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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