Oxytocinergic Innervation of the Brain of the Garden Dormouse (*Eliomys quercinus* L.)

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ABSTRACT

The oxytocinergic innervation of the brain of the garden dormouse (*Eliomys quercinus* L.) was studied by means of immunocytochemistry. In contrast to the sparse oxytocin innervation of the rat forebrain, dense fibre networks in various cortical and limbic brain areas were demonstrated in this animal. These include, e.g., the prefrontal cortex, the claustrum, the septum, and the hippocampus. A very dense innervation was also seen in the caudal regions of the garden dormouse brain; these regions are already known to have a relatively dense oxytocin fibre network in the rat.

A dense innervation of oxytocin fibres is seen in several brain regions which, in the rat, have oxytocin binding sites but no visible oxytocin innervation. This discrepancy suggests that the differences in the oxytocinergic innervation of these two rodent brains may be due to an oxytocin system in the rat brain that is more difficult to detect immunocytochemically.

Key words: immunocytochemistry, septum, claustrum, oxytocin binding sites, detection limit

Immunocytochemical localization of oxytocin (OT) and vasopressin (VP) in the rat brain revealed that these peptides are present outside the classical hypothalamoneurohypophyseal system (Swanson, '77; Nilaver et al., '80; Sofroniew and Weindl, '81; Buijs et al., '85; De Vries et al., '85). A dense VP innervation was demonstrated in many brain regions, whereas for OT the innervation turned out to be substantial only in caudal brain structures such as the nucleus of the solitary tract and the A1 area.

In order to provide additional information on the anatomical distribution of these two peptide transmitters in brains of rodents, the OT innervation in the brain of the garden dormouse was studied by means of immunocytochemistry. This animal was selected because it does not belong to the classical inbred laboratory rodent strains and lives under quite different environmental conditions, which affect the functional status of certain physiological and behavioural processes (e.g., it hibernates). These conditions have already been shown to exert a strong influence on part of the VP but not on the OT innervation in the brain of the hibernating European hamster (Buijs et al., '86).

In the present paper a detailed description of the OT innervation of the brain of the garden dormouse is given,

while its relevance for the putative OT functions and for the OT innervation of the rat brain is discussed. It turns out that the distribution of OT is entirely different from that observed in any other rodent so far. The results concerning the VP innervation and its seasonal variation will be published in a separate paper.

MATERIALS AND METHODS

Male and female garden dormice were caught in the western part of France in early October and transported to Strasbourg, where they were separated according to their sex and housed in cages (five to seven animals in a 500 × 400 × 300 mm cage) placed in the open air. The cages were filled with straw and contained a small sleeping-box.

In various periods of the year all 15 adult animals (eight males and seven females, 54–120 g) were anaesthetized intraperitoneally with sodium pentobarbital (Nembutal: 1 ml/1 kg body weight) and perfused intracardially with 0.9% NaCl followed by 250 ml of 5% glutaraldehyde in 0.1 M

Accepted February 19, 1988.

Abbreviations

7	facial nucleus	LR	lateral reticular nucleus
10	dorsal motor nucleus of vagus	LS	lateral septum
3V	third ventricle	LT	laterodorsal tegmental nucleus
ac	anterior commissure	LV	lateral ventricle
Acb	nucleus accumbens	MA	medial amygdaloid nucleus
ACg	anterior cingulate cortex	MD	mediodorsal thalamic nucleus
AOP	anterior olfactory nucleus, posterior part	ME	median eminence
Aq	aqueductus cerebri	MR	median raphe nucleus
AP	area postrema	MS	medial septum
Arc	arcuate hypothalamic nucleus	mt	mammillothalamic tract
BA	basolateral amygdaloid nucleus	oc	optic chiasm
BST	bed nucleus of the stria terminalis	PaS	parasubiculum
CA	central amygdaloid nucleus	PH	posterior hypothalamic nucleus
CA1	CA1 field of the hippocampus	pp	peripeduncular nucleus
CA3	CA3 field of the hippocampus	PR	paragigantocellular reticular nucleus
CA4	CA4 field of the hippocampus	PrS	presubiculum
cc	corpus callosum	PVN	paraventricular hypothalamic nucleus
CG	central gray	RF	rhinal fissure
Cl	claustrum	RM	raphe magnus nucleus
CLR	caudal linear raphe nucleus	RO	raphe obscurus nucleus
CM	central medial thalamic nucleus	RPa	raphe pallidus nucleus
CPu	caudate putamen (striatum)	RPN	raphe pontis nucleus
DG	dentate gyrus	RRF	retrobulbar field
DLG	dorsal lateral geniculate nucleus	S	subiculum
DMH	dorsomedial hypothalamic nucleus	SC	subcoeruleus nucleus
DPB	dorsal (lateral) parabrachial nucleus	scp	superior cerebellar peduncle
DR	dorsal raphe nucleus	SN	substantia nigra
ec	external capsule	Sol	nucleus of the solitary tract
En	endopiriform nucleus	SON	supraoptic nucleus
Ent	entorhinal cortex	SOR	supraoptic nucleus pars retrochiasmaticus
f	fornix	sp5	spinal tract of the trigeminal nerve
F	(pre)frontal cortex	sp5C	nucleus of the spinal tract of the trigeminal nerve pars caudalis
fmi	forceps minor corporis callosi	st	stria terminalis
fmj	forceps major corporis callosi	Str	striate cortex
fr	fasciculus retroflexus	TSC	septocortical tract
ic	internal capsule	TT	taenia tecta
IF	interfascicular nucleus	tz	trapezoid body
Inf	infundibular stem	VDB	nucleus of the vertical limb of the diagonal band
IO	inferior olive	VLG	ventral lateral geniculate nucleus
IP	interpeduncular nucleus	VM	ventromedial thalamic nucleus
LA	lateral amygdaloid nucleus	VMH	ventromedial hypothalamic nucleus
LC	locus coeruleus	VTA	ventral tegmental area
LPO	lateral preoptic area		

cacodylate buffer, pH 7.4 (Merck Chemicals). The brains were dissected, immersed in the same fixative for 2 hours, and stored until they were sectioned 50 μ m transversally with an Oxford Instruments vibratome in 0.05 M Tris buffer, containing 0.9% NaCl, at pH 7.4 (Tris-NaCl).

The sections were stained according to the following scheme: (1) rinsing in Tris-NaCl for 30 minutes, (2) incubation with rabbit OT antiserum (0-1-V) diluted 1:1,000 in Tris-NaCl containing 0.5% Triton X-100 (Tris-NaCl-Triton) overnight at 4°C, (3) rinsing in Tris-NaCl for 30 minutes, (4) incubation with goat antirabbit-IgG serum (Betsy) 1:100 in Tris-NaCl-Triton for 60 minutes, (5) rinsing in Tris-NaCl for 30 minutes, (6) incubation with peroxidase-antiperoxidase (PAP) 1:700 in Tris-NaCl-Triton for 60 minutes, (7) rinsing in Tris-NaCl for 30 minutes, (8) staining with 0.05% 3-3'-diaminobenzidine (DAB; Sigma) in Tris-NaCl containing 0.01% H₂O₂ for approximately 20 minutes, and (9) a final rinsing in Tris-NaCl. Next, the sections were mounted on glass slides, air-dried, dehydrated, and coverslipped with Entellan (Merck Chemicals).

To check the specificity of the staining, alternating sections were stained with (1) the OT antiserum, (2) an arginine vasopressin (AVP) antiserum (W-1), and (3) the OT antiserum 1:400 preabsorbed with agarose beads coupled with lysine to which VP or OT was conjugated with glutaraldehyde following a modified procedure of Pool et al. ('84) so as to determine the specificity of the reaction.

With the exception of the hypothalamus (Burlet, '73), no

atlas of the garden dormouse brain is available. Careful comparison with the rat brain revealed no important anatomical differences between the garden dormouse and rat. Therefore, the atlas of Paxinos and Watson ('82) was used for identification and nomenclature of the brain areas. Visualization of the anatomical distribution of the OT immunoreactivity was also performed according to this atlas (Figs. 1, 2).

RESULTS

Instead of adsorption with Sepharose beads to which VP was bound by means of CNBr conjugation (Pool et al., '84), absorption of the OT antibody was carried out by using lysine-covered Sepharose beads to which VP or OT was conjugated by means of glutaraldehyde (Pool et al., in prep.). Naturally, all staining disappeared after absorption with Sepharose OT beads. Adsorption with Sepharose VP beads (three times) resulted in removal of all detectable VP immunoreactivity in the test system (see Pool et al., '84). In the garden dormouse hypothalamus, the nonabsorbed, OT antiserum resulted in no staining of the suprachiasmatic nucleus and in a slight staining of magnocellular VP cell bodies. By using the VP-absorbed serum, this cross-reactivity on VP neurons disappeared. In extrahypothalamic brain regions, even with the non-VP-absorbed OT antiserum, no indication of staining in VP fibres was observed. Therefore,

Figs. 1,2. Line drawings of the distribution of oxytocin (OT) immunoreactive cell bodies and fibres in the brain of the garden dormouse. The sketches of transverse sections of the rat brain are selected from the atlas of Paxinos and Watson ('82).

Figure 2

both VP-absorbed and nonabsorbed anti-OT serum was used to evaluate the localisation of OT fibres. In the description of the anatomical distribution of OT, a few simplifications have been made: (1) because most OT-immunoreactive fibres appear to be varicosal, this adjective has been abandoned; (2) if the OT-immunoreactive elements are punctuated and/or perineuronally situated, they have been named terminals; and (3) appearances of OT immunoreactivity other than mentioned above will be described completely.

In the *telencephalon* a moderately dense distribution of branching fibres was observed in the medioventral part of the prefrontal cortex, from which fibres run dorsolaterally and form ramifications in front of the forceps minor corporis callosi (Fig. 3). More caudally, at the level of this last structure, the OT innervation is confined to the cortex medial to it. Ventrally, the taenia tecta receives an input at a density ranging from low to moderate.

Moderately concentrated parallel and ventromedially

running fibres are present at the border between the posterior part of the anterior olfactory nucleus and the frontal part of the nucleus accumbens.

Between the septocortical tract and the forceps minor corporis callosi, just medial to the lateral ventricle, a population of branching fibres was visible, which may be the origin of the terminals being situated along the active course of the septocortical tract.

Across the entire rostrocaudal extent of the claustrum, dense accumulations of bifurcating fibres and terminals were observed (Fig. 4). Some fibres course from the claustrum in a ventrolateral direction. Scattered fibres could be localised in the anterior cingulate cortex.

The nucleus of the vertical limb of the diagonal band and, more caudally, the organum vasculosum lamina terminalis are innervated by moderate numbers of fine fibres and terminals (Fig. 5). This population of fibres continues into the dorsal area with ramifications at the lateral border of

Fig. 3. Photomicrograph showing the distribution of OT fibres in a transverse section through the frontal cortex. F, frontal cortex. Bar = 50 μ m.

Fig. 4. A picture of the oxytocinergic innervation of the claustrum as seen through the whole rostrocaudal extent of this nucleus. Cl, claustrum; CPu, caudate putamen; Bar = 50 μ m.

Fig. 5. The nucleus of the vertical limb of the diagonal band stained for the presence of OT, visible as fibres and terminals. VDB, nucleus of the vertical limb of the diagonal band; Acb, nucleus accumbens. Bar = 75 μ m.

the medial septum, whereas the central part of the medial septum only receives a minor innervation. OT fibres were not seen in the nucleus accumbens. Scattered fibres were seen ventral to the nucleus of the vertical limb of the diagonal band.

In the lateral septum, a diverse topographic arrangement of fibres was observed: the ventromedial and medial parts receive a moderate to dense innervation, whereas the dorsal septum is virtually devoid of OT fibres. Along the lateral ventricle a cluster of OT-immunoreactive terminals was visible. More caudally, the area surrounding the descending limbs of the fornix receives a dense input of terminals (Fig. 6). Just as in the more frontal sections, this innervation is a continuation of the fibres and terminals in the remaining part of the septum.

The bed nucleus of the stria terminalis shows a light concentration of long and bifurcating fibres, both in the medial part, superior to the anterior commissure, and in the lateral part, just below the anterior commissure. The stria terminalis itself contains parallel running fibres.

In the amygdaloid complex, OT fibres mainly terminate in the medial part, where a moderate concentration of terminals can be seen. Some scattered fibres are present in the lateral and basolateral amygdaloid nucleus, mostly adjacent to the capsula externa.

The CA3 field of the hippocampus receives quite a dense innervation throughout its entire dorsoventral extent, which is most concentrated in the ventral part. Dorsally, the innervation continues into the CA4 area. A dense cluster of fibres is present in the medial part of the ventral

Fig. 6. The medioventral part of the septum around the descending limbs of the fornix, densely innervated by OT. f, fornix; LS, lateral septum; LV, lateral ventricle. Bar = 75 μ m.

Fig. 7. A cluster of fibres seen in the ventral subiculum and ventral part of the CA3 field of the hippocampus with extensions into the entorhinal

cortex and toward dorsal parts of the CA3 field. CA3, CA3 field of the hippocampus; Ent, entorhinal cortex; S, subiculum. Bar = 50 μ m.

Fig. 8. The interpeduncular nucleus containing OT fibres and terminals. Note that the midline is only sparsely innervated. IP, interpeduncular nucleus; tfp, tranverse fibres pons. Bar = 50 μ m.

subiculum, from which some fibres run into the CA1 field (Fig. 7).

The distribution of OT immunoreactivity in the dorsal subiculum is restricted to an area along the forceps major corporis callosi. The branching fibres in this area were also seen around this whole structure and, forming terminals, seem to concentrate in the deep layers of the striate cortex.

The entorhinal cortex has two vaguely arranged bands of fibres. Both seem to arise from the cluster seen in the ventral part of the subiculum. One is situated just lateral to the most posterior part of the capsula externa; the other band of branching fibres lies ventral to the former. More caudally, the innervation remains identical. In the para- and presubiculum, elongated ramifying fibres could be localised.

Apart from the known neurosecretory cells in the paraventricular hypothalamic and supraoptic nucleus and the hypothalamic islands with their thick fibres that radiate to the median eminence and neurohypophysis, a strand of parallel-running fibres was seen along the third ventricle

in the *diencephalon*. Ventrally, it bends laterally and seems to originate from the cluster of short, horizontally oriented fibres in the ventral part of the lateral preoptic area.

Long and bifurcating fibres are distributed throughout the dorsomedial hypothalamic nucleus, in the middle of the clearly distinguishable thick neurosecretory fibres arising from the paraventricular hypothalamic nucleus. The ventromedial hypothalamic nucleus is completely devoid of OT immunoreactivity.

In the thalamus, a low density of OT-immunoreactive fibres and terminals was seen in the frontal part of the mediodorsal thalamic nucleus, with some fibres running ventrally, as a continuation of the sparse innervation of the frontal part of the central medial thalamic nucleus. Ventromedial to the mammillothalamic tract, just medial to the ventromedial thalamic nucleus, a similar population of fibres was visible. The distribution of terminals in the ventral part of the lateral geniculate nucleus is moderately dense. It is confined to a laterolateral band contiguous to the dorsal part of this nucleus.

Fig. 9. The dense OT innervation of the paragigantocellular reticular nucleus with fibres seen running to the trapezoid body and raphe pallidus nucleus. PR, paragigantocellular reticular nucleus; py, pyramidal tract; RPa, raphe pallidus nucleus; tz, trapezoid body. Bar = 50 μ m.

Fig. 10. Laterolateral running fibres seen in the commissural subnucleus of the nucleus of the solitary tract. Note the perineuronal innervation of a

neuron in the dorsal motor nucleus of vagus (arrow). 10, dorsal motor nucleus of vagus; Sol, nucleus of the solitary tract. Bar = 75 μ m.

Fig. 11. The A1 area, lying just dorsal to the lateral reticular nucleus, receiving a very dense input of OT-immunoreactive fibres. LR, lateral reticular nucleus. Bar = 75 μ m.

At the level of the infundibular stem, in the diencephalon, OT immunoreactivity was seen only in the form of fibres passing to caudal brain areas, in the posterior hypothalamic nucleus and around the fasciculus retroflexus.

In the *mesencephalon*, the dorsal route (around the fasciculus retroflexus) seems to turn to the central gray and could

not be followed more caudally. In the central gray terminals could frequently be seen.

The ventral route (around the interfascicular nucleus) continues to run caudally. At the level of the ventral tegmental area a bundle of fibres leaves this route, turning to dorsolateral direction, toward the peripeduncular nucleus,

where it fans out. Some fibres turn in the direction of the central gray here. The ventral tegmental area itself contains a moderate number of branching fibres.

In the *metencephalon* a dense accumulation of OT-immunoreactive terminals and tiny, short fibres was observed in the lateral parts of the interpeduncular nucleus (Fig. 8).

The innervation of the peripeduncular nucleus becomes more concentrated at more caudal levels, being most dense in its caudal pole dorsolateral to the retrorubral fields.

The nuclei raphe, i.e., the caudal linear raphe, the median raphe, and the dorsal raphe nucleus, contain elongated ventrodorsally running fibres in their rostral parts. More caudally, dense accumulations of terminals were visible in these nuclei. The raphe pontis nucleus is only sparsely innervated. The innervation of the laterodorsal tegmental nucleus at this level is similar to that of the dorsal raphe nucleus and seems to be a continuation of it.

In the locus coeruleus, thick fibres with clearly visible intervaricosal segments could be observed. Moderately concentrated, the same fibres are present in the subcoeruleus and the rostral part of the dorsal parabrachial nucleus. They hardly form any ramifications. An occasional bundle of fibres was seen running through the superior cerebellar peduncle, in which case a dense cluster of ramifying fibres and terminals appears in the dorsal parabrachial nucleus.

In the rostrocaudal axis of the *myelencephalon*, passing fibres are distributed across the entire base of the brain. The raphe pallidus nucleus contains branching fibres.

The paragigantocellular reticular nucleus receives, in its frontal pole, a very dense input of strongly bifurcating fibres and terminals, which seem to originate from fibres seen in the midline, i.e., in the raphe magnus and raphe pallidus nucleus, which also send projections to the raphe obscure nucleus (Fig. 9). From the paragigantocellular reticular nucleus, fibres fan out dorsolaterally. In far greater numbers, however, fibres run to the ventral surface of the medulla oblongata, the trapezoid body. More caudally, fibre ramifications are present in all parts of the inferior olive and the remainder of the paragigantocellular reticular nucleus.

In the frontal part of the nucleus of the solitary tract the mediodorsal aspect is moderately to densely innervated. The other parts of the nucleus of the solitary tract receive a low input of fibres. More caudally, dense innervation was seen in the commissural subnucleus, with fibres running predominantly laterolaterally (Fig. 10). Laterally, they run for a short distance in a ventral direction. A moderate concentration of fibres and terminals was visible in the dorsal motor nucleus of vagus.

Just dorsal to the lateral reticular nucleus, the A1 area, like the paragigantocellular reticular nucleus, receives a dense innervation which consists of a dense plexus of ramifying fibres and terminals (Fig. 11). From this area, some fibres run dorsally via the lateral aspect of the caudal part of the nucleus of the spinal tract of the trigeminal nerve.

The fibre density and distribution or localization of cell bodies are independent of sex or the time of the year in which the animals are killed.

DISCUSSION

The distribution of OT cell bodies

Our results concerning the distribution of the cell bodies confirm those obtained by Oukouchoud-Marouf ('85), who made a comprehensive description of the localization of VP- and OT-containing perikarya in the hypothalamus of the

garden dormouse. She concluded that there are no major differences in the distribution and number of OT-immunoreactive cell bodies compared to the rat hypothalamus. New localizations of such neurons outside the hypothalamus have not been observed in this study.

The origin of the OT fibres in the brain still remains to be established. From studies in the rat it was initially concluded that they largely originate from the paraventricular hypothalamic nucleus (PVN) (De Vries and Buijs, '83; Lang et al., '83). However, a study by Hawthorn et al. ('85) modified this hypothesis, as it showed a substantial reduction of OT in several brain areas after lesioning of the supraoptic nucleus (SON). Moreover, they suggested additional extrahypothalamic sources for OT, since destruction of the PVN, SON, and suprachiasmatic nucleus together did not result in complete disappearance of radioimmunoassayable OT from the brain. Recently, Alonso et al. ('86) reported that the SON, which contains VP- and OT-producing neurons, not only projects to the neurohypophysis but also has axonal pathways running into the brain. Therefore it seems likely that the OT innervation of the brain is derived from more nuclei than just the PVN and that the exact origin of the OT innervation in the garden dormouse remains to be established, although in this study OT cell bodies were not detected outside the PVN and SON.

Functional aspects of OT in the rodent brain

The extensive OT innervation of many brain regions in the garden dormouse suggests the involvement of this peptide transmitter in a wide variety of functions. Studying the anatomical connections of these brain regions reveals that most of them are directly projecting to the PVN and/or SON. This holds for the lateral septum, medial amygdala, ventral subiculum, locus coeruleus, dorsal parabrachial nucleus, laterodorsal tegmental nucleus, nucleus of the solitary tract and the A1 area (Ricardo and Koh, '78; Berk and Finkelstein, '81; McKellar and Loewy, '81; Silverman et al., '81; Tribollet and Dreifuss, '81; Sawchenko and Swanson, '82; Oldfield et al., '85; Tribollet et al., '85). In addition to this neuroanatomical information it has been demonstrated that electrical stimulation in many of these brain regions influences the activity of VP and/or OT neurons in the PVN and SON (Poulain et al., '80; Hamamura et al., '82; Day et al., '84; Day and Renaud, '84). These observations suggest that OT as a neurotransmitter may be implicated in the regulation of the neurosecretory activity of these hypothalamic nuclei. Support for this hypothesis was recently obtained by infusing OT in the rat A1 area, which resulted in a pronounced increase of plasma VP values (Hermes et al., in preparation).

Otherwise, it is tempting to connect these oxytocinergic projections—in a more general sense—to physiological and behavioural processes that control VP and OT release into the periphery, such as haemorrhage, hyperosmolarity, lactation, and stressful situations. This kind of coupling seems to be a general phenomenon of hypothalamic peptides (Swaab, '82). The functional significance of the oxytocinergic innervation in certain brain regions may be inferred from a number of different experiments.

First, the fact that septal lesions result in an increased urine output and water intake, probably due to a marked decline of plasma VP (Lubar et al., '69; Iovino and Steardo, '85), combined with the known anatomical and electrophysiological connections between lateral septum and VP and OT neurones in PVN and SON (Negoro et al., '73; Garriss, '79; Poulain et al., '80; Pittman et al., '81; Cirino and

Renaud, '85; Oldfield et al., '85), suggests that the OT innervation of the septum may be involved in the regulation of water balance. Second, since the septum and hippocampus are known to selectively concentrate radiolabelled corticosterone (McEwen et al., '75; Warembois, '75), an influence of OT in the septum on the corticosterone feedback regulation of the release of adrenocorticotrophic hormone (ACTH), via a septal input in the parvicellular corticotropin-releasing factor (CRF)- and VP-containing PVN neurons, cannot be excluded. The moderate respectively dense OT innervation of the raphe magnus and paraventricular reticular nucleus, which are both known to play an important role in the induction of analgesia (Oliveras et al., '75; Akaike et al., '78), supports the hypothesis that there is a role for central OT in the response to stressful situations.

The claustrum, which receives a dense oxytocinergic input in the garden dormouse, has extensive reciprocal connections with the neocortex in various other species (Druga, '68; Carey et al., '80; Macchi et al., '83; Sloniewski et al., '86). In the cat, moreover, efferent connections with allocortical regions have been found (Markowitsch et al., '84). Interesting in this context are (1) the presence in the garden dormouse of OT fibres in neo- and allocortical areas such as the striate, anterior cingulate, retrosplenial, prefrontal, and entorhinal cortices, and (2) the observation of an OT innervation of the mediodorsal thalamic and lateral geniculate nuclei, nuclei that project to the prefrontal and striate cortex, respectively (Gilbert and Kelly, '75; Krettek and Price, '77). On the basis of tracing studies it has been proposed that the claustrum is involved in the processing of exteroceptive information (Olson and Graybiel, '80; Sherk and Le Vay, '81). Therefore, it seems likely that OT in this nucleus, and perhaps in its projection areas as well, may interfere with the reception of environmental and internal information. As this is known to possibly inhibit the OT release from the neurohypophysis induced by nipple stimulation (Taleisnik and Deis, '64; Grosvenor and Mena, '67), OT in the claustrum may act in such a way that hormonal OT can be released into the circulation (Lincoln et al., '80). Another possibility is that these sites are implicated in the observed effects of OT on maternal behaviour (Pedersen and Prange, '85). However, no sex-related OT innervation was found in this area.

Immunocytochemical considerations

Several causes can be put forward to account for the OT innervation of the brain of the garden dormouse being dissimilar from that of the rat.

First, interspecies differences (including domestication and inbreeding in the case of the laboratory rat) may underlie the presence of OT seen in additional fibre systems of many brain regions of the garden dormouse.

Second, in the garden dormouse OT may be present in the same fibre systems as in the rat, but above the detection limit of the immunocytochemical procedure. It is possible that the OT in the brain of this animal is packaged and processed in such a way that its immunocytochemical detection is easier than in the rat brain.

Since environmental factors influence the content and, as a consequence, the anatomical appearance of neurotransmitter systems (Canguilhem et al., '77; Nürnberger et al., '86; Buijs et al., '86), the fact that the animals live under quite different environmental circumstances than usual for the laboratory rat may have influenced the content of par-

ticular parts of the OT neurotransmitter system. However, the OT innervation in the brain of the European hamster living under identical conditions is similar to that found in the rat brain (Buijs, personal observation), which suggests that it is largely a species difference that accounts for the different OT innervations. Yet, several data obtained in the rat give additional arguments for an identical, although more difficult to detect, OT system. (1) Measurements done with radioimmunoassay show that in, for instance, the frontal cortex, cingulate cortex, hippocampus and nucleus parafascicularis of the rat, OT immunoreactivity is also present. (Dogterom and Buijs, '80; Hawthorn et al., '84; Valiquette et al., '85). (2) Studies of OT binding sites in the rat (Brinton et al., '84; De Kloet et al., '85; Van Leeuwen et al., '85; Ravid et al., '86; Freund-Mercier et al., '87) show labelling in the anterior olfactory nucleus, the claustrum, and the hippocampus. In contrast to the rat, the garden dormouse shows OT immunoreactivity in these regions. (3) Behavioural and electrophysiological experiments with OT in the rat brought about effects (Kovacs et al., '79; Mühlethaler et al., '84) in brain regions in which in the garden dormouse endogenous OT is present. To those former results and the present ones we can add the information that, for VP, improvement of the sensitivity of the immunocytochemical procedure resulted in an increase in the number of fibres and the number of areas in which these fibres can be detected (De Vries et al., '85). All these findings make it likely that, in the rat brain, a number of OT-containing structures indeed still remain below the immunocytochemical detection limit, which will be a matter of future investigation.

ACKNOWLEDGMENTS

The authors wish to thank Henk Stoffels and Gerben van der Meulen for their skillful assistance in preparing the illustrations; Tjitske van der Woude, Pien Boon, and Eline van der Beek for their technical assistance; Daniel Bonn for taking care of the animals; Olga Pach and Aad Janssen for revising the English and typing the manuscript; and Prof. D.F. Swaab and Dr. C.W. Pool for their suggestions. This work was supported by ATP-CNRS "Europe" and the European Community, twinning contract No. ST2 00 271.

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