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Distribution of neurotransmitters in the sheep brain

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Although the general organization of the sheep brain is similar to that of other mammals, there are species differences in the fine architecture and neurotransmitter distribution. In sheep, perikarya are generally scattered, unlike the situation in rodents where they are clustered. The same organization is observed in cows and primates. The density of neurones immunoreactive for tyrosine hydroxylase in the dorsorostral diencephalon of sheep is lower than in rodents; A14 and A15 dopaminergic cell groups do not present a dorsal part. Only one adrenergic group, C2, is observed in the dorsomedial medulla oblongata. GnRH-immunoreactive neurones are mainly found in the anterior hypothalamic–preoptic areas, a few being present in the mediobasal hypothalamus. The density of several neurones containing neuropeptides (for example vasoactive intestinal polypeptide, cholecystokinin and somatostatin) in the caudal brain of sheep is lower than in other species and in the forebrain of sheep. These differences contribute to different patterns of innervation of brain areas compared with other species. For example, the suprachiasmatic nucleus does not present a dense network of fibres immunoreactive for 5-hydroxytryptamine and neuropeptide Y as observed in rats. These morphological studies constitute information necessary for further physiological investigations.

Introduction

In sheep, as in other species, neurotransmitters in the brain are involved in the control of physiological cues through endocrine and autonomic regulation. Among the species used to study endocrine regulation, sheep present interesting and specific physiological characteristics. Sheep have been studied extensively and much data about sheep endocrinology contrast with the little information available on the sheep brain, particularly on the distribution of neurotransmitters.

The neuronal function of sheep cannot be inferred from data obtained in other species because of the large differences in the general morphology of the brain between species. Compared with rats, the sheep encephalon presents a large cortex with many circumvolutions, and compared with primates, the sheep brain does not present a flexure of the brainstem. Another large difference between the sheep and rat neuronal organization is the relative scattering of neurones in the nervous tissue. This is also observed in other large mammals such as humans and cattle (Kitahama *et al.*, 1994). Owing to these differences, distribution of neurotransmitters in the sheep brain has to be specifically studied.

The distribution of neurotransmitters in sheep has been studied by immunohistochemistry and by *in situ* hybridization and mainly concern monoamines (catecholamines and 5-hydroxytryptamine) and peptides (Table 1). Acetylcholine and amino acid distributions have not been investigated even if these compounds have been identified and sampled in nervous tissue by microdialysis and push–pull methods (Kendrick *et al.*, 1992; Clarke *et al.*, 1993; Lévy *et al.*, 1993).

In this review, only the distribution of perikarya and the main fibre bundles or terminals are considered. Terminal and fibre densities may vary according to the physiological status of the animals and with the sensitivity of the method used. Comparing their distribution from one study to another

Table 1. Extensive mapping of neurotransmitter containing structures in the sheep brain

Transmitters	Structures	Methods	References
Monoamines			
5HT	Brain[a]	IHC	Tillet, 1987
TH/DBH	Brain[a]	IHC	Tillet and Thibault (1989)
PNMT	Myelencephalon	IHC	Tillet (1988)
Neuropeptides			
βendo	Hypothalamus	IHC	Lehman and Karsch (1993)
POMC	Hypothalamus	ISH	Mc Shane *et al.* (1993)
CCK	Brain	IHC	Antonopoulos *et al.* (1987)
CCK	Hypothalamus	IHC	Marson *et al.* (1987)
CGRP	Diencephalon	IHC	Herbison *et al.* (1993a)
CRF	Hypothalamus	IHC	Kolodziejczyk *et al.* (1983)
CRF	Brain	RIA	Palkovits *et al.* (1983)
CRF	Brain	ISH	Matthews *et al.* (1991)
CRF	Hypothalamus	IHC	Paull *et al.* (1982)
DYN A	Hypothalamus	IHC	Marson *et al.* (1987)
GnRH	Hypothalamus	IHC	Hoffman *et al.* (1978)
GnRH	Hypothalamus	IHC	Polkowska *et al.* (1980)
GnRH	Hypothalamus	IHC	Dees *et al.* (1981)
GnRH	Hypothalamus	IHC	Advis *et al.* (1985)
GnRH	Hypothalamus	IHC	Glas *et al.* (1986)
GnRH	Hypothalamus	IHC	Lehman *et al.* (1986)
GnRH	Brain	IHC	Caldani *et al.* (1988)
GnRH	POA	ISH	Mc Shane *et al.* (1993)
Met-enk	Hypothalamus	IHC	Marson *et al.* (1987)
PPE	Brain	ISH	Matthews *et al.* (1992)
NPY	Hypothalamus	ISH	Mc Shane *et al.* (1993)
NPY	Brain	IHC	Antonopoulos *et al.* (1989a)
NT	Brain	IHC	Papadopoulos *et al.* (1986a)
OT	Brain	IHC/ISH	Broad *et al.* (1993b)
SRIF	Brain	IHC	Papadopoulos *et al.* (1986b)
VIP	Brain	IHC	Antonopoulos *et al.* (1987)

[a]Except cerebral hemisphere and cerebellum.
βendo: β endorphin; CCK: cholecystokinin; CGRP: calcitonin gene related peptide; CRF: corticotrophin releasing factor; DBH: dopamine β-hydroxylase; DYN A: dynorphin A; GnRH: gonadotrophin releasing hormone; IHC: immunohistochemistry; ISH: *in situ* hybridization; Met-enk: methionine enkephalin; NPY: neuropeptide Y; NT: neurotensin; OT: oxytocin; PNMT: phenylethanolamine *N*-methyl transferase; POMC: proopiomelanocortin; PPE: preproenkephalin; RIA: radioimmunoassay; SRIF: somatostatin; TH: tyrosine hydroxylase; VIP: vasoactive intestinal peptide; 5HT: 5-hydroxytryptamine.

is difficult, and for that reason they will not be considered here. Most of the studies presented in this review have been performed in the whole brain, while others have been performed only in the anterior hypothalamic–preoptic area, which contains the largest number of different neuromediators (Nieuwenhuys, 1985) and is involved in the regulation of numerous endocrine and autonomic functions.

Monoamines

5-Hydroxytryptamine

The presence of neurones containing 5-hydroxytryptamine (5HT) has been demonstrated by immunohistochemistry with antisera raised against 5HT (Tillet, 1987). 5HT-immunoreactive (IR)

perikarya are distributed in twelve neuronal groups from the caudal medulla oblongata to the caudal mesencephalon and the pineal gland (Fig. 1). Each group has been classified according to the Swedish nomenclature (Dahlström and Fuxe, 1964) and a nomenclature previously described (Tillet, 1987). Two main areas of 5HT neurones are observed: in the caudal part of the medulla oblongata (nuclei B1, B2, B3, S1 and S2) and in the rostral part of the brainstem (nuclei B5, B6, B7, B8, B9, S3 and S4).

Groups B1 and B2 are the most caudal groups situated in the nuclei raphes pallidus and obscurus. Neurones of group B1 are in the ventral half of the medulla and neurones of group B2 in the dorsal part, under the central canal around which some labelled perikarya are observed. This latter group actually presents a greater extension in sheep than in rats (Steinbusch, 1981), since 5HT neurones are observed in the dorsal vagal complex without colchicine treatment.

Group B3 extends to the same level as groups B1, B2, slightly rostrally to B2, at the level of the nucleus reticularis gigantocellularis where some 5HT neurones are observed. However, neurones of this group extend more laterally compared with those in rodents, since they are seen near the ventrolateral edge of the nucleus reticularis lateralis in sheep.

A group of 5HT neurones is not found in the nucleus vestibularis corresponding to the group B4 of rats; very few neurones are scattered in this area. This group is also absent from the squirrel monkey (Hubbard and DiCarlo, 1974).

In groups S_1 and S_2 the lateral part of the medulla presents two groups of neurones containing 5HT. The first, S1 (Fig. 2a), is located near the nucleus reticularis lateralis and ventrolateral to the nucleus ambiguus; the second, S2, is situated on each side of the nucleus reticularis gigantocellularis, dorsolateral to the pyramis. The distribution of 5HT neurones in these lateral areas is characteristic of sheep, since in rats the same structures contain only a few scattered perikarya, which do not constitute anatomical entities as in other members of group B.

Groups B5, B6, B7 and B8 contain an important population of 5HT neurones in the sheep as in other species. Group B5 extends between the fasciculi longitudinalis medialis (FLM) and the transverse pontine fibres. Dorsally to this group, between the FLM and the fourth ventricle, a small group of neurones constitutes group B6. Just rostrally to this gathering, the greatest density of 5HT neurones is found in the raphe dorsalis and in the mesencephalic central grey. In group B7, rounded neurones are stained slightly less intensely than they are in other groups. This contrasts to the neurones situated above this group in group B8 where 5HT neurones are mainly bipolar and their long axes vertical. These four groups are also observed in other mammals. However, in sheep, boundaries between these groups cannot be well delineated, and a clear-cut distinction is more difficult to observe than it is in rats. Compared with primates (Kawata *et al.*, 1984), group B8 is seen in a narrower space in sheep. Neurones of group B8 send projections to the medial preoptic area (Tillet, 1992).

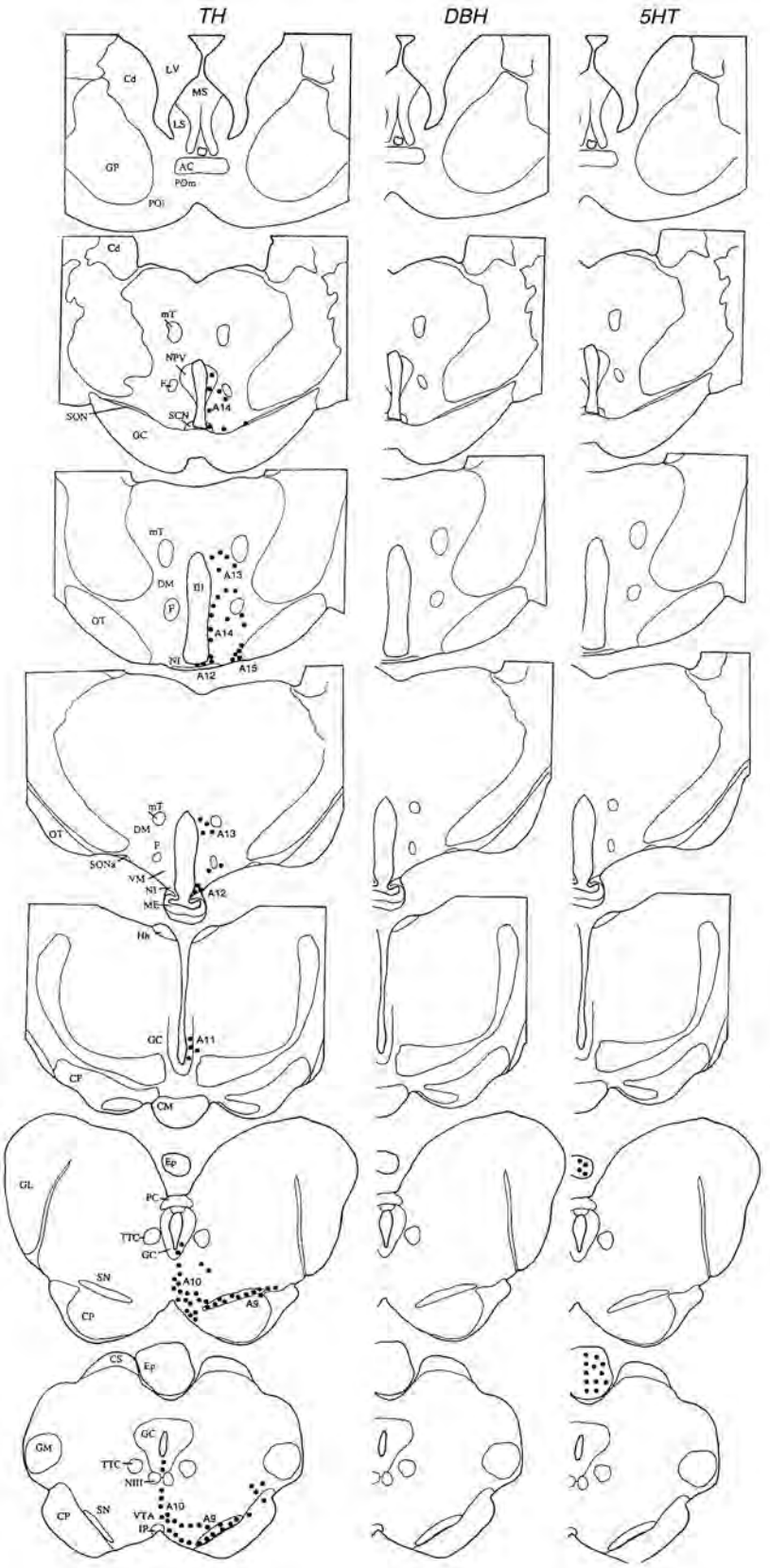
Group S3 is composed of multipolar 5HT neurones distributed in the lateral part, caudal to the locus coeruleus. This group was also observed in other species using immunohistochemistry but not using formaldehyde-induced fluorescence. Perikarya of neurones containing 5HT have a shape similar to that of noradrenergic cells but 5HT neurones are not observed in the sheep locus coeruleus, in contrast to the situation in other species (Sladek and Walker, 1977; Léger and Descarries, 1978; Léger *et al.*, 1979).

Group S4 is situated on each side of the interpeduncular nucleus (Fig. 2b) and has been observed in other species. Neurones of this group are oriented parallel to the ventral edge of the mesencephalon, in sheep and rats (Steinbusch, 1981). However, in contrast to the latter species, no perikarya are observed in the interpeduncular nucleus. These neurones are always intensely stained and some project to the medial preoptic area (Tillet, 1992).

Group B9 extends dorsolaterally to the decussation of the cerebral peduncles. In sheep it is characterized by a low density of perikarya and by a more lateral distribution than in rats (Steinbusch, 1981).

Group S5 consists of pinealocytes that are homogeneously labelled throughout the pineal gland. In sheep, the pineal almost exclusively contains pinealocytes, in contrast to many other species.

Fig. 1. Schematic drawings of successive frontal sections through the sheep brain, from rostral to caudal levels. The distribution of TH-, DBH- and 5HT-IR neurones (adapted from Tillet and Thibault, 1989 and Tillet, 1987, respectively). For abbreviations, see Table 2.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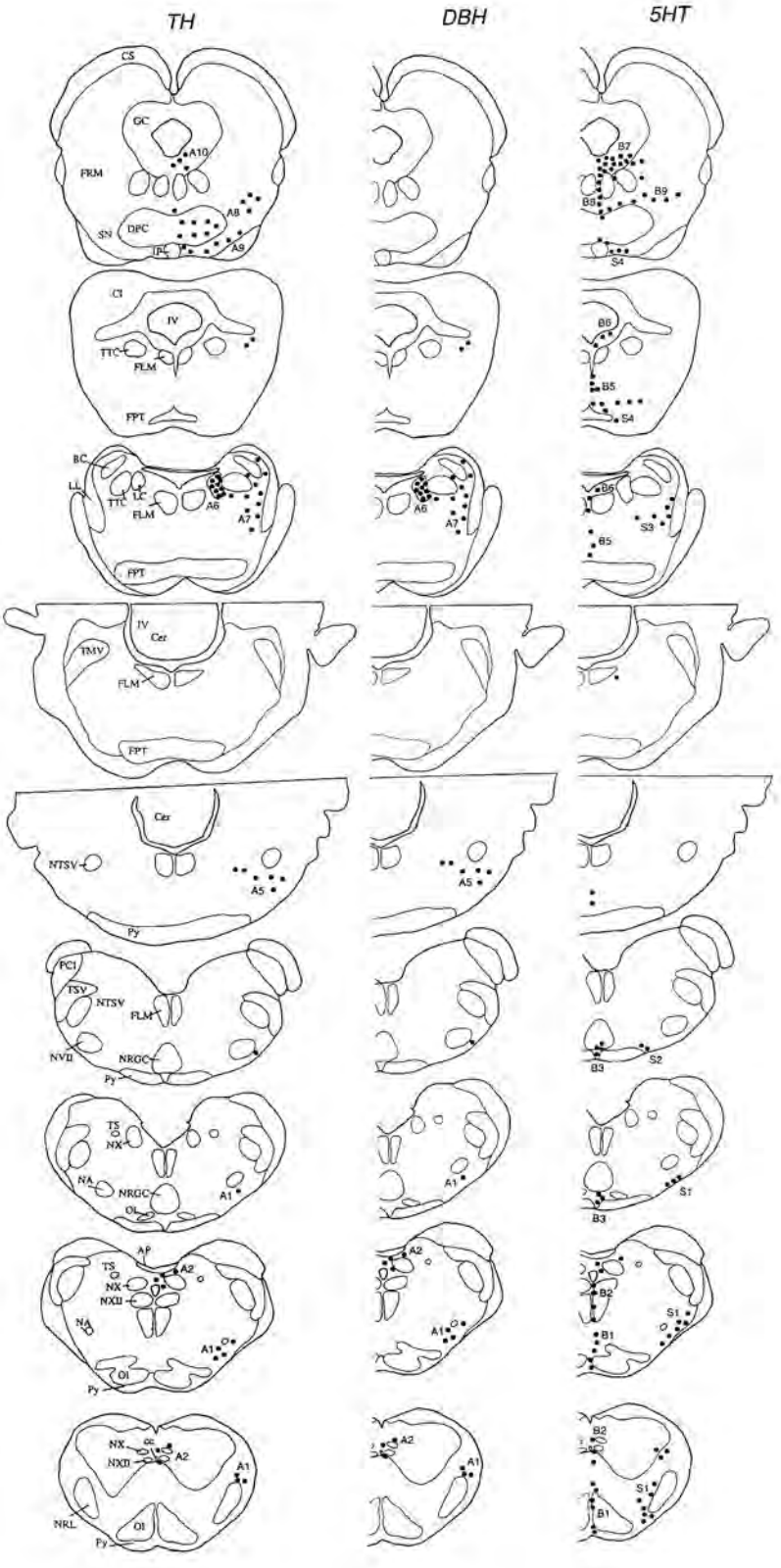
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TSV
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B1

Table 2. Abbreviations used in the figures

AC	anterior commissure	MS	medial septum
AmB	basal nucleus of the amygdala	mT	mammillothalamic tract
AmC	central nucleus of the amygdala	NA	nucleus ambiguus
AmCd	caudal nucleus of the amygdala	NCu	nucleus cuneiformis
AmCo	cortical nucleus of the amygdala	NI	infundibular nucleus
AmL	lateral nucleus of the amygdala	NIII	nucleus nervi occulomotorii
AmM	median nucleus of the amygdala	NP	nucleus pontis
AP	area postrema	NPe	nucleus periventricularis hypothalami
BC	brachium conjunctivum	NPV	nucleus paraventricularis hypothalami
BNST	bed nucleus of the stria terminalis	NRGC	nucleus reticularis paragigantocellularis
cc	canalis centralis	NRL	nucleus reticularis lateralis
Cd	caudate nucleus	NTS	nucleus tractus solitarius
Cer	cerebellum	NTSV	nucleus tractus spinalis nervi trigemini
CI	colliculi inferior	NVII	nucleus nervi fascialis
CM	mammillary nucleus	NX	nucleus nervi vagi
CP	cerebral peduncle	NXII	nucleus nervi hypoglossii
CS	colliculi superior	OC	optic chiasma
DM	dorsal hypothalamic nucleus	OI	nuclei olivares
DPC	decussatio pedunculorum cerebellarium superiorum	OT	optic tract
Ep	epiphysis	PBL	nucleus parabrachialis lateralis
F	fornix	PC	pedunculus cerebri
FLM	fasciculus longitudinalis medialis	PCI	pedunculus cerebellaris inferior
FPT	pontine transverse fibres	PH	nucleus prepositus hypoglossii
FRM	mesencephalic reticular formation	PM	nucleus premammillaris
GC	central grey	POl	lateral preoptic area
GL	lateral geniculate nucleus	POm	medial preoptic area
GM	median geniculate nucleus	Put	putamen
GP	globus pallidus	Py	pyramis
H	hippocampus	SCN	nucleus suprachiasmaticus
Hb	habenular nucleus	SN	substantia nigra (pars compacta)
III	third ventricle	SON	nucleus supraopticus
IP	interpeduncular nucleus	SONa	nucleus supraopticus accessorius
IV	fourth ventricle	TMV	tractus mesencephalicus nervi trigemini
LC	locus coeruleus	TS	tractus solitarius
LH	lateral hypothalamic area	TSV	tractus spinalis nervi trigemini
LL	lateral lemniscus	TTC	tractus tegmentalis centralis
LS	lateral septum	VM	nucleus hypothalamicus ventromedialis
LV	lateral ventricle	VTA	ventral tegmental area
ME	median eminence	ZI	zona incerta

No 5HT perikarya are observed in more rostral structures such as the diencephalon.

With respect to 5HT innervation of the diencephalon, a striking difference is observed in the sheep suprachiasmatic nucleus (SCN) which fails to present a dense innervation as in rats. The same density of fibres is found inside and outside the nucleus. The same pattern of innervation is observed in monkeys (Kawata *et al.*, 1984).

Catecholamines

The distribution of catecholamine-containing structures has been studied using antisera raised against catecholamine synthesizing enzymes (TH: tyrosine hydroxylase; AADC: aromatic L-amino acid decarboxylase; DBH: dopamine β-hydroxylase; PNMT: phenylethanolamine *N*-methyltransferase), but also with antisera raised against dopamine (DA) and noradrenaline. In sheep, classification of

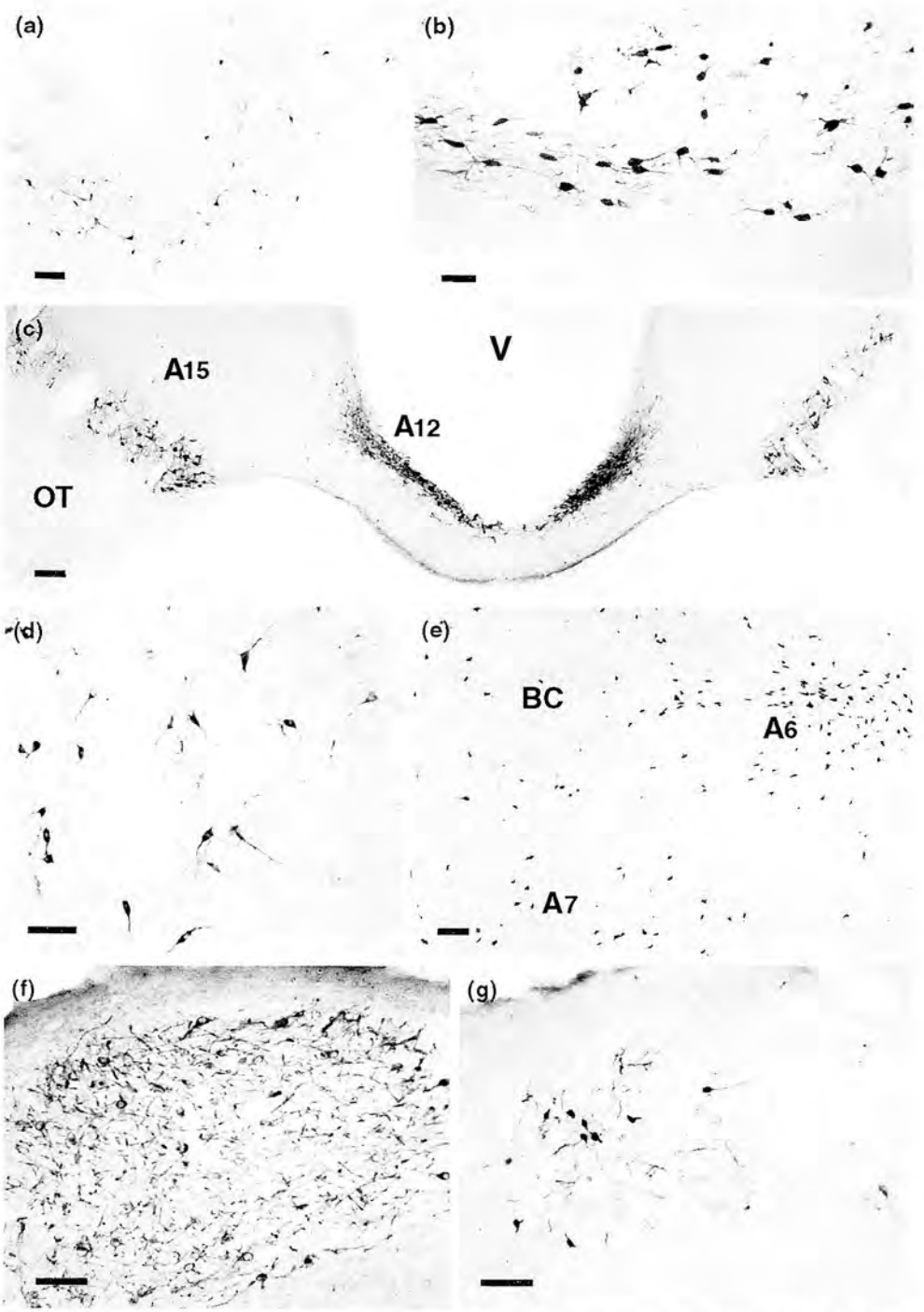

Fig. 2. 5-Hydroxytryptamine immunoreactive (5HT-IR) neurones situated in (a) group S1 in the lateral medulla oblongata and (b) in group S4 on each side of the interpeduncular nucleus. (c) Photomontage showing tyrosine hydroxylase immunoreactive (TH-IR) neurones of the infundibular nucleus (A12) and of group A15 in the ventral hypothalamus. (d) TH-IR neurones of group A10 (dorsocaudal part) situated in the nucleus raphe dorsalis. (e) Dopamine β-hydroxylase immunoreactive (DBH-IR) neurones of groups A6/A7 note the dispersion of labelled neurones in A6 and A7. (f) DBH-IR neurones in groups A2/C2 in the dorsomedial medulla; (g) serial section showing phenylethanolamine *N*-methyltransferase immunoreactive (PNMT-IR) neurones of the same area. BC: brachium conjunctivum; OT: optic tract; V: third ventricle. Scale bars in (a), (c), (e) 250 μm; (b), (d), (f), (g) 100 μm.

catecholamine cell groups can be made according to the Swedish nomenclature established in rats (Dahlström and Fuxe, 1964; Hökfelt *et al.*, 1984).

Dopamine. Cell groups containing dopamine extend to the mesencephalon and the diencephalon.

Groups A8 and A9 are the most caudal dopaminergic cell groups. Group A8 is situated dorsolaterally to the substantia nigra and is made of sparsely distributed neurones. Its boundaries with group A9 are not distinct, but are easier to observe than in A9 of primates (Felten and Sladek, 1983). In sheep, group A9 is composed of perikarya compacted in a thin layer in the pars compacta of the substantia nigra, and the bipolar neurones are oriented parallel to the ventral part of the mesencephalon. Tyrosine hydroxylase immunoreactive (TH-IR) neurones are distributed more rostrally than they are in rats and cells containing dopamine are more compactly clustered than they are in primates and rodents (Pearson *et al.*, 1983; Hökfelt *et al.*, 1984). None or very few perikarya are found in the sheep pars reticulata.

In sheep and rats (Hökfelt *et al.*, 1984), most neurones of group A10 are found in the ventral tegmental area (VTA), but others are observed in the raphe dorsalis (A10 dorsocaudal), near the habenula (A10 dorsorostral) and laterally to the mammillary bodies (A10 ventrorostral). As in rats, the presence of dopamine in these subdivisions has not been checked. Compared with rodents, this group seems to be more heterogeneous, because of the dispersion of its different components. In sheep, the dorsocaudal part (Fig. 2d) presents a higher concentration of perikarya than in rodents or primates, and in humans few neurones are visible (Kitahama *et al.*, 1994).

Groups A11 and A13 are found around the caudal part of the third ventricle and dorsolaterally to the third ventricle, respectively. Compared with rats (Hökfelt *et al.*, 1984), group A11 contains few perikarya and does not extend laterally from the ventricle. Group A13 is mainly localized in the dorsolateral hypothalamus caudal to the paraventricular nucleus; few neurones are stained in an area corresponding to the zona incerta. In contrast to their localization in rats, both groups form a unique gathering.

Group A12 is a dense cluster of TH-IR neurones in the infundibular nucleus (related to the arcuate nucleus of rodents), which is characterized in sheep by the presence of neurones containing dopamine in the dorsal area of the median eminence, in the floor of the third ventricle (Fig. 2c). In sheep and rats, a small percentage of neurones containing dopamine (less than 10%) are immunostained with anti-oestradiol receptors or concentrate [^{3}H]oestradiol (Sar, 1983; Batailler *et al.*, 1992; Lehman and Karsch, 1993).

Group A14 is found around the rostral part of the third ventricle. In sheep, neurones of this group are localized in a narrow strip along the wall of the ventricle and it does not extend as laterally as it does in rats (Hökfelt *et al.*, 1984). Neurones are mainly bipolar and oriented parallel to the wall of the ventricle. Some of the TH-IR neurones do not contain AADC (Y. Tillet, J. Thibault and M. Krieger, unpublished data), which makes the ability of these neurones to synthesize dopamine questionable.

Group A15 contains large TH-IR neurones clustered in the lateral retrochiasmatic area (Fig. 2c). In contrast to the situation in rats and cats (Kitahama *et al.*, 1990), this group presents only a ventral part: perikarya containing dopamine are not observed around the anterior commissure. In sheep, the dopaminergic nature of this group is demonstrated using anti-AADC and anti-DA (Tillet *et al.*, 1990), whereas in rats dopamine is not found and neurones appear to synthesize only L-DOPA (Tison *et al.*, 1990). In contrast to primates, dense gatherings of TH-IR neurones are not found in both supraoptic (SON) and paraventricular (PVN) nuclei of sheep (Li *et al.*, 1988; Panayotacopoulou *et al.*, 1991). In sheep and cats, group A15 sends efferent fibres towards the neural lobe of the pituitary (Luppi *et al.*, 1986; V. Gayrard, J. C. Thiéry, J. Thibault and Y. Tillet, unpublished data).

Group A16 consists of TH-IR perikarya of the olfactory bulb (Tillet *et al.*, 1987). As in other species, most dopaminergic neurones are found around glomeruli, but all other layers contain TH-IR cell bodies except the anterior olfactory nucleus, in contrast to their localization in hamsters (Davis and Macrides, 1983). The accessory olfactory bulb contains a few TH-IR cells in the glomerular layer, as in other species.

Noradrenaline. Groups A1 and A2 are the most caudal noradrenergic groups observed in the ventrolateral and dorsomedial part of the medulla oblongata, respectively, in sheep (Fig. 2f). Perikarya of group A2 are distributed around the central canal and on each side of the caudal part of the fourth ventricle near the area postrema. In sheep, a lower density of IR perikarya is found in both groups than in humans in which labelled cells in the reticular formation form a band connecting the dorsomedial and ventrolateral medulla (Pearson *et al.*, 1983, 1990). In sheep, they do not extend to the more dorsal part of the reticular formation. A similar distribution is observed in cattle and pigs (Kitahama *et al.*, 1994). In sheep and rats (Sakumoto *et al.*, 1978) group A1 sends fibres to the medial preoptic area (Tillet *et al.*, 1993).

As in cats and humans (Kitahama *et al.*, 1994), group A3 is not found in sheep, in contrast to other species; group A4 is not clearly observed in sheep. In sheep, neuronal nuclei are not well evidenced; neurones of this group could therefore be intermingled with those of the locus coeruleus complex. In primates (Felten and Sladek, 1983; Pearson *et al.*, 1983) the gatherings of groups A4, A6 and A7 appear to form a unique noradrenergic complex.

Noradrenergic neurones of group A5 are situated laterally around the emergence of the roots of the facial nerve. In sheep, this group is isolated from other noradrenergic groups, in contrast to its localization in rodents or primates, in which this group is rostrally contiguous with group A7 (Kitahama *et al.*, 1994). In sheep, no DBH-IR neurones are found at the mid-portion of the motor trigeminal nucleus.

Neurones of groups A6 and A7 constitute the most rostral noradrenergic groups (Fig. 2e). Labelled neurones are found laterally on each side of the fourth ventricle, in the locus coeruleus (A6) and in the area around and above the superior cerebellar peduncles (A7). Unlike the situation in rats, the perikarya are not clustered in the locus coeruleus, but are scattered in a large area ventrolateral to the ventricle. This pattern of distribution is also observed in primates (Pearson *et al.*, 1983), cattle and pigs (Kitahama *et al.*, 1994). According to this distribution, the different parts of the locus coeruleus described in rats are not found in sheep. As in other species, group A6 contains a greater density of cells containing noradrenaline than do other noradrenergic groups. In sheep, the boundaries between groups A6 and A7 are not clear and numerous perikarya containing noradrenaline are present between them. Most of them send their axons to the cerebral cortex, pons and medulla. Some of the neurones from group A6 project to the medial preoptic area (Tillet *et al.*, 1993).

Adrenaline. Adrenergic neurones have been studied with anti-PNMT. In most species studied, two groups of PNMT-IR cells are observed in the caudal medulla oblongata, but in sheep a different pattern of central adrenergic innervation is found. It is characterized by the presence of only one group corresponding to the group C2 of the rat. Small PNMT-IR neurones are observed clustered ventrolaterally to the area postrema (Fig. 2g), and others are scattered around the tractus solitarius. PNMT-IR neurones are not found in the ventrolateral medulla, in the area corresponding to group C1. Such an exception is also observed in guinea-pigs, which lack both groups C1 and C2 and in which adrenaline cannot be detected biochemically (Cumming *et al.*, 1986).

Neuropeptides

Cholecystokinin

In sheep, the distribution of cholecystokinin (CCK)-containing neurones (Fig. 3) has been studied with antisera raised against CCK8 which is the form commonly found in the central nervous system of other species; they are observed in all regions of the cerebral cortex, amygdala, hippocampus, lateral septal nucleus and bed nucleus of the stria terminalis (BNST) (Antonopoulos *et al.*, 1987). Scattered neurones containing CCK are observed in the medial preoptic area, periventricular nucleus, SCN, dorsocaudal hypothalamic area (Antonopoulos *et al.*, 1987) and in the supraoptic nucleus (SON) (Marson *et al.*, 1987). In the mesencephalon, CCK-IR cells are found in the central grey, laterally and ventrally to the cerebral aqueduct, in the raphe dorsalis and nucleus cuneiformis. In contrast to rats (Hökfelt *et al.*, 1980), CCK-IR neurones are not found in the VTA of sheep. This observation makes questionable the

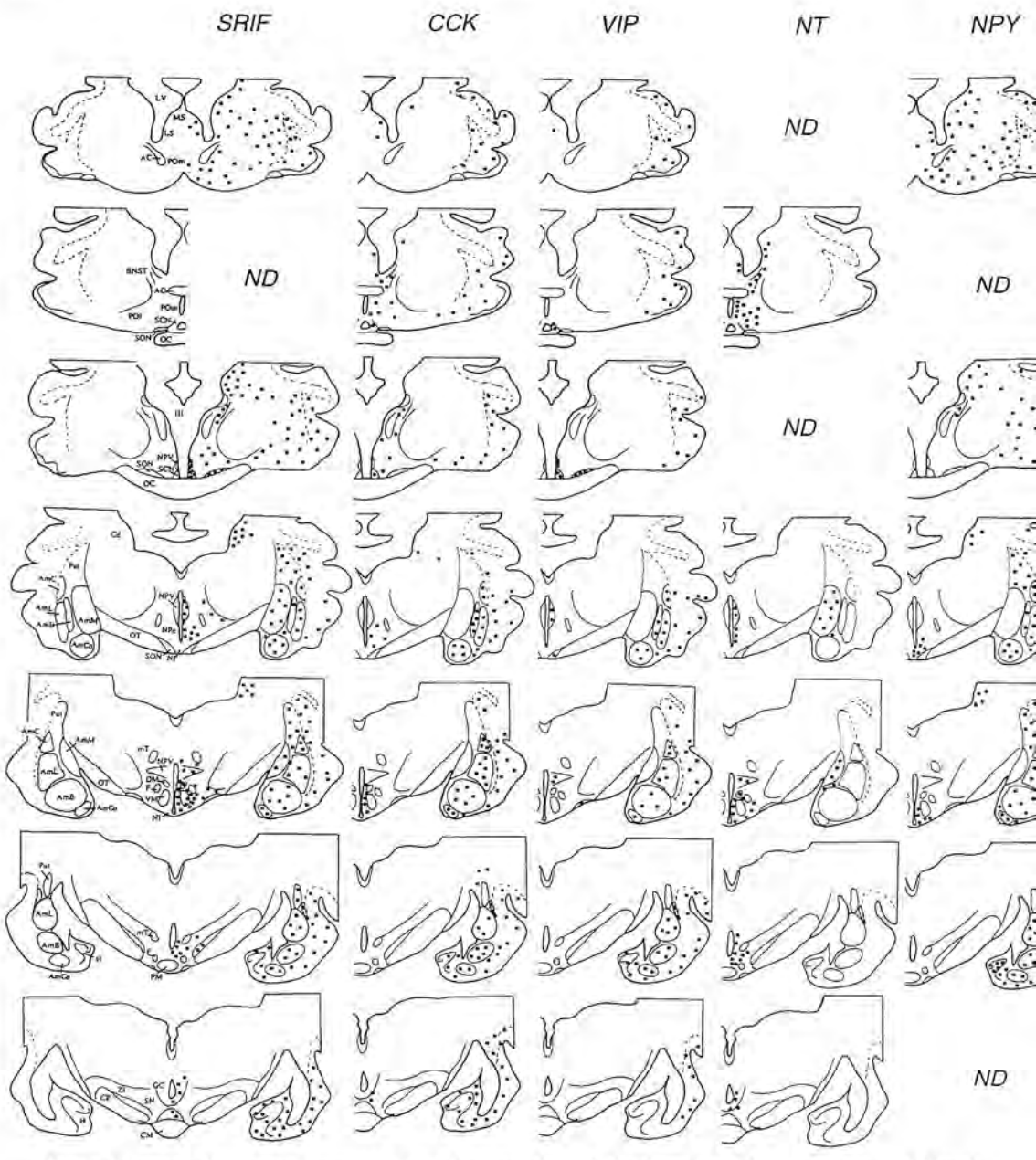

Fig. 3. Schematic drawings of successive frontal sections through the sheep brain, from rostral to caudal levels. Distribution of SRIF-, CCK-, VIP-, NT- and NPY-IR neurones (adapted from Antonopoulos *et al.*, 1987, 1989a; Papadopoulos *et al.*, 1986a,b). ND: not drawn. For abbreviations, see Table 2.

occurrence of a CCK pathway from the VTA to mesolimbic forebrain in sheep. In the sheep pons, neurones containing CCK are observed in the dorsal tegmental nucleus and in the myelencephalon; they are found only in the nucleus of the tractus solitarius (NTS).

Compared with rats, sheep (even treated with colchicine) are thus characterized by the absence of CCK-containing cells in the hypothalamic paraventricular nucleus and in the infundibular nucleus, and by a restricted distribution in other brain areas, except the cortex.

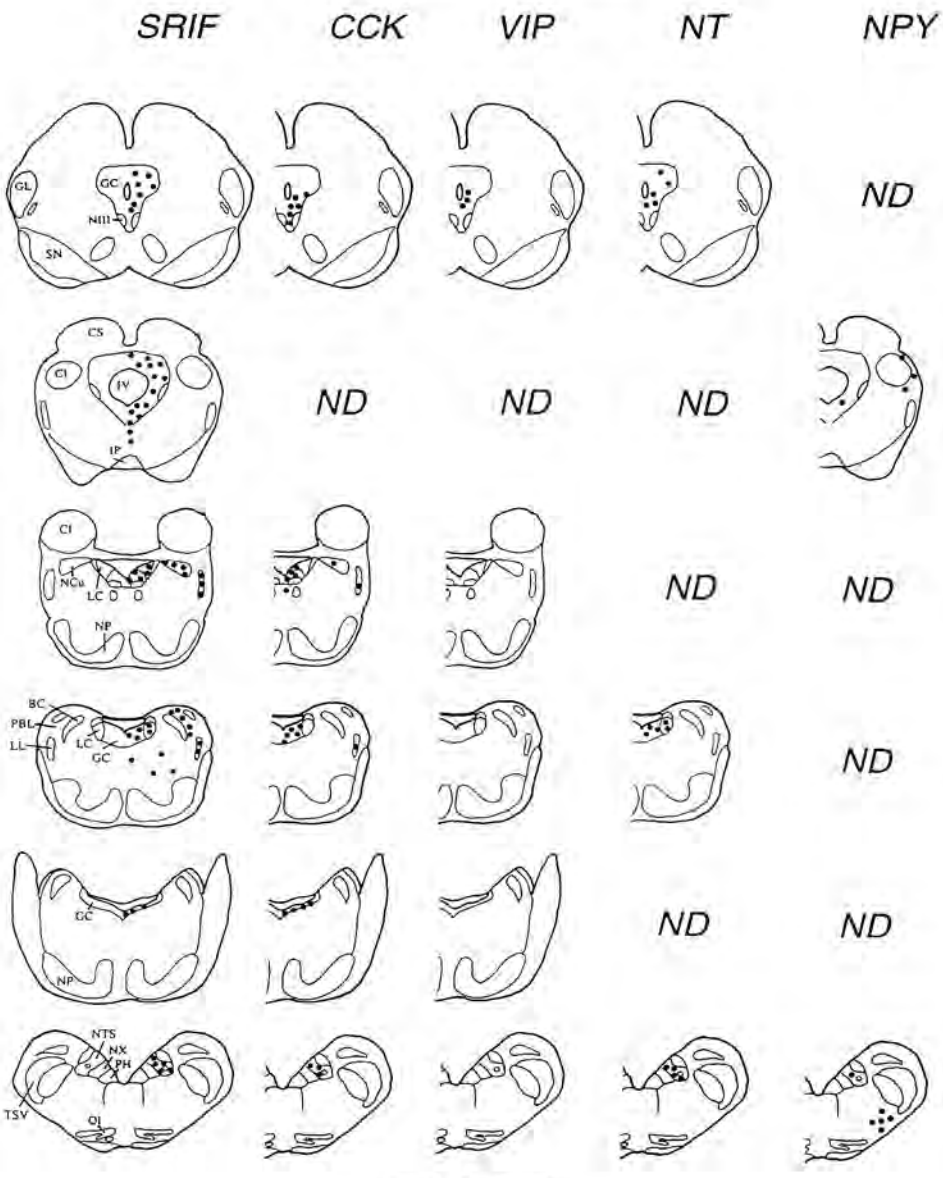

Fig. 3. Continued.

Vasoactive intestinal peptide

The distribution of vasoactive intestinal peptide (VIP) is similar to that of CCK (Fig. 3). In sheep (Table 1) as in rodents (Lorén *et al.*, 1979), all regions of the cerebral cortex contain bipolar neurones. Since they are localized in the same layers, it has been suggested that both peptides could be colocalized in the same neurones of these areas (Antonopoulos *et al.*, 1987). VIP-IR neurones are also observed in the sheep hippocampus and amygdala. In sheep, labelled neurones are observed in the SCN (Tillet *et al.*, 1989b; Tessoneaud *et al.*, 1994) as in rats, but, in addition, VIP is found in neurones of the PVN and SON as in cats (Obata-Tsuto *et al.*, 1983). In contrast to most peptides, the other diencephalic nuclei do not contain VIP-IR cells. In the mesencephalon, labelled cells are found in the central grey in sheep and rats, but in contrast to rats, the myelencephalon of sheep is devoid of VIP-IR neurones.

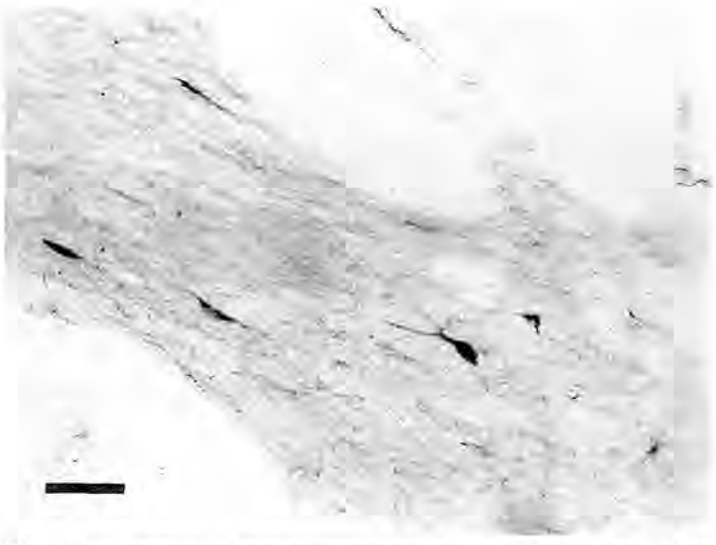

Fig. 4. Neuropeptide Y immunoreactive neurones in the bed nucleus of the stria terminalis (BNST) of colchicine-treated sheep. Scale bar represents 50 μm.

Neurotensin

In sheep, neurotensin immunoreactive (NT-IR) neurones are visible in different parts of the brain only after colchicine pretreatment (Table 1; Fig. 3). NT-IR neurones are observed in the lateral septal nucleus, BNST, ventromedial part of the caudate nucleus and medial amygdaloid nucleus. In the telencephalon, their distribution in sheep contrasts with that observed in rats (Roberts *et al.*, 1982), as the central amygdaloid nucleus of sheep does not contain NT-IR neurones, whereas in rats, a large number of labelled neurones are found in this region. Most of the hypothalamic nuclei contain NT-IR neurones and the highest density is observed in the PVN. The periventricular nucleus of the thalamus also contains labelled neurones. In the brainstem, labelled neurones are distributed in the mesencephalic central grey. In the myelencephalon, few neurones are found in the pontine central grey area, locus coeruleus and NTS. In contrast to rats, NT-IR neurones are not found in the parabrachial nucleus and in the spinal cord. Another difference between the NT system of sheep and rats concerns the organization of limbic neurotensinergic inputs to the cortex. In rats, these fibres originate from the central amygdaloid and parabrachial nuclei (Jennes *et al.*, 1982), but in sheep these nuclei are devoid of NT-IR neurones, and such afferents could have a different origin (Papadopoulos *et al.*, 1986a).

Neuropeptide Y

Neuropeptide Y immunoreactive (NPY-IR) neurones have been identified in sheep by immunohistochemistry in the different brain areas except the pons (Fig. 3) (Antonopoulos *et al.*, 1989a). This description has been confirmed by *in situ* hybridization in the hypothalamus (McShane *et al.*, 1993). Colchicine treatment increases the labelling intensity and the number of immunoreactive neurones. The cerebral cortex contains numerous labelled neurones in all laminae. In the telencephalon, immunoreactive perikarya are found in the striatum, claustrum, hippocampus, amygdaloid complex except the medial amygdaloid nucleus, septum and BNST (Fig. 4). In the diencephalon, labelled neurones are found in the infundibular nucleus and the surrounding areas using *in situ* hybridization and immunohistochemistry techniques. In the hindbrain, a lower density of immunoreactive perikarya is observed in the central grey, and in the dorsal and lateral part of the colliculi. In the medulla, immunoreactive neurones are present only in the NTS and ventrolateral medulla. Such a distribution showing a high density of immunoreactive perikarya in the forebrain and a low density in the hindbrain is also observed in rats (Chronwall *et al.*, 1985) and monkeys (Smith *et al.*, 1985).

Corticotrophin-releasing factor

In sheep, corticotrophin-releasing factor (CRF) has been identified by immunohistochemistry and by *in situ* hybridization (Table 1). CRF immunoreactivity is present in the medial part of the PVN of the hypothalamus where the highest density of labelled perikarya is observed. In addition, mRNA encoding CRF has been detected in the ovine olivary nuclei of the brainstem and in some neurones scattered in every major cortical field, in the vicinity of the LC and the parabrachial nucleus and solitary tract (Matthews *et al.*, 1991). In rats, all these structures contain CRF-IR neurones (Sakanaka *et al.*, 1987). However, the distribution in sheep differs from that of rats, since no immunoreactive cells are observed in the amygdaloid nuclei, BNST, substantia inominata, in the dorsal tegmental field, mammillary and posterior hypothalamic nuclei. Another important difference between rats and sheep is the absence of CRF in the sheep SON. In rats, both CRF and its mRNA are identified in some neurones of the SON (Lightman and Young, 1987; Sakanaka *et al.*, 1987). In sheep, CRF-IR neurones are less widely distributed than in rats. As in other species, a major projection of fibres is observed in the median eminence.

Gonadotrophin releasing hormone

In sheep, as in all the species studied, GnRH-IR neurones are not aggregated in discrete brain nuclei, but scattered throughout the preoptico–hypothalamic area (Fig. 5a). The concentration of GnRH in immunoreactive perikarya is quite high since pretreatment of the animals with colchicine is not required and does not modify the number of labelled neurones. Many studies have described the distribution of GnRH-IR perikarya (Table 1). Most of them are observed around the vascular organ of the lamina terminalis (OVLT) where 50% of the perikarya are observed (Fig. 5b) (Caldani *et al.*, 1988). The most rostral extension is identified in the accessory olfactory bulb and the most caudal extension in the anterior part of the mammillary bodies. The mediobasal hypothalamus contains about 15% of the GnRH-IR neurones. The distribution of GnRH-IR perikarya in the ovine preoptic–anterior hypothalamic area is similar to that described in rodents (Barry *et al.*, 1985). However, small species differences are observed; GnRH-IR cells are present in the SCN of guinea-pigs and hamsters but not of sheep. Compared with primates, the major difference concerns the mediobasal hypothalamus where the arcuate nucleus and surrounding areas present a large number of GnRH-IR perikarya, whereas few neurones are found here (less than 15%) in sheep (Caldani *et al.*, 1988).

GnRH perikarya in extrahypothalamic–preoptic areas represent only 5% of the population; they are observed in the amygdala and subcallosal area of ewes (Advis *et al.*, 1985). This distribution matches that found in other species more extensively described in this respect. The GnRH distribution in sheep is characterized by a lower density than in other species: in monkeys it extends more posteriorly (Barry, 1978) and in hamsters, anterior extension appears more important (Jennes and Stumpf, 1980).

As in other species, GnRH neurones send a major projection to the median eminence, but also to the OVLT. Other GnRH pathways are also observed outside the preoptic–anterior hypothalamic areas, towards the olfactory bulb, amygdala, hippocampus and mesencephalon.

Somatostatin

Neurones containing somatostatin or somatotrophin inhibiting factor (SRIF)-IR can be observed without colchicine treatment, but this treatment allows additional labelled neurones to be visualized, particularly in the myelencephalon (Papadopoulos *et al.*, 1986b) (Fig. 3). SRIF-IR neurones are observed in the different layers of all areas of the cerebral cortex. They are also found in the striatum (Fig. 6a), claustrum, different nuclei of the amygdala, hippocampus, lateral septal nucleus and BNST as in rats and humans. In the diencephalon, the periventricular nucleus of the preoptic area and the different nuclei of the hypothalamus contain various densities of labelled neurones. SRIF-IR neurones are found in the pars verticalis and horizontalis of the PVN as defined by Welento *et al.* (1969). Many labelled cells surround the ventromedial nucleus of the hypothalamus, which itself is devoid of them. The SON, SCN, infundibular nucleus, lateral preoptic area, dorsal and caudal hypothalamus also contain numerous SRIF-IR neurones. In the mesencephalon, labelled cells extend to the central grey, nucleus cuneiformis, superior central nucleus and dorsal raphe. However, their distribution in sheep is characterized by the

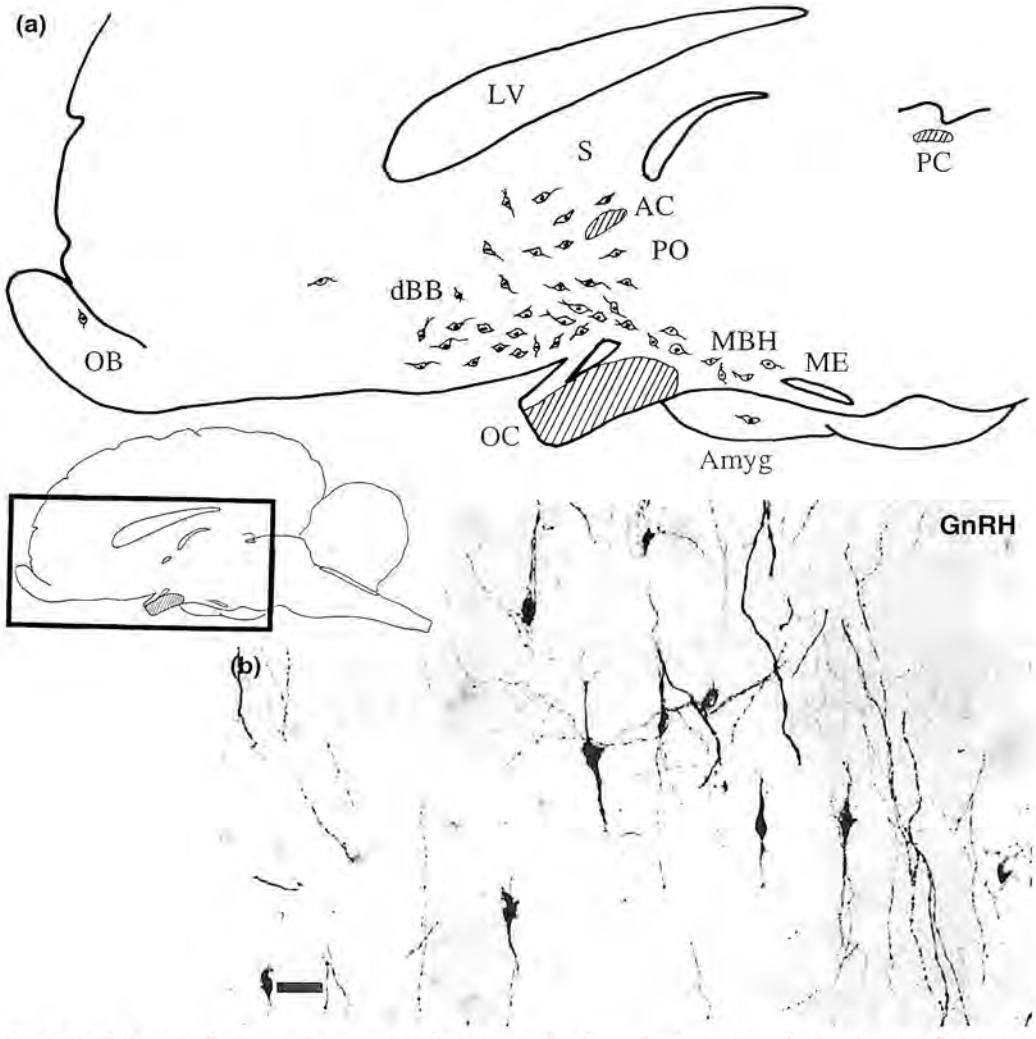

Fig. 5. (a) Schematic drawing of a parasagittal section in the sheep forebrain (boxed) showing the distribution of GnRH-IR neurones (adapted from Caldani *et al.*, 1988). (b) High density of GnRH-containing neurones in the medial preoptic area (figure kindly provided by M. Caldani). AC: anterior commissure; Amyg: amygdala; dBB: diagonal band of Broca; LV: lateral ventricle; MBH: mediobasal hypothalamus; ME: median eminence; OB: olfactory bulb; OC: optic chiasma; PC: posterior commissure; PO: preoptic area. Scale bar represents 50 μm.

lack (or very low density) of SRIF neurones in the interpeduncular nucleus, which presents numerous labelled neurones in rats (Hamill *et al.*, 1984). In the medulla, SRIF neurones are observed in the lateral lemniscus, lateral parabrachial nucleus, locus coeruleus, pontine central grey, reticular formation (but not in the lateral reticular nucleus) and nucleus tractus solitarius. In the spinal cord, the laminae II and III contain SRIF-IR neurones. Compared with rats (Johansson *et al.*, 1984), the density of SRIF neurones in the sheep is low in the caudal brainstem and medulla.

Arginine vasopressin and oxytocin

Arginine vasopressin and oxytocin are found in magnocellular neurones clustered in the SON and PVN. These nuclei present clear boundaries contrasting with other nuclei in which neurones are more

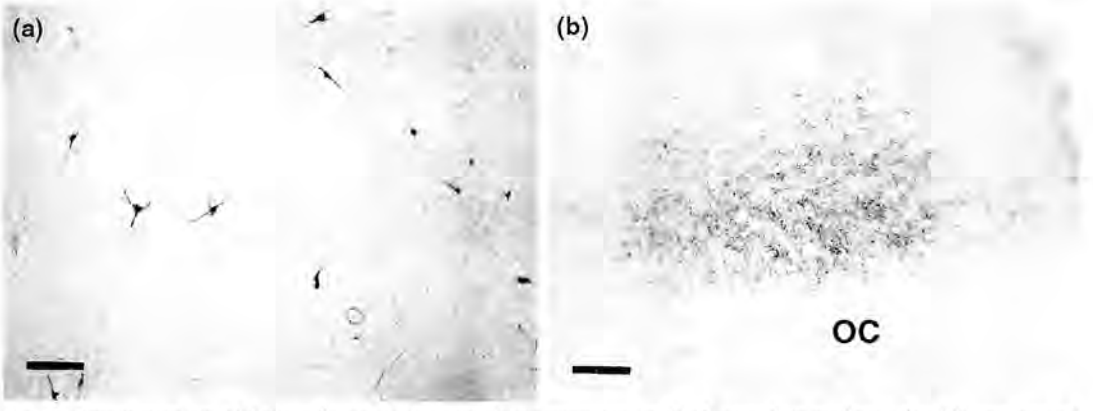

Fig. 6. Somatotrophin inhibiting factor (SRIF)-containing neurones in (a) the caudate nucleus of colchicine-treated animals. (b) The sheep suprachiasmatic nucleus (SCN) is characterized by a dense network of met-enkephalin containing fibres. OC: optic chiasma. Scale bars represent 100 μm.

scattered. The morphology of the ovine SON and PVN differs from that of rats (Welento *et al.*, 1969). In sheep the SON form a continuous structure extending along the ventral portion of the hypothalamus and covering the optic chiasma. Moreover, compared with rodents, the ovine accessory nuclei are found in the retrochiasmatic area. The sheep PVN is characterized by dorsal and ventral subgroups which join caudally in the posterior part of the nucleus. The dorsal part is similar to the PVN of rodents but the ventral part has no equivalent nucleus in rodents. The ventral part is also observed in goats and pigs (van Eerdenburg *et al.*, 1990; Hamada *et al.*, 1992).

Oxytocin. The distribution of neurones containing oxytocin has been studied by immunohistochemistry and *in situ* hybridization (Broad *et al.*, 1993b). The largest population of labelled neurones is found in the SON and PVN, and a small number in the BNST, anterior commissural nucleus, and periventricular part of the preoptic area. In the PVN, oxytocin neurones are diffusely distributed throughout the nucleus (Papadopoulos *et al.*, 1985). A similar distribution is observed in rats (Sofroniew, 1985).

Vasopressin. The AVP-IR neurones are distributed in the PVN and SON like those of oxytocin, but they constitute distinct populations (Watkins and Moore, 1983). In addition, AVP-IR neurones are found in the SCN after colchicine treatment (Tillet *et al.*, 1989b). In the latter nucleus, in contrast to the PVN and SON, labelled cells are parvicellular. Although the distribution of neurones containing AVP has not been extensively studied in the sheep brain, this peptide is not as widely distributed as it is in rats. The rat lateral septum, amygdala and locus coeruleus contain AVP-IR cells, but in sheep these areas are devoid of neurones containing AVP and of neurophysin (carrier protein of AVP and oxytocin), at least in the animals that were not treated with colchicine (Y. Tillet, unpublished data).

Calcitonin-gene related peptide

The distribution of calcitonin-gene related peptide immunoreactive (CGRP-IR) neurones has been demonstrated only in the forebrain of castrated ewes (Herbison *et al.*, 1993a). The highest density of labelled neurones (magnocellular) is observed in the ventral division of the PVN, and a few are also found in the dorsal division of the nucleus and SON. Scattered smaller neurones are also found in the preoptic area, anterior hypothalamic area, basal hypothalamus and area of the stria terminalis. Compared with rats (Herbison, 1992), the density of labelled neurones in the preoptic area of sheep is low. The lateral border of the anterior hypothalamus contains a high density of labelled neurones in rats but is devoid of immunoreactive neurones in sheep.

between TH-IR fibres and perikarya in the rat arcuate nucleus (Leranth *et al.*, 1985) and monkey periventricular nucleus (Thind and Goldsmith, 1986).

Morphological relationships involving neuropeptides

In the preoptic area, close appositions have been observed between NPY terminals and GnRH-IR neurones (Tillet *et al.*, 1989a). Ultrastructural investigations confirm the presence of synaptic inputs between these elements (Norgren and Lehman, 1989).

In the PVN, oxytocin and CRF are colocalized in only very few neurones (approximately 1% of cells contain both peptides), whereas in rodents about 10–20% of oxytocin and CRF neurone populations express both peptides (Papadopoulos *et al.*, 1985). In addition, most of the neurones containing VIP also contain CRF and SRIF is sometimes colocalized with oxytocin (Papadopoulos *et al.*, 1990). However, SRIF and VIP, VIP and oxytocin, SRIF and CRF are never found in the same neurones of the sheep PVN (Papadopoulos *et al.*, 1990). Such colocalizations are observed or suspected in other species (Hökfelt *et al.*, 1987; Sawchenko *et al.*, 1984). However, there could be interspecies variations, since CCK is not present in the sheep PVN (Antonopoulos *et al.*, 1987) and therefore cannot be colocalized with oxytocin and CRF as shown in rats (Vanderhaegen *et al.*, 1981; Mezey *et al.*, 1986). Another peptide, bombesin, the distribution of which has not been mapped in the sheep brain, is colocalized with CRF in fibres of the median eminence (Giraud *et al.*, 1987). This observation suggests the colocalization of both peptides in PVN neurones. These observations would indicate that pluripotentiality of neurones of the PVN is different in sheep and rats.

The infundibular nucleus presents extensive neurochemical heterogeneity and different neurones that exhibit a similar morphology. However, although this nucleus does not present the same compartmentation in sheep compared with rats, the different neurotransmitters are found in distinct subparts, and among the putative colocalizations studied, few are observed and concern neurones containing both SRIF and NPY (Antonopoulos *et al.*, 1989b). Most neurones from this group project to the median eminence (except β-endorphin) as observed in rats. However, some differences should be noted: if NPY, SRIF and β-endorphin innervation of the median eminence is similar to that of rodents, few NT-IR fibres are found in sheep compared with rodents (Merchenthaler and Lennard, 1991).

Conclusion

The distribution of neurotransmitters in the sheep brain is different from that of rodents or primates, which are more commonly studied: first, the architecture of the sheep brain is extensive with scattered rather than clustered neurones as seen in rodents, and the morphology of the neurohypophyseal system comprising the PVN and SON is different. Second, within these structures, the fine neurochemical organization presents some differences compared with other species: an extensive distribution of 5HT cells towards more lateral parts of the brainstem, a lower density of TH-IR neurones in the rostrodorsal diencephalon, and the absence of adrenergic neurones in the ventrolateral medulla. With respect to peptides, there are no NT neurones in the central amygdaloid and parabrachial nuclei and no CCK in the PVN and VTA, but a few GnRH-IR neurones are found in the mediobasal hypothalamus. All these differences induce variations in the projection fields and some brain areas of the sheep contain specific patterns of terminal organization. For example, the SCN exhibits a low density of 5HT- and NPY-IR fibres but a dense network of met-enkephalin fibres. Compared with that of other species, the sheep hypothalamus presents a noteworthy neurochemical diversity, and in addition to the different morphological interactions between neurotransmitters, several neuronal groups receive peripheral information through steroid hormones (see Herbison *et al.*, this supplement).

All these differences underline the difficulties in extrapolating results obtained in species other than sheep. Comparison between species (from morphological and physiological points of view) should improve our understanding of neuronal regulations and, in this way, the sheep could constitute an alternative model to rodents or primates. All these morphological observations constitute steps necessary for further physiological investigations.

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