Bilateral transection of the lateral olfactory tract but not removal of the vomeronasal organs inhibits short-photoperiod-induced testicular regression in golden hamsters*

David R. Pieper[1], Sarah W. Newman[2], Catherine A. Lobocki[1] and Gabriel Gogola[1]

[1]*Providence Hospital, Department of Physiology, Southfield, MI 48037 (U.S.A.) and* [2]*The University of Michigan, Department of Anatomy, Ann Arbor, MI 48109 (U.S.A.)*

It is now known that removal of the olfactory bulbs increases basal gonadotropin secretion and prevents short-photoperiod-induced testicular regression in Syrian hamsters. The experiments described in the present paper were an attempt to determine which neuronal systems associated with the olfactory bulbs are responsible for this influence on the reproductive neuroendocrine axis. In the first experiment, removal of the vomeronasal organ failed to influence gonadotropin secretion or testes weight in hamsters on long or short photoperiod, suggesting that the vomeronasal–accessory olfactory pathway is not individually responsible for the effect of the olfactory bulbs on gonadotropin secretion. In the second experiment, bilateral transection of the lateral olfactory tracts (LOT) did prevent short-photoperiod-induced testicular regression and the associated decrease in gonadotropin secretion. Since the nervus terminalis is confined to the surface of the medial olfactory bulb pathway, the results of LOT transection indicate that the nervus terminalis, which itself contains gonadotropin releasing hormone, does not mediate the influence of the olfactory bulbs on gonadotropin secretion. These results further suggest that the olfactory bulb influence on gonadotropin secretion is due to neural connections to the pyriform cortex, entorhinal cortex or amygdala.

INTRODUCTION

Recent studies have shown that removal of the olfactory bulbs (BX) of male golden hamsters results in an increase in basal gonadotropin secretion[4,22]. In hamsters maintained on a long photoperiod, the effect of this increase is minimal, but when BX hamsters are placed in a short photoperiod the expected testicular regression does not occur[4,22]. Within the olfactory bulbs are at least 3 different sensory systems. One is the main olfactory system, originating in the olfactory mucosa and synapsing in the main olfactory bulb (MOB). A second is the accessory olfactory pathway which originates in the vomeronasal organ (VNO) and synapses in the accessory olfactory bulb (AOB). The MOB and

AOB, in turn, give rise to separate projection pathways to the ventral forebrain[6,26,29]. In addition, the nervus terminalis, which contains, and may secrete, gonadotropin-releasing hormone[28], transmits information from the area of the vomeronasal organ to medial structures of the ventral forebrain. All of these pathways are destroyed by BX. Since the VNO and accessory olfactory pathways transmit reproductively important chemosensory information in hamsters[5,8,15,29], we hypothesized that destruction of this pathway alone would mimic the effect of BX in increasing gonadotropin secretion and preventing short-photoperiod-induced testicular regression.

Removal of the VNO, however, had no effect on gonadotropin secretion in hamsters on long photoperiod, nor did it affect testicular regression or

gonadotropin secretion in hamsters on short photoperiod. In a further attempt to identify the olfactory pathway which modulates gonadotropin secretion, we performed bilateral destruction of the lateral olfactory tracts (LOTX) caudal to the olfactory bulbs. If LOTX mimicked the effect of BX on gonadotropin secretion, this, combined with the data on VNOX, would suggest that the phenomenon was due to main olfactory pathway neurons in the lateral olfactory tract.

MATERIALS AND METHODS

Protocol for Experiment I

Forty 25-day-old male golden hamsters (*Mesocricetus auratus*, LAK:LVG (SYR) were obtained from Charles River Breeding Laboratories. Within 3 days of arrival, the animals underwent sham surgery (SH), BX or removal of the vomeronasal organ (VNOX). Immediately following surgery, some of the animals from each group were placed on a 6:18 h light:dark cycle (lights on 10.00–16.00 h) and the remaining hamsters were left on a 14:10 h light: dark cycle (lights on 06.00–20.00 h). Fourteen weeks later, the experiment was terminated. All animals were then anesthetized with ether and bled via cardiac puncture, and the serum was frozen for hormone determinations. While still anesthetized, the hamsters were perfused with 10% formalin after which the brains were removed for subsequent histologic analysis. The wet weight of the paired testes was then obtained.

Protocol for Experiment II

Sixteen 23-day-old male golden hamsters underwent either sham surgery or bilateral knife cuts to transect the lateral olfactory tract. Following surgery, all hamsters were returned to a 14:10 h light:dark photoperiod. Four weeks later, all hamsters were transferred to a 10:14 h light: dark cycle (lights on 08.00–18.00 h) for the duration of the experiment. Testicular indices were determined weekly and the paired wet testes weights and serum reproductive hormone levels were assessed at the end of the experiment. Each animal was perfused with formalin and the brains preserved for histology as described for Expt. I.

Housing

For both studies, all hamsters were group-housed 5–6 per polycarbonate cage with food and water available ad libitum. Female hamsters were present in animal rooms throughout both experiments.

Surgery

All surgery was done in animals anesthetized with sodium pentobarbital (60–80 mg/kg). Bulbectomy was performed by bilateral aspiration of the olfactory bulbs as previously described[22]. At the end of the study, gross examination indicated that the olfactory bulbs had been completely removed in all BX animals.

VNOX was done in a manner previously described for mice[30] and hamsters[5,15]. Briefly, the hamster was placed in a stereotaxic apparatus with the ventral surface up. After retraction of the soft tissues overlying the hard palate, the incisive foramina were extended approximately 2 mm rostrally to just caudal to the incisors. The narrow isthmus of the bone separating the foramina was then severed both rostrally and caudally, thereby freeing the central bone containing the vomeronasal organs. This bone with the vomeronasal organs was then gently retracted with forceps. The soft tissues were sutured in the midline after hemostasis was achieved. For Expt. I, SH surgery involved anesthetizing the animal and traumatizing the ventral surface of the hard palate.

Bilateral lateral olfactory tract transections were done as previously described by Devor[7] and Macrides et al.[14]. Briefly, the animal was placed in a stereotaxic apparatus and tilted from the horizontal at a 45° angle. The eye was gently retracted and a cranial bone chip was removed. The lateral olfactory tract was then visible and could be transected using the tip of a no. 11 scalpel blade. A cranial bone chip of about 1 mm² was then inserted at the level of the transection to inhibit neuronal anastomosis and regeneration in these young animals. SH surgery for Expt. II involved removal of the bone chip, but no LOT transection.

Histological analysis

LOT transection and VNOX were verified in tissues embedded in egg yolk mixed with 12% gelatin for sectioning on a freezing microtome as previously described[27]. In Expt. I, the forebrain and

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olfactory bulbs were carefully dissected away from the cranium. Horizontal sections of the embedded brain were cut at 40 μm and all sections containing olfactory bulb tissue were stained with Cresyl violet. In Expt. II the brain was cut in 80 mm coronal sections and stained with Cresyl violet. Every section in the series was analyzed microscopically for lesion placement. The most rostral and most caudal extent of the damage and the level of complete LOT transection were recorded for each lesion (left and right for each lesioned animal).

Testicular index

Testicular index (TI) was determined by measuring the length and width of the left testis through a moistened scrotal sac. The mm length × mm width/g body weight × 10 is an indication of testis size relative to body weight.

Radioimmunoassay

Serum luteinizing hormone (LH) was determined by double antibody RIA using ovine LH, Niswender No. 15 antibody, and National Institutes of Arthritis, Diabetes, Digestive and Kidney Diseases (NIAMDDK) rLH RP-1 as standard as previously described[17]. Serum follicle-stimulating hormone (FSH) was determined using the NIAMDDK rat FSH kit and standard FSH RP-1 for Expt. I and standard FSH RP-2 for Expt. II. Both assays have been previously validated for measurement of Syrian hamster LH and FSH[1,2]. The minimum serum LH detectable was 5 ng/ml. The minimum FSH detectable was 150 ng/ml for Expt. I and 1 ng/ml for Expt. II. The intra- and interassay coefficients of variation were 3 and 8.5% for LH and 2.6 and 9.3% for FSH, respectively. Testosterone was determined by direct RIA using a kit commercially available from Pantex (Santa Monica, CA). The minimum serum testosterone concentration detectable was 0.1 ng/ml. The intra- and interassay coefficients of variation for testosterone assays were 1.9 and 7.9%, respectively. All samples in all 3 assays were done in duplicate.

Statistics

Statistical differences in Expt. I were determined by analysis of variance (ANOVA) with post-hoc testing by Duncan's multiple range test. The TI data of Expt. II were tested by ANOVA for repeated measures and post-hoc analysis. The rest of the data in Expt. II were analyzed by unpaired t-test.

RESULTS

Experiment I

Histological analysis of the VNOX groups revealed that all but 3 animals had complete degeneration of the glomerular layer of the accessory olfactory bulbs bilaterally, indicating complete destruction of the vomeronasal nerve afferents to the AOB following removal of the vomeronasal organ. The other 3 animals had incomplete degeneration of the glomerular layer of the accessory olfactory bulb and were not included in the results. The degeneration observed was similar to that illustrated by Winans and Powers[27]. In all BX animals the olfactory bulbs had been completely removed as judged by visual inspection.

In hamsters on 14L:10D, BX increased body weight (135 ± 5.7 g; $n = 5$; x ± S.E.M.) compared to SH operated hamsters (109 ± 1.6; $n = 7$; $P <$
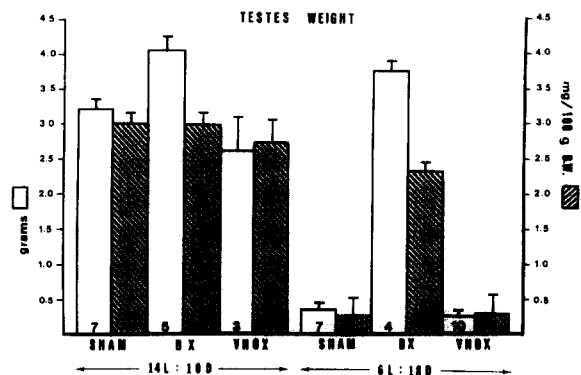

Fig. 1. Testes weight of hamsters in Expt. I. The animals were sham operated (SHAM), olfactory bulbectomized (BX) or had their vomeronasal organ removed (VNOX) and had been maintained in 14L:10D or 6L:18D photoperiods for 14 weeks. Values are presented as mean ± S.E.M. Grams wet weight is shown by the open bars while the diagonally lined bars represent the mg per 100 g body weight. The number at the base of the bar is the number of animals. BX increased testes weight in hamsters on either photoperiod ($P < 0.01$) compared to the SH group, but VNOX had no effect on testes weight in either photoperiod. For testes weight divided by 100 g body weight, there were no significant differences between the groups on 14L:10D but the BX group values were higher than the other two groups on 6L:18D ($P < 0.01$). The SH or VNOX group on 6L:18D had lower testes weights and relative testes weights than the comparable groups on long photoperiod ($P < 0.01$).

0.01) but VNOX (94 ± 6.9; $n = 4$) had no effect. The SH group in short photoperiod had higher body weights (139 ± 6.0; $n = 7$) than the SH group on 14L:10D ($P < 0.01$). BX animals on 6L:18D had higher body weights (162 ± 8.6; $n = 4$) than the SH group on 6L:18D ($P < 0.05$) but VNOX hamsters on 6L:18D had reduced body weights (94 ± 6.6; $n = 10$; $P < 0.01$) compared to the SH group on 6L:18D.

BX hamsters on long photoperiod had larger testes than the SH group on long photoperiod ($P < 0.01$), but they also had higher body weights so that the testes weight per 100 g body weight was similar to the SH group (Fig. 1). In short photoperiod, BX hamsters had much higher testes weights and testes weights per 100 g body weight than the SH group ($P < 0.01$; Fig. 1). Serum LH levels exhibited considerable variation between animals within the same group resulting in no significant differences in either photoperiod (Fig. 2). The BX group had elevated FSH levels in both photoperiods compared to the SH hamsters ($P < 0.05$; Fig. 2). In animals on short photoperiod, serum testosterone was markedly higher in the BX than SH hamsters ($P < 0.01$; Fig. 3). VNOX animals had testes weights and reproductive hormone levels similar to the SH group and this was true in animals on either photoperiod (Figs. 1–3).

Experiment II

Histological analysis indicated that the lateral olfactory tracts had been bilaterally transected in all hamsters in the LOTX group ($n = 8$). The lesions were typically large and, to a varying degree, involved other forebrain structures such as the anterior olfactory nucleus, and the primary olfactory, gustatory and agranular insular cortices. The rostrocaudal level of the transection also varied between brains and, in some cases, on the two sides of each brain. The most rostral lesion was placed in the rostral part of the anterior olfactory nucleus (Fig. 4A), and the most caudal extent of the intact LOT fibers on either side of any LOTX animal was the mid olfactory tubercle (Fig. 4C). Most of the animals, however, had relatively symmetrical lesions in the transition from the caudal anterior olfactory nucleus to the rostral olfactory tubercle and pyriform cortex (Fig. 4B).

The TI of the SH group began to decrease after about 6 weeks on 10L:14D, but the TI of the LOTX group remained high throughout the study. ANOVA with repeated measures indicated that the groups were significantly different at $P < 0.01$, and post-hoc analysis indicated that they were different at $P < 0.05$ at week 12 of the study and were different at $P < 0.001$ at the end of the study. All 8 of the sham-operated hamsters eventually underwent testicular regression in the short photoperiod but only 1 out of the 8 hamsters in the LOTX group had regressed testes ($P < 0.01$; Fig. 5). The LOTX lesion
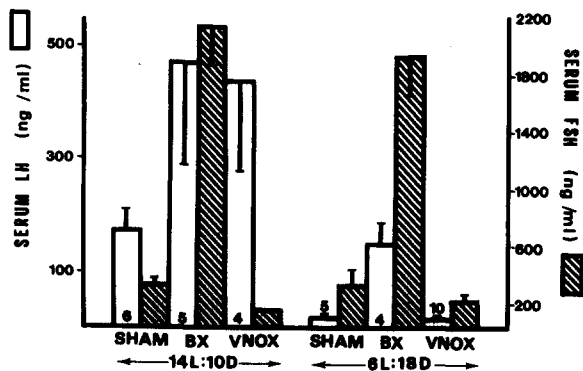

Fig. 2. Serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels at the end of Expt. I. See Fig. 1 legend for further details on figure symbols. For serum LH, there were no significant differences between groups on the same photoperiod. BX hamsters on either photoperiod had higher serum FSH levels than SH hamsters ($P < 0.01$), but VNOX had no effect on serum FSH on either photoperiod.
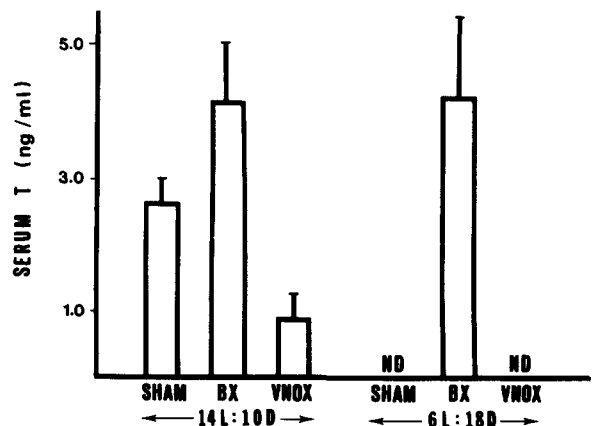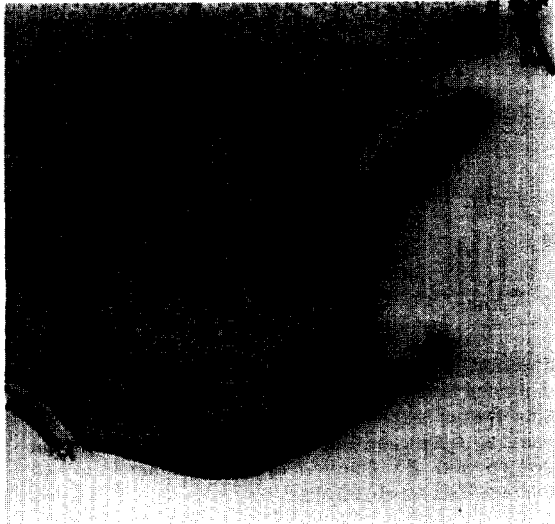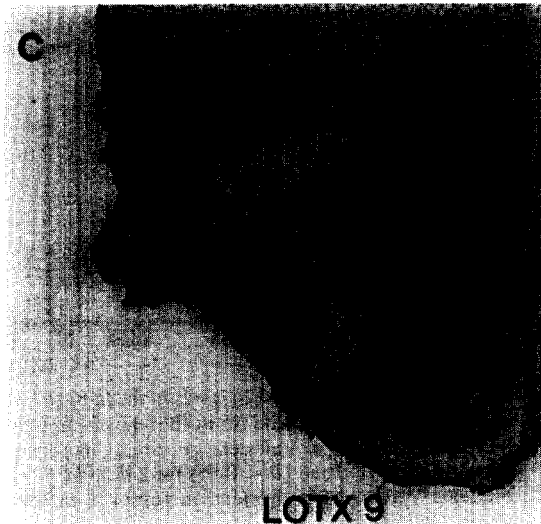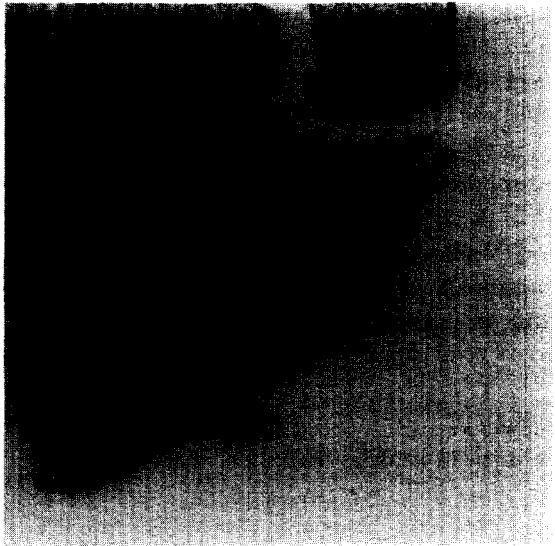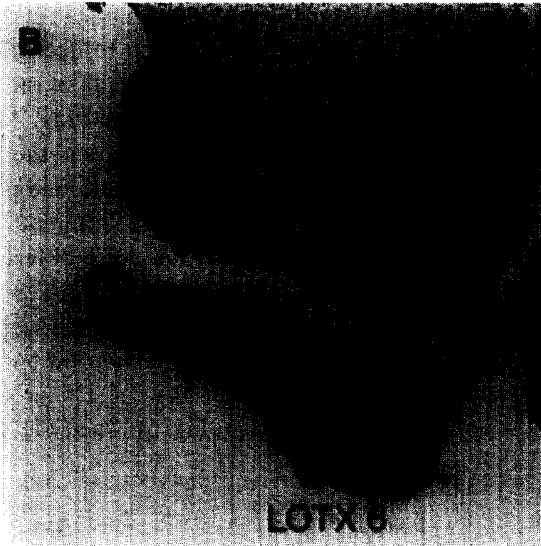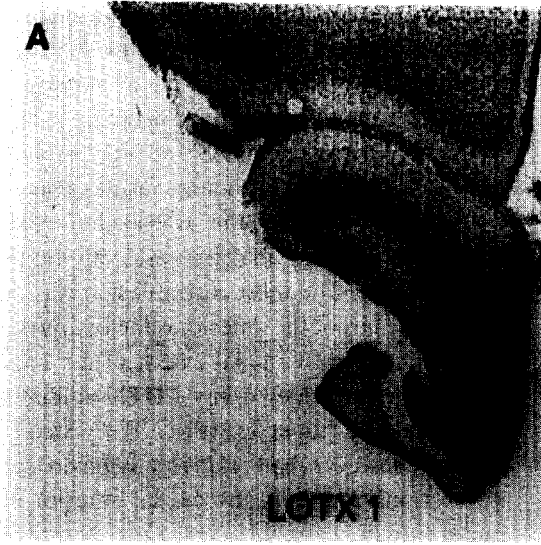

Fig. 3. Serum testosterone (T) levels in the hamsters at the end of Expt. I. See Fig. 1 legend for further details on symbols. BX increased serum T in hamsters on either photoperiod compared to the SH group ($P < 0.05$) for 14L:10D; $P < 0,01$ for 6L:18D. VNOX had no effect on serum T in hamsters on either photoperiod.
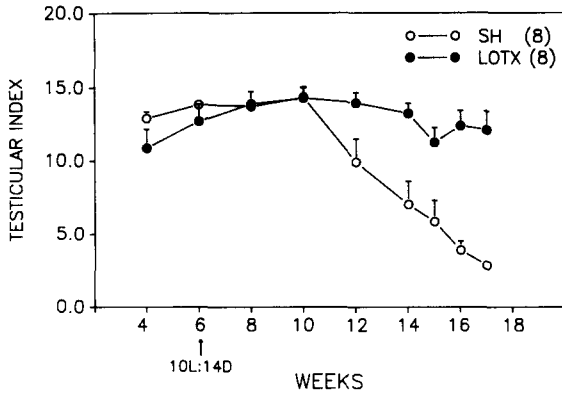
A LOTX 1

B LOTX 6

C LOTX 9

Fig. 5. Testicular index (mm length × mm width of left testis divided by g body weight × 10) of hamsters in Expt. 2. Hamsters underwent sham operation (SH) or bilateral transection of the lateral olfactory tract at 23 days of age. Six weeks later they were transferred to a 10L:14D photoperiod. The points represent the mean ± S.E.M. of 8 animals per group. ANOVA for repeated measures indicated that the groups were different at $P < 0.01$. Post-hoc analysis indicated that the two groups were different starting at week 12 of the study ($P < 0.05$) and were different at $P < 0.001$ by the end of the experiment.
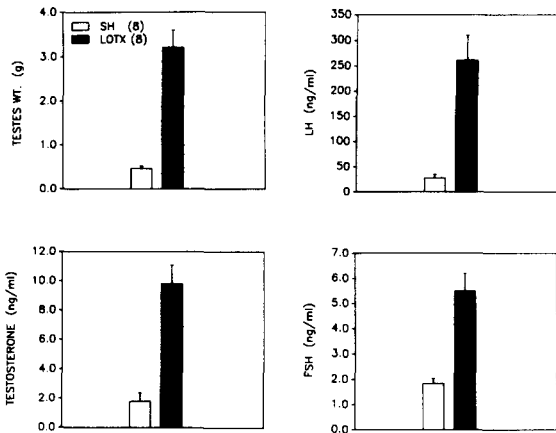

Fig. 6. Testes weight and serum LH, FSH and testosterone levels at the end of Expt. 2. Testes weight, testosterone and FSH were all greater in the LOTX group at $P < 0.001$ and LH was higher in the LOTX animals at $P < 0.01$. Number of animals per group is given in parentheses. See Fig. 1. and Fig. 5 legends for further details.

in the hamsters with regressed testes (LOTX 6) is illustrated in Fig. 4B. In placement and size this lesion was comparable to the lesions of other males that did not show regression. However, on the right side of the brain of this animal, the lesion spared more of the molecular layer of the AON than did other lesions. This spared tissue possibly could have contained scattered deep fibers of the LOT not identifiable with the Cresyl violet stain.

The reproductive hormone levels were much higher in the LOTX than SH group ($P < 0.001$; Fig. 6). The mean ± S.E.M. of the body weights for the SH group (144 ± 6.7 g) was not different than for the LOTX hamsters (156 ± 5.8 g).

DISCUSSION

BX destroys 3 separate neural systems: the main olfactory bulb, accessory olfactory bulb and nervus terminalis. The experiments presented here indicate that neither the vomeronasal organ nor the nervus terminalis are individually responsible for the effect of BX on tonic gonadotropin secretion. Vomeronasal organ removal had no effect, whereas leaving the nervus terminalis and other medial olfactory fibers intact, while transecting MOB and AOB fibers in the LOT, mimicked BX. Furthermore, the fact that a LOT lesion at the mid-rostrocaudal level of the olfactory tubercle (LOTX 9; Fig. 4C) was effective in preventing regression, suggests that MOB input to the anterior olfactory nucleus, and to the rostral pyriform cortex and olfactory tubercle is not involved.

The LOTX experiment also appears to rule out a humoral effect of the olfactory bulbs on tonic serum gonadotropin levels. The results therefore suggest that MOB, AOB or MOB plus AOB connections with the posterior pyriform cortex, amygdala or entorhinal cortex through the LOT are responsible.

It must be acknowledged that afferents to the bulb arise not only from peripheral structures (vomeronasal organ, olfactory mucosa, nervus terminalis),

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Fig. 4. Cresyl violet-stained coronal sections of the lateral olfactory tract transections (LOTX) in 3 hamsters. The level of the greatest damage to the LOT, on each side of the brain in each animal, is shown. A: LOTX animal no. 1: the most rostral bilateral lesions of the LOTX group. B: LOTX animal no. 6: bilateral lesions in the caudal anterior olfactory nucleus; representative of the level of most LOTX lesions; black arrow indicates the molecular layer in which some deep LOT fibers may have been spared only in this animal (see text). C: LOTX animal no. 9: the most caudal bilateral lesions of the LOTX group.

but also from a number of brain structures and that some of these areas send afferents to the bulb that are also contained in the LOT. Our lesion transected both sets of fibers. It is possible that transection of fibers projecting to the olfactory bulb from other areas of the brain through the LOT was responsible for the effects on gonadotropin secretion. This is particularly important when considering the organization of the connections of the AOB in conjunction with our results. The AOB in hamsters reciprocates connections with the nucleus of the accessory olfactory tract (NAOT) and the medial and posteromedial cortical nuclei of the amygdala[26]. Although the course of these fibers in hamsters has not been defined, in the rat the AOB efferents are found on the internal surface of the LOT at the level of the AON and in a compact bundle forming the dorsolateral part of the LOT at more caudal levels[26]. This segregation of AOB and MOB efferents in the LOT may explain the testicular regression in animal LOTX 6, an animal in which the LOTX lesion on the right side spared the molecular layer deep to the LOT at AON levels and thus may have spared AOB connections (Fig. 4B).

The effects of the remaining lesions in the LOTX group are also consistent with the hypothesis that AOB connections other than vomeronasal organ afferents modify the hormonal response to short days in Syrian hamsters. The most rostral termination of AOB efferents is in the NAOT at the rostral end of the amygdala. Thus, the AOB would have been disconnected from all of its targets even in animals with the most caudal LOT transections in this group. As noted above, however, our results are equally consistent with the hypothesis that MOB, or MOB plus AOB, connections with posterior pyriform cortex, amygdala and/or entorhinal cortex were maintaining this response.

Macrides et al.[14] have reported that transection of the lateral olfactory tract of hamsters had no effect on serum testosterone levels. However, these results are not directly comparable to the present data, since the animals in the Macrides et al. study underwent LOTX as adults and were then maintained on a long photoperiod, whereas we did LOTX prepubertally and maintained the animals in a short photoperiod.

It is tempting to speculate that the medial nucleus of the amygdala may be involved in the olfactory influence on the regulation of gonadotropin secretion since this nucleus has a high concentration of neurons that accumulate sex steroids[3,12,25] and many connections to the areas of the hypothalamus involved in regulating reproductive hormone release[10-12].

The lack of an effect of VNOX in the present study correlates with similar studies on the effect of BX to unmask photoperiodism in the rat. Nelson and Zucker[16] and Sanchez-Barcelo et al.[24] found that, unlike BX, VNOX did not facilitate testicular regression in blinded rats or rats maintained on short photoperiod. Interestingly, both groups found that peripheral anosmia induced by zinc sulfate treatment or deafferentation of olfactory filaments at the cribiform plate did induce sensitivity to light restriction, suggesting that the effect of olfactory bulbectomy in rats is anosmia-dependent and not due to a loss of non-olfactory functions of the olfactory bulbs. It should be emphasized that BX potentiates photoperiodic involvement in reproduction in rats but has the opposite effect in golden hamsters. It is not known whether the action of BX in rats and hamsters is related.

A recent study in which we attempted to reduce olfactory input has shown that hamsters isolated from all but their own chemosensory signals (by housing in individual cages with a filtered air supply) had similar gonadotropin levels and a similar response to short photoperiod as non-isolated animals[19]. These results suggest that the olfactory bulb effect on reproductive endocrinology in hamsters, unlike in rats may be unrelated to the sense of smell.

The presence of luteinizing hormone-releasing hormone (LH-RH) in the olfactory bulbs of hamsters[9,18] has implicated these LH-RH fibers in effects of the olfactory bulbs on LH and FSH secretion from the pituitary. However, most of these LH-RH fibers appear to be localized in the nervus terminalis[28], a fiber system which projects to the medial forebrain, and they would have been left intact in the LOTX hamsters. It therefore seems unlikely that the olfactory LH-RH containing neurons are related to the influence of the olfactory bulbs on tonic gonadotropin secretion. As noted above, however, our results do not rule out the possibility that afferents from the brain through the LOT to the nervus terminalis are the critical neural

elements. Recent studies have advanced our understanding of the nerve, but its composition, central connections and functions are not yet fully resolved.

The mechanism by which olfactory bulbectomy increases tonic gonadotropin secretion and inhibits short-photoperiod-induced reproductive regression is unknown. We know that BX prevents the antigonadotropic effect of exogenous melatonin in pineal intact[21] or pinealectomized hamsters[20]. In addition, we have shown that BX does not block the nocturnal elevation in melatonin secretion[20] and that BX results in increases in reproductive hormone secretion[21] and testes weight[21,22] in hamsters on long photoperiod. Thus BX affects the regulation of LH-RH release at a level which not only counteracts the action of melatonin, but alters serum gonadotropin levels independent of the photoperiod.

Lumia et al[13] have reported that BX reduced androgen receptor binding in the hypothalamus and amygdala in rats. A similar decrease in androgen binding in hamsters could decrease testosterone feedback on gonadotropin secretion in hamsters on long or short photoperiod[23], and lead to tonic elevation of LH and FSH. If a BX-induced decrease in androgen binding is responsible for the effect of BX, then the results of the present paper would suggest that the LOT is the pathway through which modulation of androgen receptor levels in the hypothalamus and amygdala is mediated.

The experiments of the present paper have substantially narrowed the possible neural pathways involved in the olfactory influence on reproductive hormone secretion. Further studies, however, will be necessary to delineate the specific structures and mechanisms involved in this phenomenon.

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