Connections of the Corticomedial Amygdala in the Golden Hamster. I. Efferents of the "Vomeronasal Amygdala"

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ABSTRACT   The medial (M) and posteromedial cortical (C3) amygdaloid nuclei and the nucleus of the accessory olfactory tract (NAOT) are designated the "vomeronasal amygdala" because they are the only components of the amygdala to receive a direct projection from the accessory olfactory bulb (AOB). The efferents of M and C3 were traced after injections of ^{3}H-proline into the amygdala in male golden hamsters. Frozen sections of the brains were processed for autoradiography.

The efferents of the "vomeronasal amygdala" are largely to areas which are primary and secondary terminal areas along the vomeronasal pathway, although the efferents from C3 and M terminate in different layers in these areas than do the projections from the vomeronasal nerve or the AOB. Specifically, C3 projects ipsilaterally to the internal granule cell layer of the AOB, the cellular layer of NAOT, and layer Ib of M. Additional fibers from C3 terminate in a retrocommissural component of the bed nucleus of the stria terminalis (BNST) bilaterally, and in the cellular layers of the contralateral C3. The medial nucleus projects to the cellular layer of the ipsilateral NAOT, layer Ib of C3, and bilaterally to the medial component of BNST.

Projections from M to non-vomeronasal areas terminate in the medial preoptic area-anterior hypothalamic junction, ventromedial nucleus of the hypothalamus, ventral premammillary nucleus and possibly in the ventral subiculum.

These results demonstrate reciprocal connections between primary and secondary vomeronasal areas and between the secondary areas themselves. They suggest that M, but not C3, projects to areas outside this vomeronasal network. The medial amygdaloid nucleus is therefore an important link between the vomeronasal organ and areas of the brain not receiving direct vomeronasal input.

Two separate chemosensory organs, the olfactory and vomeronasal, are found in the nasal cavities of most mammalian species (McCotter, '12; Wysocki, '80). They have been implicated in various species-specific behaviors and endocrine functions (Johns et al., '78; Fleming et al., '79; Reynolds and Keverne, '79). Earlier work involving removal of the olfactory bulbs produced deficits in male and female sexual behavior and aggressive and maternal behaviors (for review see Alberts, '74; Cain, '74). The emphasis in this laboratory has been to elucidate the role of chemosensory structures in the sexual behavior of male hamsters. The results of behavioral studies on male hamsters with peripheral deafferentation of the olfactory, vomeronasal, or both olfactory and vomeronasal organs suggest that these two systems are complementary in function and that together they provide information necessary for the maintenance of sexual behavior (Winans and Powers, '77).

Of the many areas in the ventral forebrain that receive input from the olfactory and vomeronasal pathways, the amygdala appears to be particularly important in mediating the chemosensory control of copulation in male

hamsters. Devor ('73) studied mating behavior in these animals after lesions of the lateral olfactory tract (LOT), which conveys fibers from both the main olfactory bulb and accessory (vomeronasal) olfactory bulb to the piriform lobe. When Devor cut the LOT immediately caudal to the olfactory bulbs, the males could not locate buried food pellets. They showed little sniffing investigation and no mounts with a receptive female. When the LOT was cut at the level of the caudal olfactory tubercle, the males found the buried food, investigated the receptive female and showed some aspects of sexual arousal, however these males also failed to engage in mounts, intromissions or ejaculations. Although the caudal LOT cuts in these animals spared main olfactory bulb input to the anterior olfactory nucleus, rostral piriform cortex and olfactory tubercle, they interrupted vomeronasal system efferents from the accessory olfactory bulb to the amygdala and bed nucleus of the stria terminalis, as well as main olfactory bulb fibers to the amygdala, posterior piriform cortex and entorhinal cortex. Lehman et al ('78) hypothesized that of these caudal areas, the amygdala may be of major importance in mediating olfactory and vomeronasal influences on sexual behavior. Their studies showed that male hamsters with bilateral lesions of the rostral corticomedial amygdala, particularly the medial nucleus, failed to mate, whereas animals with sham operations or lesions of only the caudal corticomedial amygdala mated normally.

Although the corticomedial amygdala receives both vomeronasal and olfactory inputs, the fibers from the two systems terminate in adjacent, non-overlapping areas. The main olfactory bulb projects to the lateral part of the corticomedial area—the anterior cortical (C1) and the posterolateral cortical (C2) nuclei—while the accessory olfactory bulb projects to the medial area—the medial nucleus (M), and the posteromedial cortical nucleus (C3) (Scalia and Winans, '75; Broadwell, '75; Devor, '76).

Thus, although behavioral evidence after deafferentation suggests that these two systems are both involved in chemosensory control of reproductive behavior, no convergence of the two pathways has been demonstrated to the level of the amygdala. The present study was initiated to determine the projections of the vomeronasal and olfactory nuclei of the corticomedial amygdala in the hamster. The studies of Krettek and Price ('77a,b '78a,b) and de Olmos ('72) have suggested that, in general, the medial/lateral segregation of vomeronasal

and olfactory areas in the amygdala is maintained in non-converging projections from those two areas in the rat and cat. The only point of interaction identified in these species was the projection of the endopiriform nucleus (which receives input from the olfactory cortex) to the vomeronasal amygdala (Krettek and Price, '77b).

The present paper describes the projections of the medial and posteromedial cortical nuclei, rostral and caudal components of the "vomeronasal amygdala." The companion paper (Kevetter and Winans, '81) examines the connections of C1 and C2 in the "olfactory amygdala."

MATERIALS AND METHODS

Electrolytic lesions were placed in 21 adult male hamsters, *Mesocricetus auratus,* anesthetized with 50 mg sodium pentobarbital/kg body weight. Anodal direct current of 0.5–1.5 mA was passed through epoxy-coated insect pins (371 μm diameter) for 5–15 seconds to produce lesions in selected parts of the amygdala.

The animals were anesthetized 2–8 days after surgery and perfused through the heart with isotonic saline followed by 10% phosphate-buffered formalin. Brains were removed, embedded in an egg-yolk-gelatin mixture, and sectioned in the coronal plane on a freezing microtome at 25 μm. Sections 125 μm or 250 μm apart were stained by the Fink-Heimer II technique (Fink and Heimer, '67). Adjacent sections were stained with cresyl violet to determine the extent and accuracy of the lesion and for cytoarchitectonic localization of degenerating fibers and terminals.

Autoradiographic studies were done on 50 male hamsters. One to five microcuries of tritiated proline [L-[3,4-³H(N)]-Proline; 25–50 Ci/mmole; New England Nuclear) in 0.01–0.05 μl physiological saline were stereotaxically injected over 45 minutes into one of the four nuclei of the corticomedial amygdala via a 1 μl or 5 μl Hamilton syringe. Survival times varied from six hours to six days. Most animals were perfused at 12, 24, or 48 hours. Perfusion with formalin and embedding of the brains were performed as described above. Twenty micrometer frozen sections, 100 or 200 μm apart, were mounted on gelatin-coated slides, coated with Kodak NTB2 emulsion, and exposed for 2–6 weeks at 4°C. The slides were then developed with Kodak D-19 developer, couterstained with cresyl violet, and observed microscopically under brightfield and darkfield illumination.

In 12 additional hamsters, electrolytic lesions were stereotaxically placed in the stria terminalis. Eight of these animals received injections of $2\,\mu\text{Ci}$ tritiated proline in 20 nl physiological saline in the amygdala two weeks after the lesion was made. The remaining four animals received identical injections three days after the stria terminalis was damaged. Twenty-four hours after the proline injections, all 12 animals were perfused, and the brains were processed for autoradiography as described above. Sections of four of these brains were also processed for Fink-Heimer impregnation.

RESULTS

The projections reported here were seen after a lesion or tritiated proline was placed in either the rostral (M) or caudal (C3) vomeronasal amygdala. Conclusions from the autoradiographic and Fink-Heimer impregnation techniques were nearly identical. The description and the illustrations in this account are primarily from autoradiographic material. The Fink-Heimer material was prepared for supplemental analysis, and proved particularly useful in distinguishing efferent fibers from terminal fields. In the following description the terms "terminals" and "terminal fields" are used interchangeably for the deposit of silver grains seen with the light microscope over cytoarchitectonically defined areas that received projections from M and C3.

The injection locus in either M or C3 was defined as the region containing cell bodies that appeared black due to heavy incorporation of tritiated proline (Cowan et al., '72). Surrounding this locus was a uniform distribution of silver grains, which diminished gradually with distance from the tip of the needle. Nuclei adjacent to M or C3 were variably included in this diffusion zone; however, the specific nuclei involved differed from brain to brain. Conclusions about the projections of a given nucleus were based on comparison of results from injections that involved that nucleus but had no other areas in common. This resulted in the elimination of projections arising from overlapping areas.

With two exceptions the projection sites described below appeared in at least ten of the 12 brains in which M was the focus of the injection and in eight of ten brains in which the injection was centered in C3. The two exceptions were terminal fields over the nucleus of the accessory olfactory tract, and contralateral bed nucleus of the stria terminalis, which were seen in only eight of the 12 brains having injections in

M. Additional brains with injections or lesions involving areas adjacent to M or C3, but not involving these target nuclei, were examined as controls.

Projections of the medial nucleus

Twelve animals had lesions or injections that primarily involved M. The maximum extent of the injection in the coronal plane in two brains is shown in Figure 1. The electrode or needle tracts commonly traversed the cortex, corpus callosum, internal capsule, optic tract, thalamic reticular nucleus, and the hippocampus. Projections from these areas could be traced directly from the electrode tract in Fink-Heimer material. All cases in which the stria terminalis was damaged were excluded from this part of the study. Projections of the medial nucleus are shown diagrammatically on the right side of Figures 2 and 3.

The pattern of terminal fields differed depending on the amount of M involved. The data suggested that neurons in the rostral and caudal parts of M project to different areas of the brain, but this could not be determined with certainty from the available material.

Efferent fibers of the medial nucleus travel medial to the central nucleus in the most medial component of the stria terminalis to a position ventral to the fornix, and continue rostrally in the medial third of the stria terminalis. At the level of the anterior thalamus, these fibers exit and course ventromedially in the middle portion of the exiting bundle to the anterior hypothalamic-medial preoptic area junction (AH-MPOA), at the level of the optic chiasm (Figs. 2D, E; 4). A dense accumulation of silver grains in the AH-MPOA indicates an area of termination at this level. Caudal to AH-MPOA the number of silver grains diminishes dramatically, although sparse and evenly spread grains are present throughout the mediobasal hypothalamus. In the core of the ventromedial nucleus of the hypothalamus (Figs. 3G, H, I; 5) and more posteriorly in the ventral premammillary nucleus (Fig. 2J), a high density of silver grains was also observed. In Fink-Heimer material degenerating fibers were found throughout the mediobasal hypothalamic area. However, fine, dust-like deposits representing degenerating boutons were seen only at the junction of the AH-MPOA, in the ventromedial nucleus of the hypothalamus, and in ventral premammillary nucleus.

Some fibers in the most medial part of the stria terminalis terminate in the medial part of the bed nucleus of the stria terminalis (BNST) (Figs. 2C, D; 6A, B). The label is seen lateral to

the stria medullaris and extends ventro-medially under this fiber bundle. Other fibers continue rostrally, decussate in the dorsal part of the anterior commissure, and terminate lateral to the stria medullaris in the contralateral BNST (Fig. 2D). This labeling is consistently lighter and more caudal than that observed in the contralateral BNST after injections of C3. After injections of M, silver grains were always seen in the anterior commissure, but the continuation of this labeling into the contralateral BNST was not always apparent.

After interruption of the stria terminalis (ST) Fink-Heimer impregnation demonstrated degenerating argyrophilic particles in the AH-MPOA, ventromedial nucleus of the hypothalamus, premammillary nucleus, and BNST. In the hamsters in which the ST was interrupted prior to the injection of tritiated proline into the amygdala, silver grains were seen in the ST up to the point of the lesion but not beyond. This was in striking contrast to the heavy labeling of fibers in the ST, which distributed to the mediobasal hypothalamus and

Abbreviations

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AC,	anterior commissure		F,	fornix
ACC,	nucleus accumbens		L,	lateral nucleus of the amygdala
AH,	anterior hypothalamus		LOT,	lateral olfactory tract
AHA,	amygdalo-hippocampal area		M,	medial nucleus of the amygdala
AOB,	accessory olfactory bulb		MPOA,	medial preoptic area
AOBgr,	internal granule cell layer, Accessory olfactory bulb		MPOA-AH,	medial preoptic area-anterior hyothalamic junction
BLa,	anterior division basolateral nucleus of the amygdala		NAOT,	nucleus of the accessory olfactory tract
BLp,	posterior division basolateral nucleus of the amygdala		NLOT,	nucleus of the lateral olfactory tract
BNST,	bed nucleus of the stria terminalis		OC,	optic chiasm
C1,	anterior cortical nucleus of the amygdala		OT, OTu,	optic tract olfactory tubercle
C2,	posterolateral cortical nucleus of the amygdala		PMNv,	ventral premammillary nucleus
C3,	posteromedial cortical nucleus of the amygdala		S, SM,	subiculum stria medullaris
CA1,	hippocampal field		ST,	stria terminalis
Ce,	central nucleus of the amygdala		tt, VMH,	tenia tecta ventromedial nucleus of the hypothalamus
CPF,	piriform cortex			
EC,	entorhinal cortex			
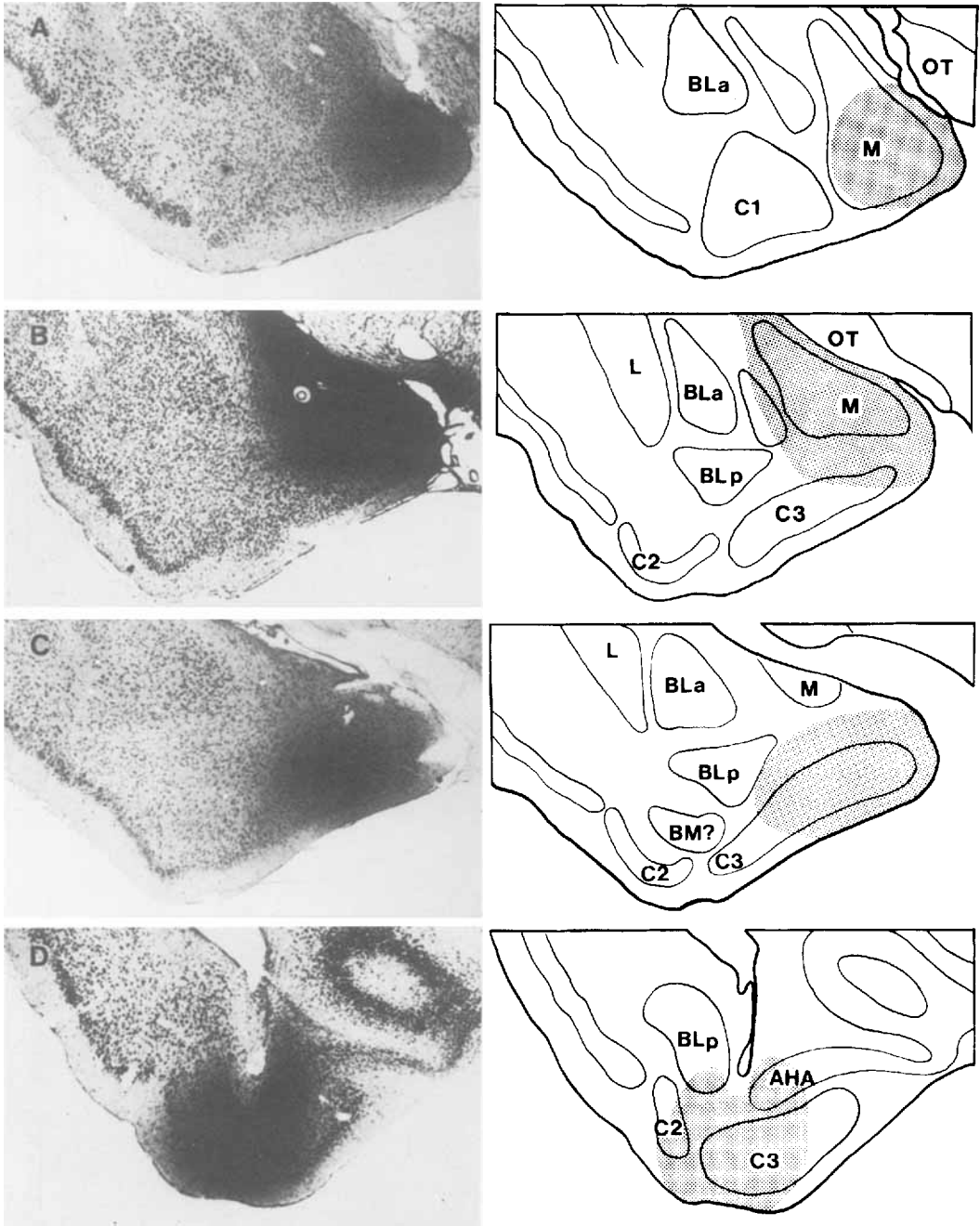
Fig. 1. Brightfield photomicrographs and matching tracing of injection sites centered in M and C3. Area of stippling corresponds to locus of the injection as defined by heavily labeled neurons. B and D show two of the largest injection sites used for this analysis. A. animal 97:2 μCi H-proline in 20 nl physiological saline. B. animal 57;5 μ ³H-proline in 50 nl physiological saline. C. animal 101; 2 μC8 ³H-proline in 20 nl physiological saline. D. animal 95; 2 μCi ³H-proline in 20 nl physiological saline. Animals 97, 57, and 95 survived 48 hours; animal 101 survived 12 hours.
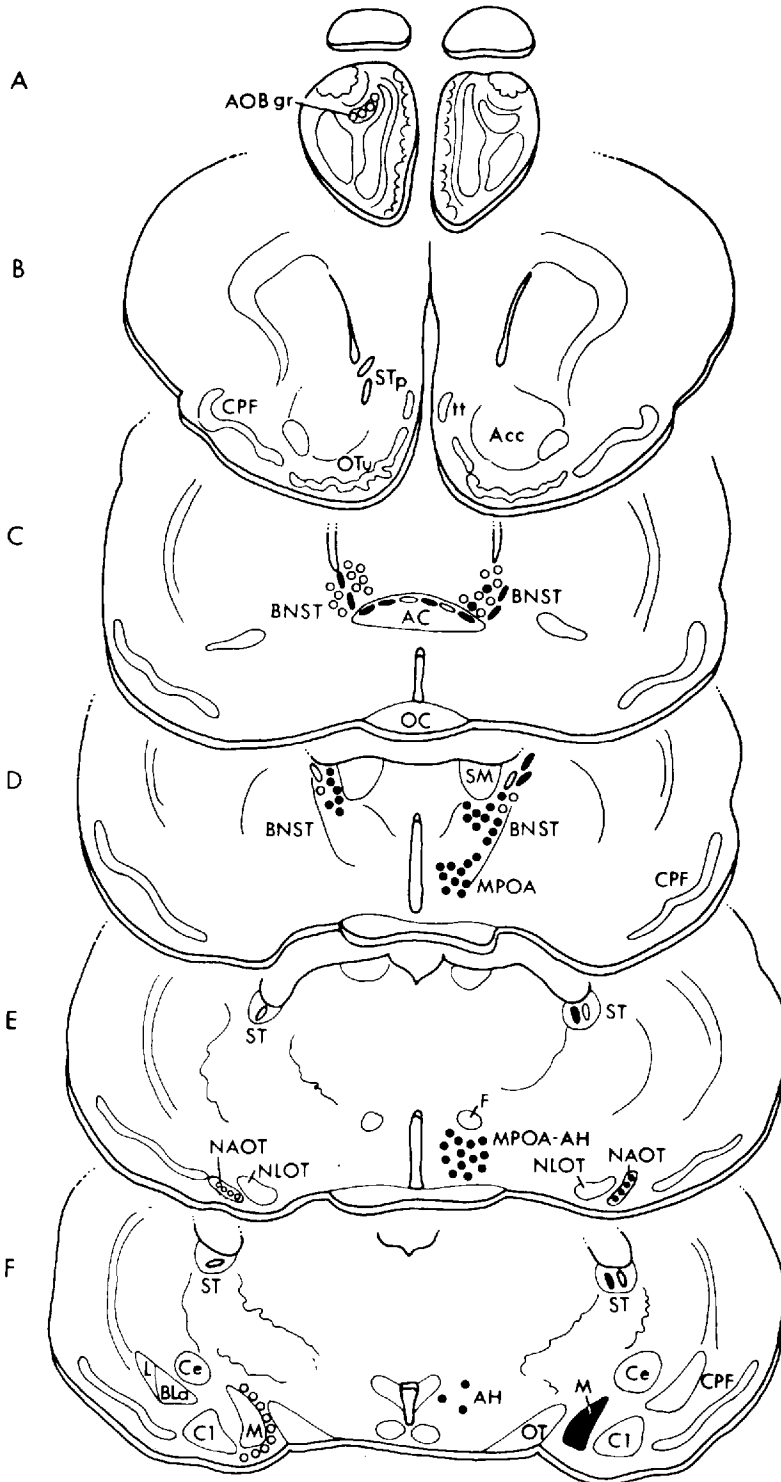
Fig. 2 and 3. Schematic drawings of coronal sections of the hamster brain showing the neuronal projections from the medial nucleus on the right (closed circles) and those from the posteromedial cortical nucleus (open circles) on the left.
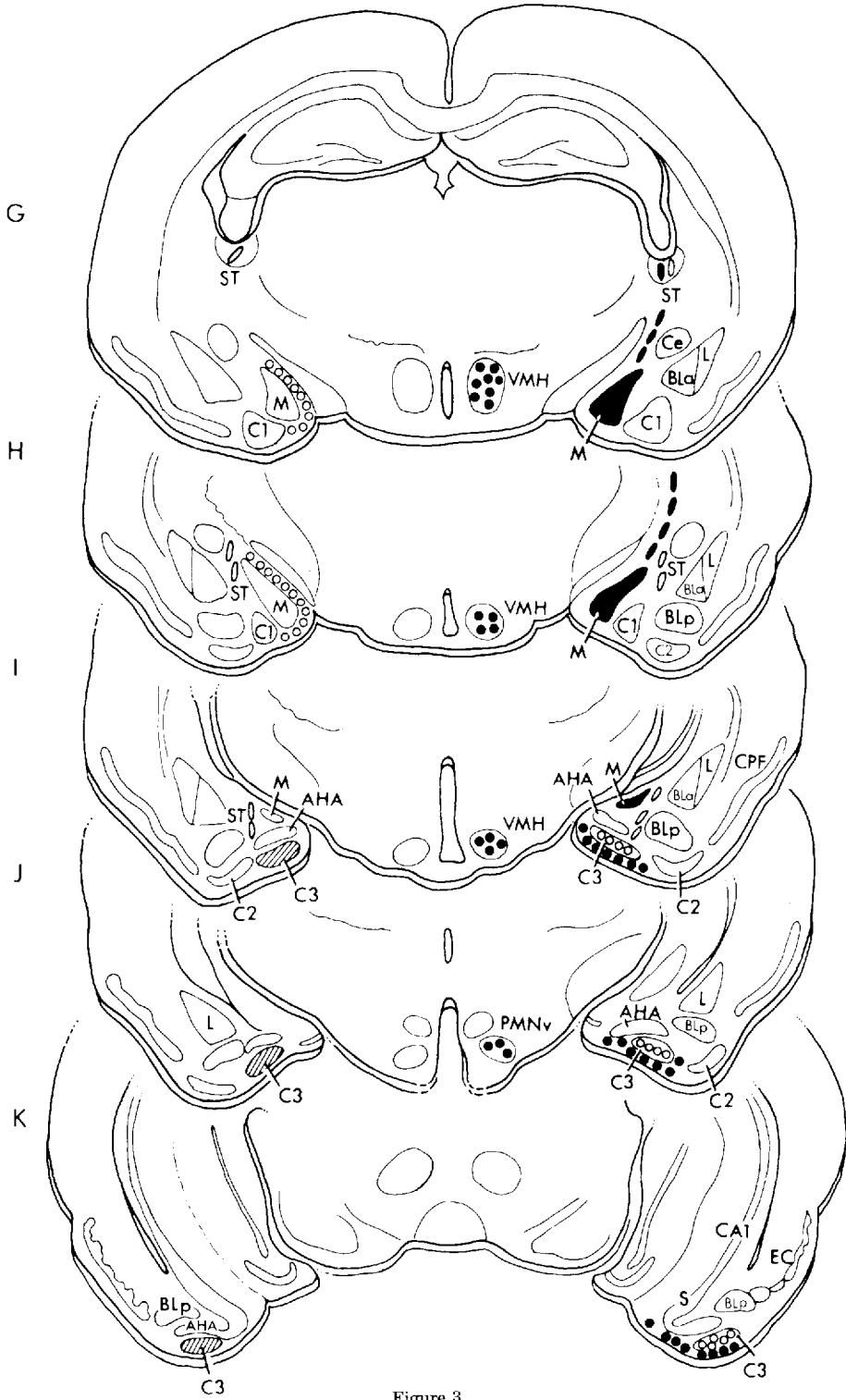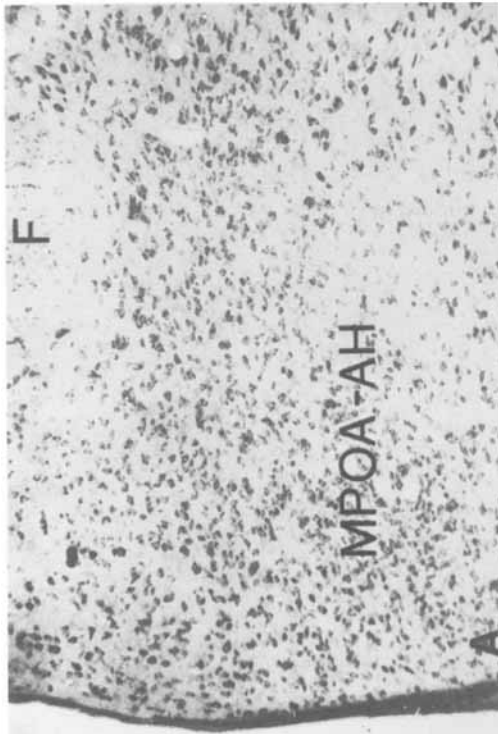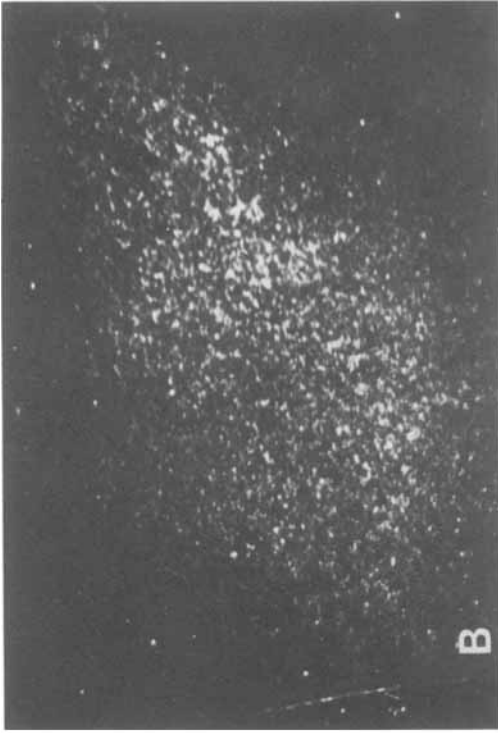
Figure 3.

BNST in experiments with injections of ³H-proline in M. These results suggested that most, if not all, of the medial hypothalamic projections from the amygdala travel through the stria terminalis.

The medial nucleus also sends efferents to C3 and may project further caudally to the subiculum. These fibers do not travel in the stria terminalis; most course caudally at the medial corner of the amygdalo-hippocampal area (AHA) and eventually terminate in layer Ib of C3 (Fig. 7A, B). Analysis of these brains revealed a lightly labeled field of silver grains in the molecular layer of the ventral subiculum. In brains in which the injection was almost entirely restricted to M, this field was faint; therefore it is likely that other amygdaloid nuclei included in the large injections contributed substantially to this projection. Fibers from M also terminate rostrally in the cellular layer of the nucleus of the accessory olfactory tract (NAOT). This nucleus is located ventral and rostral to M and therefore was often labeled by diffusion from the injection site. However, the projection from M to NAOT was apparent in both Fink-Heimer sections and in brains with limited injections of M that were processed autoradiographically.

Projections of the posteromedial cortical nucleus

Ten animals had ³H-proline injections into C3. The injection sites in two of these brains are shown in Figure 1, and the projections of C3 are diagrammed in Figures 2 and 3 on the left.

The needle tract passed through the cortex, corpus callosum, and internal capsule in all cases. The hippocampus and entorhinal cortex were also traversed in several cases. Projections of these areas were distinguished from those of C3 using the criteria described earlier.

Efferents from C3 travel medially and rostrally along the ventral surface of the brain to terminate in layer Ib on the ventral and medial surfaces of M. Some fibers from this group continue rostrally to terminate in the cellular layer of the NAOT (Figs. 2E 8). Other fibers from C3 enter the stria terminalis traveling dorsally and rostrally through the central nucleus of the amygdala to enter the sub-

Fig. 4. Junction of the anterior hypothalamus and medial preoptic area (MPOA-AH) after medial nucleus injection in hamster 70. A. cresyl violet. B. darkfield photomicrograph of coronal section in (A). Compare to Figure 2E.
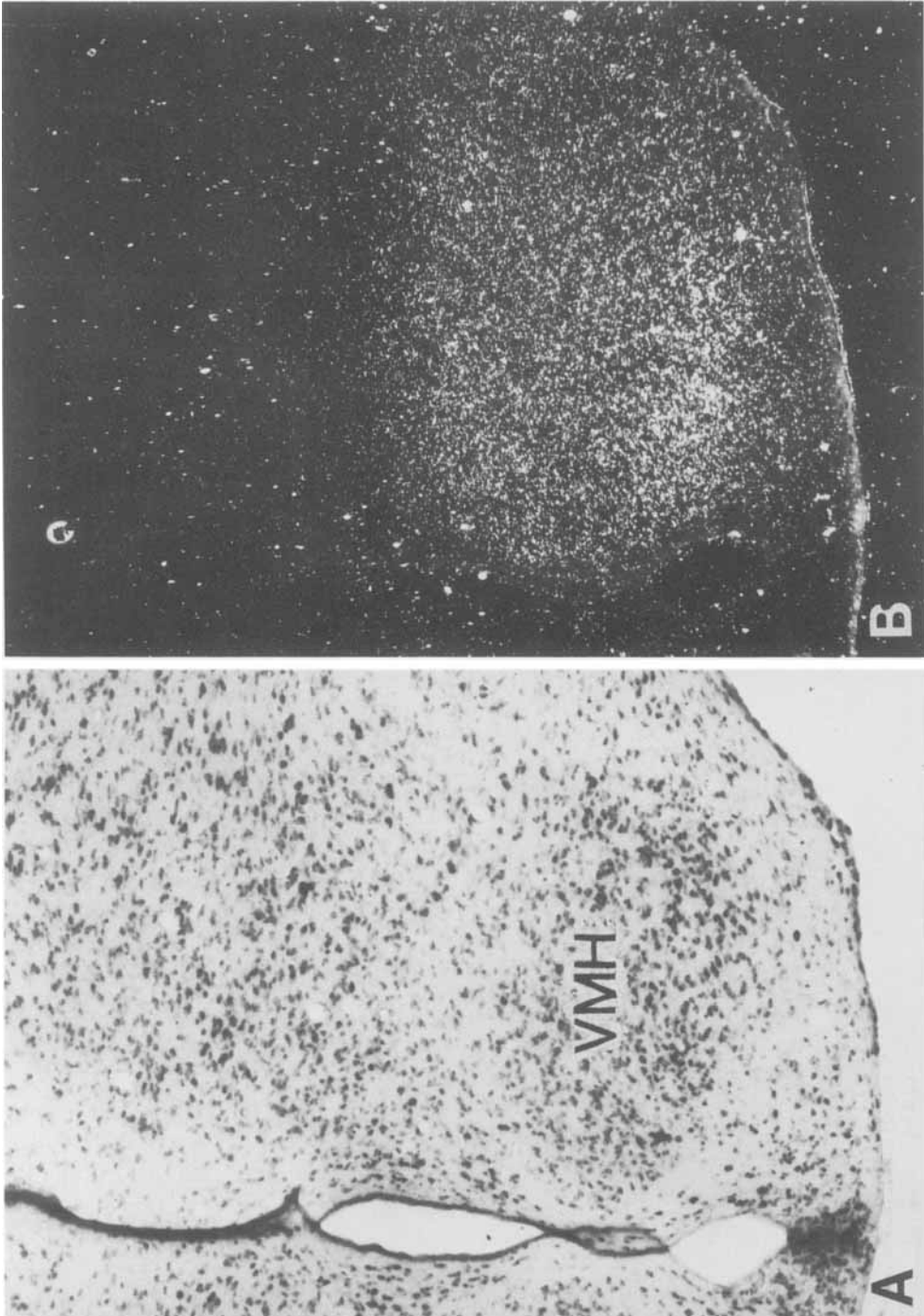
Fig. 5. Ventromedial nucleus of the hypothalamus (VMH) following a ³H-proline injection into M in hamster 70. Note that the central core of this area was labeled with silver grains. A. cresyl violet. B. darkfield photomicrograph of coronal section in (A). Compare to Figure 3H.
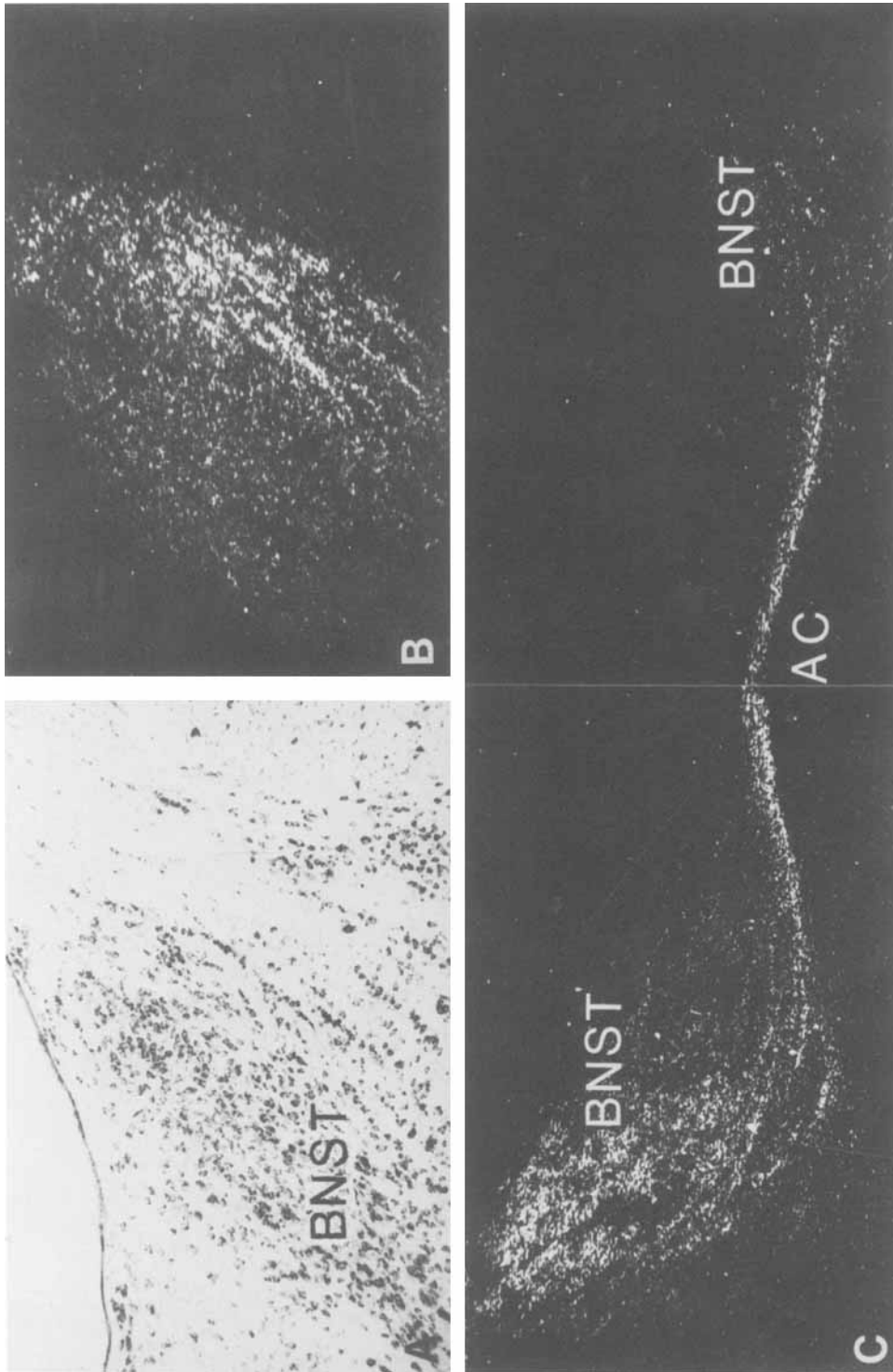
Fig. 6. The bed nucleus of the stria terminalis (BNST) labeled after injections into the vomeronasal amygdala. A. cresyl violet. B. darkfield photomicrograph of coronal section in (A) from hamster 70 after ^{3}H-proline injection into ipsilateral M. Compare to Figure 2D. C. BNST (left) labeled after injection of ipsilateral C3 in hamster 95. A projection across the anterior commissure to the contralateral BNST is seen on the right. Darkfield photomicrographs of coronal sections. Compare to Figure 2C.
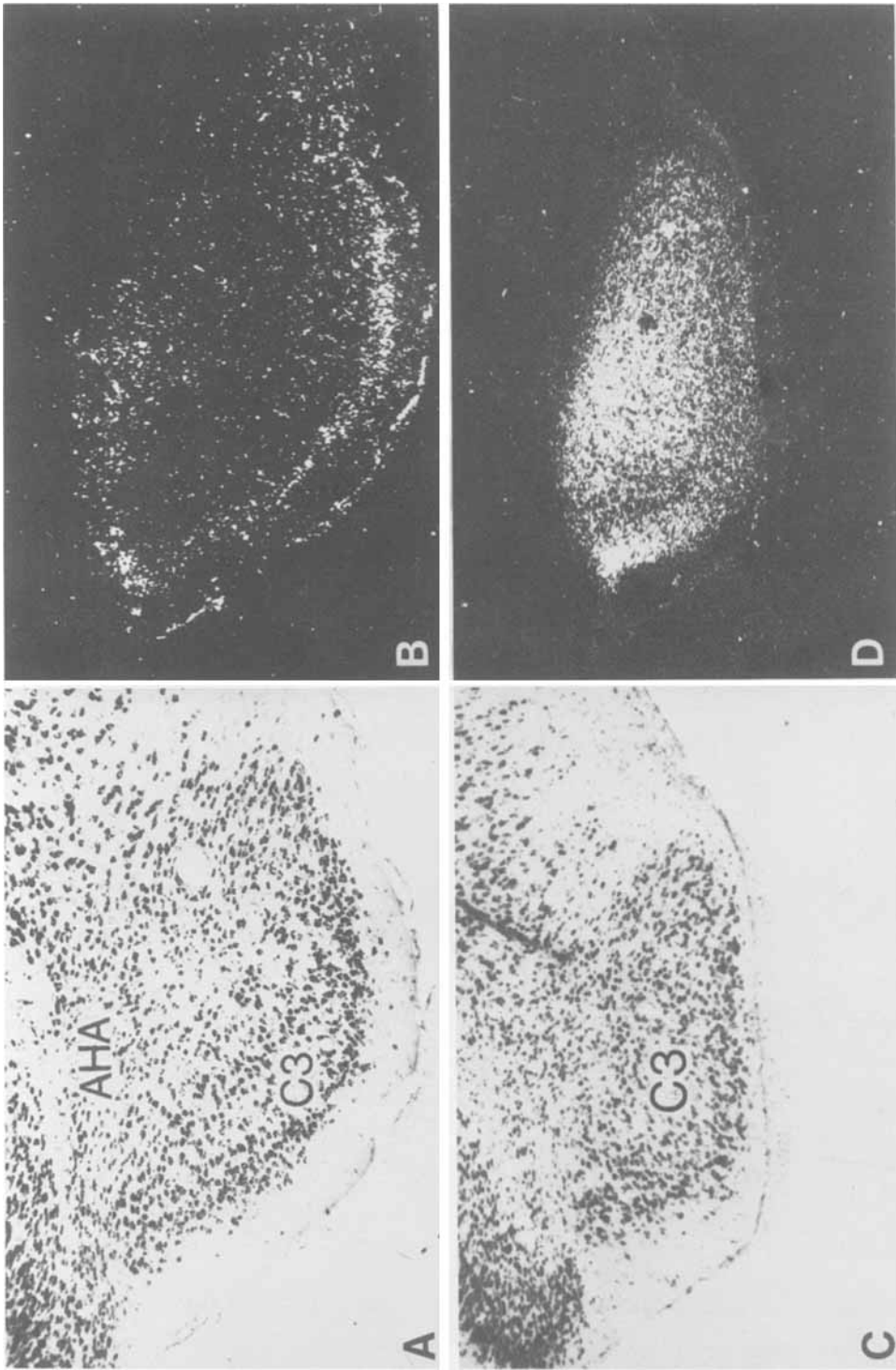
Fig. 7. The posteromedial cortical nucleus (C3) labeled after injections into the vomeronasal amygdala. A. cresyl violet. B. darkfield photomicrograph of a coronal section showing label in layer Ib after M injection in hamster 70. Compare to Figure 3J. C. cresyl violet. D. darkfield photomicrograph of coronal section in (C) showing label over contralateral C3 after injection of tritiated proline into C3 of hamster 58. Compare to Figure 3K.
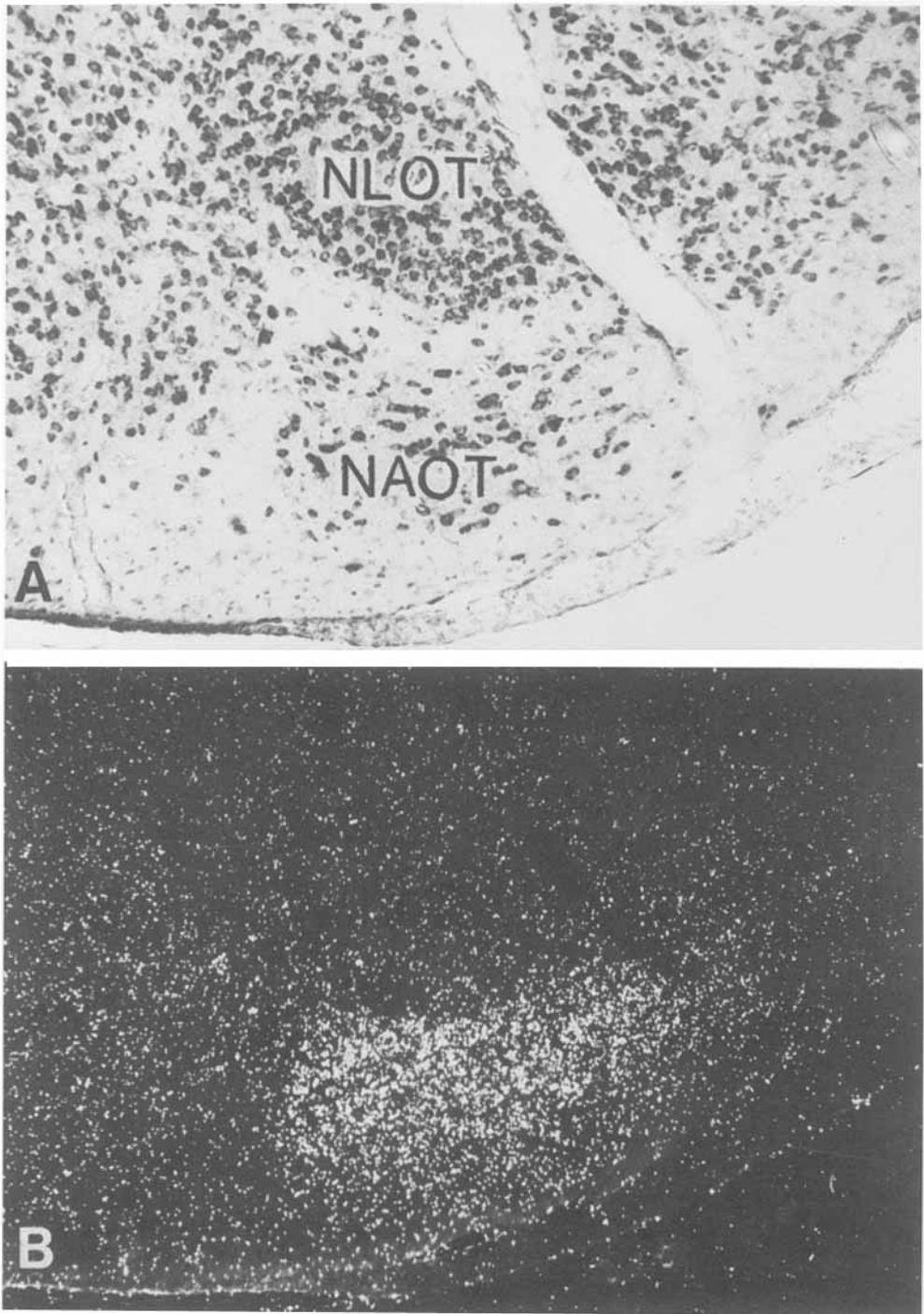
Fig. 8. Nucleus of the accessory olfactory tract (NAOT). Label which resulted from an injection of C3 in hamster 95. A. cresyl violet. B. darkfield photomicrograph of coronal section in (A). Compare to Figure 2E.

ventricular component of this bundle (de Olmos, '72). Some ST fibers end ipsilaterally in a terminal field in a lateral part of medial BNST at the level of the decussation of the anterior commissure, while others decussate in the dorsal part of the commissure and terminate contralaterally in the corresponding portion of the retrocommissural BNST (Figs. 2C, 6C, D). Additional fibers from the anterior commissure enter the central subventricular component of the contralateral stria terminalis and travel caudally to the cellular layer of the contralateral C3 (Figs. 3I, J, K; 7C, D). Ipsilateral to the injection, some fibers were observed to follow the intermediate olfactory tract, an anterior extension of the parolfactory stria terminalis (de Olmos, 72; de Olmos and Ingram, '72). These fibers continue rostrally within the olfactory peduncle ventral to the ependymal cells, and their terminals are found in the internal granule cell layer of the accessory olfactory bulb (AOB) (Fig. 9).

DISCUSSION

The AOB, which receives peripheral input exclusively from the VNO, projects to the NAOT, M, C3, and BNST. The projections described here from M and C3 provide anatomical evidence for a vomeronasal network that interconnects all of these AOB projection areas (Fig. 10). Both M and C3 project to NAOT, the ipsilateral and contralateral BNST, and to each other. In addition, C3 projects to the contralateral C3, and back to the AOB. With the exception of the medial amygdaloid nucleus, which projects to the preoptic area and medial basal hypothalamus, no area that receives from the AOB projects to areas outside of this vomeronasal network. The observation that M, but not C3, projects to the MPOA and medial hypothalamus may explain the findings of Lehman et al. ('78), that the rostral corticomedial amygdala plays a more important role in the chemosensory control of copulatory behavior in male hamsters than does the caudal corticomedial amygdala.

Although M, C3, and the AOB project to one another as well as to NAOT and BNST, the efferents from the amygdala and the AOB do not terminate in the same laminae or parts of these areas. In general, the AOB innervates the distal dendrites of the neurons in its terminal areas, while the fibers from the amygdala terminate closer to, or on, the cell bodies. For example, within C3, the AOB projects to layer Ia; M, to layer Ib; and the contralateral C3, to

the cellular layers. As illustrated in this example, the terminals from M and C3 also do not overlap. This is true in all cases except in the projections of M and C3 to NAOT, which appear to be nearly congruent. In the case of the BNST, the small subarea of BNST that receives direct input from the AOB is at the caudal edge of the area that receives from M, and this area, in turn, appears to extend only slightly into the more rostral BNST, in which fibers from C3 terminate.

Accessory olfactory bulb

In the hamster only C3 of the corticomedial amygdala was found to project to the AOB (Fig. 9).

After horseradish peroxidase injections into the AOB of the rat, de Olmos et al. ('78) identified a projection from M and NAOT, as well as from C3, back to the AOB. We did not observe this projection in the hamster, but de Olmos et al. mentioned that while HRP was located in many cells of C3 and NAOT, only a few cells were labeled in M. Thus it is possible that if this projection exists in the hamster, it was too lightly labeled in our material to be distinguished from background labeling.

The projection of C3 back to the AOB provides for feedback control of the output of the bulb. The vomeronasal nerves terminate in the glomerular layer of the AOB on dendrites of the mitral cells (Barber and Raisman, '74; Barber and Field, '75), which in turn project to the BNST, M, and C3 (Scalia and Winans, '75; Broadwell, '75; Devor, '76). Neurons of C3 project back to the internal granule cells of the AOB (Raisman, '72; Barber and Field, '75). In the MOB, the granule cells have been reported to have an inhibitory effect on the mitral cells (Rall and Shepherd, '68; Shepherd, '72). Thus if the functional connections within the AOB are similar to those of the main bulb, C3 may have an inhibitory effect on the output of the AOB.

Nucleus of the accessory olfactory tract

The nucleus of the accessory olfactory tract (NAOT) is a small cluster of cells in the anterior amygdala. Rostrally, it is ventral and lateral to the nucleus of the lateral olfactory tract, and more caudally it occupies a position ventral to M. A projection from AOB to the molecular layer Ia of NAOT (Scalia and Winans, '75; Kevetter, '78) is complemented by previously unidentified projections from both M and C3 to the deeper cellular area of this nucleus. Of these last two projections, the one from C3 to NAOT is heavier and more easily identified
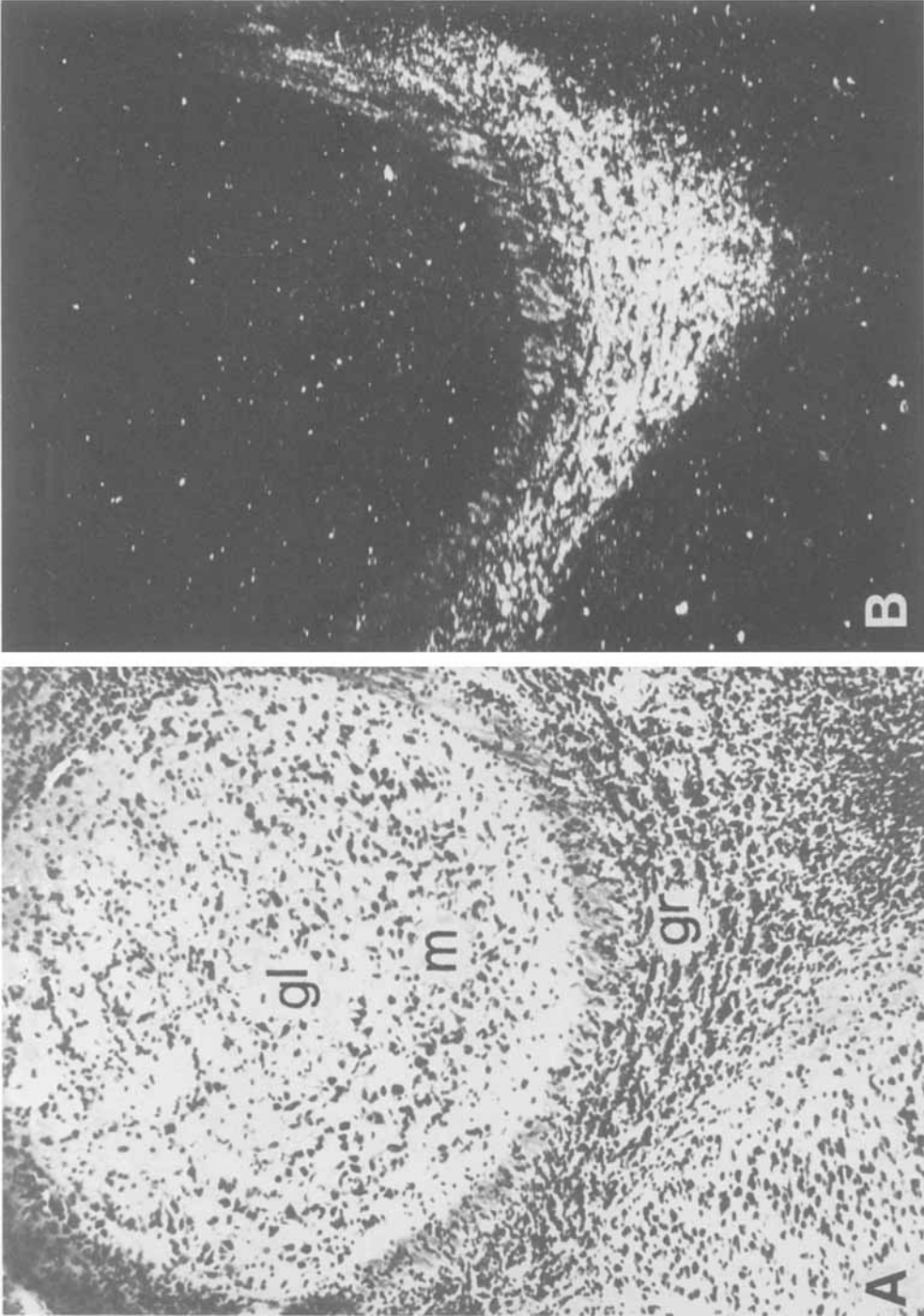
Fig. 9. Accessory olfactory bulb (AOB) after C3 injection in hamster 58. Note that the label is concentrated over the internal granule cell layer (gr) of this structure. A. cresyl violet. B. darkfield photomicrograph of (A). gl = glomerular layer; m = mitral cell layer; gr = internal granule cell layer. Compare to Figure 2A.
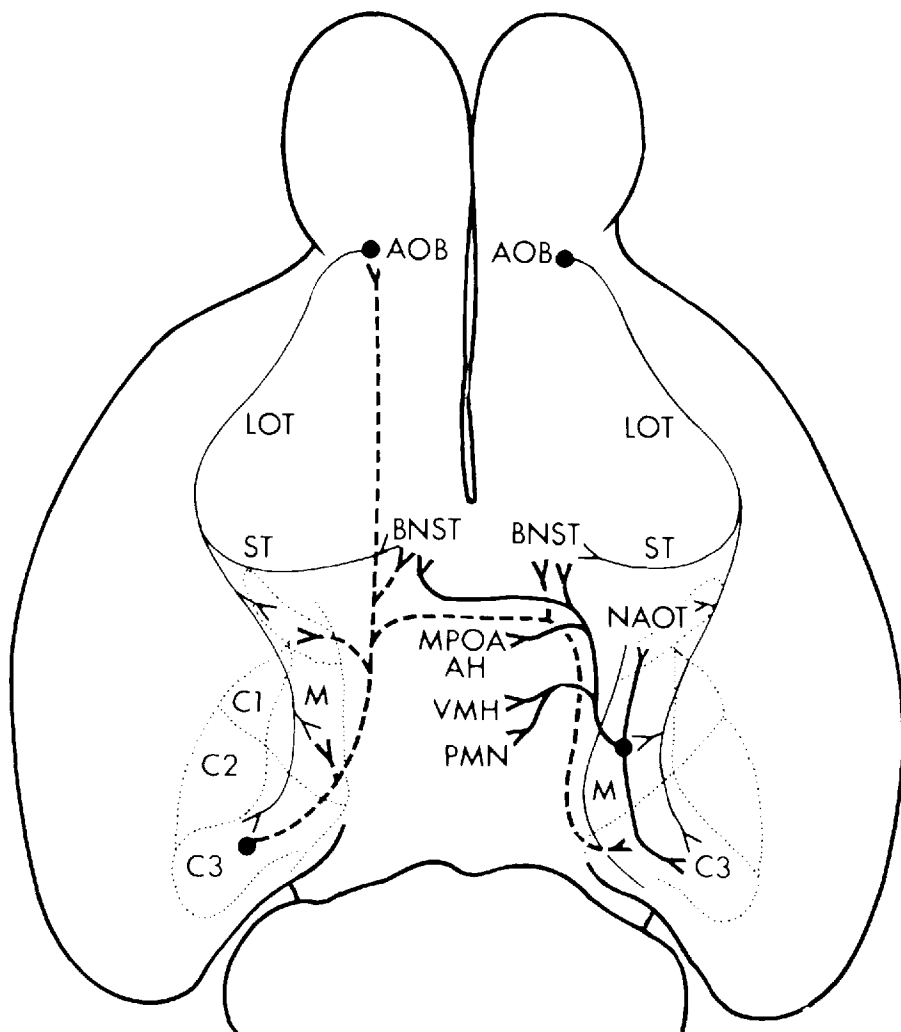
Fig. 10.   Schematic diagram of the ventral surface of the hamster brain showing the efferents of the accessory olfactory bulb (thin solid line), medial nucleus of the amygdala, M (heavy solid lines), and posteromedial cortical nucleus, C3 (dashed lines).

than that from M. The NAOT was the only area in the hamster in which the projections from M and C3 were found to share the same terminal field.

Amygdala

The nuclei M and C3 of the "vomeronasal amygdala" are reciprocally connected by intra-amygdaloid fibers, which terminate deep to the terminals of fibers from the AOB. While projections from the AOB terminate in the outer molecular layer (Ia) of both M and C3, M projects to layer Ib of C3, and fibers originating in C3 terminate in the Ib-II junction of M. Similar intrinsic connections between the two vomeronasal amygdaloid nuclei were described for the rat by de Olmos ('72). Krettek and Price ('78b) reported a projection from M to the molecular layer (I) of C3, which presumably corresponds to the results observed here in hamster, in which this projection is restricted to layer Ib. Although they could not dissociate the

intra-amygdaloid projections of C3 and AHA, Krettek and Price ('78b) did describe a projection to the cellular layers (II and III) of M from one or both of these nuclei. This differs slightly from the projection from C3 to layer Ib-II junction of M in hamster. Fibers from C3 that enter the contralateral stria terminalis continue caudally to the amygdaloid region, where they end in the cellular layers of the contralateral C3, and this commissural projection is similar to that previously reported for the rat (de Olmos and Ingram, '72) and the rabbit (Lammers, '72).

Bed nucleus of the stria terminalis

Medial and lateral subdivisions of BNST were recognized by Krettek and Price ('78a) in the rat and the cat on the basis of both cytoarchitectural differences and differences in the projections they received from the amygdala. The medial subdivision of BNST in those species receives fibers from M, C3, and the AOB (Broadwell, '75; Krettek and Price, '78a). In the hamster M projects to the caudal BNST, lateral and ventral to the stria medullaris at the level of the anterior thalamus, while C3 projects to a more rostral part of medial BNST, which is ventral to the anterior commissure. The topography of these projections differs from that observed in the rat by Krettek and Price ('78a), who described a medial to lateral (rather than a rostral to caudal) organization for the terminals from C3 and M, respectively. It is important to note that the terminals in the AH-MPOA junction after injections involving M exit more caudally and laterally from the stria terminalis than do fibers from C3 that project to this ventromedial portion of the rostral BNST.

Projections to the contralateral BNST decussate in the dorsal component of the anterior commissure and terminate in the medial division of the contralateral BNST with a rostro-caudal organization, which overlaps the input from the ipsilateral amygdala. In other words, both ipsilateral and contralateral M terminate in a more caudal area than ipsilateral and contralateral C3. Amygdaloid projections to the contralateral BNST have not been described in autoradiographic studies. After small lesions in the corticomedial amygdala, Leonard and Scott ('71) saw evidence of terminal degeneration in the anterior commissure of the rat but could not trace degeneration to the contralateral side. They concluded, as did Valverde ('65), that many of the fibers of this commissural component of the stria terminalis end on dendrites of the bed nucleus of the anterior

commissure or a component of the BNST located within the commissure. Terminals on such cells are also a possibility in the hamster, since the labeling seen in the anterior commissure, especially after injections involving M, was heavier than that seen leaving the commissure and terminating in the contralateral BNST.

Mediobasal hypothalamus

After injections or lesions of M, fibers from the ventral stria terminalis course medioventrally at the level of the anterior thalamus and terminate in the AH-MPOA junction area. This projection was seen in the hamster as previously reported in the rat by Leonard and Scott ('71), de Olmos ('72), and de Olmos and Ingram ('72). The density of labeling decreases caudal to the AH-MPOA area and increases again in the region of the ventromedial nucleus of the hypothalamus, where both the cellular core of the ventromedial nucleus of the hypothalamus and a laterally adjacent area, Diepen's nucleus tuberis lateralis, were heavily labeled after injections of M. Krettek and Price ('78a) consider this area a ventromedial extension of BNST; however, the terminal field includes the medial extent of the AH-MPOA region, adjacent to the ventricle, while the ventromedial BNST, which receives a projection from C3, is located rostral to this AH-MPOA junction. Projections from M to Diepen's nucleus have also been seen in the rat (de Olmos, '72; de Olmos and Ingram, '72; Leonard and Scott, '71; Krettek and Price, '78a) and cat (Krettek and Price, '78a).

Although input from the posterior part of the amygdala to the ventromedial hypothalamus was seen in the hamster, it appeared to arise in AHA, rather than from C3, as suggested by Leonard and Scott ('71) and de Olmos ('72). In the present study, injections of tritiated proline involving AHA resulted in heavy projections to the shell of the ventromedial nucleus of the hypothalamus, AH-MPOA, and the premammillary nucleus, whether C3 was involved or not. These findings are similar to those of Krettek and Price ('78a) in rat and cat; however, those authors suggested that the projection to the premammillary nucleus from the caudal amygdala was from C3. In the hamster material, this latter projection also appears to originate in AHA.

The projections of M to the AH-MPOA, mediobasal hypothalamus, and possibly to the subiculum were the only projections of the

corticomedial amygdala to areas not receiving a primary or secondary projection from the vomeronasal organ. Since lesions of the rostral, but not the caudal, corticomedial amygdala result in marked deficits in the sexual behavior of male hamsters (Lehman et al., '78), it is reasonable to postulate that in the hamster a necessary pathway for the expression of mating behavior includes the AH-MPOA and projections from M to the AH-MPOA and mediobasal hypothalamus. This hypothesis is supported by several other lines of evidence. Lesions of MPOA and medial hypothalamus rostral to the mammillary bodies severely disrupt copulatory behavior (Heimer and Larsson, '67; Paxinos and Bindra, '73; Caggiula et al., '73), and stimulation of the MPOA facilitates sexual performance of male rats (Malsbury, '71). In addition, estrogen and androgen concentrating neurons are found in the MPOA and in the medial nucleus of the amygdala, and M contains the heaviest concentration of androgen target areas in the amygdala of the rat (Pfaff and Keiner, '72; Grant and Stumpf, '75; Sar and Stumpf, '75; Krieger et al., '76).

The pathway by which those neurons of M that mediate male sexual behavior reach the MPOA and hypothalamus is still to be determined. Our experiments on the projections of the amygdala after severing the stria terminalis suggest that the majority of the cells that project to AH-MPOA and VMH send their axons through the stria. This conclusion is entirely in agreement with the findings of previous investigators who used degeneration techniques. In the experiments in which the ST was cut prior to injections of ³H-proline, the longest exposure time for the sections was two weeks, and the amount of ³H-proline injected in these animals was only $2 \mu Ci$—i.e., less than the amount injected in many of the animals used for identifying terminal fields. Thus, very sparse projections to the medial hypothalamus via a non-strial pathway may not have been observed in this material.

In addition, recent studies using horseradish peroxidase have provided positive evidence for the existence of a small number of neurons whose axons apparently take a non-strial route. McBride and Sutin ('77) found HRP label in cells of both the basomedial and the medial amygaloid nuclei after injecting HRP into the ventromedial hypothalamus of cats in which the stria terminalis had been cut. Preliminary studies by Lehman in our laboratory suggest that this is also the case in the hamster. A non-strial connection between the amygdala and the MPOA would be of major functional significance, since bilateral lesions of the stria terminalis, in contrast to bilateral lesions of M, do not eliminate the male's copulatory behavior.

LITERATURE CITED

Alberts, J.R. (1974) Producing and interpreting experimental olfactory deficits. Phys. and Behav., 12:657–670.

Barber, P.C., and P.M. Field (1975) Autoradiographic demonstration of afferent connections of the accessory olfactory bulb in the mouse. Brain Res., 85:201–203.

Barber, P.C., and G. Raisman (1974) An autoradiographic investigation of the projection of the vomeronasal organ to the accessory olfactory bulb in the mouse. Brain Res., 81:21–30.

Broadwell, R.D. (1975) Olfactory relationships of the telencephalon and diencephalon in the rabbit. I. An autoradiographic study of the efferent connections of the main and accessory olfactory bulb. J. Comp. Neurol., 163:329–346.

Caggiula, A.R., S.M. Antelman, and M.J. Zigmond (1973) Disruption of copulation in male rats after hypothalamic lesions: A behavioral, anatomical and neurochemical analysis. Brain Res., 59:273–287.

Cain, D.P. (1974) The role of the olfactory bulb in limbic mechanisms. Psych. Bull., 81:654–671.

Cowan, W.M., D.I. Gottlieb, A.E. Hendrickson, J.L. Price, and T.A. Woolsey (1972) The autoradiographic demonstration of axonal connections in the central nervous system. Brain Res., 37:21–51.

de Olmos, J.S. (1972) The amygdaloid projection field in the rat as studied with the cupric-silver method. In: The Neurobiology of the Amygdala. B. Eleftheriou, ed. Plenum Press, New York, pp. 145–204.

de Olmos, J., H. Hardy, and L. Heimer (1978) The afferent connections of the accessory olfactory bulb formations in the rat: An experimental HRP-study. J. Comp. Neurol., 181:213–243.

de Olmos, J.S., and W.R. Ingram (1972) The projection field of the stria terminalis in the rat brain. An experimental study. J. Comp. Neurol., 146:303–331.

Devor, M. (1973) Components of mating dissassociated by lateral olfactory tract transection in male hamsters. Brain Res., 64:437–441.

Devor, M. (1976) Fiber trajectories of olfactory bulb efferents in the hamster. J. Comp. Neurol., 166:31–48.

Fink, R.P., and L. Heimer (1967) Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Res., 4:369–374.

Fleming, A., F. VaeCarino, L. Tambosso, and P. Chee (1979) Vomeronasal and olfactory system modulation of maternal behavior in the rat. Science, 203:372–374.

Grant, L.D., and W.E. Stumpf (1975) Hormone uptake sites in relation to CNS biogenic amine systems. In: Anatomical Neuroendocrinology. W.E. Stumpf and L.D. Grant, eds. S. Karger, New York, pp. 445–463.

Heimer, L., and K. Larsson (1967) Mating behavior of male rats after olfactory bulb lesions. Phys. and Behav., 2:207–209.

Johns, M.A., H.H. Feder, B.R. Komisaruk, and A.D. Mayer (1978) Urine-induced reflex ovulation in anovulatory rats may be a vomeronasal effect. Nature, 272:446–448.

Kevetter, G.A. (1978) Efferents of the corticomedial amygdala in the golden hamster. Doctoral dissertation, University of Michigan.

Kevetter, G.A., and S.S. Winans (1981) Connections of the corticomedial amygdala in the golden hamster. II. Efferents of the "olfactory amygdala." J. Comp. Neurol., 197:99–111.

Krettek, J.E., and J.L. Price (1977a) Projections from the amygdaloid complex to the cerebral cortex and thalamus in the rat and cat. J. Comp. Neurol., 172:687–722.

Krettek, J.E., and J.L. Price (1977b) Projections from the amygdaloid complex and adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. J. Comp. Neurol., 172:723–752.

Krettek, J.E., and J.L. Price (1978a) Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. J. Comp. Neurol., 178:225–254.

Krettek, J.R., and J.L. Price (1978b) A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid connections. J. Comp. Neurol., 178:255–280.

Krieger, M.S., J.I. Morrell, and D.W. Pfaff (1976) Autoradiographic localization of estradiol-concentrating cells in the female hamster brain. Neuroendocrinology, 22:193–205.

Lammers, H.J. (1972) The neural connections of the amygdaloid complex in mammals. In: The Neurobiology of the Amygdala. B. Eleftheriou, ed. Plenum Press, New York, pp. 123–144.

Lehman, M.N., G.A. Kevetter, and J.B. Powers (1978) Sexual and aggressive behavior in male hamsters after lesions of the corticomedial amygdala. Soc. Neurosci. Prog. Abst., 251.

Leonard, C.M., and J.W. Scott (1971) Origin and distribution of the amygdalofugal pathways in the rat: An experimental neuroanatomical study. J. Comp. Neurol., 141:313–330.

Malsbury, C.W. (1971) Facilitation of male rat copulatory behavior by electrical stimulation of the medial preoptic area. Phys. Behav., 7:797–805.

McBride, R.L., and J. Sutin (1977) Amygdaloid and pontine projections to the ventromedial nucleus of the hypothalamus. J. Comp. Neurol., 174:377–396.

McCotter, R.E. (1912) The connection of the vomeronasal nerves with the accessory olfactory bulb in the opossum and other mammals. Anat. Rec., 6:299–318.

Paxinos, G., and D. Bindra (1973) Hypothalamic and midbrain neural pathways involved in eating, drinking, irritability, aggression, and copulation in rats. J. Comp. Phys. Psych., 82:1–14.

Pfaff, D.W., and M. Keiner (1972) Estradiol-concentrating cells in the rat amygdala as part of a limbic-hypothalamic hormone-sensitive system. In: The Neurobiology of the Amygdala. B. Eleftheriou, ed. Plenum Press, New York, pp. 775–785.

Raisman, G. (1972) An experimental study of the projection of the amygdala to the accessory olfactory bulb and its relationship to the concept of a dual olfactory system. Exp. Brain Res., 14:395–408.

Rall, W., and G.M. Shepherd (1968) Theoretical reconstruction of field potentials and dendrodendritic synaptic interactions in olfactory bulb. J. Neurophysiol., 31:884–915.

Reynolds, J., and E.B. Keverne (1979) The accessory olfactory system and its role in the pheromonally mediated suppression of oestrus in grouped mice. J. Reprod. Fertil., 57:31–35.

Sar, M., and W.E. Stumpf (1975) Distribution of androgen-concentrating neurons in rat brain. In: Anatomical Neuroendocrinology. W.E. Stumpf and L.D. Grant, eds. S. Karger, Basel, pp. 120–133.

Scalia, F., and S.S. Winans (1975) The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. J. Comp. Neurol., 161:31–56.

Shepherd, G.M. (1972) Synaptic organization of the mammalian olfactory bulb. Physiol. Rev., 52:864–917.

Valverde, F. (1965) Studies on the piriform lobe. Harvard University Press, Cambridge, Mass.

Winans, S.S., and J.B. Powers (1977) Olfactory and vomeronasal deafferentation of male hamsters: Histological and behavioral analyses. Brain Res., 126:325–344.

Wysocki, C.J. (1980) A review of the neurobehavioral evidence for the involvement of the vomeronasal system in mammalian reproduction. Neurosci. Biobehav. Rev. (in press).