et al., 2000; Nakajima and Patterson, 1997; Reilly, 1999; Stern and Keer, 1999).

The present results also suggest that some of the effects of HAL even at the mid (0.5 mg/kg) to high (2.5 mg/kg) doses were not necessarily due to its cataleptic effects, because male prairie voles readily moved to contact pups after administration of high doses of HAL that severely impaired licking and reduced general activity, and the intermediate dose of HAL actually *increased* self-grooming in prairie voles of both sexes. It is possible that HAL does affect parental and general locomotor behaviors via similar

mechanisms, but that the thresholds for disruption are different.

HAL affects both the D1 and D2 receptors, but the use of more specific antagonists to each of these receptors indicate that both are involved in maternal behavior in rats (Silva et al., 2001), and this is probably true in prairie voles as well. Systemic injection of HAL may not only affect parental behavior in prairie voles directly via inhibition of DA receptors, but also indirectly through other neurochemical systems. Acute treatment with HAL increases GABA release in some areas of the brain including the globus pallidus (Drew et al., 1990), decreases GABA release in the prefrontal cortex (Bourdelais and Deutch, 1994), and has no effect on it in other areas such as the striatum (Bourdelais and Deutch, 1994; Osbourne et al., 1994). Acute treatment with HAL also causes a rapid and prolonged release of pituitary prolactin in both male and female rats (e.g., Bridges et al., 1997; Hentschel et al., 2000; Horowski and Graf, 1976). Prolactin facilitates the onset of maternal responsiveness in rats and other mammals (Bridges, 1996), and is associated with ongoing paternal behavior in some male primates and rodents (Ziegler, 2000). Considering prolactin's facilitatory role in parental behaviors, it would be unlikely that an increase in circulating prolactin after HAL treatment would be responsible for the *impairment* in active parental behaviors observed in HAL-treated prairie voles of either sex.

One striking difference between the effects of HAL in lactating rats and lactating prairie voles lies in its effects on milk transfer the dam to the pups. In rats, low to moderate doses (0.2–1.0 mg/kg) of HAL increase milk letdown and litter weight gains by almost threefold within a 30-min mother–litter interaction (Stern and Taylor, 1991). This could have been due to the fact that dams in this study nursed their litters for approximately 25% more time than controls, but also could be due to direct effects of disinhibiting the D2 receptor on suckling-induced oxytocin release (Crowley et al., 1991). In lactating prairie voles, no such facilitation in milk letdown was observed at any dose of HAL, even though dams treated with the highest dose showed greater than a 25% increase in the duration of nursing. The influence of DA on oxytocin release in prairie voles under any circumstance is unknown, but the present results suggest that it differs from rats, at least in response to suckling.

4.2. Sex differences in the effects of HAL on parental behavior

The similar behavior displayed by male and female prairie voles during interactions with their pups has been described in detail previously (Lonstein and De Vries, 1999a,b; Solomon, 1993). The small number of sex differences found herein—that males lick pups more than females and that females huddle/nurse more than males—have also been reported previously (Lonstein and De Vries, 1999a,b). Sex differences in licking and huddling/nursing are likely due to the fact that males, although they spend substantial amounts of time quiescently positioned over pups (Lonstein and De Vries, 1999a,b), do not have nipples and are not subject to the same suckling-induced behavioral inhibition as females (Stern, 1996).

The present results are the first to examine a role for any monoamine in the paternal behavior of any species, and the results generally suggest that there is homology between the sexes in the influence of DA receptor activity. However, there was one striking difference between males and females in the effects of HAL on their parental behavior. In males, the 0.1- and 0.5-mg/kg doses, but not the 2.5-mg/kg dose, prolonged their latency to contact the litter. In fact, males given the 2.5-mg/kg dose were slightly *faster* to contact pups than controls not receiving any HAL at all. In females, increasing the dose of HAL produced an almost linear increase in their latency to contact the litter. The effect in males is particularly interesting considering that the 2.5-mg/kg dose disrupted males' licking of the pups, suggesting a dissociation not only between dopaminergic regulation of active and inactive (quiescent) parental behaviors in this species, but also a dissociation between the dopaminergic influence on two active behaviors in males, pup-contact seeking and licking. This is also true in lactating rats and HAL at a 0.2-mg/kg dose severely disrupts the duration of time spent licking the pups, but has no effect on contacting or retrieving them (Stern and Taylor, 1991).

The fact that increasing the dose of HAL produced a smaller disruption in the latency to contact pups in males is reminiscent of the effects of HAL on catalepsy in male rats, with increasingly higher doses producing less catalepsy than lower doses (Toru and Takashima, 1985). Higher doses of HAL can also be less effective than lower doses in other circumstances, including the ability to release prolactin from the pituitary (MacLeod and Lamberts, 1978). Why the females' latency to contact pups was not affected by HAL in a manner similar to males is unknown, but there are numerous sex differences in the effects of dopaminergic manipulations on the physiology (e.g., De la Cruz et al., 1987) and behavior (Parra et al., 1999; Field et al., 2000) of rodents. The basis of these sex differences can be found at many levels of the DA systems, from synthesis, release, and reuptake, to sex differences in receptor expression and affinity for their ligand (Becker, 1999). Changes in DA function occur in adult rats in response to changes in circulating gonadal hormones, such as those occurring during the estrus cycle (Becker, 1999). In fact, prolonged exposure to estradiol increases HAL-induced catalepsy in rats, whereas progesterone decreases it (Nicoletti et al., 1983). Sex differences in DA function can also result from naturally occurring sex differences in perinatal exposure to gonadal hormones (see Becker, 1999; Hafner et al., 1991). Therefore, not only is it likely that sex differences in parental behavior and the effects of HAL on these activities are due to the fact that adult male and females voles differ

in their circulating gonadal and pituitary hormones, which is particularly important because only females can be pregnant and lactating, but also because the sexes are exposed to different levels of gonadal hormones during development.

4.3. Conclusions

Changes in DA release and DA receptor activity during interactions with pups regulate the transition from the performance of active maternal behaviors to the display of inactive nursing behavior in rats (Stern, 1996; Stern and Lonstein, 2001). It can be seen that this simple dichotomy between the dopaminergic regulation of active and inactive maternal behaviors is conserved in female prairie voles and may extend to lactating females of other species as well. However, dopaminergic influences on milk letdown (possibly by influencing oxytocin release) appear to differ between these species. Furthermore, although the parental behavior displayed by male prairie voles is virtually identical to that displayed by their mates, some parental behaviors are differentially sensitive to dopaminergic manipulation in males and females.

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