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Rapid facilitation of ultrasound production and lordosis in female hamsters by horizontal cuts between the septum and preoptic area

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HIGHLIGHTS

- Female hamsters received horizontal cuts between the septum and preoptic area (ARD).
- As expected, ARD increased levels of lordosis, ultrasonic vocalization and weight.
- These behavioral effects first achieved reliability at 2 days after surgery.
- Such rapid changes support disinhibition as the mechanism underlying ARD effects.
- They also suggest that VMN lesions affect vocalization independently of ARD.

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ABSTRACT

Horizontal cuts between the septum and preoptic area (anterior roof deafferentation, or ARD) dramatically affect sexual behavior, and in ways that could explain a variety of differences across behavioral categories (precopulatory, copulatory), species, and the sexes. Yet little is known about how these effects develop. Such information would be useful generally and could be pivotal in clarifying the mechanism for ultrasonic vocalization in female hamsters. Ultrasounds serve these animals as precopulatory signals that can attract males and help initiate mating. Their rates can be increased by either ARD or lesions of the ventromedial hypothalamus (VMN). If these effects are independent, they would require a mechanism that includes multiple structures and pathways within the forebrain and hypothalamus. However, it currently is not clear if they are independent: VMN lesions could affect vocalization by causing incidental damage to the same fibers targeted by ARD. Fortunately, past studies of VMN lesions have described a response with a very distinctive time course. This raises the possibility of assessing the independence of the two lesion effects by describing just the development of the response to ARD. To accomplish this, female hamsters were observed for levels of ultrasound production and lordosis before and after control surgery or ARD. As expected, both behaviors were facilitated by these cuts. Further, these effects began to appear by two days after surgery and were fully developed by six days. These results extend previous descriptions of the ARD effect by describing its development and time course. In turn, the rapid responses to ARD suggest that these cuts trigger disinhibitory changes in pathways that differ from those affected by VMN lesions.

1. Introduction

Female mating behavior can be dramatically altered by horizontal cuts between the septum and preoptic area, termed anterior roof deafferentation or ARD [46]. In rats, ARD causes a clear facilitation of the lordosis responses that essentially define receptivity, or female responsiveness to male sexual advances [2,46]. Female rats also seem to respond to ARD with increases in proceptive responses such as hopping and ear-wiggling, behaviors that can set the stage for mating by attracting or arousing males [2,46]. However, these effects have not been fully described and seem less consistent across studies than those on lordosis [20,40]. Both responses to ARD are thought to reflect behavioral disinhibition triggered by the interruption of behavior-inhibiting lateral septal (LS) efferents, at least some of which project in the medial forebrain bundle to the periaqueductal gray of the midbrain and pons (PAG) [20,25,42,45–49].

Female hamsters also respond to ARD with changes in sexual behavior [10,21]. Yet these effects are quite different from those in rats, with respect to both lordosis and proceptive behavior. Specifically, the changes in lordosis produced by ARD in hamsters are much more subtle than those in rats: They can be missed altogether in tests of responses to males but are apparent when manual stimuli are used to better control the intensity and timing of the lordosis-eliciting stimulation [10,21]. This difference is allied with one in the duration of normally-elicited

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lordosis responses, this being much greater in hamsters than rats [5]. Because the system disrupted by ARD is presumed to inhibit lordosis when active, it has been suggested that a relatively weak lordosis-inhibiting system (and ARD effect) is well suited to an animal in which successful reproduction may depend on relatively prolonged lordosis responses [10,12,21].

In contrast, ARD's most powerful effect on female hamsters is the facilitation of a form of proceptive behavior involving the production of high-frequency ("ultrasonic") vocalizations. Hamsters of both sexes normally emit ultrasounds at rates that depend on levels of gonadal hormones and the availability of cues indicating the proximity of potential mates (reviews in [7,8]). The resulting calls stimulate and guide mutual approach by individuals that generally are aggressive and socially intolerant. Further evidence for a strong link between ultrasounds and sexual motivation in hamsters is evident in their ability to prolong (and sometimes trigger) lordosis. In part, the prominence of ARD's effect on ultrasound rates in female hamsters may reflect the greater dependence of solitary animals on distance signals that can promote social contact and interaction when these are most appropriate.

Part of the interest in ARD simply reflects the magnitude of its effects on behaviors of obvious biological importance. However, the evidence reviewed here suggests that ARD effects vary across species and may distinguish between receptive and proceptive forms of female-typical mating behavior. In turn, these observations suggest that studies exploiting the ARD effect have the potential to better describe how brain mechanisms for sexual behavior differ across species and category.

In addition, the mechanism tapped by ARD has been implicated in the generation of sexual differences in mating behavior. Not surprisingly, male rats normally are less prone to show lordosis than are females, a difference that seems likely to be due, at least in part, to a correlated difference in the size of the LS–PAG pathway targeted by ARD [43]. Supporting this suggestion is the ability of perinatal hormone treatments known to decrease later levels of lordosis to also decrease the size and strength of this pathway [41].

These observations suggest that the ARD effect has the potential to illuminate several central issues in the brain control of reproductive behavior. In turn, they suggest that this effect merits full description. However, though many aspects of the ARD effect have been studied, surprisingly little is known about its rate and pattern of development. Most studies have examined the impact of each combination of surgical and hormonal treatments at just a single time, often relatively long (3–5 weeks) after surgery [3,10,45–47]. Further, the one exception yielded ambiguous results. It tracked hamster ultrasound rates in weekly tests during the 3 weeks just before and after ARD [21]. Though no reliable changes were observed in postoperative (postop) patterns of vocalization, these rates nearly doubled between the first and second postop weeks. This research, then, suggests that the responses to ARD are in place by 2–3 weeks after surgery but does not permit any firm conclusion on when they first appear or how they develop during the postsurgical period.

In addition to its intrinsic value, the information required to fill this gap could illuminate a second lesion effect on ultrasonic vocalization in female hamsters. Like ARD, lesions of the ventromedial hypothalamus (VMN) can increase ultrasound rate [9,11,16]. Yet, in comparison to the limited research on the timing of responses to ARD, the time course of the changes in ultrasound production caused by VMN lesions has been thoroughly studied and found to be one of this effect's most distinctive features. In particular, previous studies of responses to VMN lesions [9,11,16] report that levels of vocalization decrease sharply by 15 min after surgery, only to recover to preoperative (preop) levels by 7–8 days. This recovery or rebound then continues, yielding levels of behavior at 2–6 weeks postop that reliably exceed baseline levels.

In rats, strong evidence supports the independence of the changes in lordosis caused by ARD and VMN lesions [24,42,45,47]. However, this does not completely resolve the ambiguity regarding the mechanism underlying ARD's effects on hamster ultrasound rates. Conventional VMN lesions could increase vocalization not by damaging VMN cells but by attacking fibers in the VMN's surround. In effect, they could act by severing the fibers that interconnect the LS and PAG, and that generate the ARD effect when cut between the LS and POA [33,38,39,42].

There may be many possible ways of assessing the independence of the increases in hamster ultrasound rates caused by ARD and such VMN lesions. But a simple test of the time course of the ARD effect can both achieve this goal and contribute to the more general objective identified earlier. If the effects of ARD and VMN lesions are due to the same mechanism, then the ARD effect on ultrasound rate should have the same distinctive time course that previous research has established for the response to VMN lesions [9,11,16]. Conversely, an ARD effect that develops very differently would suggest that ARD and VMN effects on ultrasound production, like those on lordosis, are independent.

2. Methods

2.1. Animals and their preparation

Data were collected from 17 female golden hamsters (*Mesocricetus auratus*) averaging 149.6 g (SEM = 4.4) at the start of testing. Like the sexually-experienced males used as stimuli, each was housed individually in a 34 × 18 × 18 cm wire-mesh cage with controlled temperature (20–25 °C) and photoperiod (reversed 14:10 light:dark), and with free access to food and water except during tests. All methods were approved by Bucknell University's Institutional Animal Care and Use Committee.

Subjects were bilaterally ovariectomized under sodium pentobarbital anesthesia (67 mg/kg ip, supplemented by 0.4 mg sc of the analgesic butorphanol) 7–13 days before the first in a pair of preop tests spaced 4 days apart. Each was preceded by priming with 5 μg/100 g of estradiol benzoate (EB), administered sc in 0.05–0.10 ml of peanut oil at approximately 48 h before testing. This was followed by 500 μg of progesterone injected sc in 0.05 ml of oil at least 4 h before use.

2.2. Behavioral testing

Table 1 summarizes the structure of a behavior test. As indicated there, each began with a series of test segments that focused on ultrasound production but also incorporated measures of male-elicited lordosis. This series was initiated by 2 min of adaptation to a clean 40 × 20 × 25 cm glass chamber. A QMC ultrasonic receiver tuned to 35 kHz then was used to count the subject's vocalizations during a 2-min pre-male period [13]. At its end, a male was introduced for just the time required for the elicitation of a lordosis response, defined by immobility and tail elevation to a point even with or above the top of the lower back. Once initiated, lordosis was considered to persist until the first paw movement. The lordosis responses observed at this time are referred to as male-elicited because the male was the only source of lordosis-triggering tactile stimulation and so accounted largely or fully for their occurrence, latency and duration.

Upon the cessation of lordosis, ultrasounds again were counted during a 2-min post-male interval. Electronic mimics of hamster ultrasounds were presented at a low rate (two at the end of each 30 s interval) during this period. Each consisted of a 100 ms burst of 35 kHz sine waves [13]. These are not necessary to provoke increased post-male vocalization [15]. However, the combination of two stimuli for vocalization [14,15] should be more effective than either one, and the resulting elevated baseline rate of vocalization should make any upward or downward change due to ARD more apparent than it otherwise would be.

In this design, female ultrasound rates were measured whenever possible. Early in a period of male–female contact, both animals are active and probably vocal [15]. However, their vocalizations are too similar to be distinguished on the basis of the ultrasonic receiver's output [13]. Furthermore, females are silent while in lordosis [15], excluding any such period from consideration, either during or shortly after the male's presentation.