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Axonal Projections of Medial Preoptic and Anterior Hypothalamic Neurons

Abstract. *Projections from medial preoptic area (mPOA) and medial anterior hypothalamic area (mAHA) neurons were investigated in albino rats with the use of tritiated amino acid autoradiography. Both the mPOA and the mAHA gave long-axon projections to structures in limbic forebrain and midbrain as well as short-axon projections to other hypothalamic regions. Differences between mPOA and mAHA neurons were observed in projections to the mid-septal region, ventromedial hypothalamus, premammillary region, and central gray. Further, while axons from the mPOA traveled within the medial forebrain bundle, those from the mAHA remained in a band ventromedial to the fornix. These anatomical differences may underlie functional differences between the mPOA and mAHA which have been demonstrated with other experimental techniques.*

The involvement of the preoptic area (POA) and anterior hypothalamus in a variety of physiological, endocrine, and behavioral functions is well known and intensively studied. For example, in regard to endocrine function, medial preoptic area (mPOA) and medial anterior hypothalamic area (mAHA) neurons are known to concentrate estradiol and to be involved both in production of leutinizing hormone releasing factor and in its effect on reproductive behavior (*1*). Attempts have been made to study the anatomical connections of the mPOA and mAHA (*2*), but stains for degenerating fibers, useful elsewhere in the brain, have not given satisfactory results when applied to the hypothalamus. Difficulties in staining the small-calibered axons involved and uncertainties in interpreting lesions which damage fibers-of-passage have hindered investigation of the projections from the hypothalamus and preoptic area (*2*).

With the use of tritiated amino acid autoradiography in tracing axonal connections (*3*), these problems can be circumvented. In this technique, locally injected tritiated amino acids are taken up by neuronal cell bodies, which incorporate the amino acids into protein and transport the labeled protein down their axons in an autoradiographically visible form (*3*). Using autoradiographic techniques (*3, 4*), we examined the projections from the medial preoptic area and anterior hypothalamus in a series of 35 brains from adult albino rats of both sexes with injections in these sites. Injections of tritiated leucine or proline (10 to 50 nl; 200 μc/μl) in physiological saline were made stereotaxically into mPOA (N = 13) or mAHA (N = 22); the animals survived 48 hours postoperatively. After perfusion and paraffin embedding, brains were sectioned (6-μm serial sections), and Kodak NTB-3 nuclear emulsion was applied to the mounted sections. The autoradiograms thus prepared were developed after 21 to 30 days exposure, then systematically scanned and charted. For details of the autoradiographic method, see (*3, 4*). Labeled axons were recognized by patterns of silver grains (usually above Luxol-Fast Blue stained fibers) arranged in linear (longitudinal section of fibers) or clumped fashion (cross section of fibers). This report will concentrate on a comparison of the results from mPOA and mAHA brains; a more complete analysis of mPOA and mAHA projections, including results from injection sites in the nuclei of the diagonal bands, the bed nucleus of the stria terminals, the periventricular POA and mAHA, and the dorsal and lateral mPOA and mAHA, is in preparation (*5*).

In Fig. 1, a series of charts through two brains, one with an injection site in the mPOA (case 39) and one with an mAHA site (case 34) are compared. The injection volumes (10 nl), survival times (48 hours), and exposure times (30 days) were identical in these two animals. Axons of mPOA and mAHA neurons are not restricted to short, local projections (see Fig. 1, A, E, and F) as has often been suggested. In a number of regions, we found the projections of mPOA and mAHA neurons to be similar. Both the mPOA and the mAHA sent fibers to the very anterior lateral septum under the genu of the corpus callosum. Also, both mPOA and mAHA projected to the nuclei of the diagonal bands and to the bed nucleus of the stria terminalis (Fig. 1A). Clearly labeled fibers were observed leading into the periventricular thalamus (Fig. 1, B to D); labeled fibers were also followed into the stria terminalis and the stria medullaris at the diencephalic-telencephalic junction (Fig. 1, B and C). In both mPOA and mAHA cases, the labeled fibers in the stria medullaris distributed to the habenula as diffuse grains within the lateral nucleus (Fig. 1, C and D). Labeled fibers from the mPOA and mAHA in the stria terminalis distributed in the amygdala to the anterior area and medial nucleus (Fig. 1, C and D). The stria terminalis projection to the amygdala was supplemented by labeled fibers spreading laterally into the anterior area and by labeled fibers coursing over the optic tract into the medial nucleus (Fig. 1, C and D).

From both mPOA and mAHA, labeled fiber bundles were followed into the median eminence, and label was seen in the arcuate nucleus (Fig. 1, C and D). More posteriorly, we observed a bilateral projection to the supramammillary nucleus (Fig. 1E) and labeled fibers running through the lateral mammillary nucleus into the ventral tegmental area of Tsai (Fig. 1, E and F). Fibers from this region coursed posterolaterally into the midbrain reticular formation and dorsally into the midbrain raphe nuclei (Fig. 1F).

We also noted differences in axonal projections between mPOA and mAHA neu-

Table 1. Quantitative evaluation of projections to medial and ventrolateral subdivisions of the ventromedial nucleus of the hypothalamus (VM) and the region just posterior to the VM, in matched anatomical positions. The values are mean numbers of grains per reticule square (10 μm per side) in a 100-square reticule, ± standard deviation.

Brain No. (and injection site)	Anterior-posterior level	Mean grains per reticule square	
		Medial VM	Ventrolateral VM
No. 34 (mAHA)	In VM	51.82 ± 9.49	30.66 ± 15.65
	Posterior to VM	14.50 ± 6.25*	30.32 ± 14.63
No. 39 (mPOA)	In VM	1.43 ± 1.37	8.05 ± 3.61
	Posterior to VM	3.61 ± 2.75*	5.69 ± 3.16*

*Indicates a significant difference between VM and posterior to VM at the $P < .001$ level, calculated by a two-tailed Student's *t*-test.

rons. In the septum, mAHA neurons projected most heavily to the midlateral region, below the dorsal septal nucleus and off the midline (Fig. 1A). The mPOA neurons, on the other hand, projected to the dorsal septal nucleus and showed little labeling of the midlateral septum (Fig. 1A).

Traveling posteriorly, mPOA and mAHA axons had distinctly different trajectories. Labeled fibers coursing posterolaterally from the mPOA injection site collected just lateral to the fornix, forming a dorsal-ventral band of fibers in the medial forebrain bundle (Fig. 1C). The mAHA neurons sent very few labeled axons into the medial forebrain bundle; rather, labeled fibers coursed posteriorly through the hypothalamus in a bundle ventromedial to the fornix (Fig. 1C). In the premammillary region, some of these labeled mAHA axons swept dorsomedially upward to join the periventricular fiber system to the central gray, and some spread medially into the dorsal premammillary nuclei (Fig. 1, D and E). This pattern of projections was not seen in the mPOA cases, where labeled fibers remained lateral, in the medial forebrain bundle. These different fiber trajectories had apparent correlates in areas of termination. First, the ventromedial nucleus of the hypothalamus (VM) received a heavy projection from the mAHA neurons, while in the mPOA cases only very light labeling was observed (Fig. 1C and Table 1). To test whether labeled fibers in the VM terminated there rather than simply continuing posterior, grains were counted in the VM proper and in the region just posterior to the VM. The results of this quantitative evaluation (Table 1) indicate that much of the label in the medial VM of mAHA brains terminates there. Second, while in most cases mAHA neurons projected strongly to the dorsal premammillary nuclei and only lightly to the ventral premammillary nucleus, the mPOA cases showed the opposite pattern (Fig. 1D). Finally, the mPOA brains showed a light projection to the ventrolateral quadrant of the central gray (Fig. 1, E and F). In contrast, the mAHA cases showed heavy labeling of the periventricular fiber system, which in the anterior central gray had a distinct pattern outlining the lateral border of the medial nucleus (*6*) (Fig. 1E). A diffuse but heavy projection from the mAHA was noted in all the central gray nuclei with diffuse grains spreading dorsally from the central gray (Fig. 1F).

Efferent pathways from the mPOA and the mAHA to limbic forebrain regions and to the midbrain often have been assumed on the basis of demonstrated mPOA and mAHA involvement in sexual behavior, temperature regulation, and other behavioral and autonomic functions (*7*), but they have not previously been demonstrated. The anatomical confirmation of mPOA and mAHA axons in the median eminence and the presence of projections to the arcuate nucleus (*8*) helps to clarify mechanisms for hypothalamic control of pituitary secretion. Also, anatomical differences in the projections from the mPOA and mAHA, suggested by behavioral re-

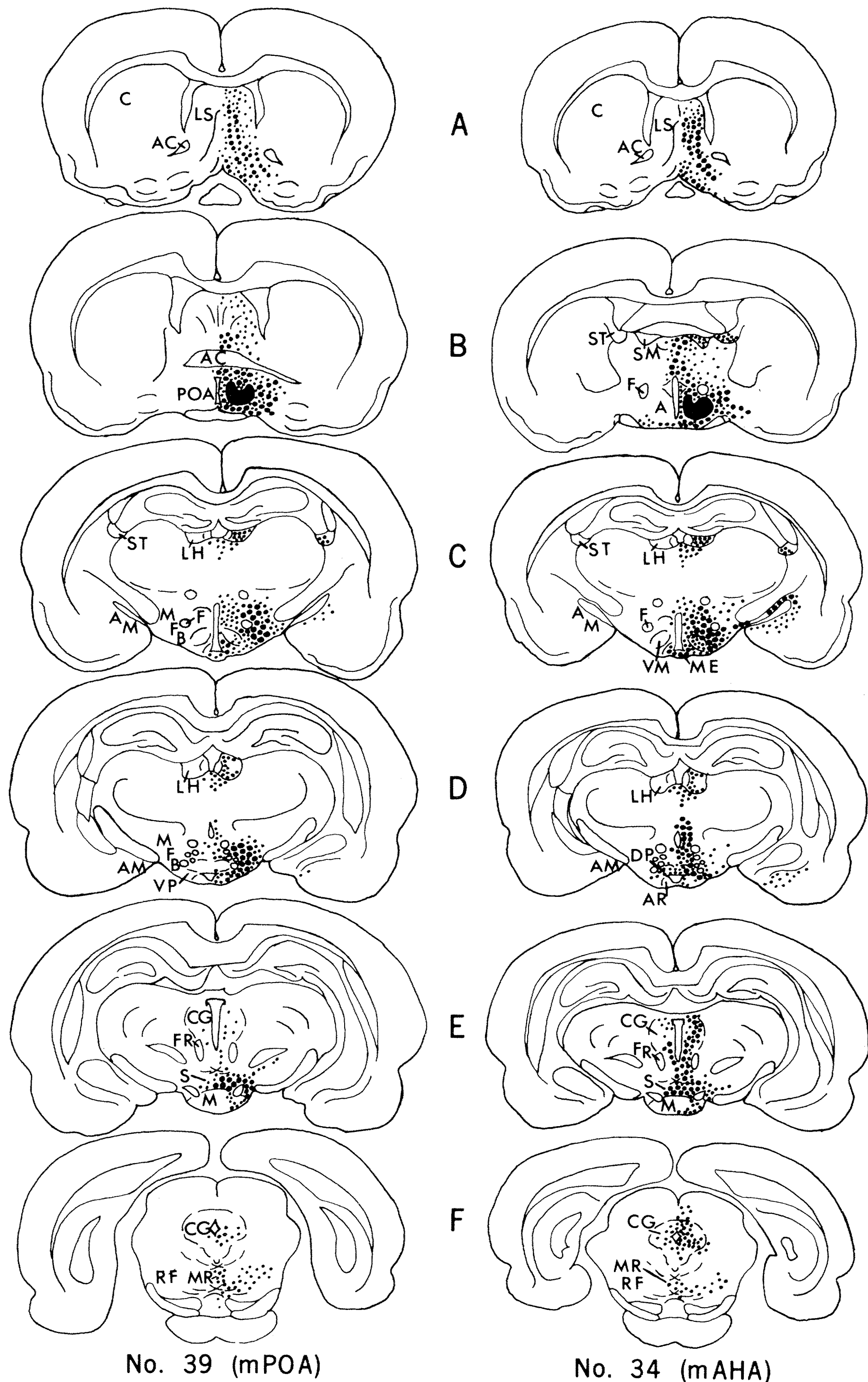

Fig. 1. Charts of the distribution of grains (projections) from autoradiograms of brains with injection sites in the mPOA (case 39) and mAHA (case 34). Sections are matched for level except in (B), where the injection sites (labeled cell bodies) are illustrated in solid black. Larger dots indicate labeled fibers, while small dots represent fields of diffuse, individual grains. Abbreviations: *A*, anterior hypothalamus; *AC*, anterior commissure; *AM*, medial amygdaloid nucleus; *AR*, arcuate nucleus; *C*, caudato-putamen; *CG*, central gray; *DP*, dorsal premammillary nucleus; *F*, fornix; *FR*, fasciculus retroflexus; *LH*, lateral habenula; *LS*, lateral septal nucleus; *M*, mammillary nuclei; *ME*, median eminence; *MFB*, medial forebrain bundle; *MR*, median raphe nucleus; *POA*, preoptic area; *RF*, reticular formation; *S*, supramammillary nucleus; *SM*, stria medullaris; *ST*, stria terminalis; *VM*, ventromedial nucleus of the hypothalamus; *VP*, ventral premammillary nucleus. See text for descriptions of the projections.

sults (*9*), have been substantiated. For example, display of masculine sexual behavior in rats has been shown to depend on the neurons of the POA (*10*) and on an intact medial forebrain bundle (*11*). The occurrence of female mating behavior, however, seems to depend on the mAHA or the VM, or both (*12*, and may actually be inhibited by the mPOA (*13*). Such results correlate well with our demonstrations of mPOA axons in the medial forebrain bundle, and both axons and terminations from the mAHA in the ventromedial region. The axonal projections demonstrated thus provide a possible anatomical substrate for separate neural systems mediating male and female sex behavior.

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Deficits in Binocular Depth Perception in Cats After Alternating Monocular Deprivation

Abstract. *Allowing very young kittens to see with only one eye at a time greatly reduces the proportion of binocular cortical cells. Compared to normal cats these specially reared animals suffer deficits in binocular depth perception while retaining normal acuity in the two eyes. Evidently, binocular cells play a crucial role in stereopsis.*

Almost all neurons in the visual cortex of a normal cat can be activated through both eyes (*1*). Many of these binocular cells have receptive fields on disparate areas of the two retinas, so that for optimal response stimuli must be positioned differently for each eye. The particular disparity which elicits the strongest response varies among cells, especially in the horizonal plane (*2*). It is generally believed that these disparity-sensitive neurons provide the neural basis for stereopsis, the uniquely binocular sense of depth perception (*3*). In support of this idea we have demonstrated that cats which lack binocular cells display marked deficits in binocular depth perception despite normal visual acuity in both eyes.

In order to disrupt the normal degree of binocular interaction among cortical neurons without affecting other receptive field properties, we subjected kittens to alternating monocular deprivation (*4*). This involves covering one of the eyes with an opaque contact lens for 1 day, then the other eye for the next day, and so on. Alternating occlusion was started when the kittens' eyes first opened and was continued until the animals were 6 months old, well beyond the end of the critical period during which visual deprivation affects the response properties of cortical neurons (*5*).

Previous electrophysiological work had shown that immediately after alternating monocular deprivation the proportion of cortical cells that can be activated through either eye is reduced significantly (*4*). In our experiments, behavioral testing was performed 1.5 to 2 years after termination of special rearing; thus each cat was allowed simultaneous use of both eyes for an extended time. To determine if this subsequent period of binocular vision altered the abnormal ocular dominance pattern seen immediately after rearing (*4*), we examined the response properties of single neurons in the visual cortex of one alternately occluded cat allowed 17 months of normal binocular experience prior to recording. We also studied a control animal that was reared normally. Prior to recording, the cats were paralyzed (Flaxedil) and anesthetized lightly (thiopental sodium); residual eye movements were reduced further by suturing the eyes to support frames. Contact lenses were used to focus the eyes on a tangent screen onto which a variety of visual stimuli could be rear-projected. Varnished tungsten microelectrodes were used to isolate individual units in visual cortex, the majority from area 17. The receptive field size, stimulus specificity, and ocular dominance of each cell was studied carefully.

In many respects neurons in the normal cat and the alternately occluded cat were indistinguishable. Most cells responded vigorously to a moving slit oriented properly and did not respond at all to a line orthogonal to this preferred orientation; receptive field size was related systematically to retinal eccentricity; and no unresponsive units were encountered. There was, however, a notable difference between the two animals in the pattern of ocular dominance. Specifically (Fig. 1A), the majority of cells studied in the normal cat were binocular and were placed into eye-dominance categories 2 to 6, while most cells in the alternately occluded cat were monocular exclusively, and thus were assigned either to category 1 or to category 7 (Fig. 1B). It is unlikely that the conspicuous absence of binocular units in the deprived cat resulted from degeneration, for each electrode penetration was rich in activity, with no regions of silent cortex. There was an obvious asymmetry in ocular dominance favoring the contralateral eye in both hemispheres of the alternately oc-