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Evidence for a ventral non-strial pathway from the amygdala to the bed nucleus of the stria terminalis in the male golden hamster

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Male hamsters in which the stria terminalis (ST) had been interrupted either by electrolytic lesions or knife cuts, or normal control males, received iontophoretic injections of horseradish peroxidase in either the bed nucleus of the stria terminalis (BNST) or the medial preoptic-anterior hypothalamic area (MPOAH). Comparison of intact and ST-lesioned brains revealed the existence of a ventral non-strial pathway, from cells in the medial amygdaloid nucleus (M) to the preoptic portion of the BNST but not to the MPOAH. Since bilateral lesions of M completely eliminate male hamster mating behavior, but ST lesions do not, we suggest that the ventral pathway to the BNST may be an important route by which M influences male copulatory behavior.

The bed nucleus of the stria terminalis (BNST) is a major efferent target of the amygdala[4,9–11,28] and, like the amygdala, has been shown to play an important role in both behavioral and neuroendocrine aspects of reproduction. Specifically, bilateral destruction of the BNST in male rats severely disrupts copulatory behavior[7,27]. In addition, this area is a major target for the actions of androgens in the mammalian brain[22,23,26] and electrochemical stimulation of the BNST modulates the release of luteinizing hormone (LH) by the anterior pituitary[2]. Recently, the discovery of a high concentration of opiate receptors in the BNST[8] has led to the suggestion that this brain area may be a site where endogenous or exogenous opiates exert their influence on the release of LH and, more generally, on the display of sexual behavior[20].

The medial nucleus of the amygdala (M) projects to the preoptic portion of the BNST[9,11,28] and this amygdaloid nucleus has also been implicated in the control of sexual behavior[16] and neuroendocrine events[1]. Evidence in the male golden hamster suggests that M, which, like the BNST, is an androgen-binding brain area[6], may process olfactory and vomeronasal sensory stimuli critical for the display of copulatory behavior. Vomeronasal sensory input reaches M directly via efferents of the accessory olfactory bulb[3,24], while olfactory information may reach this nucleus indirectly by way of afferents from olfactory-related areas of the ventral forebrain[11]. Peripheral deafferentation of both vomeronasal and olfactory systems[29], or bilateral lesions of M[15,16], completely eliminate male hamster copulatory behavior, and severely diminish the male's investigation of the female's anogenital odor cues during mating tests. The behavioral importance of M is not surprising in light of its neuroanatomical projections, not only to the BNST, but also to the medial preoptic-anterior hypothalamic junction (MPOAH)[9], another androgen-binding brain area[6,22,23] which has been strongly implicated in the control of male copulatory behavior in almost all vertebrates[12,18].

Neuroanatomical studies have demonstrated that efferents from M to the BNST and MPOAH travel in topographically organized fascicles within the ventromedial portion of the stria terminalis (ST)[4,11]. Given the importance of M,

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BNST and MPOAH in mediating copulatory behavior we were therefore surprised to find that male hamsters with bilateral destruction of the ST, unlike males with bilateral lesions of M, continued to display mating behavior during postoperative tests, although each of these animals did show distinct alterations in the temporal sequence of their copulatory behavior pattern[13]. These results led us to suggest the existence of a non-strial pathway from M to the BNST or MPOAH.

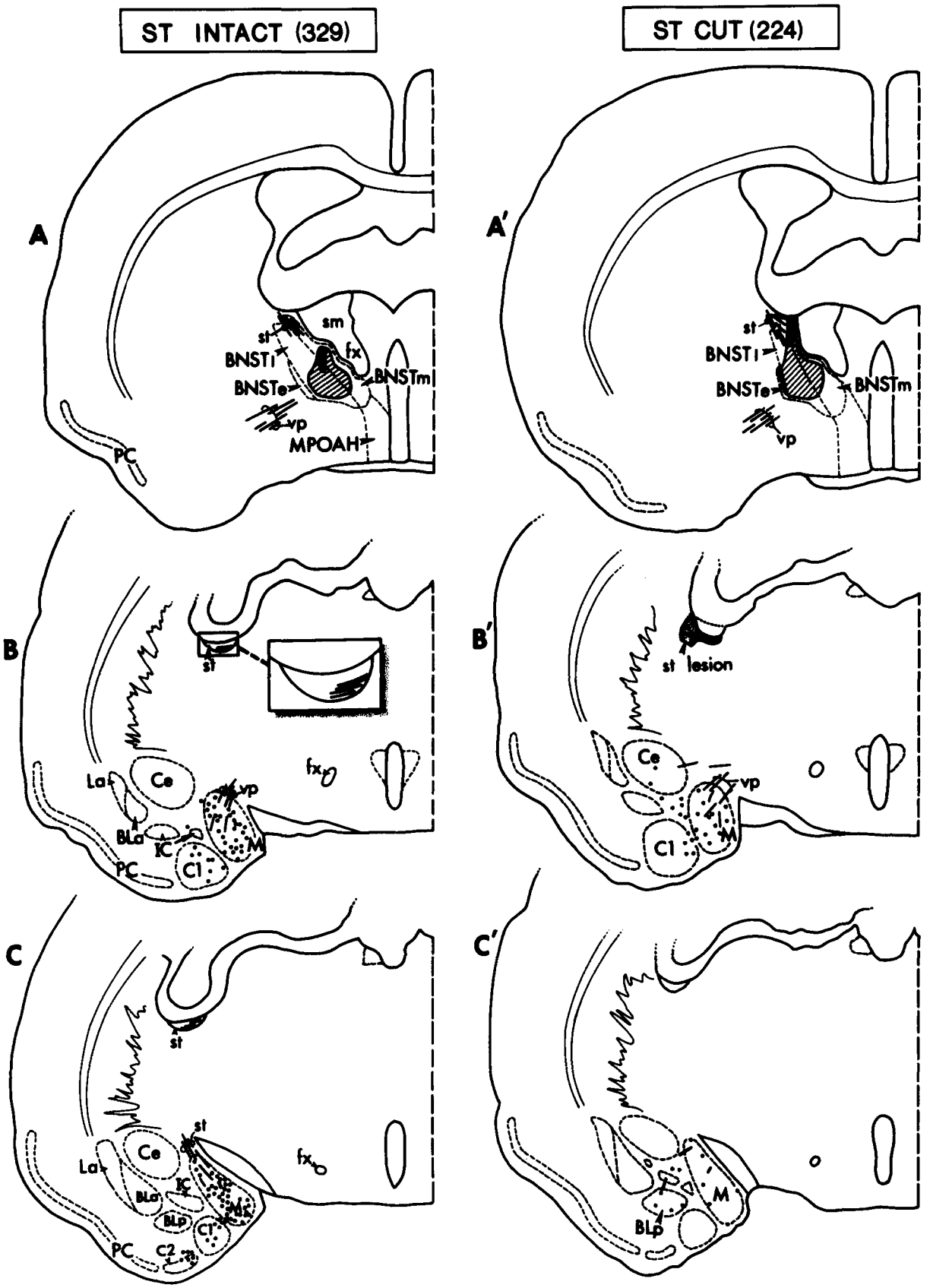
In order to test this hypothesis, we iontophoretically applied horseradish peroxidase (HRP) in the BNST or MPOAH of 15 male hamsters in which we had previously interrupted the ST with either bilateral electrolytic lesions or knife cuts. Nine of these hamsters received mating behavior tests before the HRP application as part of a behavioral study[13], so that the interval between the time of the ST lesions and the HRP surgery was approximately one month. Results from these animals were compared with those from 6 hamsters in which the ST lesion was made two days prior to the HRP application. Stria terminalis lesions and knife cuts were made according to procedures described previously[13]. In addition, we iontophoretically applied HRP in the BNST or MPOAH of 5 control animals in which the ST was intact.

Horseradish peroxidase (Miles Laboratories) was introduced into the brains of anesthetized male hamsters (Nembutal, 75 mg/kg) by stereotaxically lowering a glass micropipette (tip diameter 20–30 μm) filled with HRP in Tris buffer at pH 8.6 (500 mg/ml) and delivering square-wave DC pulses of 1.0–1.5 μA (1 Hz, 500 ms duration) for 8–10 min. Forty-eight hours following HRP surgery, animals were perfused and their brains removed and prepared for HRP histochemistry according to previously described procedures[21]. Brains were cut coronally in 50 μm sections on a freezing microtome, and every fourth section was reacted histochemically for HRP histochemistry using the tetramethylbenzidine (TMB) procedure of de Olmos et al.[5]. Adjacent sections were stained with cresyl violet. Sections treated with TMB were studied microscopically using bright-field and dark-field illumination, and the location of labeled cells and fibers were recorded with the aid of a Leitz drawing tube. The area of active uptake of HRP within each deposit was defined using criteria described by Newman and Winans[21].

The overall pattern of labeled cells seen in the amygdala after HRP applications in the BNST allowed us to confirm earlier descriptions of amygdaloid projections to this area in the hamster using autoradiographic and silver degeneration techniques[9,10]. A complete description of the topographic pattern of projections from individual amygdaloid nuclei to different subdivisions of the BNST, obtained in part from the results of this experiment, will be presented in a future paper. This report will deal primarily with BNST afferents from the medial nucleus of the amygdala. The data presented here are from animals in which HRP was introduced into the medial division of the preoptic portion of the BNST, that subdivision of the BNST shown in previous autoradiographic studies to be the major target of M, although not the only area of the BNST that receives input from this nucleus[9,28].

Fig. 1 illustrates the maximum extent of HRP application sites in the preoptic BNST and the pattern of labeled amygdaloid cells observed in hamsters 329 and 224, which were representa-

Fig. 1. Tracings of coronal brain sections from two hamsters, representative of those in which the stria terminalis was intact (A–C) or interrupted by lesion or knife cut (A′–C′), depicting HRP application sites in the preoptic portion of the bed nucleus of the stria terminalis and the location of labeled fibers (solid dark lines) in the stria terminalis (st) and the ventral pathway (vp), and labeled cells (black dots, one dot = one cell) in the amygdala. Solid black area = zone 1 of the application site (as defined in ref. 21); hatched area = zone 2 of the application site; stippled area = stria terminalis lesion. Abbreviations: BLa, basolateral nucleus of the amygdala (anterior division); BLp, basolateral nucleus of the amygdala (posterior division); BNSTe bed nucleus of the stria terminalis (extensor division); BNSTl, bed nucleus of the stria terminalis (lateral division); BNSTm, bed nucleus of the stria terminalis (medial division); C1, anterior cortical nucleus of the amygdala; C2, posterolateral cortical nucleus of the amygdala; Ce, central nucleus of the amygdala; fx, fornix; IC, intercalated masses of the amygdala; La, lateral nucleus of the amygdala; M, medial nucleus of the amygdala; PC, piriform cortex; sm, stria medullaris; st, stria terminalis; vp, ventral amygdaloid pathway to the bed nucleus of the stria terminalis.


ST INTACT (329)
ST CUT (224)
A
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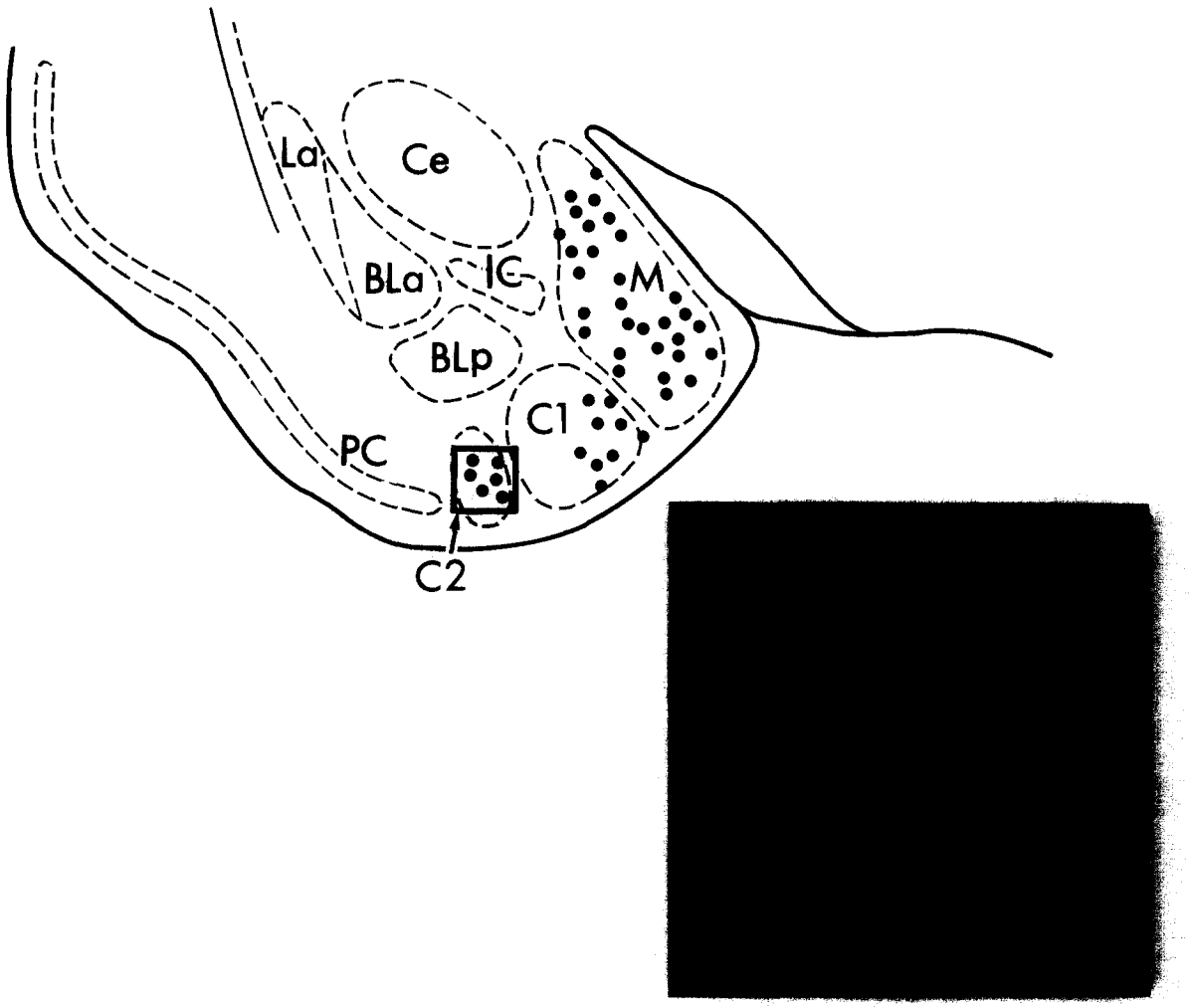

Fig. 2. Tracing of a coronal brain section depicting the location of labeled cells (black dots, one dot = one cell) in the medial (M), anterior cortical (C1), and posterolateral cortical (C2) nuclei of the amygdala following an HRP application into the bed nucleus of the stria terminalis in hamster 329, an animal in which the stria terminalis was intact. Dark-field photograph (lower right) corresponds to the area enclosed by the box, and shows a cluster of HRP-filled cells in the posterolateral cortical nucleus. See Fig. 1 for additional abbreviations.

tive, respectively, of control animals in which the ST was intact (A–C) and animals in which the ST had been interrupted either by electrolytic lesions or knife cuts (A′–C′). In both animals labeled fibers could be traced from the application site, centered in the medial division of the preoptic BNST (Fig. 1A, A′), dorsolaterally toward the ST and ventrolaterally, crossing the internal capsule. In hamster 329, in which the ST was intact, labeled fibers could be traced caudally in the ventromedial portion of the ST (see inset, Fig. 1B) and heavily labeled cells were found throughout the rostral-caudal extent of M (Fig. 1B, C). Scattered clusters of labeled neurons were also found in the anterior cortical nucleus (C1) and in the rostral part of the posterolateral cortical nucleus (C2) (Figs. 1B, C and 2).

In hamster 224, in which the ST had been destroyed, labeled fibers in the ST could be traced from the HRP application site in the preoptic BNST to the rostral edge of the electrolytic lesion (Fig. 1B′), but could not be found in the ST caudal to the lesion (Fig. 1C′). Nevertheless, labeled cells could still be seen in the rostral part

Fig. 3. Dark-field photomicrograph of a heavily labeled cell in the rostral part of the medial amygdaloid nucleus following an HRP application into the bed nucleus of the stria terminalis in hamster 224, an animal in which the stria terminalis was interrupted. Arrows indicate the course of a labeled dendrite extending from the cell body ventromedially toward the superficial plexiform layer of this nucleus.

of M and C1, as well as scattered throughout the central nucleus (Ce) and the posterior division of the basolateral nucleus (BLp) (Figs. 1B′, C′ and 3). In this animal, as in hamster 329, labeled fibers could be traced from the iontophoresis site ventrolaterally and caudally toward the rostral amygdala (Fig. 1A′, B′). These observations demonstrated the existence of a ventral non-strial pathway (VP) from cells in M to the preoptic BNST. Furthermore, labeling of this ventral pathway was observed in both groups of lesioned animals; those in which the ST lesion was made two days and those in which it was produced one month before the HRP application. The presence of labeled fibers in the VP in both lesioned groups and in the normal control animals indicates that this non-strial projection to the BNST is a component of amygdaloid efferents present in the normal animal, and is not a result of lesion-induced changes in the efferent projections of neurons in M.

In contrast, we found no evidence of a ventral non-strial projection from M to the MPOAH. HRP was introduced into the MPOAH at approximately the coronal level shown in Fig. 1A, A′ – that rostro-caudal level of MPOAH which corresponds to that of the terminal field observed after injections of [^{3}H]proline in M[9]. Following iontophoresis of HRP into the MPOAH of control hamsters in which the ST was intact, we observed labeled fibers coursing dorsolaterally from the application site, and traced them caudally in the ST. In these animals we saw heavily labeled cells scattered throughout the dorsocaudal portion of M, though not as many cells were observed as after iontophoresis of HRP into the preoptic BNST of normal animals. However, in hamsters in which the ST was cut

before HRP was deposited in the MPOAH, no labeled cells were seen in the amygdala. These observations confirm the previously described strial projections from M to MPOAH in the hamster[9] and provide no evidence for a non-strial amygdaloid projection to MPOAH.

Although we have focused here on the projections of cells in M, it should be noted that the evidence of this study, and other preliminary results in our laboratory from animals with HRP deposits in other areas of the BNST, indicate that those amygdaloid cells which send efferents to the BNST via the ventral non-strial pathway may be found within almost every amygdaloid nuclear group, and notably, also in those areas between definable nuclear groups. For example, the presence of scattered labeled cells in the central and basolateral nuclei of hamster 224 (Fig. 1B′, C′), in which the ST was cut, suggests that these nuclei, like M, also send efferents to the BNST via a ventral non-strial projection. That we observed labeled cells in the central and basolateral nuclei of hamster 224, but not in those nuclei of hamster 329, is probably because the HRP application site in hamster 224, but not 329, included the external lateral division of the BNST (BNSTe in Fig. 1A, A′). This portion of the BNST has been shown to receive input specifically from the central and basolateral nuclei in autoradiographic studies of amygdaloid efferents in the rat[11].

The evidence presented here confirms that M is a major source of amygdaloid afferents to the BNST in the hamster, and demonstrates for the first time a ventral non-strial pathway by which cells in M (primarily in its rostral portion) send efferents to the preoptic BNST. The existence of a ventral amygdaloid pathway to the BNST in the hamster complements evidence in the cat for a ventral non-strial pathway by which neurons in rostral M send efferents to the ventromedial nucleus of the hypothalamus[19]. The ventral amygdaloid pathway described here is distinct from the classically defined ventral amygdalofugal pathway[4,17] through which efferents of the central and basolateral nuclei, travelling at a more caudal level, course over the optic tract toward the lateral hypothalamus.

Since bilateral lesions of M completely eliminate male hamster mating behavior, but ST lesions do not, we suggest that the ventral pathway to the BNST may be an important route by which M influences the display of male copulatory behavior. In fact, preliminary results in our laboratory indicate that bilateral knife cuts which interrupt *both* strial and non-strial pathways from M lead to mating deficits similar to those seen after bilateral destruction of M[14].

Evidence from previous studies of those neural pathways which mediate chemosensory control of male hamster mating behavior[15,16,29] have led us to suggest that M is a site of integration of both olfactory and vomeronasal sensory information, even though this nucleus primarily receives vomeronasal input via afferents from the accessory olfactory bulb[3,24]. There are several indirect routes by which olfactory information might reach M which we have discussed previously[15,16]. One of these is by way of intra-amygdaloid connections to M from nuclei of the 'olfactory amygdala' namely the anterior (C1) and posterolateral (C2) cortical nuclei which receive olfactory afferents directly from the main olfactory bulb[3,24]. Our description, in this report, of a projection from clusters of cells in C1 and C2 labeled after HRP applications in the medial part of the preoptic BNST (see Figs. 1B, C and 2) suggests the possibility that portions of both the 'olfactory' and 'vomeronasal' amygdala project to a common area of the BNST, and that the BNST may also be an important area for chemosensory integration.

Another way by which olfactory input may reach M is by way of a small olfactory terminal field in the most rostral part of M, which we previously described in the hamster[15], and has also been reported in the rat and opossum[24,25]. Although, as mentioned, the predominant input to this nucleus in the hamster is that of vomeronasal afferents from the accessory olfactory bulb, this small olfactory input may allow for some integration of direct olfactory and vomeronasal information within M itself. In this light it is interesting to note that in this study, many of the labeled cells in M found to project to the preoptic BNST via the ventral non-strial pathway were

located in the most rostral part of M. In some of those heavily labeled neurons, we could, in fact, observe dendrites extending ventrally toward the superficial plexiform layer of this rostral portion of M (see Fig. 3), where in previous work we have localized projections of the main olfactory bulb[15]. Therefore, it is possible that the few cells in M which receive olfactory information directly from the main olfactory bulb, may send efferents to the preoptic BNST via the ventral non-strial pathway described in this report. The BNST may then be an area of integration of olfactory and vomeronasal information, not only from different amygdaloid nuclei, but also possibly from individual cells within M that receive either olfactory or vomeronasal information.

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