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15 March 1971

Sexual Dimorphism in the Preoptic Area of the Rat

Abstract. *A quantitative evaluation of the relative distribution of synapses on dendritic shafts and spines serves to differentiate the neuropil of the preoptic area from that of the ventromedial hypothalamic nucleus; it also shows that the neuropil of the preoptic area is sexually dimorphic.*

There is considerable evidence that the tuberal part of the hypothalamus is involved in the maintenance of the basal level of output of gonadotrophins, and that the preoptic area is essential for initiation of the preovulatory surge of gonadotrophins which is typical of the adult female but which does not occur in the male (*1, 2*). The amygdala and the hippocampus together constitute the major source of fibers from the limbic forebrain to the hypothalamus (*3*). The amygdala has two principal efferent tracts; these are the stria terminalis, which among other areas projects to the preoptic area and to the ventromedial nuclei of the tuberal hypothalamus, and the ventral amygdalofugal pathway (*4*). There is evidence to suggest that the stria terminalis may be of special importance as a route for the effects of amygdaloid stimulation on ovulation (*5, 6*) and for the effects of the amygdala on the timing of puberty in the female rat (see *7*). Anatomically, therefore, the stria terminalis links the amygdala with the preoptic area and the tuberal hypothalamus, all areas that have been implicated in the control of gonadotrophin release. The aim of our investigations was to examine the synapses in the neuropil of those parts of the preoptic area and tuberal hypothalamus which receive strial projections and to identify the terminals of amygdaloid origin by taking advantage of the reaction of orthograde degeneration.

Lesions were made in the stria terminalis in the rat by use of a stereotaxically guided knife blade entering from the dorsal aspect of the brain, at a level 2.2 mm behind and to a depth of 5.5 mm below the bregma with the rat in a nose-down position. This lesion also destroys the fimbria and damages some adjacent structures. A previous study of the efferent projection of the hippocampus has established that these incidental parts of the lesion do not give rise to degeneration in either of the two specific areas examined in this study (*8*). Two days later the animals were killed, and the brains were fixed and processed for electron microscopy. Ultrathin sections from the levels of the preoptic area and the ventromedial nucleus were mounted on uncoated grids, whose mesh served to divide the region up into convenient sampling units of about 1800 μm^2. All the grid squares from the regions containing degenerating amygdaloid fibers were counted, and for each square every synapse was classified according to its site of termination on the postsynaptic element as well as by the presence or absence of degeneration of the presynaptic terminal. In general, the samples consisted of about 20 grid squares (700 synapses) taken from a single section, although in a few instances the same procedure was repeated at successive levels about 100 μm apart to ensure that there was no systematic variation. In these cases the ratios given in Table 2 are the means of the ratios at each level.

The maximum number of recognizably degenerating terminals is reached at about 2 days after section of their parent fibers, whereas the process of astrocytic phagocytosis is not sufficiently advanced to cause a major reduction in the number of terminals still in contact with their postsynaptic sites. Terminal degeneration is indicated by collapse and increased electron opacity of the axonal endings, and may be used as a reliable and quantitatively consistent marker of those terminals

Table 1. Total numbers of synapses of amygdaloid and nonamygdaloid origin on dendritic shafts (SH) and spines (SP) in samples taken from the preoptic area and the ventromedial nucleus of male (M) and female (F) rats. The total number of synapses counted was 28,184. The numbers of animals used were M = 10 and F = 8 for the preoptic area, and M = 6 and F = 6 for the ventromedial nucleus.

Sex	Amygdaloid		Nonamygdaloid	
	SH	SP	SH	SP
	Preoptic area			
M	258	170	10,802	574
F	254	227	8,779	954
	Ventromedial nucleus			
M	71	233	2,102	498
F	87	371	2,235	569

Table 2. Ratio of the number of nonamygdaloid synapses on dendritic shafts to the number on dendritic spines. Mann-Whitney U test for significance of difference between male and female: for the preoptic area, $P < .001$; for the ventromedial nucleus, difference is not significant ($P = .5$).

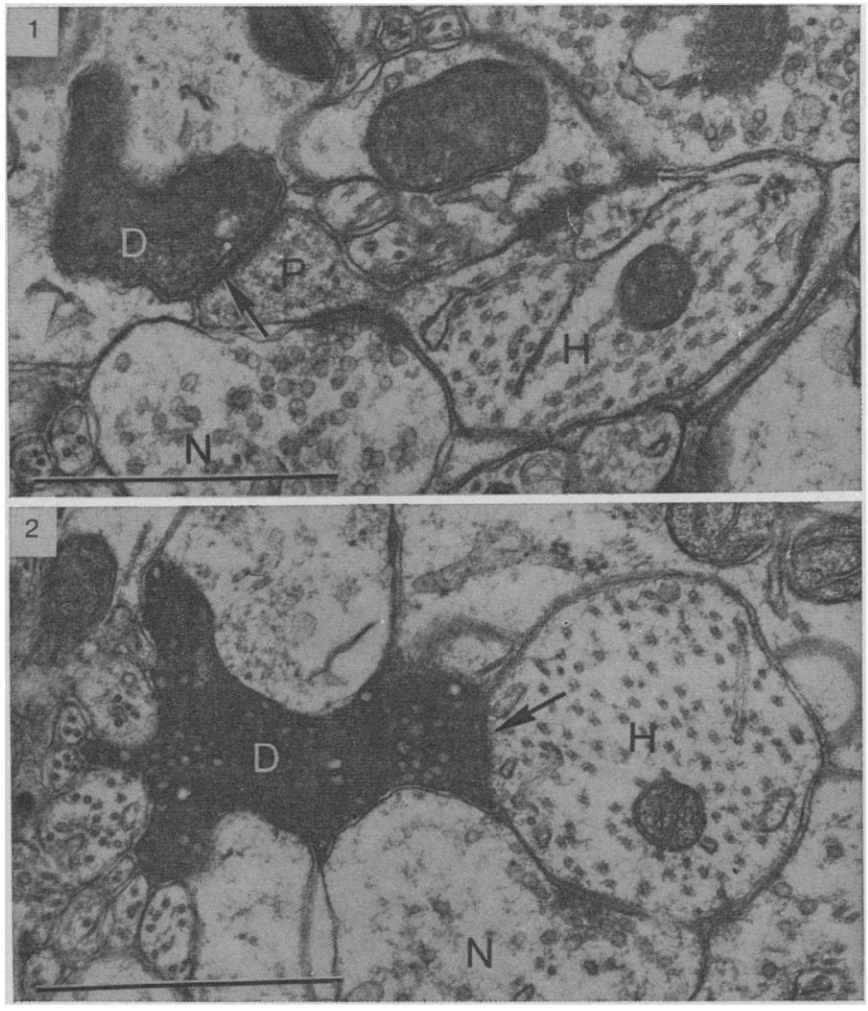
Rat number	Preoptic area		Ventromedial nucleus	
	Male	Female	Male	Female
M1	32.3			
M4	27.2			
M27	23.8			
M26	23.4			
M53	22.6			
M54	20.9			
M28	18.2			
M49	14.6			
F11		13.9		
F42		13.5		
M51	13.4			
M50	12.9			
F44		11.7		
F12		10.7		
F45		10.5		
F46		9.7		
F43		9.1		
F6		6.2		6.2
M4			6.1	
EF6				5.1
M54			4.8	
M27			4.7	
F46				4.5
F45				4.0
M50			3.9	
F26				3.8
M28			3.7	
M26			3.4	
F12				3.3

whose axons have been sectioned in the stria terminalis. The use of the electron microscope is necessary to provide such information about the terminals as their site of termination; it also has the further advantage that the surviving, nondegenerating (nonamygdaloid) axon terminals may also be recognized and classified. As a result, information is gained not only about how the amygdaloid fibers terminate, but also about the composition of the neuropil in the regions where they terminate. In the preoptic area and the tuberal hypothalamus, the two main sites of termination of afferent fibers are the cell bodies (axosomatic synapses) and the dendrites (axodendritic synapses). Axosomatic synapses form only a small minority of the total (between 2 and 3 percent). Axodendritic contacts are of two types, those made directly upon the shafts of dendrites and those made upon dendritic spines (Figs. 1 and 2, Table 1).

In both the preoptic area and the ventromedial nucleus the majority of nonamygdaloid fibers form synapses upon the shafts of dendrites. However, in the ventromedial nucleus the proportion of synapses upon dendritic spines is considerably higher than in the preoptic area. The fibers of the stria terminalis make synaptic contacts in both the preoptic area and the ventromedial nucleus, but their mode of termination is different in the two regions. In the preoptic area they form contacts upon dendritic shafts and upon dendritic spines in roughly equal numbers (slightly more upon dendritic shafts), whereas in the ventromedial nucleus they are preferentially distributed upon dendritic spines with only a small proportion terminating directly upon the shafts of the dendrites. In both areas the amygdaloid terminals account for a large proportion of the total number of synapses upon dendritic spines (Table 1).

In the preoptic area of the male, the synapses of nonamygdaloid origin almost all make contacts directly upon dendritic shafts. In the female the preoptic dendrites receive a significantly larger proportion of the afferent input upon dendritic spines (in the Mann-Whitney U test, $P < .001$; Table 2). Whether the amygdaloid terminals in the preoptic area are also sexually differentiated is not clear at present, because the number of synapses is smaller and the variation in the ratio of shafts to spines is larger. The finding that the anatomical differences between the preoptic areas in the male and female is best seen in the nonamygdaloid terminals is in contrast to the regional difference between the preoptic area and the ventromedial nucleus, where the difference is most marked for the amygdaloid terminals.

The regional and sexual differences found in this study apply to the ratios of one type of synapse to another and not to the total numbers of any particular type of synapse. Furthermore, our results give little information about the nature of the postsynaptic neurons to which the dendritic shafts and spines belong. The strial fibers to the ventromedial nucleus form a shell around the nucleus, and the long dendrites of the ventromedial neurons project out from the central part of the nucleus into this shell (*9*). Our own studies of Golgi material from the preoptic area indicate that a large proportion of the dendrites are orientated at right angles to the fibers of the stria terminalis, suggesting that in this area too there is an important interaction between the strial fibers and the dendrites. In the preoptic area there are clearly two different types of dendrites, one bearing fairly numerous dendritic spines and the other being beaded or varicose and having very few spines. This heterogeneity in the preoptic area must be taken into account when considering the electron microscopic counts, in which we have aggregated



Figs. 1 and 2. Axon terminals (*D*) in the preoptic area, showing collapse and increased electron opacity associated with orthograde degeneration 2 days after a lesion of the stria terminalis. (*N*), Adjacent nondegenerating axon terminals. In Fig. 1 the degenerating terminal makes synaptic contact with a dendritic spine (*P*) which is connected by a narrow neck to the dendritic shaft (*H*), a configuration commoner in the female. In Fig. 2 the contact which is directly onto the dendritic shaft (*H*) is of a type commoner in the male. Arrows mark synaptic thickenings. Dendritic shafts are identified by their content of microtubules, cut in transverse or oblique section. Scale: 1 μm.

all types of synapses, regardless of potential differences between the postsynaptic neurons.

Endocrine experiments suggest that although the tuberal hypothalamus is essential for the tonic control of the basal secretion of gonadotrophins, the cyclic trigger for the preovulatory surge, which is present in the adult female but not in the male, requires the integrity of the preoptic area (*1*). The preoptic area is also important for both male and female sexual behavior (*10*). There is also evidence that the amygdala may have effects on gonadotrophin control, and that the stria terminalis is a crucial pathway for these effects (*5*). Our anatomical findings show that the axons of the stria terminalis differ in their mode of termination in the preoptic area and in the tuberal hypothalamus. In addition, the neuropil of the preoptic area (but not of the ventromedial nucleus) is different in the male rat from that in the female. This difference is seen, however, in the terminals of fibers which do not originate in the amygdala, and whose origins are at present unknown. An ovulatory surge of gonadotrophins can be elicited by stimulation of the preoptic area in the male rat or in the female rat treated with androgens as a neonate; neither possesses a spontaneous trigger for the induction of ovulation (*11*). This therefore raises the possibility that the functional differences between the male and the female preoptic areas are due to some difference in the neural connections rather than some intrinsic property of the neurons of the preoptic area, a conclusion which is in agreement with our anatomical demonstration of sexual dimorphism in the mode of termination of the afferent fibers to the preoptic area.

The fact that sexual dimorphism occurs in a part of the brain does not, of course, prove that such dimorphism is related to sexually differentiated functions such as the ability to produce an ovulatory surge of gonadotrophins or sexual behavior. However, the location of the anatomical difference in the preoptic area, which has been shown to be essential for these functions, is persuasive circumstantial evidence.

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12. We thank Dr. K. Brown-Grant for advice, and the Medical Research Council (G 970/-668/B) and the Foundations Fund for Research in Psychiatry (grant 70-472) for financial assistance.

27 May 1971

Stimulation-Dependent Alterations in Peroxidase Uptake at Lobster Neuromuscular Junctions

Abstract. *The uptake of cytochemically demonstrable horseradish peroxidase into small vesicles within nerve endings in lobster stretcher muscles can be enhanced by electrical stimulation of transmitter release by the endings. This is observed particularly if stimulation is interrupted periodically and the nerves permitted to rest.*

It is widely, though not universally, assumed that neurotransmitters are released from nerve endings by processes resembling *exocytosis*, that is, by the fusion of synaptic vesicles with the plasma membrane delimiting the axon. However, as dramatically illustrated by recent work of Bittner and Kennedy (*1*), such a process should add considerable amounts of membrane to the axon surface during periods of sustained rapid firing. In gland cells and some other tissues, the addition of membrane to the surface through exocytosis appears to be compensated for by processes resembling *pinocytosis*, that is, by the budding back into the cell of small vesicles and tubules (see *2*).

Pinocytosis by nerve cells, including by axon endings, has been shown to occur in a wide variety of experimental systems (*3–6*) by use of several macromolecular tracers, including Thorotrast, ferritin, and horseradish peroxidase. But it has proved difficult to demonstrate that tracer uptake rates respond to increases in rates of transmitter release, as might be expected if pinocytosis-like formation of tubules and vesicles were a compensatory or membrane retrieval mechanism. Thus, Birks (*4*) reports that in frog neuromuscular junctions there is little or no change in the frequency of Thorotrast-containing vesicles when the endings are stimulated to prolonged repetitive firing by raising the K^+ concentration of the medium. Among other conceivable explanations, Birks advances the possibility that the tracer-containing tubules and vesicles are artifacts, derived during fixation by alteration of membrane systems that are actually continuous with the plasma membrane of the living cell. Elsewhere (*6*) we have outlined evidence, from studies with lanthanum on axon endings in the rat adrenal medulla, indicating that such an artifactitious origin is unlikely to explain the presence of macromolecular tracers in more than a very few of the vesicles in a given ending. Below we report evidence that there are experimental conditions under which the number of peroxidase-containing

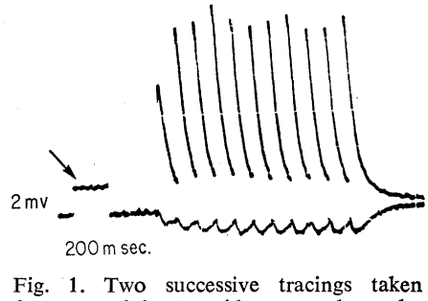

Fig. 1. Two successive tracings taken from one of the peroxidase-treated muscles that was given three 10-minute periods of stimulation alternating with 10-minute rest periods. The arrow indicates a calibrator pulse with the parameters noted (2 mv, 200 msec). In the upper trace, the excitor nerve was stimulated, and in the lower, the inhibitor nerve was stimulated. Corresponding EPSP's and IPSP's are seen (*7*).