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Responses to central oxotremorine and scopolamine support the cholinergic control of male mating behavior in hamsters

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ABSTRACT

The responses of hamsters to intracranial injections of the cholinergic agonist oxotremorine (OXO) implicate cholinergic mechanisms in the medial preoptic area (MPOA) in the control of male mating behavior. To extend these observations, we ran three studies of responses to cholinergic drugs delivered singly or in combination to the vicinity of the MPOA. The first tested responses to OXO, confirming its ability to reduce the postejaculatory interval. The second complemented the first by examining responses to MPOA microinjections of the cholinergic antagonist scopolamine (SCO). These caused several changes revolving around intromission. These included increases in intromission frequency and ejaculation latency. They also included a change in the patterning of intromissions, marked by continuous strings without the usual separation by dismounts. The final study resembled the others in examining the effects of MPOA injections of OXO and SCO but focused on the ability of each drug to antagonize responses to the other. Most of the responses to OXO and SCO individually replicated earlier findings, though the measures examined here also permitted the description of effects on some noncopulatory sexual behaviors, specifically the male's inspection of the female. However, the most interesting results may be those suggesting asymmetry in the responses to the addition of the second drug: Whereas responses to OXO tended to be antagonized by SCO, OXO was less effective at counteracting responses to SCO. Though the explanation of this asymmetry is not completely clear, it is consistent with previous suggestions of differences in the affinities of these drugs for subtypes of muscarinic receptors. Therefore, it suggests that the cholinergic synapses and circuits controlling distinct elements of male behavior could differ in their dependence on these receptors.

1. Introduction

The mating behaviors of male rodents are structurally complex but composed of elements that are stereotyped and relatively easy to measure. The modal pattern seems to be one in which males show one intravaginal thrust per intromission, multiple intromissions prior to an initial ejaculation and multiple ejaculations in the course of an interaction (Dewsbury, 1972, 1975). Accordingly, the methods used to study this behavior typically begin by distinguishing mounts, intromissions and ejaculations, often clustering them into copulatory series, each defined by an ejaculation and the series of behaviors immediately preceding it. These elements then are described by a combination of latency, interval and frequency measures. Some, such as the ratio of intromissions to all mounts, seem to relate directly to the quality of performance whereas others, such as the time or absolute number of intromissions required to achieve ejaculation, are considered to relate inversely to overall quality.

Because of their biological importance and the ease with which they can be elicited and described, these behaviors have long attracted the attention of behavioral neuroscientists. Some of the resulting studies have examined the underlying neurochemical mechanisms, in the process implicating several neurotransmitters, including acetylcholine (ACh).

The cholinergic control of behavior commonly is studied by observing the responses to muscarinic drugs such as the agonist oxotremorine (OXO) and the antagonist scopolamine (SCO). Responses to systemic treatments with these 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) and hamsters (Floody, 2011b). These effects seem especially pronounced in hamsters, in which every conventional index of male behavior was affected by one or both drugs.

Such results suggest a central neurochemical system that is important and likely to be distributed across multiple sites. However, tests of this inference require more direct brain manipulations, often involving the observation of responses to treatments delivered directly to areas of special interest. In the case of male-typical mating behavior, there are many areas that deserve to be studied in this way (Meisel

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and Sachs, 1994). However, most studies using this approach have focused on the medial preoptic area (MPOA), reflecting the wealth of data that suggest for it an especially influential role in male behavior (Meisel and Sachs, 1994).

Studies of responses to MPOA injections of cholinergic drugs have advanced farthest in male rats (Hull et al., 1988a, 1988b). These studies have described several effects, each with implications for the underlying mechanism. First, microinjections of OXO produce very specific effects: When appropriate measures are taken to minimize the spread of drug through the ventricles, the only reliable response to central OXO consists of a facilitatory reduction in intromission frequency (Hull et al., 1988a, 1988b). In contrast, SCO microinjections have produced effects that are more disruptive and all-or-none, often involving reductions in the incidence of intromission and ejaculation (Hull et al., 1988b). Though these effects confirm the responsiveness of male behavior to cholinergic manipulations of MPOA circuits, they seem much less specific than the response to OXO, raising questions about the range of behaviors that is subject to cholinergic control. Last, despite any dissimilarity in the responses to OXO and SCO individually, the simultaneous administration of the two reinstates baseline levels of behavior, with each drug apparently able to counteract any response to the other (Hull et al., 1988b). This suggests that the responses to these drugs really are attributable to their effects on cholinergic neurotransmission. In addition, it suggests that the muscarinic receptors activated by OXO and blocked by SCO are of the same type (but also see Retana-Marquez and Velazquez-Moctezuma, 1993).

From even this brief review, it should be clear how the assessment of ACh's role in male behavior benefits from knowledge of the responses to multiple drugs, administered singly and in combination. We have begun to extend this research program to hamsters, in the process suggesting an important role for cholinergic synapses in or near the MPOA (Floody, 2011b; Floody et al., 2011). However, significant gaps remain in the body of data required for the analysis of this mechanism. This report seeks to fill the most important of these gaps with three studies, each corresponding to one of the types of data described earlier. Specifically, the first extends our earlier studies of the impact on male behavior of OXO treatments aimed at the MPOA (Floody et al., 2011). The second provides a complementary analysis of responses to central SCO. Finally, the third compares the responses to OXO and SCO when administered individually or together.

2. Experiment 1

In our work with systemic treatments, OXO disrupted male behavior in many ways, affecting measures including mount, intromission and ejaculation latencies along with the durations of the interintromission and postejaculatory intervals (Floody, 2011b). These effects were the first to document the responsiveness of male behavior in hamsters to manipulations of central cholinergic activity. But they could reflect drug actions at any or many brain sites.

Some information on the roles of cholinergic synapses in or near the MPOA was provided by a report describing responses to central microinjections of OXO (Floody et al., 2011). The responses to these injections were much more specific than those to the systemic treatments. Furthermore, consistent with previous work on rats (Hull et al., 1988a), these treatments tended to facilitate male behavior rather than disrupting it. In particular, the most consistent effect was a reduction in the postejaculatory interval.

These results identify at least some of the elements of male behavior in hamsters likely to be controlled by a cholinergic mechanism associated with the MPOA. However, it seemed possible that the specificity of the effects reflects the limited range of OXO doses employed. Therefore, it seemed desirable to revisit the effects of OXO treatments, but increasing the dose range so as to better define the dose–response relationship.

2.1. Methods

2.1.1. Animals and drug treatments

The subjects were 12 male golden hamsters (LVG:Lak strain) that averaged 165.1 g in weight (SEM = 4.4) at the time of surgery. Each was sexually experienced, having passed a screening process requiring ejaculation within 10 min in each of 4 tests spaced at 4–6 day intervals. 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 stimuli were 13 adult females, each of which had been bilaterally ovariectomized at least 1 week before use. Each 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 subject was anesthetized with an intraperitoneal injection of 75 mg/kg of sodium pentobarbital (Sigma-Aldrich). This was supplemented by additional sodium pentobarbital, as necessary, and by a subcutaneous (sc) injection of 0.4 mg of the analgesic butorphanol tartrate (Henry Schein). While anesthetized, each male was implanted with a single 26 G stainless-steel guide cannula (Plastics One) aimed at a point approximately 1 mm dorsal to the anteromedial MPOA. This was angled 15° toward the midline and cemented to the leveled skull at a point representing a compromise between coordinates based on skull features (1.5 mm anterior to bregma, 1.3 mm to the left of the midline, 6.2 mm below the skull at bregma) and interaural line (7.2 mm anterior, 2.5 mm dorsal). This placement was designed to balance four goals: (a) sufficient proximity to the MPOA to ensure that structure's exposure to the infused drug; (b) sufficient proximity to the midline to permit a single cannula and infusion to yield bilateral drug exposure; (c) sufficient distance from all ventricles (lateral and third) to minimize the chances of any ventricular diffusion of drug to targets potentially distant from the MPOA region; and (d) a sufficiently small intrusion of cannulae into the MPOA to minimize incidental damage to the very tissue expected to mediate behavioral responses to the infused drug. It should be noted that a placement that is optimal in the context of these goals must target the anteromedial MPOA and may even be located slightly dorsal or anterior to the substance of the MPOA within this region.

After at least 7 days of recovery, each male began a series of four behavioral tests at 4–7 day intervals. Each was preceded by a single intracranial injection of 0.5 μl of a 0.9% NaCl solution containing 0 (Placebo), 1, 3 or 5 μg of OXO (oxotremorine sesquifumarate, Sigma-Aldrich). The order of exposure to the doses was counterbalanced across subjects, and all injections and tests were conducted without knowledge of the solution in use. This selection of doses significantly extended the range of treatments used in our previous studies of responses to OXO microinjections (Floody et al., 2011). The results of pilot testing excluded the possibility of any wider range: Of the four animals receiving 10 μg OXO injections, two were completely inactive and two attacked their female partners, suggesting that these treatments were excessive.

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. The needle was left in place for 30 s after the infusion to reduce the amount of solution backing up along the needle track. 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 ovariectomized after similar anesthetic and analgesic treatments. To ensure sexual responsiveness during 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 10–15 μg of estradiol benzoate and the second at approximately 5 h before use and containing 500 μg of progesterone (both from Steraloids).