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Cholinergic control of male mating behavior in hamsters: Effects of central oxotremorine treatment

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ABSTRACT

The responses of rats to intracranial injections of cholinergic drugs implicate acetylcholine in the control of male mating behavior and suggest specific brain areas as mediators of these effects. In particular, past work has linked the medial preoptic area (MPOA) to the control of intromission frequency but implicated areas near the lateral ventricles in effects on the initiation and spacing of intromissions. Studies of responses to systemic cholinergic treatments suggest that acetylcholine is even more important for the control of mating behavior in male hamsters but provide no information on the relevant brain areas. To fill this gap, we observed the effects of central injections of the cholinergic agonist oxotremorine that approached the MPOA along contrasting paths. Both studies suggest that increased cholinergic activity in or near the MPOA can facilitate behavior by reducing the postejaculatory interval and possibly affecting other parts of the mechanisms controlling the initiation of copulation and the efficiency of performance early in an encounter. In addition, oxotremorine caused other changes in behavior that could not be tied to the MPOA and may reflect actions at more dorsal sites, possibly including the bed nucleus of the stria terminalis and medial septum. These effects were notably heterogeneous, including facilitatory and disruptive effects on male behavior along with a facilitation of lordosis responses to manual stimulation. These results emphasize the number and diversity of elements of sexual behavior in hamsters that are under the partial control of forebrain cholinergic mechanisms.

1. Introduction

Studies of responses to systemically administered cholinergic drugs have implicated central muscarinic mechanisms in the control of mating behavior in male rats (Ahlenius and Larsson, 1985; Bignami, 1966; Leavitt, 1969; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997; Soulairac, 1963). At the same time, these studies have not completely resolved the bases of these effects, in part because of responses that seem poorly correlated with drug categories. Muscarinic agonists such as oxotremorine have tended to facilitate copulation by causing decreases in intromission frequency and ejaculation latency (Ahlenius and Larsson, 1985; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997). Together, these effects suggest that acetylcholine (ACh) facilitates mating by reducing the amount of somatosensory stimulation required to trigger ejaculation, or the "ejaculatory threshold" (Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997). In contrast, the most consistent response to treatment with the muscarinic antagonist scopolamine involves a more global disruption of behavior that prevents copulation altogether in many individuals (Ahlenius and Larsson, 1985; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997).

Some of this asymmetry could reflect interactions among drug effects at multiple brain sites. To avoid such complexities, and also clarify the sites that do mediate specific drug effects, some studies have described the responses of male rats to centrally-administered oxotremorine or scopolamine (Hull et al., 1988a, 1988b). Uniformly, these studies have focused on the medial preoptic area (MPOA), reflecting the wealth of evidence implicating this area in the control of male-typical sexual behavior (review in (Meisel and Sachs, 1994)). However, this target has been approached in different ways, with distinctive behavioral effects. When cannulae have been angled so as to avoid any penetration of the lateral ventricle, the behavioral response to oxotremorine treatment has been very specific, amounting to a decrease in intromission frequency (Hull et al., 1988a). But cannulae that penetrate a ventricle can have additional effects, including an increase in intromission latency, possibly accompanied by increases in mount latency or interintromission interval (Hull et al., 1988a). These results have been taken to suggest that cholinergic neurons in or near the MPOA help to control the ejaculatory threshold whereas others, in tissue adjacent to some part of the lateral ventricle, are part of the mechanism regulating sexual arousal (Hull et al., 1988a).

Collectively, these studies reveal much about the role of ACh in the control of mating behavior in male rats. However, they have not

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addressed the extent to which these effects generalize across species. Further, there are several reasons to suspect that their generality may be limited. Some of these emerge from a companion study of hamsters exposed to systemic oxotremorine or scopolamine treatments (Floody, in press). This revealed a set of behavioral responses to cholinergic manipulations that seemed considerably broader than that in rats. For example, whereas oxotremorine treatments of rats have tended to affect just intromission frequency and ejaculation latency (Ahlenius and Larsson, 1985; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997), similar treatments in hamsters affected measures including the latencies to mount, intromit and ejaculate along with the interintromission and postejaculatory intervals and even the incidence of copulatory behavior. This study also suggested species differences in the directions of the changes in performance caused by cholinergic treatments. In rats, the decreases in intromission frequency and ejaculation latency that follow oxotremorine treatment suggest the facilitation of copulation by ACh. In contrast, male hamsters responded to this drug with a set of disruptive changes including a decrease in the incidence of copulation and increases in each of the other measures mentioned just above.

Together, these responses to systemic treatments suggest the dependence of male behavior in hamsters on a cholinergic mechanism that may include links at multiple brain sites and that may differ significantly from the corresponding mechanism in rats. At the same time, these results provide no information on how or where this mechanism controls any specific behavioral element. The following studies address this issue, in the process permitting the comparison of responses to centrally-applied cholinergic agonists in rats and hamsters.

2. Experiment 1

In the first of these studies, we compared the responses of male hamsters to several doses of oxotremorine aimed at the MPOA. This drug seems an appropriate focus because of its use in the previous studies that have most strongly suggested links between specific brain areas and specific elements of male mating behavior (Ahlenius and Larsson, 1985; Hull et al., 1988a, 1988b; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997). Similarly, the MPOA seems to be an appropriate brain target because of the previous results implicating it in the cholinergic control of male behavior (Hull et al., 1988a) and the wealth of other data suggesting for it a central role in the control of male behavior (Floody, 1989; Meisel and Sachs, 1994). In this study, we tried to exploit the strategy that has been successful in past studies of rats (Hull et al., 1988a), of delivering oxotremorine to the MPOA using cannulae that are angled to reduce the chances of intruding into any part of the lateral ventricle.

Based on previous studies of rats, we expected successful infusions into the MPOA to have very specific effects (Hull et al., 1988a). These seemed likely to be limited to the measures that proved most sensitive to systemic oxotremorine treatments in hamsters, including the postejaculatory interval and a cluster of measures related to the initiation of copulation (Floody, in press). In each case, the systemic effects predict disruptive changes (e.g., increases in postejaculatory interval). In contrast, we expected a wider set of changes to accompany any treatments that extended beyond the MPOA (Hull et al., 1988a). The systemic results suggest that these might include increases in the interintromission interval and the latencies to mount, intromit and ejaculate (Floody, in press).

Throughout this work, we have focused on the changes in male-typical behavior caused by central oxotremorine treatment. However, our behavioral tests also included manipulations and measures designed to test drug effects on female-typical responding. In part, this reflects pilot work in which we were surprised to occasionally observe oxotremorine-treated males responding to stimulus females with unmistakable lordosis responses. But concern for possible effects of cholinergic treatments on female-typical responses by male hamsters also is appropriate in view of results documenting the relative ease with which lordosis can be elicited in untreated male hamsters (Kow et al., 1976) and the ability of cholinergic treatments to affect lordosis in female rats and hamsters (Dohanich et al., 1984; Dohanich and Clemens, 1981; Dohanich et al., 1990, 1991; Menard and Dohanich, 1989). Studies of the latter have documented the facilitation of lordosis by central applications of agonists and the disruption of this response by similar treatments with antagonists (Dohanich et al., 1984; Dohanich and Clemens, 1981; Dohanich et al., 1990, 1991; Menard and Dohanich, 1989). Nevertheless, the brain areas that mediate these effects remain uncertain. Lordosis can be facilitated by infusions of an agonist into the lateral ventricles, the MPOA, or, to a lesser extent, the ventromedial hypothalamus. As for some elements of male-typical behavior, MPOA implants that are angled to avoid the lateral ventricles fail to support drug effects on lordosis, at least in rats (Dohanich et al., 1984). The net effect is to suggest the mediation of these effects by tissue in proximity to some part of the lateral ventricle. Whether this is as true for lordosis by male hamsters as it is in female rats is another issue that may be illuminated by these studies.

Despite the existence of some gaps in the literature describing cholinergic effects on lordosis in female hamsters, the net effect of this work is to suggest that rats and hamsters are more similar than different in the cholinergic control of this response. Accordingly, we expected that oxotremorine would facilitate lordosis in our male hamsters, and that this would be equally true of infusions that were and were not restricted to the MPOA.

2.1. Methods

2.1.1. Animals

Complete data were collected from 11 male golden hamsters (LVG: Lak outbred strain) that averaged 172.5 g in weight (SEM = 6.8) at the time of surgery. Each was individually housed in a 43 × 21 × 20 cm plastic cage in a colony maintained at 20–25 °C and on a reversed 14:10 light:dark cycle. The experimental stimuli included 11 adult female hamsters, each of which had been bilaterally ovariectomized approximately 5 months before use. Each female was housed in a 34 × 18 × 18 or 31 × 21 × 21 cm stainless steel cage in the same colony. All animals had free access to food and water except during behavioral tests. Housing conditions and all procedures were approved by Bucknell University's Institutional Animal Care and Use Committee.

2.1.2. Surgical and drug treatments

Each male subject was anesthetized with an intraperitoneal injection of 75 mg/kg of sodium pentobarbital. This was supplemented with additional sodium pentobarbital, as necessary, and by a subcutaneous (sc) injection of 0.4 mg of the analgesic butorphanol tartrate (both from Henry Schein, Inc). During the period of anesthesia, each male was implanted with a 26 G stainless-steel guide cannula (Plastics One, Roanoke, VA) aimed at a point 1 mm dorsolateral to the lateral edge of the MPOA. This was angled 20° toward the midline and cemented to the leveled skull at a point representing a compromise between coordinates based on skull features (1.8 mm anterior to bregma, 3.3 mm to the left of the midline, 6.0 mm below the skull at bregma) and interaural line (7.0 mm anterior, 2.1 mm dorsal).

After at least 7 days of recovery, each male began a series of 4 behavioral tests at 4–6 day intervals. Each was preceded by an intracranial injection of 0.5 μl of a 0.9% NaCl solution containing 0 (placebo), 0.75, 1.5 or 3.0 μg of oxotremorine (oxotremorine sesquifumarate, Sigma-Aldrich, Inc.). The order of exposure to the 4 doses was counterbalanced across subjects, and all injections and tests were conducted without knowledge of the solution in use.

Each treatment was infused over a period of 30 s using a 33 G injection needle that extended 1 mm beyond the guide cannula and was

attached to a 5 μl Hamilton microsyringe driven by a Razel A-99 syringe pump. This needle was left in place for 30 s after the infusion to reduce the amount of solution backing up along the needle track. Taking into consideration the 1 mm extension of the needle and the placement of the guide cannula, injections were aimed at points in or just lateral to the MPOA, so as to maximize the target's exposure to the treatment while minimizing any damage due to repeated injections. Upon the completion of an infusion, the male was placed in a 40×20×25 cm glass test chamber. Behavioral testing began 5 min later.

Each stimulus female was bilaterally ovariectomized after similar anesthetic and analgesic treatments. To ensure sexual responsiveness during behavioral tests, each was primed with two sc injections of gonadal hormone in 0.05 ml of peanut oil, the first at approximately 48 h before use and containing 7.5 μg of estradiol benzoate and the second at approximately 5 h before use and containing 500 μg of progesterone (both from Steraloids, Inc).

2.1.3. Behavioral tests

Each test included periods of exposure to a sexually receptive female and to stimulation by the experimenter. The first served primarily to test the effect of treatment on levels of male-typical mating behavior: Though we were prepared to score female-typical responses during this period, none were observed then. Consequently, the subsequent manual-stimulation test segment served as the primary source of insights into any drug effects on female-typical behavior.

The initial test phase began with the introduction of a stimulus female into the test chamber. The timing of the ensuing encounter began with the first social contact. Mating tests then continued through 2 copulatory series (2 ejaculations plus the first intromission thereafter). The data collected during each test included the timing of the first mount and intromission in each copulatory series, the timing of each ejaculation, and the total numbers of mounts and intromissions in each series. From these scores we derived each of the 14 dependent variables that typically would be used to describe male behavior in encounters of this type (Bunnell et al., 1977). These include 2 measures that are considered to initiate the interaction as a whole and so are not tied to a copulatory series, i.e., mount latency (ML, the delay between the initiation of contact and the first mount), and intromission latency (IL, the corresponding delay for the first intromission). The remaining 12 measures include 6 dependent variables, each of which is determined for each of the 2 copulatory series. These include ejaculation latency (the interval separating the first intromission of a series from the ejaculation that concludes that series, identified here as EL-1 for the first series and EL-2 for the second), mount frequency (the number of mounts in a series; MF-1, MF-2), intromission frequency (the number of intromissions in a series; IF-1, IF-2), intromission ratio (the proportion of all mounts and intromissions in a series that are intromissions, or IF/(MF + IF) for the relevant series; IR-1, IR-2), interintromission interval (the average interval separating successive intromissions in a series, or EL/IF for the series; III-1, III-2), and postejaculatory interval (the interval separating the ejaculation of a focal series from the first intromission of the next series; PEI-1, PEI-2).

With 3 exceptions, these behavioral elements and measures were defined in standard ways (Bunnell et al., 1977). First, we relied entirely on overt patterns of pelvic thrusting and hindlimb movement to distinguish mounts, intromissions and ejaculations. Second, both ML and IL were measured from the initiation of contact rather than the female's introduction. Third, we scored mounts without regard for a male's orientation rather than requiring initiation from the rear. Though these do represent methodological differences between this and some previous studies, there is good reason to think that these definitions are valid, these procedures reliable, and that even this entire set of changes is likely to have had little impact on the results (Floody, 2011a).

Upon the completion of the initial test phase, each male was exposed in rapid succession to 2 stimuli with the potential to elicit female-typical responses (Kow et al., 1976). The first was intended to be of low-moderate intensity and consisted of 30 s of light brushing with a 1 cm wide camel's hair brush that alternated between the flanks at a rate of 1–2 strokes/s. The second was more intense and consisted of 30 s of bilateral manual stimulation of the flanks. During each period, we recorded the incidence and total duration of lordosis, a posture that is closely associated with receptivity in females and defined by the cessation of locomotion, the absence of head movements exceeding 1 cm in any direction, and an elevation of the tail to at least the horizontal.

2.1.4. Histological analysis

Upon the completion of this testing, each subject was deeply anesthetized and perfused transcardially with 8.5% sucrose in deionized water followed by 7.5% sucrose in 10% formalin. Frozen frontal sections through the region surrounding the MPOA were cut at 50 μm and stained with formol thionin. The resulting sections were examined microscopically to locate the injection sites (points of deepest penetration by the injection needle) on reference sketches through the hypothalamus (Morin and Wood, 2001).

2.1.5. Analysis of individual measures of male behavior

Drug effects on male-typical behavior were assessed by analysis of variance (ANOVA), using transformations as necessary to reduce heterogeneity of variance. For the 2 measures that relate to entire encounters rather than copulatory series (ML, IL), ANOVA treated oxotremorine Dose as a within-subjects factor. For all other measures, the ANOVAs also included copulatory Series as a within-subjects factor. Throughout, drug effects were assessed primarily on the basis of previously planned analyses. Specifically, the presence of a drug effect was recognized on the basis of a reliable ($p \leq 0.05$) Dose main effect or Dose×Series interaction (suggesting an effect specific to one series). The shape of the dose response curve was assessed on the basis of the presence and type of within-subject contrast: If the analysis of a dose effect revealed a reliable linear contrast but no reliable higher order (quadratic or cubic) effect, we inferred an orderly dose-related drug effect on the relevant measure. In isolated cases, these analyses required post hoc tests for clarification, i.e., Dunnett's test for comparisons of each drug dose with the control (placebo) treatment and Tukey's test to compare drug responses to each other (Winer, 1971).

2.1.6. Analysis of factor scores

A recent study identifies several significant differences between hamsters and rats in the organizations of their mating behaviors, as revealed by factor analysis (Floody, 2011a). The application of this method to rats (Dewsbury, 1979; Pfaus et al., 1990; Sachs, 1978) suggests the operation of 3–4 conceptual variables, including ones related to how quickly copulation is initiated, its rate once initiated, and its efficiency. But similar analyses of hamsters (Floody, 2011a) arrive at quite a different structure, highlighted by the absence of a copulatory rate factor and the existence of initiation and efficiency factors that differ from those in rats. Further, some of the responses to systemic cholinergic treatments seem to reflect these behavioral differences. For example, though the individual measures affected by oxotremorine in rats suggest an effect restricted to the Intromission factor, those in hamsters seem instead to involve changes in Initiation and Efficiency factors (Floody, in press).

In view of these differences, we assessed possible drug effects on both individual measures and the coherent sets of measures revealed by factor analysis. As suggested briefly above, the factor analysis of male behavior in hamsters described 5 conceptual variables, each a potential target of drug action (Floody, 2011a). One was defined largely by ML, IL and PEI-1, and seems to relate to the Initiation of copulation. Two others seem to relate to copulatory efficiency. These clustered by copulatory series, so that the efficiency of behavior in the first series

(Efficiency-1) was defined largely by the combination of MF-1, III-1, IR-1 and EL-1, while that in series 2 (Efficiency-2) was defined mainly by MF-2, IR-2 and III-2. The last 2 factors suggest a link between intromissions and ejaculations, again in a series-specific way. The intromission-focused factor for the first series (Intromissions-1) emphasized IF-1 and EL-1, whereas that for the second (Intromissions-2) was determined mainly by IF-2, EL-2 and PEI-2.

To assess drug effects on these conceptual variables, we applied ANOVA to the corresponding factor scores. Each such score is calculated by (a) converting all of the dependent variables for an animal and condition to standard scores, (b) multiplying each standard score by the coefficient describing the relationship between the relevant dependent variable and conceptual variable, and then (c) summing the resulting weighted standard scores (further discussion and example in (Floody, in press)). The results of these analyses were interpreted as were those of the individual measures of male behavior.

2.1.7. Analysis of female behavior

Possible drug effects on female-typical responses were assessed primarily by using the Cochran Q test (Siegel, 1956) to compare the incidences of lordosis across treatments. For treatments associated with positive lordosis responses, ANOVA then was used to assess possible effects on the duration, or strength, of these responses.

2.2. Results

2.2.1. Histology

Histological analysis established the injection sites depicted in Fig. 1 and in the upper half of Fig. 2. As intended, these were concentrated in or just lateral to the MPOA, at anterior–posterior levels approximating the crossing of the anterior commissure. Unfortunately, 9 of these implants impinged upon a lateral ventricle, though at distances from the injection sites (mean = 1.8 mm, SEM = 0.2) approaching the maximum over which infused solutions are estimated to diffuse along such a cannula track (Hull et al., 1988b). In cases in which the ventricle was penetrated, this must increase the chance that some of the infused oxotremorine entered the ventricular circulation. However, penetration

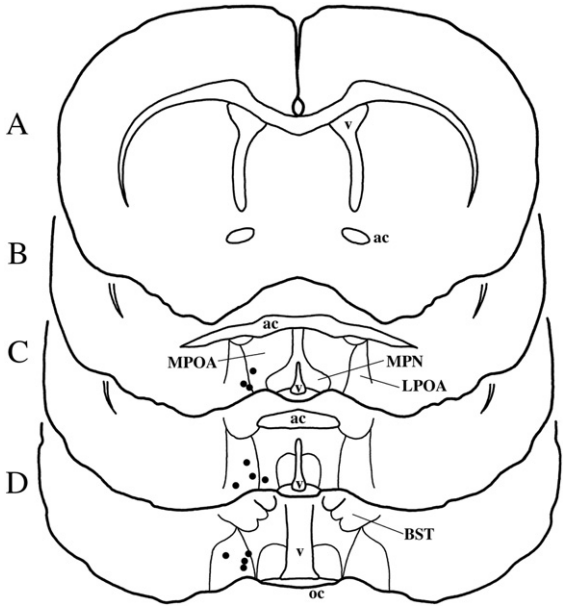

Fig. 1. The sites of the microinjections described in Experiment 1 are indicated by filled circles on sketches of published reference sections (Morin and Wood, 2001). The 4 panels depict sections spaced 300–400 μm apart from anterior (A) to posterior (D). Abbreviations: ac, anterior commissure; BST, bed nucleus of the stria terminalis; LPOA, lateral preoptic area; MPN, medial preoptic nucleus; MPOA, medial preoptic area; oc, optic chiasm; v, ventricle.

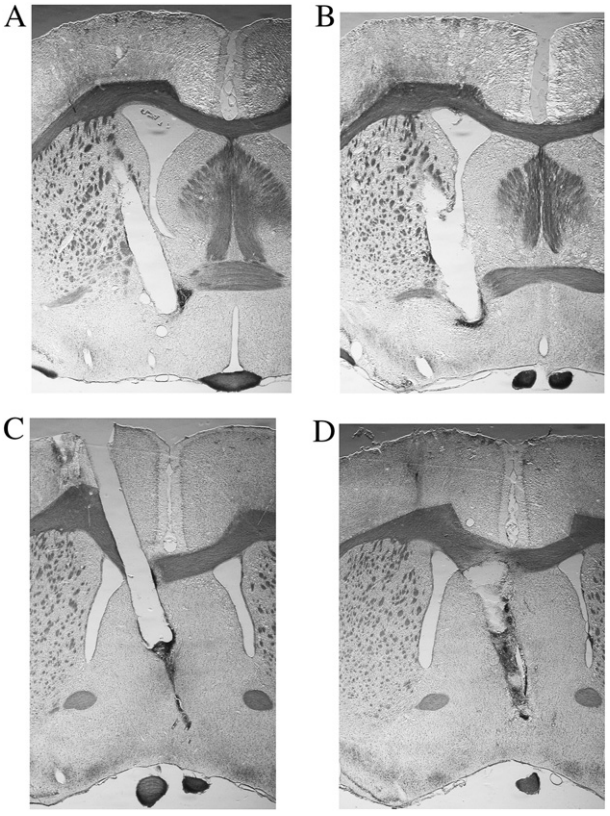

Fig. 2. These photomicrographs show illustrative implants from Experiment 1 (panels A and B) and Experiment 2 (panels C and D). Each of the panels on the left depicts a cannula that did not penetrate a lateral ventricle at any point along its path. Each of those on the right shows a cannula that did penetrate a ventricle, though the penetration by the track depicted in panel D occurred more anteriorly and dorsally than the level shown here.

at such a distance does not guarantee such circulation (Hull et al., 1988b), which would have to be monitored directly (e.g., using a visible dye) to be established with confidence.

2.2.2. Effects on male behavior

Two related effects emerged from the analyses of drug effects on individual behavioral elements and broader conceptual variables. First, the application of ANOVA to the individual elements revealed a highly reliable Dose main effect on PEI ($F(3,30) = 7.14$, $p = 0.001$, Fig. 3A). The attribution of this to an orderly dose-related decrease in PEI is supported by a highly significant linear effect of Dose on this measure ($F(1,10) = 27.99$, $p < 0.001$). Second, ANOVA revealed a reliable Dose effect on Initiation factor scores ($F(3,30) = 4.62$, $p = 0.009$, Fig. 3B). A combination of linear ($F(1,10) = 5.75$, $p = 0.037$) and cubic ($F(1,10) = 11.02$, $p = 0.008$) Dose effects on these scores suggested a need for clarification by post hoc tests. These revealed that Initiation factor scores were reliably depressed by oxotremorine treatments of 0.75 and 3 μg (each $p < 0.05$, Dunnett's test), but not by the intermediate treatment of 1.5 μg.

Though the focus of this report is on drug effects, levels of male behavior also varied as a function of copulatory series. Thus, ANOVA revealed main effects of Series on MF, IF, IR, EL and PEI ($F(1,10) \geq 9.05$, $p \leq 0.012$). In each case, performance improved over series, as indicated by reliable decreases in MF, IF, EL and PEI and a reliable increase in IR (upper half of Table 1).

2.2.3. Effects on female behavior

The same treatments that facilitated male behavior facilitated female-typical responses to tactile stimuli delivered by the experimenter. In particular, the incidence of lordosis was significantly

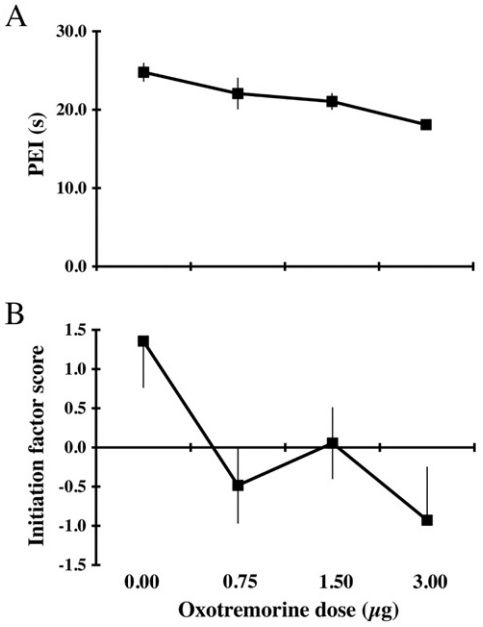

Fig. 3. Panel A describes the mean (and SEM) postejaculatory intervals (PEIs) observed in Experiment 1 after oxotremorine microinjections of 0, 0.75, 1.5 or 3.0 μg. Because the analysis of these data revealed just a reliable Dose main effect ($F(3,30)=7.14$, $p=0.001$), these levels of PEI have been averaged across copulatory series. Panel B describes the impact of the same manipulations on the mean (and SEM) factor scores for Initiation, one of the 5 conceptual variables revealed by the factor analysis of hamster mating patterns (Floody, 2011a). The analysis of these scores also revealed a reliable Dose effect ($F(3,30)=4.62$, $p=0.009$), with declines in factor score suggesting a dose-related facilitation of this aspect of copulation.

elevated by the combination of central oxotremorine treatment and exposure to brushing or manual stimulation of the flanks ($Q(3)\geq 9.22$, each $p<0.05$, Cochran test, 2-tailed, upper half of Table 2). The incidences and durations summarized in Table 2 indicate that these responses were common and prolonged, extending over much of the available 30 s. At the same time, it is clear that they were not dose-related: At least over the range of doses tested, they reflect the presence or absence of oxotremorine treatment, not its extent.

2.3. Discussion

These results document the responsiveness to central oxotremorine treatment of specific male- and female-typical sexual responses in male hamsters. First, they show that oxotremorine treatments aimed at the MPOA can shorten the PEI, thereby helping to facilitate the set of responses identified by factor analysis with the initiation of copulation

Table 1
Mean (and SEM) levels of male behavior across copulatory series.

Measure	Series 1	Series 2
After more lateral oxotremorine treatments (Experiment 1)		
MF	1.1 (0.2)	0.2 (0.1)
IF	6.3 (0.4)	1.6 (0.1)
IR	0.87 (0.03)	0.93 (0.02)
EL	43.8 (2.0)	11.6 (1.0)
PEI	19.3 (1.1)	23.7 (1.0)
After more medial oxotremorine treatments (Experiment 2)		
MF	2.5 (0.5)	0.3 (0.1)
IF	7.0 (0.6)	1.7 (0.1)
IR	0.78 (0.03)	0.92 (0.02)
EL	58.8 (3.6)	13.4 (1.1)

Notes: See text for definitions and units. For these measures, all main effects of series were significant by ANOVA, $F(1,10–13)\geq 9.05$, $p\leq 0.012$.

Table 2
Incidence and duration (s) of lordosis responses to manual stimulation.

	Oxotremorine dose (μg) in Experiment 1		
Measure and stimulus	0.75	1.50	3.00
% tests positive for lordosis to			
Brush*	27.3	45.5	45.5
Hand*	45.5	54.5	36.4
Mean (SEM) lordosis duration in positive tests using			
Brush	17.9 (4.9)	15.9 (1.9)	15.8 (3.9)
Hand	15.5 (5.3)	20.9 (2.5)	23.7 (1.2)

	Oxotremorine treatment and dose (μg) in Experiment 2			
	Dorsal injection		Ventral injection	
Measure and stimulus	0.5	3.0	0.5	3.0
% tests positive for lordosis to				
Brush	14.3	21.4	21.4	14.3
Hand*	35.7	28.6	35.7	14.3
Mean (SEM) lordosis duration in positive tests using				
Brush	14.6 (5.9)	7.5 (2.4)	14.2 (5.0)	12.1 (3.9)
Hand	17.3 (4.9)	18.3 (4.6)	17.8 (2.2)	24.5 (0.3)

Note: Placebo treatments are omitted because of their uniform failure to affect lordosis. For each treatment with an asterisk, including each injection site in Experiment 2, oxotremorine significantly increased the incidence of lordosis relative to that observed after placebo treatment, Cochran Q test, $Q(2 \text{ or } 3)\geq 7.00$, each $p<0.05$ (2-tailed).

in male hamsters (Floody, 2011a). Second, the same treatments facilitated species-typical female acts in males, increasing the likelihood of lordosis responses to light brushing or manual stimulation of the flanks.

These effects on male-typical behavior resemble previous descriptions of responses to centrally-administered ACh agonists in suggesting that cholinergic synapses in or near the MPOA control specific elements of male behavior. At the same time, they disagree with previous reports on the specific elements controlled by this mechanism, possibly reflecting the different species examined in these studies. Previous descriptions of rats suggest that the only elements sufficiently sensitive to ACh to be affected by unilateral exposure to an agonist are IF and the related conceptual variable of ejaculatory threshold or intromission count (Hull et al., 1988a; Sachs, 1978). The present results suggest a species difference in this mechanism by instead describing drug-related changes that, in hamsters, seem confined to the PEI and related dimension of Initiation (Floody, 2011a).

But are these effects in hamsters specific to the MPOA? The previous studies of rats effectively exploited comparisons of groups with implants that penetrated or avoided the lateral ventricles (Hull et al., 1988a): Treatments likely to be restricted to the MPOA affected just IF whereas those with the potential to circulate more widely through the ventricles affected other measures as well. Our results are more complicated, by differences in cannula placement across subjects: Whereas some cannulae penetrated a ventricle, others did not. Nevertheless, the histological evidence permitted us to distinguish 2 subgroups of males, one of 7 with ventricular intrusions at distances less than the 2 mm estimated upper limit on diffusion along a cannula (Hull et al., 1988b), and one of 4 with no intrusions or with nicks well beyond the same distance. Whereas some ventricular circulation of the applied drug seems possible in the first subgroup, it would seem less likely in the second. This contrast then permitted a Group × Dose analysis of PEI that revealed no reliable effect, and thus no suggestion of the modulation of the drug effect by implant trajectory. Though these results are not conclusive, they suggest that the oxotremorine effects on PEI and initiation described here for hamsters are mediated by the MPOA.

The effects on female-typical behavior that we describe are consistent with previous reports emphasizing the relative ease of eliciting such responses in male hamsters (Kow et al., 1976) and establishing the responsiveness of lordosis in female rats and hamsters to systemic or central cholinergic treatments (Dohanich et al., 1984; Dohanich and

Clemens, 1981; Dohanich et al., 1990, 1991; Menard and Dohanich, 1989). They extend these earlier results by integrating them, showing that lordosis in male hamsters is subject to cholinergic control similar to that operating in females.

As in the case of male behavior, previous studies have attempted to localize cholinergic effects on lordosis in female rats by comparing responses to cannulae that penetrate or avoid the ventricles (Dohanich et al., 1984). Here, however, it has not been possible to identify an implant site that is more effective than the ventricles themselves. Consequently, the results argue against mediation by the MPOA or ventromedial hypothalamus, and support the involvement of some tissue flanking a ventricle, though without specifying that tissue. Again, our results seem consistent with these analyses. As previously noted, we were able to identify subgroups differing in the likelihood of drug diffusion sufficient to access a ventricle. This permitted a Group × Dose analysis of lordosis durations during flank stimulation. Though the results of this analysis were not conclusive, they suggested shorter lordosis responses to brushing in the males with implants that should have reduced the chances of ventricular circulation ($F(1,9) = 3.33$, $p = 0.10$). In turn, this is consistent with the suggestion that the oxotremorine effect on female-typical behavior is not localized to the MPOA, and instead is mediated by other tissue, possibly dorsal or dorsolateral to the MPOA, or possibly even more distant and flanking some part of the lateral ventricle.

3. Experiment 2

It would be desirable to better localize these cholinergic effects on both male- and female-typical forms of behavior. Previous studies of rats suggest that the remedy lies in implants that are more severely angled and thus more likely to avoid the ventricles (e.g., (Dohanich et al., 1984; Hull et al., 1988a)). However, we have been unable to increase this angle sufficiently to achieve this end and still reach the MPOA. Therefore, we have reverted to a more dorsal approach, but one in which the guide cannula is angled and placed so as to avoid both the superior sagittal sinus and the medial (nearest) corner of the ipsilateral lateral ventricle. This approach seems to offer 4 potential advantages. First, it should reduce the likelihood of any penetration of a ventricle. Second, any penetrations that do occur should be located at or near the dorsal edge of the ventricle, placing them too far from the MPOA for the effective diffusion of injected fluid to a ventricle (Hull et al., 1988b). Third, this approach offers the opportunity to compare responses to treatments that vary in proximity to the MPOA while all being relatively distant from the lateral ventricles. Past work has shown that behavior is affected differently by implants that approach the MPOA from directly above and pierce a ventricle as opposed to approaching the MPOA more dorsolaterally and avoiding the ventricle (e.g., (Dohanich et al., 1984; Hull et al., 1988a)). However, it is not completely clear if these outcomes reflect the different impacts on the ventricles or the different opportunities for drug diffusion to structures immediately dorsal to the MPOA. Last, the approach to be pursued here simply is very different from that in Experiment 1. Any effects attributed to the MPOA by both studies may be further strengthened by this difference and the resulting convergence of evidence. The expected effects of these manipulations on measures of male behavior and lordosis are the same as specified earlier for Experiment 1.

3.1. Methods

3.1.1. Animals, treatments and testing

Complete data were collected from 14 male hamsters that averaged 172.0 g (SEM = 3.9) at the time of surgery. The stimuli included 15 females that averaged 157.3 g (SEM = 4.4) at the time of their first use. Each had undergone bilateral ovariectomy at least 10 days before the start of testing and was brought into hormone-induced estrus by a series of injections similar to that used in Experiment 1. All animals were housed and maintained as previously described.

Each male underwent surgery for the implantation of a 26 G stainless-steel guide cannula using methods similar to those described previously. However, these cannulae were aimed at points 2.5 mm dorsomedial to their ultimate targets in the MPOA and so required different medial–lateral (1.7 mm to the left of the midline) and dorsal–ventral (compromise between 4.7 mm below bregma and 4.0 mm above the interaural line) coordinates.

After at least 6 days of recovery, each male began a series of 3 behavioral tests at 5–6 day intervals. Each was preceded by an intracranial microinjection of 0.5 μl of a 0.9% NaCl solution containing 0 (placebo), 0.5 or 3.0 μg of oxotremorine. The 33 G needle used to administer these injections extended 0.5 mm beyond the guide cannula, consistent with our intention to direct an initial series of treatments at targets well dorsal to the POA. With this exception, the methods used to administer injections and conduct behavioral tests were identical to those in the first study. This initial series of tests was followed by a second series that was identical with one important exception. Specifically, the needle used to administer these injections extended 2.5 mm beyond the guide cannula, consistent with our goal of directing some treatments at targets in or near the MPOA.

This ordering of dorsal and ventral treatments deviates from an ideal factorial design. Under other conditions, it might have been desirable to counterbalance the entire series of treatments. However, one of our goals was that of limiting the extent to which effects of treatments at one site can be explained by the diffusion of drug, especially along a cannula track. By the present design, treatments targeting the more ventral site could possibly affect behavior after having diffused to a more dorsal target. But the opposite is not true and this should help distinguish behavioral effects that are and are not likely to be mediated by the MPOA.

3.1.2. Analysis

The targets of ventral treatments were determined as previously described. However, the later injections eliminated the cues that ordinarily would have been used to localize the initial series of injection sites. Consequently, these dorsal injections were taken to be concentrated at points 2.0 mm dorsally along the cannula track from the sites of the later injections.

Drug effects on male-typical behavior were assessed much as in the preceding study, but recognizing the fact that there were 2 series of treatments distinguished by Locus (dorsal vs. ventral injections). Therefore, ANOVAs on factor scores or behavioral elements that relate to an encounter as a whole (ML, IL) treated Locus and oxotremorine Dose as within-subjects factors. For all of the other measures of male behavior, the ANOVAs also included copulatory Series as a third within-subjects factor. Drug effects then were inferred from reliable main effects or interactions involving Dose. Possible drug effects on female-typical responses were assessed primarily through the use of the Cochran Q test to compare the incidences of lordosis across treatments, as in Experiment 1.

In addition to identifying drug effects, we sought to tie these effects to specific loci, at least by identifying those most likely to reflect changes in cholinergic activity in or near the MPOA. In particular, the attribution of a drug effect to the MPOA was considered here to require a difference across oxotremorine doses that was limited to the more ventral injections or was significantly greater in that series than after the more dorsal treatments. Conversely, effects that were observed only, or to a greater extent, after dorsal injections were taken to suggest mediation by structures other than the MPOA.

3.2. Results

3.2.1. Histology

The dorsal and ventral injection sites for each male are described in Fig. 4 and in the lower half of Fig. 2. All were concentrated at anterior–posterior levels approximating the crossing of the anterior commissure.

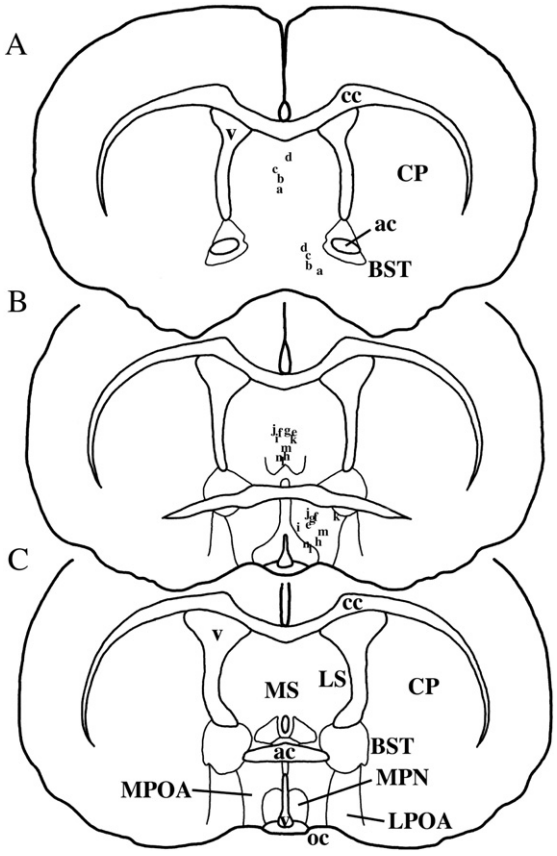

Fig. 4. The sites of the microinjections described in Experiment 2 are indicated by small lower case letters on sketches of reference sections (Morin and Wood, 2001) spaced 300–400 μm apart from anterior (A) to posterior (C). Each letter corresponds to a subject, permitting the comparison of the dorsal and ventral injection sites in each individual. Abbreviations: ac, anterior commissure; BST, bed nucleus of the stria terminalis; cc, corpus callosum; CP, caudate putamen; LPOA, lateral preoptic area; LS, lateral septum; MPN, medial preoptic nucleus; MPOA, medial preoptic area; MS, medial septum; oc, optic chiasm; v, ventricle.

Dorsal injections were concentrated on or near the midline, in the medial septum. Ventral sites were concentrated in or slightly anterior to the MPOA. The latter sites clearly are consistent with our primary goal of further testing the impacts on male- and female-typical sexual responses of exposing the MPOA to a cholinergic agonist.

More generally, the entire set of placements in Fig. 4 is consistent with our goal of comparing responses to treatments at pairs of sites that were relatively distant, both from each other and the lateral ventricles. At the same time, it may be worth noting that guide cannulae did pass near the medial end of the border between the left lateral ventricle and the corpus callosum. In 2 cases, guides penetrated the ventricle at this point. In several others, the cannula track and ventricle were separated by just a narrow band of tissue. Accordingly, we cannot completely exclude the possibility that some of the oxotremorine delivered at dorsal injection sites entered a lateral ventricle and circulated to tissues other than those immediately surrounding these sites. Even so, it would seem that the level of drug exposure that could be achieved in this way would be low both in absolute terms (especially for the lower oxotremorine dose) and relative to that experienced closer to the needle tip. Further, it would seem that the likelihood of such spread would be negligible in the case of POA injections, as these were separated from the point of greatest proximity between cannula track and ventricle by distances approximating 3 mm (mean = 2.9 mm, SEM = 0.1), much greater than the upper limit estimated for this sort of diffusion (Hull et al., 1988b).

3.2.2. Effects on male behavior

The analysis of individual elements of male behavior revealed Locus × Dose × Series interactions affecting MF and IR ($F(2,26) = 6.88$ and 3.40, respectively, $ps = 0.004$ and 0.049). Further analysis showed that the drug effects on these measures were confined to the first copulatory series, for which there were reliable Locus × Dose interactions ($F(2,26) = 6.11$ and 7.17 for MF-1 and IR-1, respectively, $ps = 0.007$ and 0.003). In turn, these seem attributable to contrasting responses to the highest oxotremorine dose ($F(1,13) = 10.59$ and 13.26 for MF-1 and IR-1, respectively, $ps = 0.006$ and 0.003). When applied to the more dorsal site, this disrupted behavior by increasing MF-1 and decreasing IR-1 (Fig. 5). But when applied to the POA, the same treatment facilitated behavior by causing each measure to change in the opposite direction.

Other drug effects on individual measures of male behavior were suggested by a Dose × Series effect on IF ($F(2,26) = 5.14$, $p = 0.013$) and a Locus × Dose × Series effect on PEI ($F(2,26) = 4.38$, $p = 0.023$). The former was found to reflect changes confined to the first copulatory series ($F(2,26) = 4.19$, $p = 0.027$). At that time, dose-related reductions in IF-1 by drug delivery to either implant site were supported by a linear Dose effect ($F(1,13) = 11.37$, $p = 0.005$, Fig. 6A). In contrast, further analysis of the PEI scores revealed a reliable Dose effect for just the more ventral treatments, targeting the MPOA ($F(2,26) = 21.11$, $p < 0.001$). The ability of treatments there to cause dose-related decreases in both PEI-1 and PEI-2 was supported by a linear Dose effect specific to the ventral site ($F(1,13) = 41.93$, $p < 0.001$, Fig. 6B).

As before, drug effects on male behavior also were sought in analyses of the conceptual variables revealed by factor analysis (Floody, 2011a). These revealed a Locus × Dose interaction affecting Efficiency-1 ($F(2,26) = 3.91$, $p = 0.033$, Fig. 7A) and a Dose main effect on Intromissions-1 ($F(2,26) = 3.42$, $p = 0.048$, Fig. 7B). Considering the measures that define these (Floody, 2011a), it is not surprising that the effect on Efficiency-1 resembles those on MF and IR whereas the effect on Intromissions-1 resembles that on IF. Efficiency-1 was increased (suggesting a disruption of copulation) by dorsal treatments but decreased (facilitated) by ventral ones. However, only for the latter were dose-related changes supported by reliable Dose main ($F(2,26) = 3.87$,

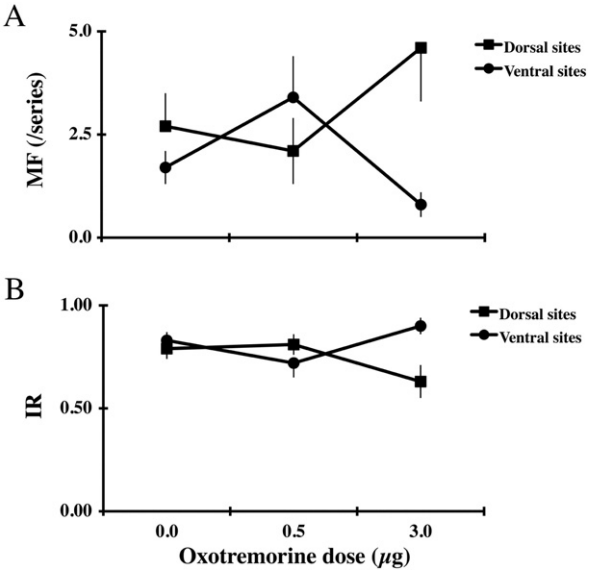

Fig. 5. Panel A describes the mean (and SEM) mount frequencies for the first copulatory series (MF-1s) that were observed in Experiment 2 after the delivery of oxotremorine doses of 0, 0.5 or 3.0 μg to the dorsal (square symbols) or ventral (circles) sites depicted in Fig. 3. Panel B does the same for intromission ratio (IR-1s). For each of MF and IR, ANOVA revealed a reliable Locus × Dose × Series interaction ($F(2,26) \geq 3.40$, $p \leq 0.049$). Further analysis revealed reliable differences in MF-1 and IR-1 across injection sites at the highest oxotremorine dose ($F(1,13) \geq 10.59$, $p \leq 0.006$).

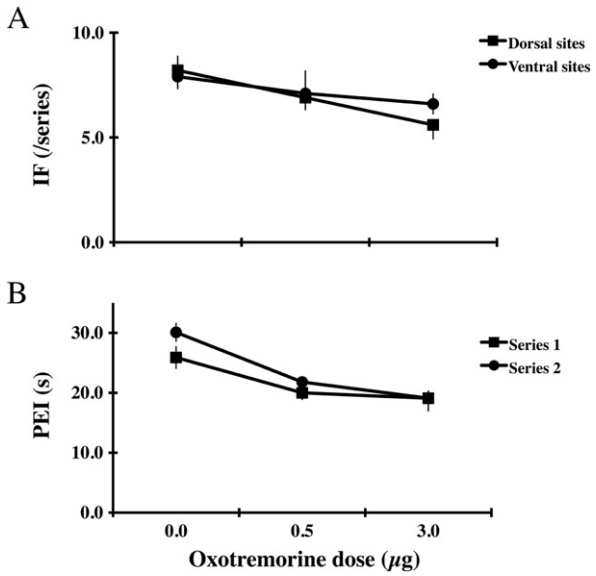

Fig. 6. Panel A describes the mean (and SEM) intromission frequencies for the first copulatory series (IF-1s) that were observed in Experiment 2 after the delivery of oxotremorine doses of 0, 0.5 or 3.0 μg to the dorsal (square symbols) or ventral (circles) sites depicted in Fig. 3. The analysis of these data revealed a reliable Dose × Series interaction ($F(2,26) = 5.14$, $p = 0.013$), reflecting a reliable Dose effect in the first copulatory series ($F(2,26) = 4.19$, $p = 0.027$). Panel B describes the mean (and SEM) postejaculatory intervals (PEIs) in the first and second copulatory series (squares and circles, respectively) observed in Experiment 2 after the delivery of oxotremorine doses of 0, 0.5 or 3.0 μg to the ventral sites in Fig. 3. The application of ANOVA to these data revealed a reliable Locus × Dose × Series interaction ($F(2,26) = 4.38$, $p = 0.023$). Further analysis revealed a reliable Dose effect at the ventral injection site ($F(2,26) = 21.11$, $p < 0.001$).

$p = 0.034$) and linear effects ($F(1,13) = 10.19$, $p = 0.007$). Facilitatory dose-related decreases in Intromissions-1 by treatment at either site were supported by a linear Dose effect ($F(1,13) = 7.79$, $p = 0.015$).

Though the focus of this report is on responses to drug treatments, levels of male-typical behavior also responded to differences in copulatory series and injection site. With respect to the first, ANOVA revealed reliable main effects of Series on MF, IF, IR and EL ($F(1,13) \geq 13.45$, $p \leq 0.003$). For each, performance improved over series, with reliable decreases in MF, IF and EL and a reliable increase in IR (see lower half of Table 1). Effects of implant site included Locus main effects on ML, IL, Initiation and Efficiency-2 ($F(1,13) \geq 7.12$, $p \leq 0.019$) and Locus × Series interactions affecting III and EL ($F(1,13) \geq 4.85$, $p \leq 0.046$). For several reasons, these seem most reasonably interpreted as practice effects, reflecting our inability to counterbalance the dorsal and ventral treatments. First, these effects uniformly reflect improvements in performance in the second series of tests. Second, Rabedeau (Rabedeau, 1963) explicitly tested for experiential effects on ML, IL and III and documented such effects for the first 2 of these. Third, this interpretation seems consistent with the results of analyses that examined scores on the 4 individual measures over successive test days. For most of these measures, these analyses confirmed small but consistent improvements in performance over days (for ML and IL, $F(1,13) \geq 7.12$, $p \leq 0.019$; for III, $F(5,65) = 2.63$, $p = 0.031$, Fig. 8).

3.2.3. Effects on female behavior

As in the preceding study, these drug treatments facilitated female-typical responses to tactile stimuli delivered by the experimenter, even in tests immediately following vigorous male-typical behavior. In particular, the incidence of lordosis was significantly elevated by bilateral flank stimulation after either dorsal or ventral drug treatments ($Q(2) \geq 7.00$, each $p < 0.05$, Cochran test, 2-tailed, lower half of Table 2). As before, these responses were robust and facilitated to similar degrees by the different oxotremorine doses.

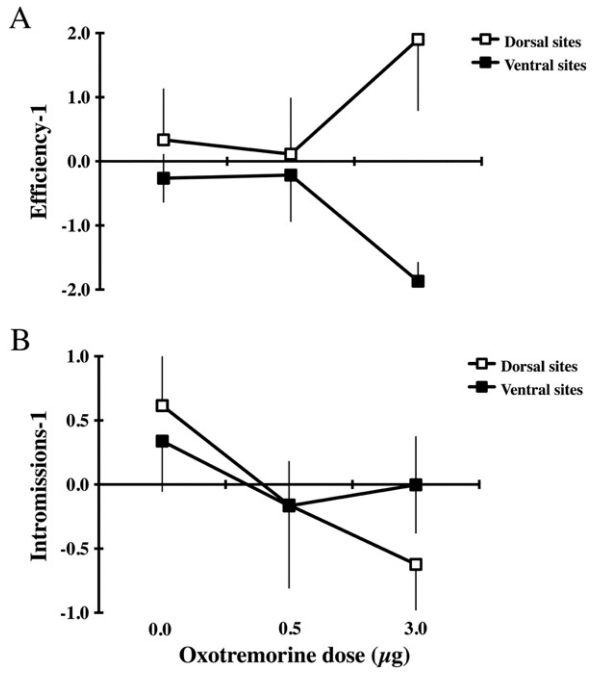

Fig. 7. Panel A describes the impact of the dorsal (open symbols) or ventral (filled symbols) oxotremorine injections in Experiment 2 on the mean (and SEM) factor scores for Efficiency-1, one of the 5 conceptual variables revealed by the factor analysis of hamster mating patterns (Floody, 2011a). Panel B does the same for Intromissions-1, another of these conceptual variables. The analysis of Efficiency-1 scores revealed a reliable Locus × Dose interaction ($F(2,26) = 3.91$, $p = 0.033$). Further analysis revealed just a reliable Dose effect on the scores associated with ventral injections ($F(2,26) = 3.87$, $p = 0.034$). The analysis of Intromissions-1 scores revealed a reliable Dose main effect ($F(2,26) = 3.42$, $p = 0.048$). For each factor score, a decrease due to treatment suggests a drug-related facilitation of the relevant aspect of copulation.

3.3. Discussion

These results confirm some of those described in Experiment 1 (Table 3). In particular, both studies describe decreases in PEI and increases in the incidence of lordosis, documenting the ability of central

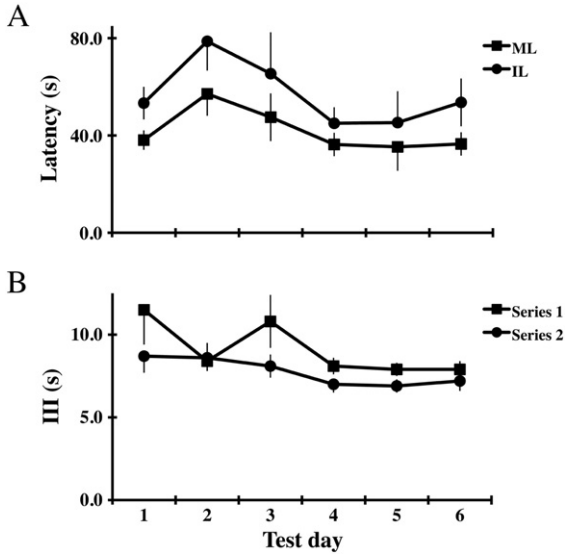

Fig. 8. Panel A describes the mean (and SEM) mount and intromission latencies (squares and circles, respectively) observed over successive days of testing in Experiment 2. Panel B does the same for the interintromission intervals observed in the first and second copulatory series (squares and circles, respectively) in that study. In each case, ANOVA confirmed a reliable improvement in performance over days ($F(1,13) \geq 7.12$, $p \leq 0.019$ for ML and IL; $F(5,65) = 2.63$, $p = 0.031$ for III).

Table 3
Summary of reliable drug effects.

Experiment #	Measure	Direction of change	Active site	Linear dose effect?
1	PEI	↓	–	Y
	Initiation	↑	–	N
	Lordosis	↑	–	N (all-or-none)
2	IF-1	↓	Both	Y
	IR-1	↓	Dorsal	N (high only)
	IR-1	↑	Ventral	N (high only)
	MF-1	↑	Dorsal	N (high only)
	MF-1	↓	Ventral	N (high only)
	PEI	↓	Ventral	Y
	Efficiency-1	↓	Ventral	Y
	Intromissions-1	↓	Both	Y
	Lordosis	↑	Both	N (all-or-none)

Notes: Measures include elements of male behavior, factor scores combining these elements, and the incidence of lordosis. All of these changes in factor scores reflect the facilitation of male behavior. Dose-related changes in some measures were suggested by linear dose effects. However, other measures responded only to the highest oxotremorine dose (high only) or were affected equally by the oxotremorine treatments (described here as an all-or-none effect).

oxotremorine treatments to facilitate both male- and female-typical behavior in male hamsters. They also extend the earlier results. First, they describe additional responses to the drug treatments. These include reductions in IF due to injections within or dorsal to the MPOA and related changes in MF and IR, each of which reversed direction as a function of injection site. Second, they create new opportunities to examine the relationship between a behavioral effect and the site or method of drug delivery. It was hoped that these comparisons could clarify the structures mediating specific effects, and involved in the cholinergic control of male- or female-typical reproductive behavior.

In exploring these comparisons and relationships, we have focused on brain areas that have been implicated in the control of mating behavior and thus seem well positioned to directly mediate neurochemical effects on this behavior. However, cholinergic drugs can affect, many behaviors and aspects of behavior (e.g., (Klinkenberg and Blokland, 2010)). Therefore, though the behavioral effects described here may all be mediated by some part of the brain system for reproductive behavior, it also is possible that some arose indirectly, through cholinergic effects on other aspects or forms of cognition or behavior.

Discussions of forebrain mechanisms for sexual behavior have tended to focus on a few hypothalamic structures including the MPOA. However, many other brain areas have been implicated in the control of these responses. Several of these may be especially relevant here, including the caudate putamen, bed nucleus of the stria terminalis, medial septum, and lateral septum. Each has been implicated in the control of both male- (Emery and Sachs, 1976; Gogate et al., 1995; Powers et al., 1987; Rodríguez-Manzo and Pellicer, 2010) and female-typical (Acosta-Martinez and Etgen, 2002; Bradley et al., 2005; Dudley and Moss, 1994; King and Nance, 1986; López and Carrer, 1982; Nance and Myatt, 1987) forms of mating behavior. Further, each is found dorsal or dorsolateral to the MPOA, on or near one or both of the injection routes used in these studies. Finally, all contain at least moderate densities of muscarinic receptors, suggesting the potential to mediate responses to oxotremorine and cholinergic influences on male- or female-typical behavior (Gattu et al., 1997; Kubieta and Frey, 1993; Levey et al., 1991; Vilaró et al., 1994). These areas and considerations relate differently to the 3 types of effects described here, including effects unique to treatments targeting the MPOA, effects of either MPOA or more dorsal injections, and effects unique to the dorsal injections. Each will be discussed in turn.

3.3.1. Effects specific to treatments of the POA

In principal, it seems possible for drugs microinjected into the vicinity of the MPOA to act in one or more of 3 ways. First, they could operate on cholinergic synapses in the MPOA. Second, they could act on another target that is close to the cannula track and exposed to drug that has diffused along this track from its point of delivery. The most likely targets of this type should depend on an implant's trajectory. For implants angled toward the MPOA from a point lateral to the lateral ventricle, as in Experiment 1, they include the bed nucleus and caudate putamen. For those approaching the MPOA from more directly above, as in Experiment 2, the most likely other targets include the medial septum, lateral septum, and, again, the bed nucleus. Third, it may be possible for drug aimed at the MPOA to act on a periventricular structure that it accesses through the ventricular circulation. The likelihood of this, too, should depend on implant site and trajectory (Hull et al., 1988b). Many of the implants in Experiment 1 penetrated a lateral ventricle at a point sufficiently close to the injection site to possibly permit an effect of this type. At the opposite extreme, the MPOA injections in Experiment 2 were delivered so far from the cannula's closest approach to a lateral ventricle that significant ventricular circulation seems unlikely. But this issue is more difficult to resolve for the dorsal injections in the same study. Most of these cannulae avoided the ventricle and terminated near the midline, sufficiently far from the ventricles to make diffusion through the interposed neural tissue unlikely. At the same time, many passed very close to a ventricle at a point roughly 1 mm from the site of drug delivery, making us reluctant to completely exclude the possibility that some part of the response to these treatments reflects ventricular circulation to a periventricular target.

Of the drug effects observed in Experiment 2, the ones most likely to reflect mediation by the POA would seem to be those that appeared only when oxotremorine was applied to this target. In terms of individual elements of male behavior, these include drug-related decreases in PEI and MF-1, along with an increase in IR-1 that itself is due largely to the change in MF (Table 3). With regard to conceptual variables, POA treatments tended to facilitate initiation and the efficiency of performance in the first copulatory series, no doubt reflecting the effects on PEI, MF-1 and IR-1 (Floody, 2011a).

Of all these effects, only that on PEI appeared in both studies, providing especially strong evidence for the control of this element by a cholinergic mechanism identified with the MPOA. In contrast, an effect on Initiation was found just in Experiment 1 whereas those on MF-1, IR-1 and Efficiency-1 were limited to Experiment 2. These differences across measures could reflect multiple cholinergic mechanisms that differ in sensitivity, the less sensitive requiring bilateral cholinergic changes and thus a more medial point of drug delivery. This suggestion is consistent with the fact that the effects on PEI and Initiation were dose-related whereas most of those on MF-1, IR-1 and Efficiency-1 were confined to the highest oxotremorine dose. Alternatively, these differences could reflect cholinergic mechanisms occupying different parts of the POA, including one that is concentrated laterally and controls PEI, and one that is located more medially and influences MF and IR. A division of the POA into regions linked to specific elements of male behavior is consistent with some past results in rats (McGinnis et al., 2002).

However, these results also suggest that the neurochemistry of POA mechanisms for male behavior differ across species, as the behavioral elements affected here clearly differ from those most responsive to central cholinergic treatments in rats. In the previous work, the oxotremorine treatments most specific to the MPOA affected just IF, and, by extension, ejaculatory threshold (Hull et al., 1988a). A broader range of cholinergic effects was suggested by the decreases in intromission and ejaculation incidence caused by bilateral scopolamine treatments targeting the MPOA (Hull et al., 1988b). Nevertheless, even that work provides no suggestion of effects specific to PEI, MF or IR, the elements most strongly linked to cholinergic control by the POA in hamsters.

3.3.2. Effects common to dorsal and MPOA treatments: Male behavior

Experiment 2 revealed a facilitatory effect of oxotremorine on IF-1 that was equally strong for injections in and dorsal to the MPOA (Table 3). Related to this was a similar effect on Intromissions-1, a

conceptual variable strongly influenced by IF-1 (Floody, 2011a). Each pattern could reflect separate drug effects in and dorsal to the MPOA. Alternatively, they both could originate at a dorsal site, responding either to drug delivered in its immediate vicinity (by one of the more dorsal injections) or drug that has diffused to it along the cannula track (from one of the MPOA injections).

Though the evidence is not decisive, we favor the first of these alternatives for two reasons. First, all of the relevant behavioral effects were dose-related. Considering the dose disparity and the spatial separation of injection sites, this seems difficult to reconcile with the mediation of all effects by a single dorsal target, which might be expected to respond only to the highest drug dose when applied to the MPOA. Second, the MPOA in rats mediates cholinergic effects on IF (Hull et al., 1988a), suggesting that this structure could be among those that do the same in hamsters.

But if the MPOA mediated responses to oxotremorine delivered there, what structure mediated those to the more dorsal treatments? The fact that IF and Intromissions-1 were affected only in Experiment 2 points to a target that is along the more vertical cannula tracks and thus directly dorsal to the POA. Specific candidates then include the bed nucleus, the medial septum, and the lateral septum. We are not aware of any effects of septal manipulations that seem at all specific to IF. For example, though lesions of the lateral septum can affect IF, much or all of this effect seems to reflect more global disruptions, reducing the incidence, not just frequency, of intromission (Gogate et al., 1995; Kondo et al., 1990). In contrast, the evidence relating the bed nucleus to the control of IF seems stronger in several respects. First, lesions of the bed nucleus or stria terminalis have been shown to affect elements of male behavior including IF in both rats (Emery and Sachs, 1976; Liu et al., 1997; Sachs, 1978) and hamsters (Lehman et al., 1983; Powers et al., 1987) (but also see (Been and Petrulis, 2010)). Second, even in some of the cases in which several elements of male behavior were affected by these lesions, special importance was attached to the effect on IF, reflecting both its consistency and potential impact on other measures (Emery and Sachs, 1976; Lehman et al., 1983; Sachs, 1978). Third, even the effects on IF in rats of oxotremorine injections targeting the MPOA were interpreted more expansively, as reflecting the control of IF and ejaculatory threshold by an integrated system including the amygdala, stria terminalis and MPOA (Hull et al., 1988a).

3.3.3. Effects common to dorsal and MPOA treatments: Female behavior

As in the first study, the oxotremorine treatments in Experiment 2 reliably increased the incidence of lordosis, and without regard to dose or injection site. While confirming the ability of cholinergic manipulations to affect lordosis in male hamsters, these results provide no clear answer to the question of the site(s) mediating this effect.

Studies of female rats have compared lordosis responses to oxotremorine injections into the lateral ventricles, POA, and ventromedial hypothalamus (Dohanich et al., 1984). The results of this work suggest that the cholinergic facilitation of lordosis is attributable to a periventricular site, not the POA or ventromedial hypothalamus. However, studies of female hamsters have not taken this analysis quite as far (Dohanich et al., 1990). Ventricular treatments have been shown to be effective, but have not been compared to injections at other sites. Because of this, it seems premature to exclude the MPOA from consideration as a possible mediator of some cholinergic effects on lordosis in hamsters.

At the same time, it seems clear that at least some of these effects were mediated elsewhere. In this case, some specific candidates include the ventrolateral septum and bed nucleus, both of which contain muscarinic receptors (Gattu et al., 1997; Kubieta and Frey, 1993; Levey et al., 1991; Vilaró et al., 1994) and are near our injection sites or cannula tracks. The first of these has been clearly implicated in the control of lordosis in rats and hamsters (King and Nance, 1986; Nance and Myatt, 1987). The evidence suggesting a similar role for the bed nucleus is more limited. Part of it revolves around the alteration of activity in the bed nucleus by the exposure of female rats to mating stimulation (Dudley and Moss, 1994; López and Carrer, 1982). Further, this structure helps to mediate chemosensory effects on sexual behavior in male hamsters (Been and Petrulis, 2010; Lehman et al., 1983; Powers et al., 1987), and evidence suggesting a similar role in females emerges from the recent observation that blocking oxytocin receptors in either the POA or bed nucleus inhibits a type of precopulatory scent marking normally shown by female hamsters upon exposure to the odors of a potential mate (Martinez et al., 2010).

The consideration of the lateral septum and bed nucleus as mediators of cholinergic effects on lordosis does not exclude the involvement of some other, as yet unknown, periventricular site. Nor would roles for one or both of these structures be incompatible with one for the MPOA. As previously discussed, the bed nucleus and MPOA are viewed as components of an integrated system for the chemosensory control of sexual responses in hamsters (e.g., (Been and Petrulis, 2010; Powers et al., 1987)). Similarly, studies of lordosis in rats and hamsters have emphasized the coordinated control of this response by the cells in, and the fibers interconnecting, the lateral septum and POA (Floody, 1993; Hoshina et al., 1994; Takeo et al., 1993; Yamanouchi and Arai, 1990).

3.3.4. Effects specific to treatments dorsal to the MPOA

Against a background of generally facilitatory effects, it was intriguing to observe the disruptive changes in MF-1 and IR-1 caused by the application of 3 μg of oxotremorine to the more dorsal of the injection sites in this study. These suggest the existence of a mechanism that specializes in the control of MF, but is of limited sensitivity. It seems clearly distinct from any mechanism associated with the MPOA because its activation changes MF in the direction opposed to that produced by otherwise identical MPOA treatments.

Possible mediators of this effect include the bed nucleus, lateral septum and medial septum. The evidence relating the first of these to the control of male behavior has been reviewed. It is clear that lesions of the bed nucleus can affect a variety of measures of male behavior including MF (Emery and Sachs, 1976; Sachs, 1978). However, we are not aware of any evidence suggesting that lesions of the entire bed nucleus, or of any of its subdivisions (Been and Petrulis, 2010; Claro et al., 1995; Finn and Yahr, 2005; Parfitt and Newman, 1998), produce effects concentrated on MF or opposed to the effects of MPOA lesions. Lesions of the lateral septum also are well known to affect multiple measures of male behavior including MF (Gogate et al., 1995; Kondo et al., 1990). In direction, these effects resemble the responses to MPOA lesions, and there seems to be nothing in this evidence to suggest a focus on MF. However, the control of male behavior by this structure may be more complex than some accounts suggest. Noradrenergic manipulations of the lateral septum that generally facilitate male behavior seem to increase MF, both in absolute terms and relative to IF (Gulia et al., 2002). This raises some possibility of lateral septal effects on MF that are both specific and negative. Last, the medial septum would seem to be the structure that our dorsal injections would have most affected. However, evidence suggesting an effect on male behavior due to this drug exposure is sparse, partly due to disagreement on whether lesions of the medial septum have any reliable effect on male behavior (Gogate et al., 1995; Kondo et al., 1990). Still, one study found medial septal lesions to facilitate male behavior generally, thereby creating a contrast with the effects of POA lesions (Gogate et al., 1995). Further, the relevant changes were focused on mounting, raising the possibility that a cholinergic mechanism concentrated in this area could selectively affect MF.

4. Conclusions

This report completes a pair examining the effects of systemic, then central, applications of muscarinic drugs on sexual behavior in male golden hamsters. The initial report, on systemic treatments, described a very broad pattern of cholinergic effects (Floody, in press). Of the 12 measures considered there, 8 were affected by oxotremorine and

all were affected by it or scopolamine. These results suggest that ACh powerfully influences male-typical behavior in hamsters. Further, they suggest that ACh may be more influential in hamsters than rats, in which oxotremorine most consistently affects just IF and EL and scopolamine seems to affect primarily ML and IL (e.g., (Ahlenius and Larsson, 1985; Retana-Marquez et al., 1993)).

The effects of central oxotremorine treatments that are described here also extend across multiple measures of male behavior, including 4 individual elements (IF, IR, MF, PEI) and as many as 3 conceptual variables (Initiation, Efficiency-1, Intromissions-1). In addition, these treatments affected lordosis, a response ordinarily associated with sexual receptivity in females but that can be elicited with relative ease in male hamsters (Kow et al., 1976). Though these add up to a substantial number of measures, this is considerably smaller than that linked to systemic treatments. This presumably reflects the fact that systemic treatments can access and affect many brain areas whereas central treatments must act relatively near their point of delivery.

The focus of this study was the MPOA, which we approached in 2 ways so as to better test its involvement. Together, our results suggest that cholinergic synapses in the MPOA influence several aspects of male behavior in hamsters. The best supported of these was a facilitatory effect on PEI that extended across copulatory series. Facilitatory effects on MF, IR and behavioral efficiency in the first copulatory series also seem likely, though they may require larger changes in cholinergic activity or changes concentrated more medially within the POA. Cholinergic synapses in the MPOA may facilitate IF early in an encounter as well, though this could require the coordinated control of this parameter by a larger cholinergic system, possibly including links in both the bed nucleus and POA.

These results suggest species differences in the cholinergic control of male behavior by the MPOA. Oxotremorine treatments targeting the MPOA in rats seem to affect just IF, reducing it, possibly in just the first copulatory series (Hull et al., 1988a). The fact that IF was affected and the facilitatory nature of all these changes represent possible points of overlap with the effects we describe in hamsters. But we cannot be sure that any part of the effect on IF in hamsters is attributable to the MPOA and know of no evidence suggesting effects in rats that resemble the drug effects on PEI, MF and IR that were observed here in hamsters.

Our second study also included treatments at sites 2 mm dorsal to the MPOA. These were intended to permit a more critical assessment of MPOA involvement in specific behavioral effects. In effect, these comparisons were designed to function much as comparisons of implants that did and did not penetrate a lateral ventricle have in some previous studies (e.g., (Dohanich et al., 1984; Hull et al., 1988a)). At the same time, most of our central injections should have had limited or no effective access to a lateral ventricle. Consequently, they may be superior to earlier comparisons in suggesting specific alternative or auxiliary sites of drug action.

The measures that responded to the dorsal treatments here included IF-1, the related conceptual variable of Intromissions-1, and the female-typical lordosis response, all of which were facilitated by oxotremorine. In addition, dorsal injections caused disruptive changes in MF-1, the related behavioral element of IR-1, and the related conceptual variable of Efficiency-1. We have suggested that the effects allied to IF may reflect, wholly or in part, a mechanism identified with some part of the bed nucleus whereas those allied to MF may stem from drug action upon the medial septum. One curious aspect of these results is the apparent failure of any of these measures to respond to ventricular injections of oxotremorine in rats (Hull et al., 1988a). This is consistent with other evidence (here and in (Floody, in press)) for a substantial species difference in the cholinergic mechanisms helping to control male behavior. But the difference might instead relate to the fact that some or all of the mediators suggested here (at least the medial septum, possibly some parts of the bed nucleus) are not in immediate contact with a lateral ventricle, possibly limiting their exposure to any drug circulating there in previous studies.

Finally, throughout this paper we have suggested regions of the POA or sites outside the POA that might mediate specific effects of oxotremorine on male or female behavior. These suggestions follow from the behavioral differences we observed across treatments differing in trajectory or target. However, it should be clear that these suggestions are just that, and that most or all require further study using appropriately targeted infusions.

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