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Cholinergic control of male mating behavior in hamsters: Effects of systemic agonist or antagonist treatment

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ABSTRACT

Sexual behavior in male rats is thought to depend in part on central cholinergic activity. In particular, previous studies of responses to systemically administered cholinergic drugs suggest that male rat behavior can be facilitated by the muscarinic agonist oxotremorine but is disrupted by the muscarinic antagonist scopolamine. However, it is not clear how broadly these effects generalize across species. To address this issue, we observed the impact on sexual behavior in male hamsters of systemic treatment with oxotremorine or scopolamine. In each case, the peripheral muscarinic antagonist methylscopolamine was used as an auxiliary or control treatment to better isolate central cholinergic effects. Both oxotremorine and scopolamine disrupted male behavior in hamsters. For example, both increased the likelihood of failure to achieve intromission or ejaculation. Further, even on completed tests oxotremorine treatment led to changes including increases in mount latency and postejaculatory interval while scopolamine treatment caused changes including increases in ejaculation latency and intromission frequency. The many changes caused by these treatments suggest that acetylcholine helps to control many elements of male behavior, probably by acting at multiple brain sites. The generally similar responses to a cholinergic agonist and antagonist suggest the dependence of efficient mating behavior on optimal levels of central cholinergic activity.

1. Introduction

The mating behaviors of male animals are structurally complex and of obvious biological importance. At the same time, they are easy to elicit and composed of behavioral elements that are stereotyped and relatively easy to measure. Because of this combination of attributes, these behaviors have long attracted the attention of behavioral neuroscientists. Some of the resulting studies have examined the neurochemical mechanisms underlying this behavior, in the process implicating several neurotransmitters, including acetylcholine (ACh).

Early studies of acetylcholine's role in copulation described the responses of male rats to systemic treatments with cholinergic drugs, including the agonists nicotine and physostigmine and the antagonists atropine and scopolamine (Bignami, 1966; Leavitt, 1969; Soulairac, 1963). The results of these studies suggest that, in comparison to drugs affecting ACh's nicotinic receptors, muscarinic drugs are both more powerful and selective in their effects on mating behavior. However, these studies were limited in their ability to distinguish centrally- and peripherally-mediated muscarinic effects. Possibly as a consequence, some of the effects they describe extend across many measures or involve other wholesale, sometimes clearly nonspecific, changes.

Some more recent studies have continued to use systemic muscarinic treatments but have focused on central effects by exploiting the fact that the blood–brain barrier permits scopolamine to move from the general circulation into the brain while preventing such movements of the otherwise similar methylscopolamine. For instance, this difference can be exploited simply by directly comparing responses to scopolamine and methylscopolamine: Effects limited to the first seem likely to reflect central cholinergic changes (Klinkenberg and Blokland, 2010). In complementary studies, the properties of methylscopolamine are used to help isolate the central effects of muscarinic receptor mimics such as oxotremorine: Though such drugs can act both centrally and peripherally, combining them with methylscopolamine should reduce or cancel any peripheral effects, highlighting the more central ones.

Using this strategy, Ahlenius and Larsson (1985) showed that oxotremorine causes a dose-related facilitation of mating in male rats, specifically by reducing intromission frequency and ejaculation latency. These observations were extended by Retana-Marquez and colleagues (Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997), who found both of these effects to be restricted to the first copulatory series (the behaviors leading to the first ejaculation) and to be accompanied by increases in ejaculation frequency and, in relatively inexperienced males, the incidence of ejaculation. These effects were taken to suggest that ACh facilitates male behavior, specifically by reducing the "ejaculatory threshold" (the amount of stimulation required to trigger ejaculation) and

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increasing the "copulatory potential" (the number of ejaculations that can be achieved in a fixed time).

However, these inferences were supported only in part by the observed responses to scopolamine treatment. Ahlenius and Larsson (1985) detected no reliable response to scopolamine doses up to 0.4 mg/kg whereas Retana-Marquez et al. (1993) described several disruptive effects of such treatments, including decreases in the incidence of intromissions and ejaculations, decreases in ejaculation frequency, and increases in mount and intromission latencies. From this, they drew further support for the cholinergic control of copulatory potential. In addition, they inferred a possible facilitory effect of ACh on sexual motivation.

Though these results do not agree perfectly, they offer strong support for the dependence of male mating behavior on central muscarinic mechanisms. At the same time, it should be noted that all of this work has been restricted to male rats, raising the issue of the generality of the effects and conclusions.

I recently have used factor analysis to compare male hamsters and rats on purely behavioral grounds, in terms of the organization of their mating behaviors (Floody, 2011). In general, factor analysis begins by comparing individuals in terms of performance on a number of measures. Patterns of high interindividual correlation are used to identify clusters of measures (factors or conceptual variables) that may represent the products of coherent behavioral or physiological mechanisms. By conventional practice, these factors are named and described on the basis of the few measures that most strongly "load on" or determine them, though all measures enter into each factor to some degree.

These comparisons of factor structure in hamsters and rats are potentially relevant because of the species differences in the organization of mating behavior that they suggested. In fact, they revealed reliable species differences on nearly every important dimension. For instance, a factor identified with the rate of copulatory behavior (a cluster in which all major elements measure some aspect of the rate of this behavior) is one of the most consistent findings across factor analytic studies of rats (Dewsbury, 1979; Pfaus et al., 1990; Sachs, 1978). Yet a factor such as this was notably absent from the pattern observed in hamsters. This is not due to the absence of individual measures of rate. Instead, it reflects the failure of these measures to cluster together. More generally, this and other similar findings do not prove that the neurochemical mechanisms for sexual behavior differ across rats and hamsters. But they do raise the possibility of such differences and suggest the value of directly examining these mechanisms in a variety of animals. These studies take one small step in this direction by using systemically administered cholinergic drugs to examine the role of the central muscarinic system in male hamster mating behaviors.

2. Experiment 1

In our first study, we examined the effects of the cholinergic agonist oxotremorine on the mating behavior of male hamsters. As in the corresponding work on rats (Ahlenius and Larsson, 1985; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997), pretreatment with methylscopolamine was used to limit oxotremorine's effects to central cholinergic synapses and mechanisms. In other respects, past studies of rats vary in methods including the amount of prior experience provided to subjects and the durations of mating tests (Ahlenius and Larsson, 1985; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997). Because we routinely begin with animals that have been screened to ensure sexual competence and then observe these subjects through a fixed number of copulatory series, the most relevant prior effects of oxotremorine are the decreases in intromission frequency and ejaculation latency that this drug consistently has caused in male rats (Ahlenius and Larsson, 1985; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997).

3. Methods

3.1. Animals and drug treatments

Complete data were collected from 13 adult male golden hamsters (*Mesocricetus auratus*, LVG:Lak outbred strain) that averaged 149.5 g in weight (SEM = 5.0) at the time of their first behavioral test. Stimuli included 9 adult females, each of which was bilaterally ovariectomized 1 month before the start of testing. Each animal was housed in a 34×18×18 or 31×21×21 cm stainless steel cage in a colony maintained at 20–25 °C and on a reversed 14:10 light:dark cycle. All had free access to food and water except during behavioral tests. Conditions of housing and all experimental procedures were approved by Bucknell University's Institutional Animal Care and Use Committee.

Each of the stimulus females was ovariectomized under sodium pentobarbital anesthesia (65 mg/kg, intraperitoneal (ip)) supplemented by a subcutaneous (sc) injection of 0.4 mg of the analgesic butorphanol tartrate (both from Henry Schein, Inc). To ensure sexual responsiveness during behavioral tests, each female 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 10 μg of estradiol benzoate and the second at 4–6 h before use and containing 500 μg of progesterone (both from Steraloids, Inc).

Each male received two ip injections shortly before each behavioral test. The first occurred at 45 min pretest and contained 1 mg/kg of methylscopolamine (scopolamine methyl bromide, Sigma-Aldrich, Inc) in a volume of physiological saline equal to (body weight)/1000 ml. The second was administered 15 min later (30 min pretest) and contained 0, 0.2 or 0.4 mg/kg of oxotremorine (oxotremorine sesquifumarate, Sigma-Aldrich) in the same vehicle and volume. These doses are comparable to those that in past work on rats have affected copulation without preventing it in any significant fraction of the population (Ahlenius and Larsson, 1985; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997). Whereas methylscopolamine treatments were held constant over tests, the dose of oxotremorine was varied within-subject over a series of 6 tests at weekly intervals. To determine treatments on the first 3 tests, the 6 possible orders of treatment were randomly assigned to subjects with the constraint that each be equally represented to the extent possible (2–3 subjects/order). For each subject, the second series of 3 tests duplicated the first. Tests were staged and scored without knowledge of the drug treatment.

3.2. Behavioral tests

Each test began with the introduction of a male into a 40×20×25 cm glass aquarium. After 1–2 min of adaptation, a female was presented, the timing of the encounter beginning with the first social contact. 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 copulatory behavior in encounters of this length (e.g., see Arteaga-Silva et al., 2005; Bunnell et al., 1977; Dewsbury et al., 1979; Miernicki et al., 1990 for previous descriptions of male behavior in hamsters). This set includes 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 social 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).

As suggested previously, most of these elements and measures were defined in standard ways (e.g., as in Arteaga-Silva et al., 2005; Bunnell et al., 1977; Dewsbury et al., 1979; Miernicki et al., 1990). However, we departed from some previous approaches in several ways. First, we did not confirm penile insertion or sperm transfer and instead relied entirely on overt patterns of pelvic thrusting and hindlimb movement to distinguish mounts, intromissions and ejaculations. Second, to reduce the chances of mistaking a failure to detect the female for a disinclination to initiate copulation, 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 changes do create methodological differences between this and some previous studies, there is good reason to think that these definitions are appropriate and valid, and that even this entire set of changes is likely to have had little impact on the results (see further discussion in Floody (2011)). Further, appropriate analyses suggest very high levels of intra- and inter-observer reliability for these measures and procedures (Floody, 2011).

3.3. Analysis

Our analyses distinguished the likelihood of failing a behavioral test (failing to intromit within 10 min, ejaculate within 15 min or complete 2 copulatory series within 20 min) from the quality of behavior on successful tests. Treatments were compared for possible effects on the likelihood of failure, and on the incidences of intromission and ejaculation, using the Cochran Q and sign tests (Siegel, 1956).

Effects on other measures were assessed by analysis of variance (ANOVA), using logarithmic transformations as necessary to reduce heterogeneity of variance. For the 2 measures that relate to a mating encounter as a whole rather than a specific 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 second within-subjects factor.

Throughout this project, 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 Dose main effect or Dose × Series interaction (if the effect was 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 the dose effect revealed a reliable linear contrast but no reliable quadratic effect, we inferred an orderly dose-related drug effect on the relevant measure. This approach is consistent with recent thought in statistics (e.g., Howell, 2010; Keppel and Wickens, 2004) and requires no post hoc comparisons of individual means. To be complete, however, we also have followed each reliable ANOVA main effect or interaction with appropriate post hoc tests. Specifically, we have compared the response to each drug dose with that to the control (placebo) treatment using Dunnett's test and have compared the two drug responses to each other using Tukey's test (Winer, 1971). The results of these supplementary comparisons are provided just in the tables and figures, using asterisks to identify reliable differences. As in all of the analyses reported here, a probability of 0.050 was used to define reliability and effects with a probability between 0.051 and 0.070 were considered to have approached significance.

In addition to examining individual measures, we considered the possibility of drug effects on the coherent sets of measures revealed by factor analysis (Floody, 2011). This analysis described 5 factors or conceptual variables, each of which seems to represent a possible target of drug action. One was defined mainly by ML, IL and PEI-1, and thus seems to relate to the Initiation of copulation. Two others seem to relate more closely to the efficiency of copulatory performance. The measures that clustered most strongly here did so by copulatory series, so that efficiency in the first series (Efficiency-1) was defined largely by the combination of MF-1, III-1, IR-1 and EL-1, while the corresponding factor for series 2 (Efficiency-2) was defined mainly by MF-2, IR-2 and III-2. The clusters defining the last 2 factors seemed to revolve around the 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) depended most heavily on IF-2, EL-2 and PEI-2.

Though drug effects on these factors could be estimated from changes in the individual dependent variables, the number of variables that define each factor inevitably would limit the quality of these estimates. Therefore, to objectively assess drug effects on the conceptual variables, we calculated the factor scores corresponding to each. Each factor score is a unitless composite measure that combines *all* measures, including both those that loaded strongly and those that loaded weakly on the factor in question. After having been converted to standard scores, each individual measure is multiplied by the coefficient describing the strength of the relationship between it and the relevant factor. Once this has been done for each measure, the resulting weighted scores are summed to yield the factor score for that factor and condition. For example, consider the calculation of the Initiation factor score for one animal and condition. A prior factor analysis (Floody, 2011) tells us the loadings on this factor of *each* of the 14 standard measures of male copulatory behavior. For example, the loadings of ML and EL-1 on the Initiation factor approximated 0.92 and 0.15, respectively. Accordingly, the 14 values that were summed in calculating an animal's Initiation score for a particular condition included the appropriate ML standard score multiplied by 0.92 and the appropriate EL-1 standard score multiplied by 0.15 (along with 12 other standard scores, each representing a different measure and each weighted by the appropriate loading). Once calculated in this way, factor scores were subjected to ANOVA using drug Dose as a within-subjects factor.

4. Results

Whereas no male failed either of his tests after placebo treatment, 24% of the population failed to complete one of their 2 tests after the oxotremorine treatment of 0.2 mg/kg and 46% failed one of the tests after the 0.4 mg/kg treatment. The comparison of the incidence of such failures across all 3 treatments revealed a reliable drug effect ($Q(2) = 7.71$, $p < 0.05$, Cochran test, 2-tailed). Further analysis suggests that this is due to a reliable difference between the extreme treatments, the higher oxotremorine dose causing more failures than the placebo ($p = 0.032$, sign test, 2-tailed). Though tests could be failed in several ways, most of these (56%) involved a failure to achieve intromission and all but one (89%) involved a failure to achieve ejaculation. Therefore, the effects on failure seem likely to reflect drug-related decreases in the incidence of intromission and ejaculation.

In addition to affecting the likelihood of a successful test, oxotremorine treatment affected performance on these positive tests. Such effects are suggested, first, by reliable Dose main effects on ML, IL and PEI ($F(2,24) = 5.40$, 4.20 and 9.28, respectively; $ps = 0.012$, 0.027 and 0.001; Fig. 1). For each of these, dose-related

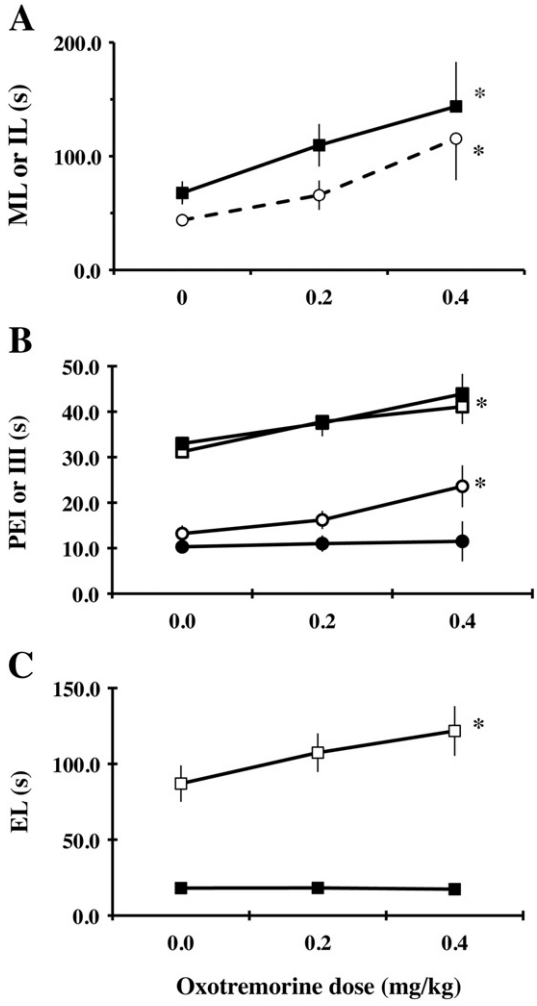

Fig. 1. Panel A describes the mean (and SEM) mount latencies (ML, open circles connected by dashed lines) and intromission latencies (IL, filled circles and solid lines) observed after treatment with oxotremorine doses of 0, 0.2 or 0.4 mg/kg. Panel B does this for postejaculatory intervals (PEI, squares) and interintromission intervals (III, circles), and Panel C does the same for ejaculatory latency (EL). In each of the lower 2 panels, scores in the first copulatory series are described by open symbols and those in the second series are described using filled symbols. The application of ANOVA to levels of ML, IL and PEI revealed reliable Dose main and linear effects on each. Analyses of III and EL revealed reliable Dose × Series interactions, reflecting drug effects confined to the first copulatory series and thus to III-1 and EL-1. In each of these cases, there was a reliable linear effect of Dose for that series. Asterisks indicate scores that differ reliably from those observed after placebo treatment ($p<0.05$, Dunnett's test).

increases in latency or interval are supported by significant linear effects of Dose ($F(1,12)\geq 7.46$, $p\leq 0.018$), supporting orderly dose-related effects of oxotremorine on each measure.

Additional drug effects are suggested by reliable Dose × Series interactions affecting EL and III ($F(2,24)=4.71$ and 3.51, respectively; $ps=0.019$ and 0.046). To clarify these, separate ANOVAs were used to examine the effects of drug treatment on performance in each copulatory series. In each case, reliable drug effects were confined to the first series ($F(2,24)=3.77$ and 4.07 for EL and III, respectively; $ps=0.038$ and 0.030). At this time, dose-related increases in each measure are supported by significant linear effects of Dose ($F(1,12)=5.67$ and 5.21 for EL and III, respectively; $ps=0.035$ and 0.042; Fig. 1).

Taken together, these drug effects on individual measures suggest effects on the conceptual variables relating to the initiation of copulation and the efficiency of performance in the first copulatory series. Analyses of factor scores confirmed these suggestions. These revealed reliable Dose effects on each of these factors ($F(2,24)=9.41$, $p=0.001$ for Initiation; $F(2,24)=4.18$, $p=0.028$ for Efficiency-1). Additional evidence for dose-related increases in each factor score (corresponding to increasing delays in initiation and decrements in efficiency) is provided by reliable linear Dose effects on each ($F(1,12)=15.95$, $p=0.002$ for Initiation; $F(1,12)=6.21$, $p=0.028$ for Efficiency-1; Fig. 2).

Though the focus of this report is on responses to cholinergic drugs, the performance of subjects also varied as a function of copulatory series. Two series effects modulated the impacts of oxotremorine on EL and III and have already been described. For several other measures, copulatory series affected performance independently of drug treatment, resulting in reliable main effects of Series. Combining these subsets, the entire set of measures with reliable Series main effects includes EL, III, MF, IF and IR ($F(1,12)\geq 8.95$, $p\leq 0.011$). In each case, the quality of performance increased over series, signaled by reliable decreases in EL, III, MF and IF, and a reliable increase in IR (see uppermost section of Table 1).

5. Discussion

These results suggest reliable effects of systemic oxotremorine treatment on the incidence and quality of male mating behavior in hamsters. Such treatments increased the likelihood that males would fail to mate. In most cases, such failures reflected decreases in the incidence of intromission or ejaculation. In addition, oxotremorine disrupted performance even in completed tests. The most reliable of these changes were increases in ML and PEI, but less consistent increases also were observed in IL, EL-1 and III-1. These individual changes may reflect more global effects on 2 of the 5 conceptual

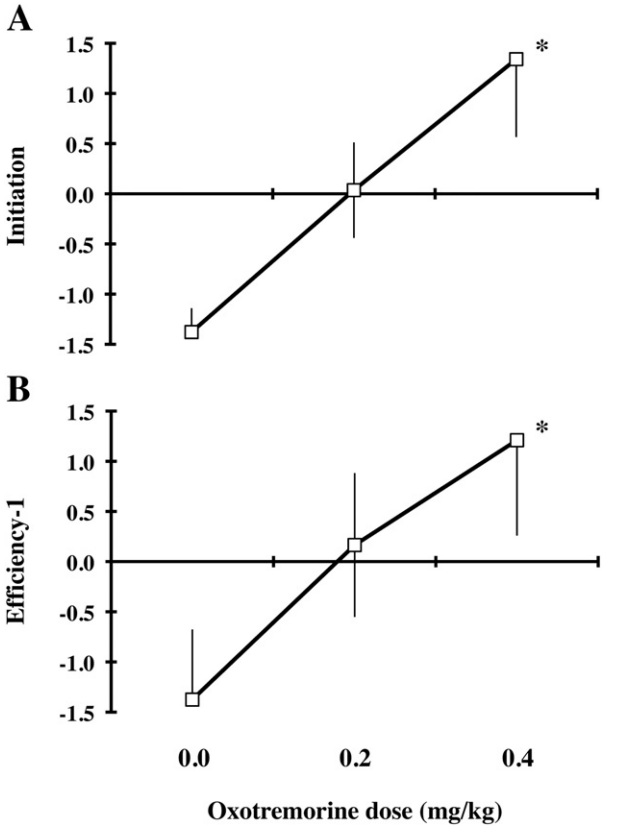

Fig. 2. The factor analysis of hamster mating patterns revealed 5 conceptual variables, including one related to the initiation of copulation (Initiation) and one related to the efficiency of performance in the first copulatory series (Efficiency-1). The impact of oxotremorine treatment on Intiation (panel A) and Efficiency-1 (panel B) is described here using mean and SEM factor scores calculated as described in the text. The analysis of each of these variables revealed reliable Dose main and linear effects. Asterisks indicate scores that differ reliably from those observed after placebo treatment ($p<0.05$, Dunnett's test).

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

Measure	Series 1	Series 2
After systemic placebo or oxotremorine treatment (Experiment 1)		
MF	2.9 (0.5)	0.2 (0.1)
IF	6.7 (0.6)	1.6 (0.1)
IR	0.72 (0.04)	0.95 (0.02)
III	17.7 (2.2)	10.9 (0.7)
EL	105.4 (11.7)	17.9 (2.2)
After systemic placebo or methylscopolamine treatment (Experiment 2)		
MF	2.3 (0.4)	0.3 (0.1)
IF	7.0 (0.6)	1.7 (0.2)
IR	0.79 (0.03)	0.93 (0.03)
III	12.2 (1.1)	8.9 (0.5)
EL	82.8 (7.6)	15.2 (2.1)
After systemic placebo or scopolamine treatment (Experiment 2)		
MF	2.8 (0.5)	0.7 (0.2)
IF	9.4 (1.1)	2.3 (0.3)
EL	114.8 (12.9)	25.3 (4.3)

Notes: See text for definitions and units. For each of these measures, the main effect of copulatory series was significant.

variables that seem to organize male behavior in hamsters. Specifically, oxotremorine reliably slowed the initiation of copulation and also decreased the efficiency of performance in the initial copulatory series. Importantly, all of these effects seem likely to reflect changes in central cholinergic activity: All experimental treatments were preceded by methylscopolamine treatments that were held constant at levels considerably above those of oxotremorine itself, and that therefore should have been adequate to negate peripheral responses to the agonist.

These effects differ in several important respects from recent descriptions of the responses of male rats to oxotremorine (Ahlenius and Larsson, 1985; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997). In particular, the two sets of results differ in the identity of the individual measures affected, the identity of the conceptual variables that were affected and may mediate effects on individual measures, and the directions of these influences. Each of these differences will be discussed in turn.

First, the specific behavioral elements that have been affected most consistently in rats are EL-1 and IF-1. Consequently, EL-1 has been shown to respond to oxotremorine treatment in both rats and hamsters. In contrast, IF-1 has been affected only in rats whereas ML, IL, PEI and III-1 were affected just in hamsters. This suggests some species similarities, but a larger number of species differences, in the individual elements of copulatory behavior that are subject to cholinergic control.

Second, effects that extend across multiple measures raise the possibility of changes in broader aspects of behavior, such as the conceptual variables revealed by factor analysis. For the present purposes, the most relevant study of rats is that of Dewsbury (1979), which describes interactions extending over multiple copulatory series. This revealed several factors, including an Intromission count factor defined largely by the combination of EL and IF. This clearly is compatible with the effects of oxotremorine on individual behavioral elements in rats. In turn, this coincidence suggests that ACh in rats may specialize in the control of this aspect of mating behavior. This interpretation resembles earlier analyses that used oxotremorine's impact on EL and IF to suggest a cholinergic focus on the control of the ejaculatory threshold (Ahlenius and Larsson, 1985; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997).

In hamsters as in rats, there seems to be some consistency between the clusters of measures affected by oxotremorine and those linked by factor analysis to specific aspects of male behavior. However, the affected clusters seem to differ across species. For hamsters, these results suggest the partial cholinergic control in hamsters of behavioral efficiency, at least early in a sexual interaction. In addition, they suggest cholinergic involvement in the mechanism that initiates copulation, a relationship consistent with oxotremorine's effects on both Initiation factor scores and the incidence of intromission. Mating behavior in male hamsters depends heavily on chemosensory processing (Murphy and Schneider, 1970; Powers and Winans, 1975), suggesting that some part of oxotremorine's effect on Initiation could be mediated by effects on chemoinvestigatory behavior. However, the likelihood of this is unknown: Such behaviors have not been included in the relevant factor analytic studies and I am not aware of any studies assessing their dependence on ACh.

Even on their own, the contrasting factor structures described for male rats and hamsters suggest that it would be inappropriate to interpret drug effects in either species in terms of the factor structures of the other. The results of this and past studies of responses to oxotremorine reinforce this point, suggesting significant neurochemical differences between the mechanisms controlling copulatory behavior in these species.

Third, and perhaps most importantly, the effects of oxotremorine treatment on mating behavior in male rats and hamsters differ in direction. In rats, oxotremorine tends to facilitate performance (Ahlenius and Larsson, 1985; Retana-Marquez et al., 1993; Retana-Marquez and Velazquez-Moctezuma, 1997) whereas similar treatments produced disruptive effects in hamsters. The reason for this difference is unclear. It could reflect a straightforward species difference in the role played by ACh in the brain system controlling mating behavior. Such a difference could be viewed as a simple extension of the other species differences described earlier in this section. However, at least two other interpretations of this difference seem possible.

One of these focuses on the receptors that mediate oxotremorine's effects. Though this drug is thought to interact with all five types of muscarinic receptor, there is some suggestion of a special affinity for the M2 subtype (Bräuner-Osborne and Brann, 1996; also see Sánchez et al., 2009; Thomsen et al., 2010). Further, there is evidence suggesting that M2 receptors are especially likely to be located presynaptically, where they can decrease ACh synthesis or release when activated (Gattu et al., 1997; Levey et al., 1991; Murakami et al., 1996; Vilaró et al., 1994, 1992). These observations raise the possibility that oxotremorine could, perhaps at just some sites in the brain, act to decrease net levels of cholinergic activity. In turn, this could provide a way of reconciling behavioral disruption by oxotremorine with the existence of a cholinergic system that facilitates the same behavior. This suggestion resembles one interpretation of the sometimes opposed effects on rat mating behavior of low and high doses of the dopamine agonist apomorphine (reviews in Bitran and Hull, 1987; Meisel and Sachs, 1994). To be effective here, however, this suggestion also requires a species difference of some sort, perhaps in the brain systems in which M2 receptors (or autoreceptors) predominate.

Alternatively, it is possible that rats and hamsters differ in the regulation of central cholinergic activity, with basal levels in hamsters more closely approximating those that are optimal for mating behavior. By this argument, changes in cholinergic activity in rats might be able to move the system controlling sexual behavior toward or away from its optimum, whereas the same manipulations in hamsters would uniformly cause disruptive changes of one sort or another (from near-optimal to excessively low or high levels; cf., Bitran and Hull, 1987; Leavitt, 1969). It is the case that a comparison of sexual behavior in male rats and hamsters reveals several instances of much more rapid performance in the latter (Floody, 2011). However, if hamsters thereby approach ceiling levels of performance, they seem not to actually attain them, since further gains can be achieved after some drug treatments (Arteaga et al., 2002).

Finally, all of these interpretations assume that comparable drug treatments in hamsters and rats present the same cholinergic stimulus to behavioral mechanisms that differ across species. However, all drug effects depend on a variety of pharmacokinetic

and pharmacodynamic factors that determine a drug's absorption, distribution, elimination and impact (Bourne, 1998; Gringauz, 1997; Holford and Benet, 1998). Few direct comparisons of such processes in hamsters and rats seem to exist and I am not aware of any suggesting consistent species differences in the processing of cholinergic drugs (Chen et al., 1945; Kajbaf et al., 1992). Nevertheless, it is possible that pharmacokinetic or pharmacodynamic differences could enter into the species-specific responses to oxotremorine and the other cholinergic drugs that are a focus of this report.

6. Experiment 2

The results of Experiment 1 suggest that mating behavior in male hamsters is disrupted by the muscarinic receptor mimic oxotremorine. The fact that these changes were observed in animals pretreated with the peripheral antagonist methylscopolamine suggests that they were mediated by central muscarinic receptors and mechanisms.

As previously explained, this disruption of behavior could reflect increased activity in a cholinergic system that normally inhibits elements of sexual behavior in male hamsters. Alternatively, it could reflect change in a system that depends on a specific level of activity for effective behavior: In such a system, changes in activity in either direction could produce disruptive effects. By the first of these interpretations, treatment with a cholinergic antagonist such as scopolamine should facilitate mating behavior in hamsters. By the second, such treatment should disrupt this behavior.

To test these predictions, and further assess the cholinergic control of mating behavior in hamsters, we observed the behavioral impact of systemic scopolamine treatment. In order to highlight centrally-mediated effects, we also observed responses to the similar, but exclusively peripheral, receptor blocker methylscopolamine. For behavioral elements that are affected only by scopolamine, it would seem reasonable to attribute drug effects to changes in central cholinergic mechanisms (Klinkenberg and Blokland, 2010).

7. Methods

7.1. Animals and treatments

Data were collected from 16 adult male hamsters that averaged 149.6 g in weight (SEM = 2.7) at the time of their first behavioral test. Eight of these also served as subjects in Experiment 1. However, preliminary analyses confirmed that this variable had no impact on responses to drug treatment. Stimuli included 11 ovariectomized females, 5 of which also served in this capacity in the previous study. All animals were housed and maintained as previously described.

Stimulus females were brought into hormone-induced estrus by a series of estrogen and progesterone treatments similar to that used in Experiment 1. Each male received a single ip injection 45 min before each of 5 weekly behavioral tests. In each case, the vehicle was saline and the volume was the male's (body weight)/1000 ml. Otherwise, the contents of this injection varied, yielding 5 experimental treatments: saline only (placebo treatment), 0.2 or 0.5 mg/kg of methylscopolamine, 0.2 or 0.5 mg/kg of scopolamine (scopolamine hydrochloride, Sigma-Aldrich). These drug doses were selected to resemble the scopolamine treatments emphasized in previous studies (Ahlenius and Larsson, 1985; Bignami, 1966; Leavitt, 1969; Retana-Marquez et al., 1993). The order of treatment was counterbalanced across subjects.

7.2. Behavioral tests and analysis

Behavioral tests were conducted and scored as in Experiment 1. Likewise, the initial step in the statistical analysis focused on the incidence of test failures. However, this immediately revealed treatment effects with implications for all subsequent analyses. Whereas all 16 males completed both tests preceded by methylscopolamine treatment, only 11 completed both scopolamine tests. Because of this and the fact that there was just a single placebo test, the analysis of performance on successful tests followed 3 steps. First, the impact of methylscopolamine treatment was assessed using all 16 males and ANOVAs with Dose (ML, IL) or Dose and copulatory Series (all other measures) as within-subject factors. Second, the impact of scopolamine treatment was assessed in the same way but using just the data from the 11 males that completed this series of tests. Third, for measures that were affected reliably by both drugs, the relative magnitudes of these effects were assessed in the same 11 males using ANOVAs that were limited to the drug tests (excluding those after placebo treatment) and treated Drug (methylscopolamine, scopolamine), Dose (0.2, 0.5 mg/kg) and Series as within-subject factors.

8. Results

8.1. Responses to methylscopolamine

As indicated earlier, methylscopolamine treatment had no impact on the incidence of mating behavior. However, ANOVA revealed a reliable Dose main effect on III ($F(2,30) = 4.97$, $p = 0.014$). Further support for a dose-related increase in III is provided by a reliable linear effect of Dose on this measure ($F(1,15) = 7.42$, $p = 0.016$; Fig. 3A). ANOVA also revealed a reliable Dose × Series interaction affecting EL ($F(2,30) = 4.09$, $p = 0.027$). Further analysis confirmed the restriction of the drug effect to the first copulatory series, in which a dose-related increase in EL is supported by a reliable linear effect ($F(1,15) = 6.24$, $p = 0.025$; Fig. 3B). These changes were not sufficient to reliably alter the factor scores for the relevant conceptual variables implicated in the organization of hamster behavior.

In addition to these drug effects, performance in tests after placebo or methylscopolamine treatment revealed many of the expected facilitory effects of copulatory series. The modulation of methylscopolamine's effect on EL has been described. Reliable main effects of series also were common, affecting III, MF, IF and IR, all as in the first study ($F(1,15) \geq 19.04$, $p \leq 0.001$; middle section of Table 1).

8.2. Responses to scopolamine

As previously suggested, scopolamine treatment reduced the incidence of sexual behavior. At the same time, this effect was confined to the higher drug dose and a minority of the population. No subject failed his placebo test and only 1 (6%) failed after treatment with 0.2 mg/kg of scopolamine. However, 5 males (31%) failed tests at the higher, 0.5 mg/kg, dose of this drug. The comparison of all 3 doses revealed a reliable drug effect ($Q(2) = 8.40$, $p < 0.02$, Cochran test, 2-tailed). Further analysis suggested that this effect was due largely to the higher scopolamine dose by revealing a nearly significant difference between the incidence of failure after it as opposed to the placebo treatment ($p = 0.062$, sign test, 2-tailed). In addition, these analyses linked test failures to drug-related decreases in the incidences of both intromission and ejaculation ($Q(2) \geq 6.50$, $p < 0.05$, Cochran test, 2-tailed).

Beyond affecting the completion of tests by some males, scopolamine affected the performance of the 11 males that completed all of the tests in this series. Such effects are suggested, first, by reliable Dose main effects on III and IR ($F(2,20) = 6.72$ and 4.05, respectively; $ps = 0.006$ and 0.033). For each, dose-related changes (increases in III and decreases in IR) are supported by significant linear effects ($F(1,10) = 12.60$ and 23.08 for III and IR, respectively; $ps = 0.005$ and 0.001; Fig. 3A). In addition, ANOVA revealed a Dose main effect on MF that approached significance ($F(2,20) = 3.40$, $p = 0.054$). This was accompanied by a highly reliable linear Dose effect on the same measure ($F(1,10) = 10.47$, $p = 0.009$). Together, these suggest an orderly increase in MF with increasing scopolamine dose (Fig. 4A).

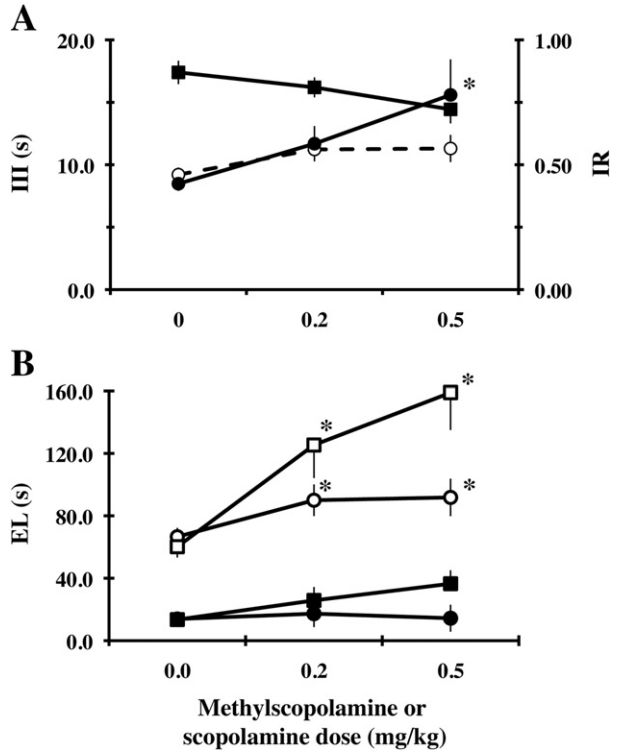

Fig. 3. Panel A describes the mean (and SEM) interintromission intervals (III, circles) and intromission ratios (IR, squares) observed after treatment with methylscopolamine (open circles, describing just effects on III) or scopolamine (filled squares, describing effects on both measures) doses of 0, 0.2 or 0.5 mg/kg. Note that the scale for IR is on the right. Each of these 3 drug effects was reliable and accompanied by a reliable linear effect of Dose. Because each of the initial effects was a main effect of drug treatment, the scores depicted here have been averaged across copulatory series. Panel B describes the ejaculatory latencies (EL) observed after treatment with methylscopolamine (circles) or scopolamine (squares) for both the first and second copulatory series (open and filled symbols, respectively). For each of these treatments, analysis revealed a reliable Dose × Series interaction, reflecting drug effects confined to the first copulatory series. For each treatment, further analysis revealed a reliable linear effect of Dose for that series. Asterisks indicate scores that differ reliably from those observed after placebo treatment ($p<0.05$, Dunnett's test). The asterisk in Panel A refers to the levels of III observed after scopolamine treatments of 0.5 mg/kg.

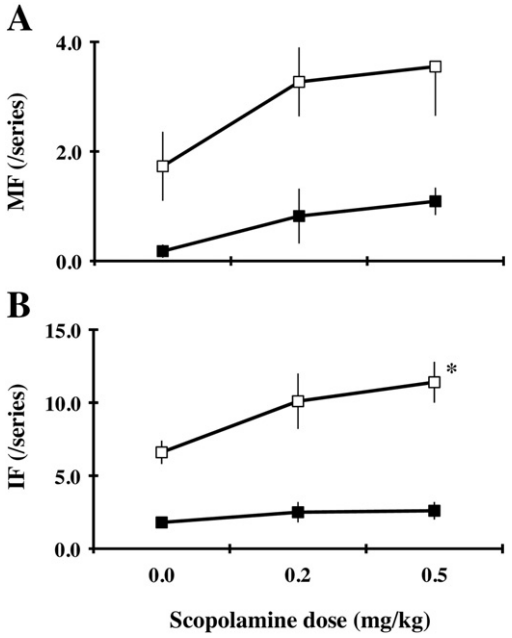

Fig. 4. Panels A and B describe the impact of scopolamine treatment on mean (and SEM) levels of mount frequency (MF) and intromission frequency (IF), respectively. In each case, scores in the first copulatory series are described by open symbols and those in the second series are described using filled symbols. The analysis of MF revealed a nearly reliable Dose main effect and a reliable linear effect of Dose. That of IF revealed a reliable Dose × Series interaction, reflecting a drug effect confined to the first copulatory series. A drug effect at that time is supported by a reliable linear effect of Dose for that series. Asterisks indicate scores that differ reliably from those observed after placebo treatment ($p<0.05$, Dunnett's test).

Further effects of scopolamine treatment were indicated by reliable Dose × Series interactions affecting EL and IF ($F(2,20)=8.97$ and 6.46, respectively; $ps=0.002$ and 0.007). To clarify these effects, separate ANOVAs examined the effects of drug treatment on performance in each copulatory series. In each case, reliable drug effects were confined to the first series ($F(2,20)=8.80$ and 4.43 for EL and IF, respectively; $ps=0.002$ and 0.026), for which dose-related increases in each measure are supported by significant linear effects ($F(1,10)=19.09$ and 14.44 for EL and IF, respectively; $ps=0.001$ and 0.003; Figs. 3B and 4B).

As these results show, EL and III were reliably extended by treatment with either methylscopolamine or scopolamine. This raises the issue of the relative magnitudes of these effects. This was addressed by ANOVAs that directly assessed the impacts of Drug identity (methylscopolamine, scopolamine) and Dose (0.2, 0.5 mg/kg) for the 11 subjects that completed all of the relevant tests. Neither of these analyses distinguished copulatory series. Because both of the effects on EL were confined to the first series, only the data from it were included in the new analysis. Because both of the earlier drug effects on III were main effects, the scores on this measure were averaged across copulatory series prior to further analysis. Each of these analyses revealed a reliable main effect of Drug identity ($F(1,10)=29.86$ and 5.31 for EL and III, respectively; $p<0.001$ for EL, $p=0.044$ for III). In each case, these reflect a greater extension of the relevant interval by scopolamine (Fig. 5).

Taken together, these responses to scopolamine seem consistent with the organization of male behavior in hamsters suggested by factor analysis (Floody, 2011). In particular, the individual measures affected by scopolamine agree perfectly with those linked by factor analysis to the efficiency of performance in the first copulatory series (Efficiency-1), efficiency in the second series (Efficiency-2), and the impact of intromissions in the first series (Intromissions-1). It may not be surprising, then, that analyses of factor scores confirmed reliable scopolamine Dose effects on each of these factors ($F(2,20)=5.65$, 4.49 and 3.69 for Efficiency-1, Efficiency-2 and Intromissions-1, respectively; $ps=0.011$, 0.025 and 0.043). More unexpected was a nearly reliable Dose effect on Intromissions-2 ($F(2,20)=3.15$, $p=0.064$), suggesting an additional conceptual variable responsive to scopolamine. For all of these, orderly dose-related increases in factor score (suggesting drug-induced declines in the quality of behavior) were supported by highly reliable linear Dose effects ($F(1,10)=21.33$, 11.61, 11.22 and 12.25 for Efficiency-1, Efficiency-2, Intromissions-1 and Intromissions-2, respectively; each $p\leq0.007$; Fig. 6).

Finally, as has been true throughout this project, effects of scopolamine treatment were accompanied by effects of copulatory series. The modulation of the drug effects on EL and IF already has been described. A reliable main effect involving consistent reductions in MF across series also was apparent ($F(1,10)=19.52$, $p<0.001$, lower section of Table 1).

9. Discussion

This study compared responses to methylscopolamine and scopolamine so as to isolate the consequences of blocking central muscarinic receptors. If and when methylscopolamine and scopolamine affect a behavior to the same degree, it would seem logical to attribute the effect to their shared ability to block peripheral muscarinic receptors. At the opposite extreme, changes effected just by scopolamine would seem attributable to this drug's unique ability to cross the blood–brain

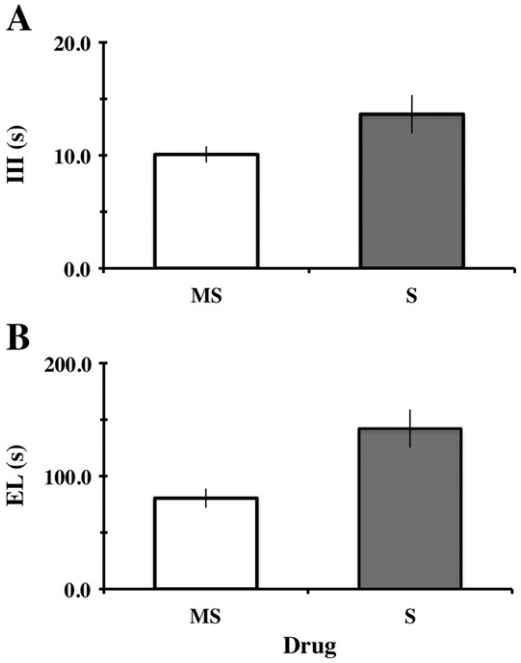

Fig. 5. Panels A and B describe the impact of methylscopolamine (MS) and scopolamine (S) on mean (and SEM) interintromission intervals (III) and ejaculation latencies (EL), respectively. For reasons explained in the text, these III scores were averaged across copulatory series while these EL scores were restricted to the first series. For each measure, analysis revealed a reliable main effect of drug treatment. Because both were main effects, the scores described here for each drug have been averaged across its 0.2 and 0.5 mg/kg doses.

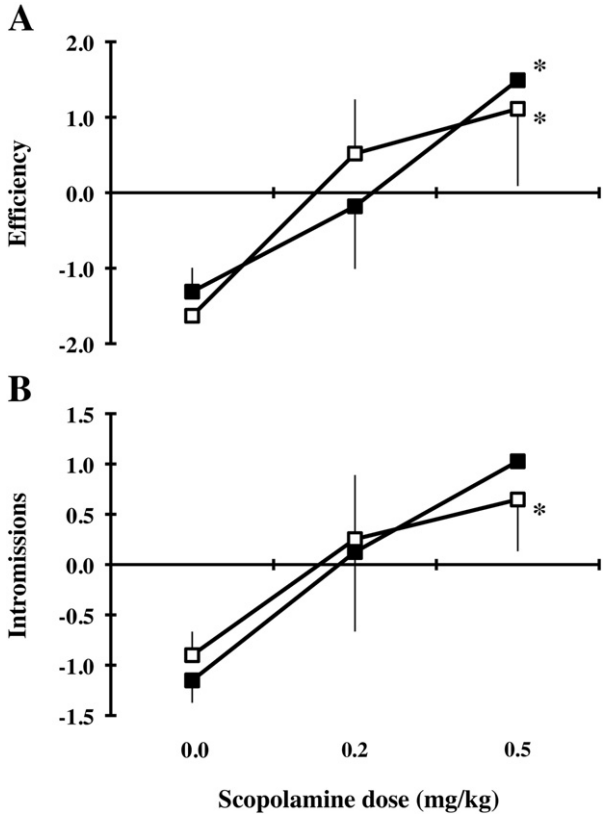

Fig. 6. The factor analysis of hamster mating patterns revealed conceptual variables related to the efficiency of performance in the first and second copulatory series (Efficiency-1, Efficiency-2) and revolving around the role of intromissions in these series (Intromissions-1, Intromissions-2). The impact of scopolamine treatment on Efficiency (panel A) and Intromissions (panel B) is described here using mean and SEM factor scores calculated as described in the text. In each case, the factor related to the first copulatory series is described using open symbols and that for the second series is described using filled symbols. The analysis of Intromissions-2 revealed a nearly reliable Dose effect and a reliable linear effect of Dose. For each of the other factors, ANOVA revealed reliable effects of each of these types. Asterisks indicate scores that differ reliably from those observed after placebo treatment ($p<0.05$, Dunnett's test).

barrier and block central, as well as peripheral, muscarinic receptors (Klinkenberg and Blokland, 2010). More difficult to interpret is a case of partial overlap, in which methylscopolamine produces some fraction of the response to scopolamine. Even such effects would seem to depend at least partly on central responses to scopolamine, though the full behavioral effect could reflect an interaction of central and peripheral cholinergic effects.

The results presented here suggest that scopolamine disrupts mating behavior in male hamsters, sometimes to the extent of preventing intromission or ejaculation. However, only a minority of tests were affected to this extent. More commonly, scopolamine's disruptive effects took the form of quantitative changes in performance. The most reliable of these included a decrease in IR that extended across copulatory series and increases in EL and IF that were specific to the first series (EL-1, IF-1). In addition, scopolamine-treated animals tended to show increases in III and MF relative to controls. Of these changes in male behavior, most would seem clearly attributable to central actions of scopolamine. The only exceptions are the effects on EL and III, which could reflect some interaction of central and peripheral effects.

As was true for oxotremorine, these effects in hamsters can be compared to those in rats on each of several levels, including those of the individual measures and conceptual variables affected, and the directions of the behavioral change. With respect to the first, studies of responses to scopolamine in rats disagree on the aspects of behavior affected, the extents of the effects, and the doses required to produce them. A study by Ahlenius and Larsson (1985) establishes scopolamine's ability to prevent copulation, but found this only at doses of 0.8 mg/kg or more and observed no quantitative changes in performance in positive tests at doses up to 3.2 mg/kg. In contrast, other studies report much larger effects on the incidence of mating, and at much lower scopolamine doses (Bignami, 1966; Leavitt, 1969; Retana-Marquez et al., 1993). In some cases, these changes were sufficient to prevent any further analysis of performance. In others, quantitative analyses were possible and suggested drug-related increases in ML and IL, along with a decrease in ejaculation frequency (Bignami, 1966; Retana-Marquez et al., 1993). Yet none of these studies reports both a positive response to scopolamine and a negative one to methylscopolamine (i.e., a reliable effect limited to the first). To a large extent, the attribution of scopolamine's effects to central cholinergic changes seems based on the failure of Ahlenius and Larsson to observe a reliable response to methylscopolamine. However, the same authors also saw little or no response to scopolamine except at very high doses. Some additional evidence bearing on the localization of scopolamine's effects was provided by Bignami (1966), who compared the responses to several cholinergic antagonists and suggested a direct relation between their behavioral impacts and their abilities to act centrally.

On balance, these results suggest that scopolamine reduces intromission and ejaculation incidence in both hamsters and rats. Nevertheless, the extents of these effects seem to differ across species: Whereas Retana-Marquez et al. (1993) observed intromission in just 20% of the rats they treated with 0.4 mg/kg of scopolamine, this behavior appeared in 75% of the hamsters that we exposed to a slightly higher dose of the same drug (0.5 mg/kg; $p=0.021$, Fisher test, 2-tailed). Consistent with this difference were others in the individual measures affected by scopolamine on successfully-completed behavioral tests. In rats, the best-supported drug effects include increases in ML and IL (Bignami, 1966; Retana-Marquez et al., 1993). In hamsters, they include decreases in IR and increases in III, EL-1, IF-1 and possibly MF. Together, these results emphasize species

differences in the elements of behavior affected by scopolamine. At the same time, the difference in the incidence of copulation complicates this issue by possibly obscuring other effects that rats might exhibit under conditions permitting more thorough testing.

On the level of conceptual variables, all of scopolamine's effects on mating in rats suggest cholinergic control of the initiation of this behavior. This seems consistent with the link between ACh and sexual arousal that originally was proposed on the basis of these data (Retana-Marquez et al., 1993). In hamsters, the responsiveness of initiation to scopolamine is less clear. Such a change is suggested by the drug effect on the incidence of intromission but questioned by the data from positive tests, which suggest Initiation as the only conceptual variable able to resist scopolamine treatment. Further, scopolamine's effects on the efficiency of performance and the impact of intromissions in each copulatory series (Efficiency-1 and -2, Intromissions-1 and -2) suggest unambiguous differences between the neurochemical mechanisms controlling mating behavior in rats and hamsters. They also suggest that ACh exerts an influence over mating behavior in male hamsters that is both powerful and pervasive, affecting all or nearly all of its aspects or dimensions.

Finally, studies of rats and hamsters agree on the direction of the change in mating behavior produced by scopolamine: All reliable effects described here or previously have been disruptive or inhibitory (Ahlenius and Larsson, 1985; Bignami, 1966; Leavitt, 1969; Retana-Marquez et al., 1993). Changes of this sort could reflect reduced activity in a cholinergic system that exerts a net facilitory influence over copulatory behavior. Alternatively, they could reflect a departure in cholinergic activity from the levels required for effective performance. However, they seem unlikely to reflect effects that are concentrated on M2 autoreceptors and have the ultimate effect of increasing ACh release and causing an increase in postsynaptic cholinergic activity that goes on to inhibit behavior: No evidence that I know of suggests a special affinity of scopolamine for M2 receptors, let alone M2 autoreceptors (e.g., see Ehlert and Tran, 1990; Pilar-Cuéllar et al., 2008). These considerations do not completely resolve the issue of how ACh relates to mating behavior in hamsters but they do strengthen the case for a net facilitory influence, as has been suggested to operate in rats.

10. Conclusions

On the levels of both individual measures and conceptual variables, these studies have described numerous changes in the mating behavior of male hamsters treated systemically with the cholinergic agonist oxotremorine or the cholinergic antagonist scopolamine (Table 2). These results are of interest partly due to the number and variety of variables affected by these drugs. Such broad effects suggest a brain system that depends on ACh, and probably at numerous sites, assuming that there is some spatial separation of the subsystems controlling specific behavioral elements. It could be that these sites and effects are direct, that is fully contained within a brain system dedicated to the control of male-typical mating 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 direct, it also is possible that some arose indirectly, through cholinergic effects on other aspects of cognition or behavior.

These results also raise the possibility of significant differences between hamsters and rats in the neurochemical mechanisms controlling male behavior. They suggest, first, that ACh is a more prominent part of this mechanism in hamsters than in rats. In addition, they suggest numerous more specific species differences in the behavioral elements subject to cholinergic control. In comparison to the effects summarized in Table 2, oxotremorine treatment in rats tends to positively affect the intromission count factor by decreasing EL-1 and IF-1, whereas scopolamine tends to negatively affect initiation by increasing ML and IL.

Table 2
Summary of significant responses to systemic oxotremorine or scopolamine treatment in hamsters.

Measure	Oxotremorine	Scopolamine
Individual measure		
Incidence	↓	↓
ML	↑	
IL	↑	
MF		↑
IF		↑*
IR		↓
III	↑*	↑
EL	↑*	↑*
PEI	↑	
Conceptual variable		
Initiation	↓	
Efficiency	↓*	↓
Intromission		↓

Note: For individual measures, upward- and downward-pointing arrows indicate increases and decreases, respectively. For conceptual variables, they indicate facilitatory and inhibitory changes, respectively. An asterisk indicates that the change was limited to the first copulatory series or the conceptual variable associated with that series.

Though interesting, these results are perplexing in at least two ways. First, oxotremorine and scopolamine both are classified as cholinergic drugs. Therefore, one might expect them to affect similar or identical sets of behavioral elements. They obviously did not in these studies: Though the results summarized in Table 2 indicate some overlapping effects, the two profiles are more different than similar. Though the number of measures affected may make this disparity especially pronounced in hamsters, there also is no overlap between the sets of measures or conceptual variables affected by these drugs in rats (Ahlenius and Larsson, 1985; Bignami, 1966; Leavitt, 1969; Retana-Marquez et al., 1993).

Such departures from expectations might make sense if either drug were having important noncholinergic effects, perhaps along with the expected cholinergic ones. However, this seems unlikely to be true of either of these drugs. In general, both are widely viewed and used as selective muscarinic drugs (e.g., see Bräuner-Osborne and Brann, 1996; Ehlert and Tran, 1990; Gattu et al., 1997; Levey et al., 1991; Murakami et al., 1996; Pilar-Cuéllar et al., 2008; Sánchez et al., 2009; Thomsen et al., 2010; Vilaró et al., 1994, 1992). Further, previous studies of mating behavior have documented the ability of each to prevent any detectable response to the other, strongly suggesting that their effects on this behavior are attributable to their effects on cholinergic transmission (Ahlenius and Larsson, 1985; Hull et al., 1988).

Other factors that could enter into this disparity in behavioral profiles include a difference in the distributions of drug within the brain or a difference in affinity for the multiple types of muscarinic receptor. As previously discussed, there is evidence suggesting some special affinity by oxotremorine for M2, or M2 and M4, receptors: It may even show some preference for M2 autoreceptors, at least in some brain areas (Bräuner-Osborne and Brann, 1996; Gattu et al., 1997; Levey et al., 1991; Murakami et al., 1996; Vilaró et al., 1994, 1992). As described in the literature, these differences do not seem sufficient by themselves to account for the behavioral differences at hand. Perhaps they could do so, however, if combined with subtle differences in drug distribution or some other factor.

Second, whereas oxotremorine is a muscarinic agonist, scopolamine is a muscarinic antagonist. Therefore, one might expect these drugs to affect their endpoints in opposite directions. The results summarized in Table 2 again refute this. On the most general level, all of the observed responses to either drug involved some disruption or decline in the quality of performance. Further, though this departure from expectations may be especially clear in hamsters, similar disparities have been noted in both rats and rabbits (review in Meisel and Sachs, 1994).

Possible explanations of this disparity have been discussed previously. One possibility would rely on a difference in the receptors bound by the two drugs. Specifically, an agonistic effect of oxotremorine that is concentrated on M2 autoreceptors could have the net effect of reducing cholinergic transmission. This would converge on the same ultimate result as the block of postsynaptic muscarinic receptors expected to follow scopolamine treatment. Alternatively, levels of cholinergic activity in hamsters could be maintained at levels close to those required for effective behavior. If these levels are caused to depart in either direction from this near-optimal baseline, behavior could suffer. Such changes possibly are more likely in a system that depends heavily on ACh, as such a system might incorporate multiple cholinergic links that could be affected by drugs in a cumulative fashion.

Unfortunately, as indicated previously, there seems to be no evidence strongly supportive of either of these possibilities. Thus, it is clear that further work will be required to resolve these issues. However, the powerful effects of cholinergic drugs that are described here suggest that ACh is a more important part of the neurochemical mechanism controlling male behavior in some animals than previously has been recognized. In turn, this suggests that the future work required to more completely describe its role in these mechanisms will repay the effort.

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References

Ahlenius S, Larsson K. Central muscarinic receptors and male rat sexual behavior: facilitation by oxotremorine but not arecoline or pilocarpine in methscopolamine pretreated animals. Psychopharmacology 1985;87:127–9.

Arteaga-Silva M, Márquez-Villanueva Y, Martínez-García R, Hernández-González M, Bonilla-Jaime H, Retana-Márquez S. Effects of hormonal replacement with androgens and estrogens on male sexual behavior and plasma levels of these steroids in gonadectomized golden hamsters (*Mesocricetus auratus*). Physiol Behav 2005;85:571–80.

Arteaga M, Motte-Lara J, Velázquez J. Effects of yohimbine and apomorphine on the male sexual behaviour pattern of the golden hamster (Mesocricetus auratus). Eur Neuropsychopharmacol 2002;12:39–45.

Bignami G. Pharmacologic influences on mating behavior in the male rat. Psychopharmacologia 1966;10:44–58.

Bitran D, Hull EM. Pharmacological analysis of male sexual behavior. Neurosci Biobehav Rev 1987;11:365–89.

Bourne HR. Drug receptors & pharmacodynamics. In: Katzung BG, editor. Basic & clinical pharmacology. 7th edition. Stamford, CT: Appleton & Lange; 1998. p. 9–33.

Bräuner-Osborne H, Brann M. Pharmacology of muscarinic acetylcholine receptor subtypes (m1-m5): high throughput assays in mammalian cells. Eur J Pharmacol 1996;295:93–102.

Bunnell BN, Boland BD, Dewsbury DA. Copulatory behavior of golden hamsters (*Mesocricetus auratus*). Behaviour 1977;61:180–206.

Chen KK, Powell CE, Maze N. The response of the hamster to drugs. J Pharmacol Exp Ther 1945;85:348–55.

Dewsbury DA. Factor analysis of measures of copulatory behavior in three species of muroid rodents. J Comp Physiol Psychol 1979;93:868–78.

Dewsbury DA, Lanier DL, Oglesby JM. Copulatory behavior of Syrian golden hamsters in a one-male two-female test situation. Anim Learn Behav 1979;7:543–8.

Ehlert FJ, Tran LLP. Regional distribution of M1, M2 and non-M1, non-M2 subtypes of muscarinic binding sites in rat brain. J Pharmacol Exp Ther 1990;255:1148–57.

Floody OR. Organization of mating behavior in male hamsters. J Comp Psychol 2011;125:185–93.

Gattu M, Pauly JR, Urbanawiz S, Buccafusco JJ. Autoradiographic comparison of muscarinic M1 and M2 binding sites in the CNS of spontaneously hypertensive and normotensive rats. Brain Res 1997;771:173–83.

Gringauz A. Introduction to medicinal chemistry: how drugs act and why. New York: Wiley-VCH; 1997.

Holford NHG, Benet LZ. Pharmacokinetics & pharmacodynamics: dose selection & the time course of drug action. In: Katzung BG, editor. Basic & clinical pharmacology. 7th edition. Stamford, CT: Appleton & Lange; 1998. p. 34–49.

Howell DC. Statistical methods for psychology. 7th edition. Belmont, CA: Wadsworth, Cengage Learning; 2010. p. 364–6.

Hull EM, Pehek EA, Bitran D, Holmes GM, Warner RK, Band LC. Brain localization of cholinergic influence on male sex behavior in rats: antagonists. Pharmacol Biochem Behav 1988;31:175–8.

Kajbaf M, Jahanshahi M, Lamb JH, Gorrod JW, Naylor S. Bioanalytical applications of tandem mass spectrometry in the in vitro metabolism of the anticholinergic drug cimetropium bromide to detect differences in species metabolism. Xenobiotica 1992;22:641–55.

Keppel G, Wickens TD. Design and analysis: a researcher's handbook. 4th edition. Upper Saddle River, NJ: Pearson Prentice Hall; 2004. p. 91–2.

Klinkenberg I, Blokland A. The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioural studies. Neurosci Biobehav Rev 2010;34:1307–50.

Leavitt F. Drug-induced modifications in sexual behavior and open field locomotion of male rats. Physiol Behav 1969;4:677–83.

Levey AI, Kitt CA, Simonds WF, Price DL, Brann MR. Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J Neurosci 1991;11:3218–26.

Meisel RL, Sachs BD. The physiology of male sexual behavior. In: Knobil E, Neill JD, editors. The physiology of reproduction, 2nd edition, Vol 2. New York: Raven Press; 1994. p. 3–105.

Miernicki M, Pospichal MW, Powers JB. Short photoperiods affect male hamster sociosexual behaviors in the presence and absence of testosterone. Physiol Behav 1990;47:95–106.

Murakami Y, Matsumoto K, Ohta H, Watanabe H. Effects of oxotremorine and pilocarpine on striatal acetylcholine release as studied by brain dialysis in anesthetized rats. Gen Pharmacol 1996;27:833–6.

Murphy MR, Schneider GE. Olfactory bulb removal eliminates mating behavior in the male golden hamster. Science 1970;167:302–4.

Pfaus JG, Mendelson SD, Phillips AG. A correlational and factor analysis of anticipatory and consummatory measures of sexual behavior in the male rat. Psychoneuroendocrinology 1990;15:329–40.

Pilar-Cuéllar F, Paniagua MA, Díez-Alarcia R, Dos_Anjos S, Montori S, Pérez CC, et al. Muscarinic receptor changes in the gerbil thalamus. Brain Res 2008;1243:38–46.

Powers JB, Winans SS. Vomeronasal organ: critical role in mediating sexual behavior of the male hamster. Science 1975;187:961–3.

Retana-Marquez S, Salazar ED, Velazquez-Moctezuma J. Muscarinic and nicotinic influences on masculine sexual behavior in rats: effects of oxotremorine, scopolamine, and nicotine. Pharmacol Biochem Behav 1993;44:913–7.

Retana-Marquez S, Velazquez-Moctezuma J. Cholinergic–androgenic interaction in the regulation of male sexual behavior in rats. Pharmacol Biochem Behav 1997;56:373–8.

Sachs BD. Conceptual and neural mechanisms of masculine copulatory behavior. In: McGill TE, Dewsbury DA, Sachs BD, editors. Sex and behavior: status and prospectus. New York: Plenum Press; 1978. p. 267–95.

Sánchez G, Colettis N, Vázquez P, Cerveñansky C, Aguirre A, Quillfeldt JA, et al. Muscarinic inhibition of hippocampal and striatal adenylyl cyclase is mainly due to the M_4 receptor. Neurochem Res 2009;34:1363–71.

Siegel S. Nonparametric statistics for the behavior sciences. New York: McGraw Hill; 1956.

Soulairac M-L. Étude expérimentale des régulations hormono-nerveuses du comportement sexuel du rat mâle. Ann Endocrinol 1963;24:1–98.

Thomsen M, Wess J, Fulton BS, Fink-Jensen A, Caine SB. Modulation of prepulse inhibition through both M_1 and M_4 muscarinic receptors in mice. Psychopharmacology 2010;208:401–16.

Vilaró MT, Palacios JM, Mengod G. Multiplicity of muscarinic autoreceptor subtypes? Comparison of the distribution of cholinergic cells and cells containing mRNA for five subtypes of muscarinic receptors in the rat brain. Mol Brain Res 1994;21:30–46.

Vilaró MT, Wiederhold K-H, Palacios JM, Mengod G. Muscarinic M_2 receptor mRNA expression and receptor binding in cholinergic and non-cholinergic cells in the rat brain: a correlative study using in situ hybridization histochemistry and receptor autoradiography. Neuroscience 1992;47:367–93.

Winer BJ. Statistical principles in experimental design. New York: McGraw Hill; 1971.