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Review

The vertebrate diencephalic MCH system: A versatile neuronal population in an evolving brain

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ABSTRACT

Neurons synthesizing melanin-concentrating hormone (MCH) are described in the posterior hypothalamus of all vertebrates investigated so far. However, their anatomy is very different according to species: they are small and periventricular in lampreys, cartilaginous fishes or anurans, large and neuroendocrine in bony fishes, or distributed over large regions of the lateral hypothalamus in many mammals. An analysis of their comparative anatomy alongside recent data about the development of the forebrain, suggests that although very different, MCH neurons of the caudal hypothalamus are homologous. We further hypothesize that their divergent anatomy is linked to divergence in the forebrain – in particular telencephalic evolution.

1. Introduction

Most brain divisions were delineated according to specific cytoarchitectonic features and named with regard to their location, particular shape or color. These divisions very often correspond to functionally relevant neurological entities. However, some brain structures contain specific neuron populations that ignore these clear anatomical borders. As an example, we can cite magnocellular cholinergic neurons that are distributed in ventral telencephalic nuclear masses (pallidum, septum, substantia innominata) and that send topographically organized cholinergic projections throughout the cortical mantle in mammals (Mesulam et al., 1983; Risold, 2004). In the mammalian hypothalamus, neurons containing melanin-concentrating hormone (MCH) or producing the hypocretins/orexins (Hcrt) are also two examples of such populations. They are co-localized in posterior hypothalamic regions regardless of cytoarchitectonic boundaries and both are made of large cells projecting throughout the brain, including the whole cortical mantle. At functional levels, both cell groups have been implicated in a large range of responses and behaviors (from sleep to feeding and reproductive behaviors) (Bittencourt, 2011; Boutrel et al., 2010; Griffond and Baker, 2002; Peyron et al., 2009; Sapin et al., 2010).

MCH was first isolated from the salmon pituitary (Kawauchi et al., 1983). The structure of the precursor (preproMCH = ppMCH) was elucidated by cDNA cloning in chum salmon (Kawauchi et al., 1983), as well as in Chinook salmon, Coho salmon, rainbow trout, tilapia (for review see Griffond and Baker, 2002; Gröneveld et al., 1993; Kawauchi and Baker, 2004; Nahon, 1994). The ppMCH was also characterized in the rat, mouse and primates like human (Breton et al., 1993; Nahon, 1994; Nahon et al., 1989).

In mammals, potential cleavage sites suggest that three putative peptides might be generated from the precursor. MCH is located at the carboxyterminus of the prohormone. The peptide directly preceding MCH is called neuropeptide glutamic acid-isoleucinamide (NEI) in mammals, neuropeptide glutamic acid-valine (NEV) in salmonids and MCH gene-related peptide (Mgrp) in tilapia. In the mammalian MCH prohormone, another putative peptide named neuropeptide glycine-glutamic acid (NGE) or neuropeptide proline-glutamic acid (NPE) precedes NEI (see Baker et al., 1995; Gröneveld et al., 1993, 1995; Nahon, 1994).

MCH exhibits considerable structural conservation from cyclostomes to mammals (Cardinaud et al., 2004). In all species studied, it is a heptadecapeptide or a nonadecapeptide with a disulfide bridge forming a 10-amino-acid ring. MCH antibodies (against salmon MCH, synthetic MCH, or rat MCH) have been largely used to describe the neurons synthesizing this peptide in many non-mammalian vertebrates including agnathans, several teleosteans and holosteans (see Baker and Kawauchi, 1997), in the elasmobranch *Scyliorhinus canicula* (Vallarino et al., 1989), the dipnoan *Protopterus annectens* (Vallarino et al., 1998), anuran amphibians (Andersen et al., 1986; Francis and Baker, 1995; Lázár et al., 2002), reptiles

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(Cardot et al., 1994) and several bird species (Cardot et al., 1998, 1999). The MCH system has also been described in mammalian species including human (Bresson et al., 1989; Griffond and Baker, 2002; Nahon, 1994).

Little correlation seems to exist between the periventricular neurons of the lamprey, the large magnocellular neuroendocrine neurons in the nucleus lateralis tuberis of the teleostean ventral hypothalamus, or the large lateral hypothalamic group reported in rodents. This stands in sharp contrast with the remarkable structural conservation of the MCH peptide as well as the fact that it is synthesized in posterior hypothalamic neurons in all vertebrate species (Baker, 1991).

One goal of this work is to survey the available data on the comparative anatomy of these neurons to verify if the great diversity of their anatomical organization is compatible with these neurons being homologous, and understand the origin of the large hypothalamic cells of the mammalian posterior lateral hypothalamus.

In rodent, we showed recently that the connection patterns of adult MCH neurons intimately reflect the anatomical organization of the embryonic brain at the stage at which these neurons are generated (Croizier et al., 2010, 2011). Some of the molecules involved in the embryonic differentiation of the MCH phenotype, such as sonic hedgehog, are known to play key roles in the forebrain evolution. Therefore, we also hypothesized that differences in the MCH system anatomy across vertebrate species reflect some key aspects of the evolution of the prosencephalon, especially concerning connections between telencephalon and hypothalamus. All the data reviewed in this work show the exceptional plasticity of this system and may explain the large panel of functions in which these neurons are involved and that are sometimes clade specific.

2. Anatomy of the MCH system in non-mammalian vertebrates

2.1. Lampreys

Lampreys are often considered as living representative examples of the most primitive groups of vertebrates. These jawless (agnathans) animals live in water and are predacious or parasites, feeding over larger organisms by sucking blood and tissues. Their brain is of a very small size and the spinal cord is by far the largest part of the CNS. The basic parts from the telencephalon to rhombencephalon are nevertheless identified (Nieuwenhuys et al., 1998; Osorio and Rétaux, 2008). The hypothalamus takes the largest part of the diencephalon. It contains a preoptic/anterior region separated from the posterior hypothalamus by the optic chiasm and a large supraoptic commissure. The posterior hypothalamus is formed of a compact periventricular layer of neurons, but a dorsal and a ventral nucleus can be distinguished. The thick lateral neuropil is made of white matter and contains axons from the neuronal layer and large bundles connecting the telencephalon and the brainstem.

2.1.1. Perikarya

Few studies have reported the distribution of the MCH system in these species. Nevertheless, the article by Bird et al. (2001) provides a good description in several species of lampreys. The present work is largely based on this article, completed by observations from material processed in our laboratory (see Appendix).

In most species, MCH is exclusively expressed in cell bodies of the dorsal hypothalamic nucleus (Fig. 1A, D and E). In *Lampetra planeri* and *Lampetra fluviatilis* few cells are also observed in the basal telencephalon of sexually maturing specimens. They sit in the deep primordium piriform (following the nomenclature of Nieuwenhuys et al. (1998)) and, as described by Bird et al. (2001), are few in number and faintly labeled. The perikarya in the posterior dorsal hypothalamus are far more numerous and intensely labeled. They are bipolar and sit in the ependyma and subependyma (Fig. 1D). Few can be seen at some distances from the third ventricle. All MCH cells are very similar in shape and size to surrounding unlabeled neurons.

The posterior hypothalamic region is characterized by abundant monoaminergic neurons, in particular histaminergic and serotoninergic neurons (Brodin et al., 1990; Weigle and Northcutt, 1999). Dopaminergic neurons are observed throughout the hypothalamus but it is worth mentioning that the dopaminergic afferents to the lamprey striatum originates mostly from the posterior tuberculum which sit at the diencephalic/mesencephalic limit (Pombal et al., 1997). Mesencephalic A8, A9 or A10 dopaminergic cell groups are not clearly recognized in cyclostomes (Reiner et al., 1998; Yamamoto and Vernier, 2011). Hypothalamic MCH neurons are localized in the periventricular posterior hypothalamic nucleus, which means that they are just rostral to dopaminergic neurons in the posterior tuberculum and at the caudal end of the hypothalamus. In their nicely illustrated paper, Brodin et al. (1990) showed histaminergic cell bodies in the ventral and dorsal posterior hypothalamus as well as in the postinfundibular commissural nucleus, indicating that some MCH cell bodies are adjacent to dorsal histaminergic neurons (Fig. 2A). Finally, MCH perikarya are caudal to the neuroendocrine magnocellular cells that sit in the preoptic region and project in the neurohypophysis. These neurosecretory vasotocinergic neurons are densely packed and their projections form a clear tract which first reaches the neuropil lateral to the neurons and adjacent to the optic tract before running caudo-ventrally to the neurohypophysis (Fig. 3). Using an antibody against vasotocin, we observed a few labeled cells in the ventral and dorsal posterior hypothalamus. These cells have also been observed by Bird et al. (2001) and cannot be mistaken for the magnocellular cells located in the anterior hypothalamus (Goossens et al., 1977).

2.1.2. Fibers

MCH expansions can be traced to the ventricular surface suggesting that MCH neurons are cerebrospinal fluid- (CSF-)contacting cells, which is common in these species (e.g. histaminergic cells in dorsal and ventral hypothalamus, Brodin et al. (1990)). Axons are also observed away from the ventricular surface in the lateral hypothalamic region. These projections follow ascending and descending directions (Fig. 2B). Bird et al. (2001) reported MCH axons mainly in the ventral half of the brain, from the olfactory bulb to the spinal cord. These authors particularly emphasized MCH projections in the neurohypophysis observed in *L. fluviatilis* but not in other species. These projections were also described by Al-Yousuf and Mizuno (1991). They are illustrated in Fig. 1B and C but, as described by Bird et al. (2001), they are far less abundant than vasotocinergic projections (Fig. 3). MCH axons are observed with some abundance in the striatum and the preoptic region, but they are very scarce in the pallium, including the olfactory bulb and the primordium hippocampi. In the posterior hypothalamus, fibers are very abundant in the lateral neuropil, but some seem to innervate periventricular perikarya in the dorsal and ventral regions. Dorsally directed fibers are seen in a periventricular position, providing a moderate to weak innervation of cell bodies in the thalamus and the habenular nuclei. Caudal to the diencephalon, MCH axons are still abundant in the lateral neuropil of the tegmentum but the frequency of putative axo-somatic synapses decreases as few fibers are seen in the immediate vicinity of cell bodies. Further caudally, the number of MCH axons decreases rapidly, and only few fibers are observed in the rhombencephalon. MCH projections in the spinal cord are negligible in lampreys.

2.1.3. Conclusions

In cyclostomes, like *L. fluviatilis*, the MCH cell group of the posterior dorsal hypothalamus constitutes the main source of MCH in

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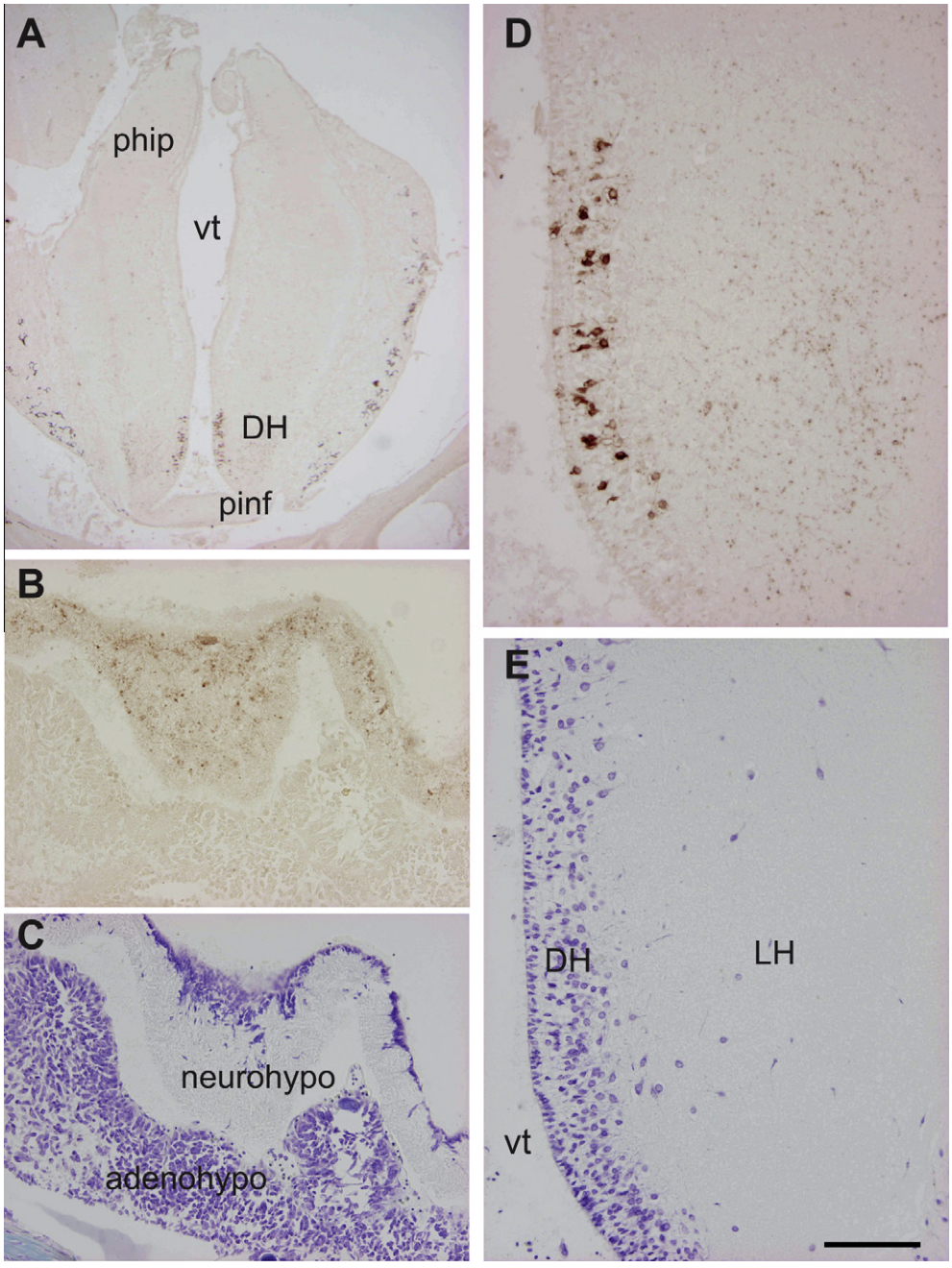

Fig. 1. MCH in *Lampetra fluviatilis*. (A) Low magnification of an oblique section (angle is shown in Fig. 2) through the diencephalon and impar telencephalon to illustrate the distribution pattern of MCH neurons in the posterior hypothalamus. These neurons are detected using an anti-salmon MCH antiserum and revealed using the peroxidase–antiperoxidase method. (B and C) MCH axons in the neurohypophysis. Note in (B) the moderate innervation by MCH axons. Figure (C) shows an adjacent section stained with toluidin blue for cytoarchitectonic purpose. (D and E) Photomicrographs showing the periventricular distribution of MCH perikarya in the caudal–dorsal hypothalamus (D) and an adjacent section stained with toluidin blue (E). All MCH perikarya are in the periventricular layer, but axons are very abundant in the cell poor lateral zone. Note that MCH labeled elements reach the ventricular surface. Abbreviations: adenohypo: adenohypophysis; DH: dorsal hypothalamus; LH: lateral hypothalamus; neurohypo: neurohypophysis; phip: primordium hippocampi; pinf: nucleus post infundibularis; vt: ventricle. Scale bar (*E*) = 500 μm in A; = 50 μm in (B–E).

the brain, with another small cell group not consistently observed in the basal telencephalon. MCH projections innervate mostly the basal telencephalon and the hypothalamus itself, and provide a moderate input to the thalamus and mesencephalon. This hodological organization resembles the patterns of histamine projections arising from neurons located in the posterior hypothalamus (Brodin et al., 1990). Furthermore, MCH may also be released in the CSF, and act through general volume transmission. In *L. fluviatilis*, these neurons could be neuroendocrine as they innervate the neurohypophysis. Contrasting with the patterns described in mammals (see below), few projections reach the pallium and the spinal cord.

The functions of MCH in lampreys are unknown. MCH neurons do not seem to respond to salinity challenges (Bird et al., 2001). The pattern of their projections suggests that already in these species, MCH may intervene in several processes including modulatory and neuroendocrine roles.

2.2. Fishes

Fishes represent more than half of vertebrate species. Their brain shows an astonishing diversity in form and organization. For example, the telencephalon of ray-finned fishes undergoes

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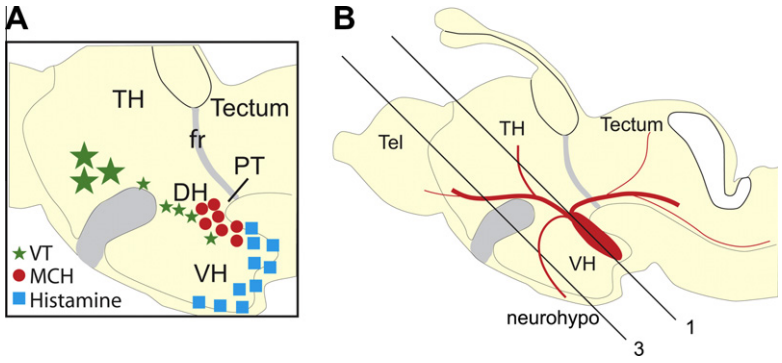

Fig. 2. Schematic distribution of MCH neurons in lampreys. (A) Line drawing of a sagittal section of a lamprey hypothalamus to illustrate the distribution of MCH perikarya (dots) compared to the distribution of vasotocin-containing cells (stars, large stars are for magnocellular neuroendocrine neurons) and histaminergic soma (squares). (B) Line drawing to illustrate the main pathways for MCH projections in the lamprey brain (sagittal section). MCH axons course rostrally to reach the ventral telencephalon, but do not extend in the pallium. Some run caudally in the midbrain and others reach the deep tectum. Dorsally and ventrally directed axons are found respectively in the thalamus or, in *Lampetra fluviatilis*, in the neurohypophysis. Oblique lines illustrate the plane of sections of Figs. 1 and 3 respectively. Abbreviations: DH: dorsal hypothalamus, fr: fasciculus retroflexus, neurohypo: neurohypophysis; PT: posterior tuberculum; Tel: telencephalon; TH: thalamus; VH: ventral hypothalamus.

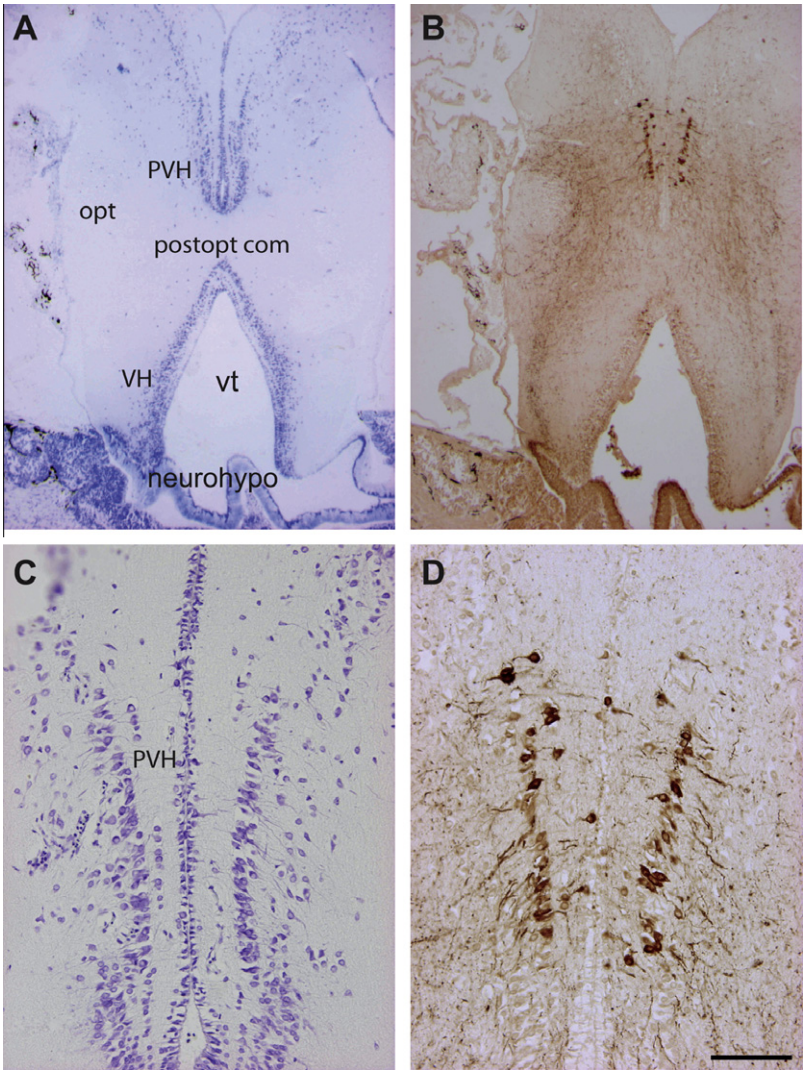

Fig. 3. Vasotocin in *Lampetra fluviatilis*. (A–D) Photomicrographs showing the distribution of neuroendocrine magnocellular neurons in the anterior hypothalamus labeled by an anti-vasotocine antiserum and revealed by the peroxidase anti-peroxidase method (B and D), and the adjacent toluidin blue stained sections (A and C). Note in (D) the large size of the vasotocin-labeled perikarya. In (B), the path of the labeled axons leaving laterally the PVH is clear. Axons abut the optic tract, and then course in the ventral hypothalamus toward the neurohypophysis. Abbreviations: neurohypo: neurohypophysis; opt: optic tract; PVH: paraventricular hypothalamic nucleus; Postopt com: post optic commissure; VH: ventral hypothalamus; vt: ventricle. Scale bar (*D*) = 500 μm in (A and B); = 40 μm in (C and D).

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pallial eversion (see Northcutt (2008) for a review about the forebrain evolution in bony fishes), while the telencephalon of most cartilaginous fishes is partly impar and bilobed (Butler and Hodos, 2005; Nieuwenhuys et al., 1998; Northcutt, 2008). The hypothalamus varies considerably among species, with cell masses that seem specific of fishes. It is beyond the scope of this work to describe in detail the anatomy of the fish hypothalamus; we refer again to the very useful work of Nieuwenhuys et al. (1998) for additional information and references.

2.2.1. Perikarya

The comparative anatomy of the MCH system in fishes is very complex. Several research groups have analyzed the distribution of MCH somata in many species of cartilaginous or bony fishes. These works reflected the great interest raised by the neuroendocrine melanin-concentrating action of the peptide in teleosteans (Baker, 1993). After a careful review of the comparative anatomy of the MCH system in fishes, Baker and Bird (2002) formulated the hypothesis that this system differentiates specific molecular/anatomical features and neuroendocrine functions along the fish evolutionary scale (Fig. 4A) (see as well Sherbrooke and Hadley (1988)). Briefly, in cartilaginous fishes, which are thought to be representative of primitive fishes, MCH cell bodies are found in a periventricular position in posterior and dorsal hypothalamic regions, very much like in lamprey. In holosteans and teleosteans, an additional group of cells is located away from the ventricular surface in the nucleus lateralis tuberis (NLT) (Fig. 4B). These cells are very large and project into the posterior neurohypophysis. In some of these species, this lateral neuroendocrine group represents 80% of the whole hypothalamic MCH neuron population (Baker and Bird, 2002). In zebrafish and other teleost fishes, MCH is encoded by two genes, one of which would be the structural and functional ortholog of the mammalian gene (Baker et al., 1995; Berman et al., 2009). Neurons that express these genes are largely co-distributed in the NLT and the dorsal hypothalamus.

Magnocellular MCH cells in the NLT cannot be mistaken for the rostral magnocellular vasotocinergic and isotocinergic cell bodies which are described in the preoptic region (Batten et al., 1990; Duarte et al., 2001; Saito et al., 2004). Using a dual immunohistochemical approach we found that MCH perikarya in the NLT lie within the path of the hypothalamo-neurohypophyseal tract labeled by a vasotocin antiserum (Fig. 4B and C). Histaminergic neurons are described in structures that are immediatly caudal to those containing the MCH cell bodies, and in particular in the nucleus of the posterior recess (Ekström et al., 1995; Inagaki et al., 1991). Finally, MCH perikarya in the NLT are not near dopaminergic neurons.

2.2.2. The case of the lungfish

Lungfishes belong to the lobe-finned fishes, and are considered the closest living relative of tetrapods. Only one work has been devoted to the study of MCH in the African lungfish (Vallarino et al., 1998). As in other species, MCH perikarya are abundant in the post-chiasmatic region of the hypothalamus. However, they do not project in the neurohypophysis and are not neuroendocrine. Perikarya are observed in periventricular position, but also in the peripheral layer of the ventral hypothalamus. This peripheral layer corresponds to a sheet of migrated cells in contact with the dorsal periventricular hypothalamus (Nieuwenhuys et al., 1998) and could have a dorsal origin. In this species, the subpallial telencephalon also contains additional groups of MCH cell bodies.

2.2.3. Fibers

A clear distinction can be made in the distribution of MCH projections, depending on the existence of the magnocellular neuroendocrine group in the NLT. Therefore, in cartilaginous and

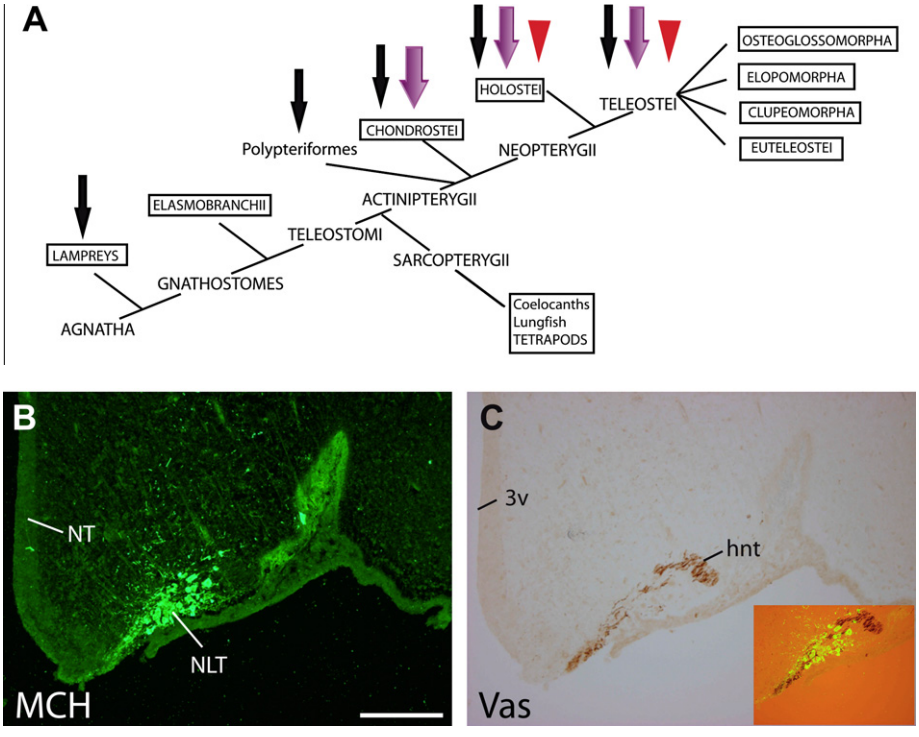

Fig. 4. MCH in fishes. (A) Functions of MCH on a fish evolutionary scale, adapted with permission from Baker and Bird (2002). Thin arrows: adenohypophysial regulation; large arrows: neurohypophyseal secretion; arrowheads: melanophore regulation. Baker and Bird have only proposed an adenohypophyseal function in lampreys, but even if not suggested here, neurohypophyseal secretion cannot be excluded, at least in *Lampetra fluviatilis*. (B and C) MCH neurons in the nucleus lateralis tuberis (NLT) in *Oncorhynchus mykiss* (trout). Photomicrographs of a double immunolabeling for MCH (immunofluorescence) and vasotocin (Vas) (peroxidase anti-peroxidase method) on the same transverse section of the hypothalamus. Both signals are superimposed in the inset in (C). Note that the NLT lies within the hypothalamo-neurohypophyseal tract. Abbreviations: hnt: hypothalamo-neurohypophyseal tract; NLT: nucleus lateralis tuberis; NT: nucleus tuberis; 3v: third ventricle. Scale bar (*B*) = 700 μm in (B and C).

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lobe-finned fishes, the overall MCH projection pattern resembles the one described in lampreys, with ascending axons in the ventral telencephalon, a dense network of fibers in the hypothalamus and descending projections in the reticular core, including deep tectal layers. Projections in the medial pallium are observed, and they are scarce in the spinal cord.

MCH projections in the anterior neurohypophysis (median eminence) are observed in all fishes but lungfishes, and MCH is thought to control hormonal pituitary secretions in cartilaginous and bony species. However, projections to the posterior neurohypophysis are only obvious in some chondrosteans, in holosteans and in teleosteans, with a proved role on the skin color only in holosteans and teleosteans. We strongly encourage readers to refer to the work of Baker and Bird (2002) that reviews these projections.

2.2.4. Conclusions

The basic feature of a dorsal periventricular hypothalamic cell group in the posterior hypothalamus is found in all fishes with a projection pattern similar to that in lampreys. However, in teleost fishes, which are believed to be last on the fish evolutionary line, a group of lateral magnocellular neuroendocrine neurons exists which is responsible for the melanin-concentrating effect of the peptide. Ontogenetic evidence has not convincingly shown that the cells in the NLT and those observed more dorsally have the same origin and are generated in the same germinal region (Amano et al., 2003; Mancera and Fernandez-Llebrez, 1995), but this hypothesis cannot be excluded (Pandolfi et al., 2003). A second MCH gene (*pmch2*) has been discovered and both genes are expressed in distinct neurons that are co-localized in the dorsal hypothalamus and the NLT. These neurons seem to be involved in different functions: *pmch1*-producing cells in skin color, and *pmch2*-containing neurons in feeding behavior (Berman et al., 2009). Therefore, the MCH story in fishes is far from being done, and it would be very interesting, among many other topics, to learn more on when a second gene (*pmch1*) arose in fishes, or if it was lost in tetrapods. Nevertheless, hypothalamic MCH neurons migrate away from the ventricular surface in fishes, and differentiate into magnocellular neuroendocrine cells.

2.3. Batrachians

MCH expression has been analyzed quite exclusively in anurans, including frogs, toads and Xenopus. To date, urodela have been largely ignored. Anurans undergo through a restructuring metamorphosis after an aquatic tadpole stage. During this process, the animal changes diet and behaviors: from a legless aquatic algae eater, it becomes a predatory terrestrial tetrapod that is able to reproduce.

As in lampreys, the hypothalamus is easily divided into a preoptic/anterior and tuberal/posterior region by the optic chiasm. Most of the cell bodies are periventricular in the preoptic/anterior hypothalamus where several nuclei are distinguished, such as the nucleus preopticus magnocellularis which contains the large neuroendocrine vasotocinergic neurons. The tuberal hypothalamus also contains several distinguishable periventricular nuclei. Some authors recognize a complex set of nuclei in the posterior hypothalamus including mammillary and supra-mammillary nuclei (Puelles et al., 1996). However, for practical reasons, we will mostly refer to the nomenclature of Nieuwenhuys et al. (1998) which recognize a dorsal periventricular nucleus (DH), a ventral periventricular nucleus (VH) and a paraventricular organ (PVO) (see as well Neary and Northcutt (1983)). The border between the dorsal and ventral nuclei is very clear on Nissl stained material (Fig. 5A, C and E); these nuclei show different cytoarchitectonic features with neurons forming a compact nucleus in the VH, while the DH is loosely organized. Within the DH, the PVO forms a clear cell condensation adjacent to the ependyma.

2.3.1. Perikarya

MCH cell bodies were mostly reported in the posterior (tuberal) dorsal hypothalamic region and a few more ventrally at the level of the infundibular recess. Francis and Baker (1995) and Lázár et al. (2002) described quite well their distribution and our own present observations agree with these previous reports (Figs. 5B, D, F and 6A). These neurons form a long and thin band of cells close to the ependyma and just dorsal to the border of the ventral nucleus. Cell bodies are not seen within the borders of the PVO. Furthermore, MCH cells are not observed in the lateral hypothalamus proper. In *Rana temporaria*, Francis and Baker (1995) described cells in more ventral caudal parts of the hypothalamus. However, this observation was not confirmed in *Rana esculenta* (Lázár et al., 2002), and we did not see them in *R. temporaria*. MCH neurons do not display any specific morphology. They are medium-sized and direct contact with the CSF is not obvious. In the vicinity of MCH cell bodies, some cells labeled for vasotocin are observed, like in lampreys, but they are clearly not part of the magnocellular neurosecretory cell group which is located rostrally (Fig. 6C). Lázár et al. (2002) observed MCH perikarya in the posterior tuberculum. In frog, this nucleus is related to the ventral thalamus according to some authors (Neary and Northcutt, 1983) or to the caudal-most hypothalamus following others (part of it is referred to as the retromammillary nucleus by Puelles et al. (1996)). In Anurans, the dopaminergic innervation of the striatum arise predominantly from the posterior tuberculum; a clear nigral or ventral tegmental region is not described in these species (Smeets and Gonzalez, 2000). Using an antiserum to tyrosine hydroxylase, we labeled these dopaminergic neurons that are located immediately caudal and dorsal to MCH neurons (Fig. 7A and B).

MCH perikarya have also been found in other cerebral sites, including a few cells during the reproductive period in the lateral septal nucleus and in the periventricular preoptic nucleus (*R. temporaria*) (Francis and Baker, 1995; Lázár et al., 2002).

2.3.2. Projections

The important new feature about the distribution of MCH axons in frogs as compared to that in fishes and lampreys, is the innervation of the spinal cord. In general no or few projections were reported in the spinal cord of aquatic vertebrates, while MCH projections are abundant in the ventral telencephalon and the hypothalamus. Conversely in anurans, MCH spinal projections are significant; these projections might be correlated to tetrapody and the fact that frogs spend most of their adult life outside the water. These projections are unlikely to play a direct role in locomotion since axons are mostly observed in the dorsal horn of the spinal cord. They could more probably play a role in the modulation of somatosensory information. Indeed, our own observation in *R. temporaria* (Fig. 7C) is favoring a modulatory role of sensory information in frogs, including olfactory, somatosensory and auditory information (for example the deep layers of the tectum), often linked with the expression of social behaviors. Finally, projections to the medial, dorsal and lateral pallium are observed, but they are sparse (Francis and Baker, 1995; Lázár et al., 2002).

2.3.3. Conclusions

As in lampreys and fishes, MCH neurons form a conspicuous cell group in the posterior hypothalamus that projects in many brain sites. These neurons appear to have lost a direct contact with the ventricular surface as they lie outside the paraventricular organ (PVO). They do not project in the posterior pituitary and are not neuroendocrine. In frogs, their projections significantly innervate the basal telencephalon, the deep tectum, the reticular formation and extend caudally to reach the spinal dorsal horn.

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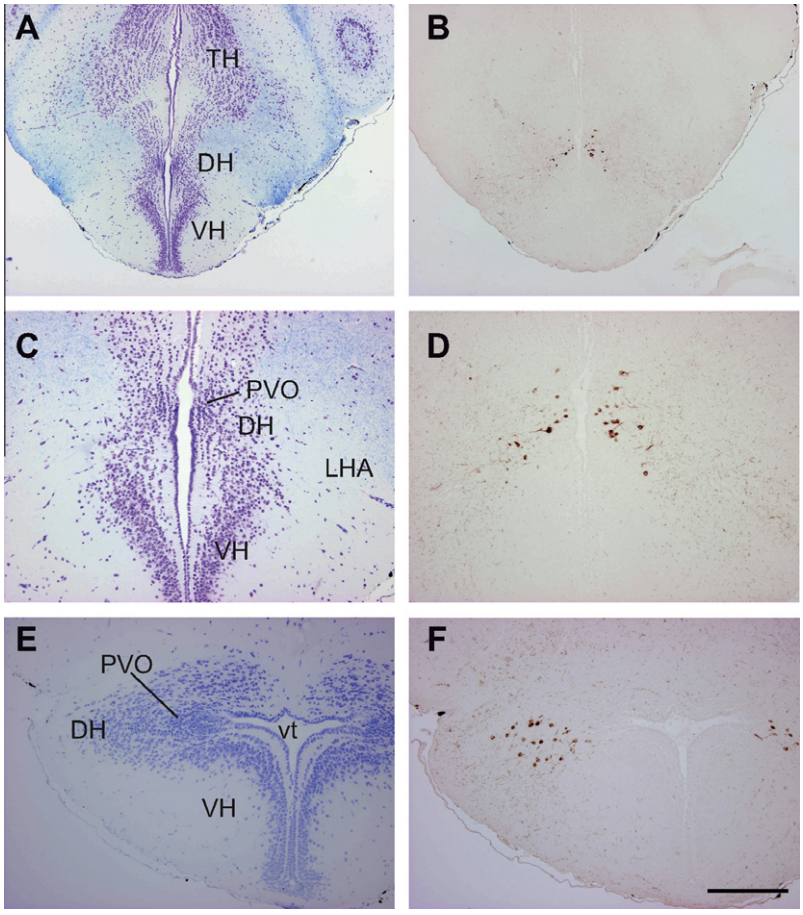

Fig. 5. MCH in *Rana temporaria;* transverse sections. (A–F) Distribution of MCH neurons in the dorsal hypothalamus at rostral (A–D) or caudal (E and F) levels. MCH is detected using the peroxidase anti-peroxidase method in (B, D and F). Adjacent sections were stained using the Kluver–Barrera method (A and C) or toluidin blue (E) for cytoarchitectonic purpose. Note that the dorsal hypothalamus is made of loosely organized neurons close to the PVO, while neurons are densely aggregated in the ventral hypothalamus. All MCH cellbodies are observed in the periventricular cell layer. None are seen further laterally in the LHA. Abbreviations: DH: dorsal hypothalamus; LHA: lateral hypothalamic area; PVO: paraventricular organ; TH: thalamus; VH: ventral hypothalamus; vt: ventricle (third). Scale bar (*F*) = 400 μm in (A and B); = 200 μm in (C–F).

2.4. Sauropsids

Sauropsids and mammals are amniotes, and, unlike amphibians, their full reproductive cycle occurs outside standing water. Birds, among other physiological properties, are homeothermous animals, resembling to mammals and differing from presently living reptiles with respect to this character.

Sauropsids have developed strong motor skills (terrestrial locomotion, flight), and their brain shows a marked increase of the tectum, cerebellum and forebrain as compared to amphibians.

The hypothalamus of reptiles or birds is very complex, made of dozens of nuclear masses, but rostral (anterior, preoptic) and caudal (postchiasmatic) regions are distinguished. In these species, a paraventricular organ with CSF-contacting cells is described in the tuberal periventricular region, like in anurans. A clear mediolateral differentiation exists, and the hypothalamus of reptiles shares many similarities with that of mammals. For example, a well-differentiated ventromedial hypothalamic nucleus exists (Fig. 8A) and the medial forebrain bundle (mfb) passes through a differentiated lateral hypothalamic area (LHA) that contains neuronal perikarya (Nieuwenhuys et al., 1998).

2.4.1. Perikarya

Only one study in reptiles (Cardot et al., 1994) and two in birds (Cardot et al., 1998, 1999) have been devoted to the distribution of the sauropsidian MCH system. In all sauropsids, MCH perikarya were seen only in the dorsal posterior hypothalamus (Fig. 8). In several species of reptiles, including turtles, lizards and snakes, MCH cell bodies are observed in the periventricular hypothalamus, ventral to the paraventricular organ and lateral to the hypothalamic sulcus. In *Podarcis muralis*, only periventricular MCH perikarya are found (Fig. 8). MCH neurons are described in the LHA of turtles (*Chrysemis scripta elegans*) and in snakes (*Netrix netrix*), although they are few in number (Cardot et al., 1994).

In two other works, Cardot et al. (1998, 1999) studied several species of birds (including coks – *Gallus domesticus*, hens – *Numida meleargis*, quails – *Corturnix coturnix*, gosling – *Anser domesticus*, ducks – *Cairina moschata*, and one coot – *Fulica atra*). In all these species, perikarya are observed mostly in the periventricular caudal hypothalamus, around the paraventricular organ, and a less abundant group is reported in the lateral hypothalamic nucleus, in an 'arc-shaped fashion' extending from the periventricular nucleus and in the infundibular tract.

2.4.2. Projections

In sauropsids, MCH axons course rostrally in the mfb and abundantly innervate the septal region (mostly the medial septal nucleus). These projections can be traced into the olfactory bulbs. In reptiles, scattered projections are found in all parts of the pallium, including the dorsal ventricular ridge. However, these projections are absent in birds and Cardot et al. (1999) did not observe MCH axons entering the pallium in any of the analyzed brains. In birds, the medial septal nucleus contained abundant pericellular nets (Cardot et al., 1999). Descending axons are seen in all parts

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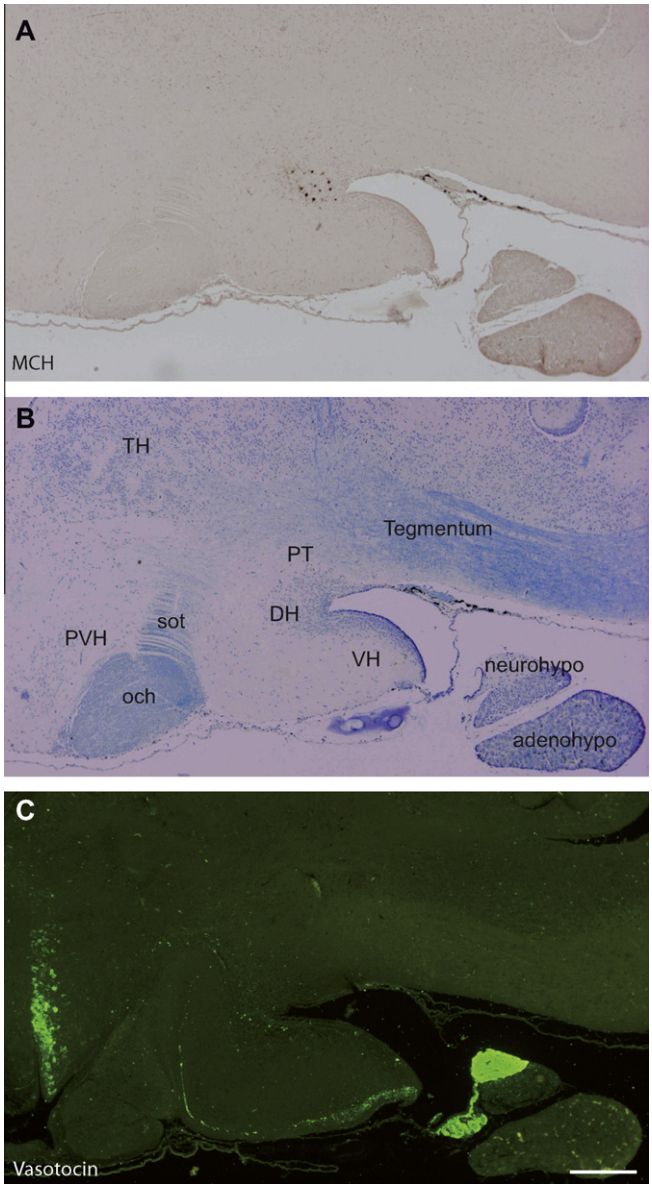

Fig. 6. MCH in *Rana temporaria;* parasagittal sections. (A) MCH neurons labeled using the peroxidase anti-peroxidase method in the dorsal hypothalamus. (B) Section adjacent to (A) and stained by the Kluver–Barrera method for cytoarchitectonic purpose. (C) Medially neighboring section labeled using immunofluorescence to reveal vasotocin. Abbreviations: Adenohypo: adenohypophysis; DH: dorsal hypothalamus; neurohypo: neurohypophysis; och: optic chiasm; PT: posterior tuberculum; PVH: paraventricular hypothalamus; sot: supraoptic commissure; TH: thalamus; VH: ventral hypothalamus. Scale bar (*C*) = 400 μm.

of the brainstem and innervate the spinal cord (dorsal horn). The optic lobes appeared particularly well innervated in reptiles.

2.4.3. Conclusions

Although the distribution of MCH perikarya and axons appears similar to that in other species, three features draw our attention in sauropsids. Firstly, in reptilian species, such as turtles and snakes, and in birds, MCH perikarya are noted in the LHA (even if they are few in number), while in other species, as *P. muralis*, they are only observed in the periventricular dorsal region. Secondly, the paucity of pallial innervations is also a striking feature, especially in birds in which Cardot et al. (1999) did not observed any MCH projections. Finally, no extrahypothalamic group of MCH neurons has been described.

3. Anatomy of the MCH system in mammals

The general cytoarchitecture of the mammalian hypothalamus is widely known, with three longitudinal zones (periventricular, medial and lateral) and four rostrocaudal regions (preoptic, anterior, tuberal and mammillary – see Swanson (1987) or Toni et al. (2004) for the human hypothalamus). However, a very attractive scheme of the morphofunctional organization of the hypothalamus has been proposed more recently in the rat, based on cytoarchitectonic, hodological and experimental evidences: several intrahypothalamic circuits initiating defensive, reproductive and feeding behaviors interconnect medial zone nuclei. These nuclei interact with a visceromotor pattern generator network (VMPGN) and with the hypothalamic lateral zone. Several periventricular nuclei as well as internuclear regions (i.e. the capsule of the ventromedial nucleus) form the VMPGN and coordinate neuroendocrine and vegetative responses, while the LHA is mostly involved in reward and motivation (Swanson, 2003; Thompson and Swanson, 2003), or sleep (Peyron et al., 2009; Sapin et al., 2010).

Within the mammalian hypothalamus, the anatomy of the MCH system has been mostly studied in the laboratory rat. For example, MCH projections have been reported in full extent only in rat. However, the description of the MCH neuron distribution has also been made in primates (including human), carnivores (dog, cat) and herbivores (sheep) (Krolemski et al., 2010; Tillet et al., 1996; Torterolo et al., 2006).

Usually, MCH neurons are mostly located in the lateral hypothalamic region, but detailed comparative analyses reveal that their distribution is much more complex, shows interspecific differences, and does not respect cytoarchitectonic borders.

3.1. Perikarya

3.1.1. Overall distribution in the rat caudal hypothalamus

Rostral-most MCH perikarya are found in the medial zona incerta, dorsal to the posterior paraventricular nucleus of the hypothalamus. Caudal to this level, cells are progressively observed more laterally in the zona incerta and ventrally in the LHA. In the rat, the distribution of these cell bodies was very well illustrated and carefully compared with that of the co-localized neurons producing the hypocretins/orexins (Hcrt) (Hahn, 2010; Swanson et al., 2005). A lateral hypothalamic parceling was proposed on the basis of their distribution patterns. Overall, MCH cell bodies are observed in the rat lateral hypothalamus in which they lie adjacent to the cerebral peduncle or settle in the perifornical regions. Many are in the rostromedial zona incerta, in and around the posterior part of the anterior hypothalamic nucleus, in the periventricular nucleus at the level of the dorsomedial nucleus, in the capsule of the ventromedial nucleus, and in the posterior part of the periventricular nucleus (Bittencourt, 2011; Bittencourt et al., 1992; Hahn, 2010; Swanson et al., 2005; Zamir et al., 1986).

Based on the observation of sagittal, horizontal or oblique sections, a further analysis of the distribution of MCH perikarya in the rat hypothalamus is proposed here. The goal of this approach is less to analyze in detail this distribution (as described above) but more to apprehend a general pattern within the hypothalamus as done for other vertebrates. Indeed, on horizontal sections, MCH perikarya distribute within a rostro-caudally restricted region, like Hcrt-producing cell bodies (Fig. 9). This region corresponds quite well to the dorsal tuberal hypothalamic region that is just caudal to the anterior hypothalamus. As in other vertebrates, the anterior hypothalamus contains neuroendocrine magnocellular cell bodies (synthesizing prepro-vasopressin and -oxytocin, and labeled by an anti-neurophysin antiserum in our illustrations) (Figs. 9 and 10D–F). Obviously, MCH neurons are distributed over the entire

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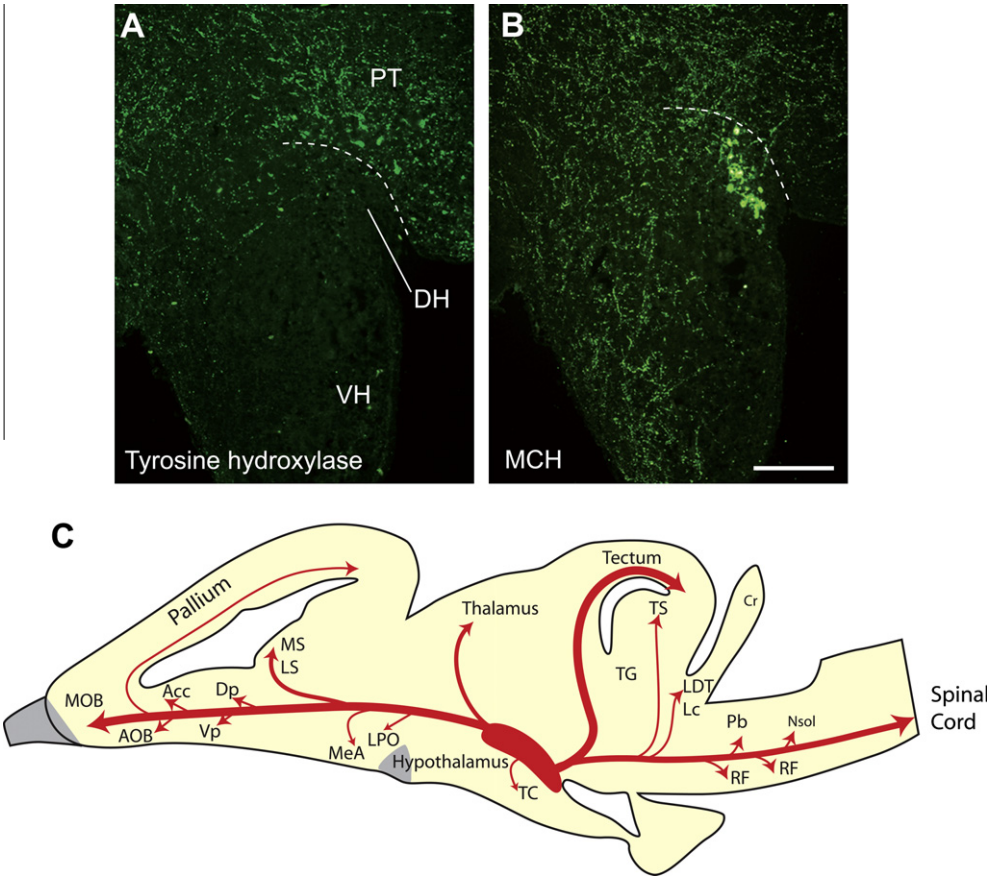

Fig. 7. MCH in *Rana temporaria:* sagittal plane. (A and B) Distribution of tyrosine hydroxylase and MCH in the posterior diencephalon; immunofluorescence on two adjacent frozen sections. Tyrosine hydroxylase-labeled neurons are abundant in the posterior tuberculum. MCH cell bodies are just rostral and ventral to these dopaminergic neurons (see text for details). (C) Diagram summarizing the projection pattern of MCH neurons of the dorsal hypothalamus in frog. MCH projections run from the dorsal hypothalamus in two main directions: (i) rostrally toward the basal telencephalon with some projections observed through the pallium; (ii) caudally to terminate in the deep tectal layers, the reticular formation and the spinal cord. Abbreviations: Acc: nucleus accumbens; AOB: accessory olfactory bulb; Cr: cerebellum; DH: dorsal hypothalamus; Dp: dorsal pallidum; Lc: locus coeruleus; LDT: laterodorsal nucleus of the tegmentum; LPO: lateral preoptic area; LS: lateral septal nucleus; MeA: medial nucleus of the amygdala; MOB: main olfactory bulb; MS: medial septal nucleus; Nsol: nucleus of the solitary tract; Pb: parabrachial nucleus; PT: posterior tuberculum; RF: reticular formations; TC: tuber cireneum; TG: tegmentum; TS: torus semicircularis; VH: ventral hypothalamus; Vp: ventral pallidum. Scale bar (*B*) = 150 μm.

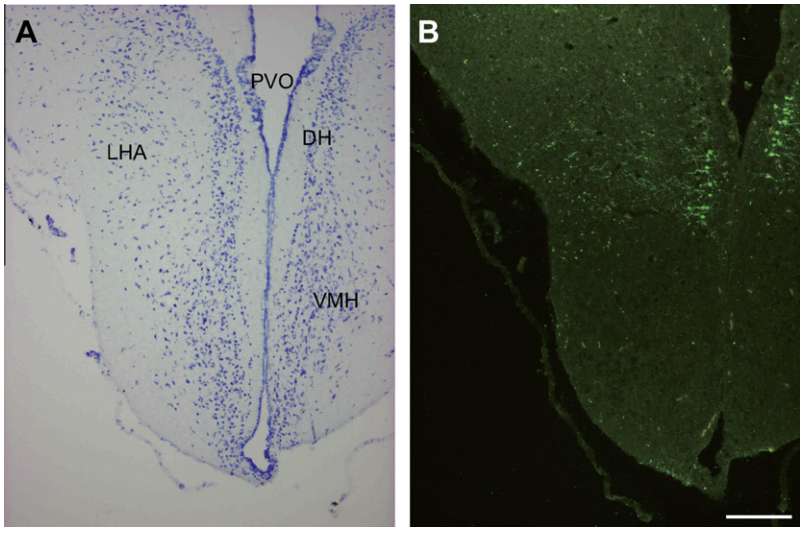

Fig. 8. MCH in *Podarcis muralis:* transverse sections. (A and B) Immunofluorescence for MCH on a frozen section through the posterior hypothalamus (B), and the adjacent section stained with toluidin blue for cytoarchitectonic purpose (A). MCH neurons are only observed in the periventricular cell layer in this species. MCH axons are followed laterally in the LHA. Note the large VMH. Abbreviations: DH: dorsal hypothalamus; LHA: lateral hypothalamic area; PVO: paraventricular organ; VMH: ventromedial hypothalamic nucleus. Scale bar (*B*) = 150 μm.

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mediolateral extent of the dorsal tuberal region, and not only in the lateral hypothalamus. On horizontal and sagittal sections, MCH cell bodies are caudal and mostly dorsal to the fornix, while magnocellular elements are rostral and ventral to this tract (Figs. 9 and 10D–F). The MCH pattern is particularly clear on oblique sections of the rat hypothalamus. These sections were cut 10 degrees to the horizontal plane which is broadly parallel to the path of the fornix in the hypothalamus (we named this plane 'parafornical') (Fig. 10A–C). Dorsomedial MCH perikarya in the medial zona incerta (ZI, around the dopaminergic cell group A13) and many caudoventral cell bodies in the LHA can be observed on the same section. The hypothalamic dorsomedial nucleus forms a cell mass mostly devoid of MCH cell bodies, while only few small sized MCH perikarya are observed caudal to it. MCH perikarya are observed in the caudal half of the hypothalamus and spread in a band of tissue extending from the third ventricle to the cerebral peduncle. In rats, MCH-containing region is clearly separated from the anterior/ventral hypothalamic regions by the fornix, while the mammillothalamic tract edges dorsally the MCH-containing area (Brischoux et al., 2001, 2002).

The rat MCH population is not homogeneous: several sub-populations exist. These sub-populations are characterized in part by the expression of the cocaine- and amphetamine-regulated transcript (CART) peptide and the NK3 receptor, but also by hodologic and developmental evidence. We have clearly established that the time of birth of these neurons determines their neurochemical nature as well as their projections in rat and mouse (Brischoux et al., 2002; Croizier et al., 2011; Cvetkovic et al., 2004).

MCH neurons contain other recognized or putative neurotransmitters/neuromodulators. It is now admitted that most if not all MCH neurons are GABAergic (Elias et al., 2001; Meister, 2007; Sapin et al., 2010). A variable proportion of MCH neurons also express other neuropeptides. Among them we can cite nesfatin, or substance P (Cvetkovic et al., 2003; Fort et al., 2008). Very few of them express calbindin or parvalbumin in adult animals while by contrast many if not all Hcrt neurons express one or both proteins (Cvetkovic et al., 2003). This may be related to the fact that Hcrt neurons are maintained in a depolarized state while MCH neurons are not in adult animals (Bayer et al., 2003; Cvetkovic-Lopes et al., 2010; Hassani et al., 2009).

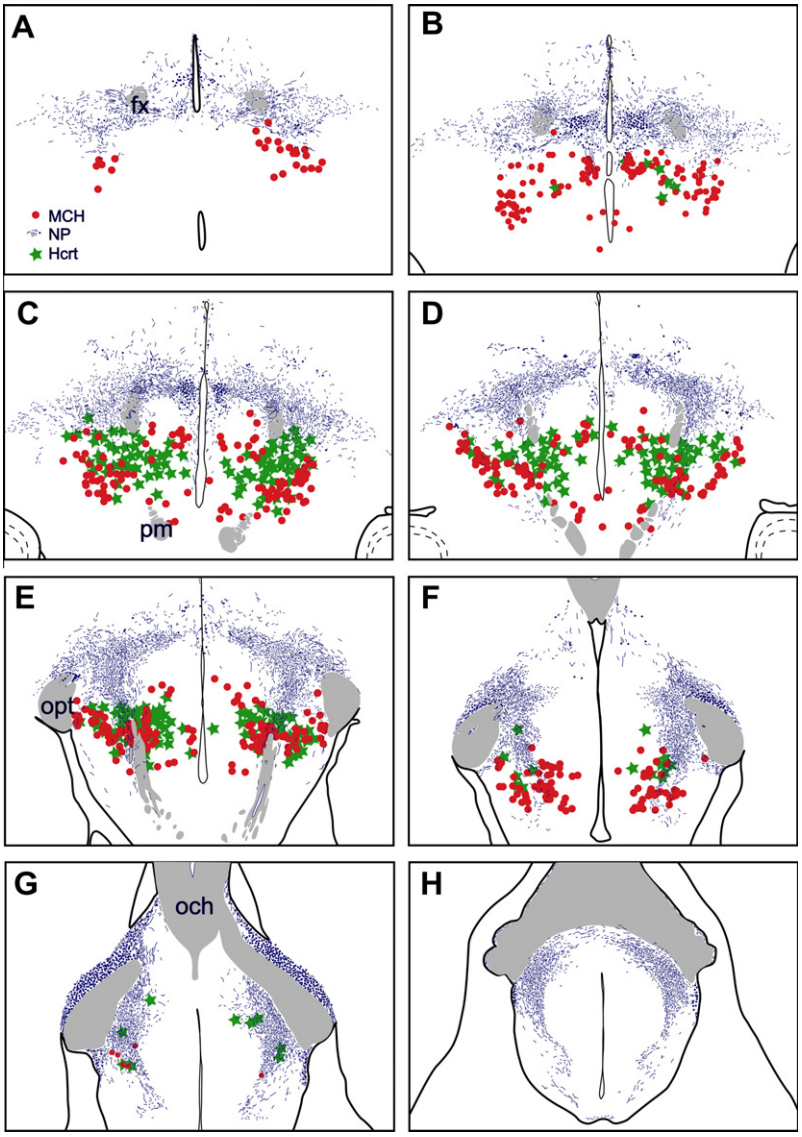

Fig. 9. MCH in *Rattus norvegicus*; horizontal plane. MCH (large dots), hypocretin (stars) perikarya and vasopressin/oxytocin-containing perikarya and axons (small dots and lines; detected by an anti-neurophysin 1 and 2) are plotted on line drawings of horizontal sections of the hypothalamus arranged from dorsal (A) to ventral (H). There is little overlap between the distribution patterns of MCH/Hcrt neurons and magnocellular neurons or projections. They clearly delineate tuberal and anterior hypothalamic compartments. Abbreviations: fx: fornix; NP: neurophysin; och: optic chiasm; opt: optic tract; pm: principal mammillary tract.

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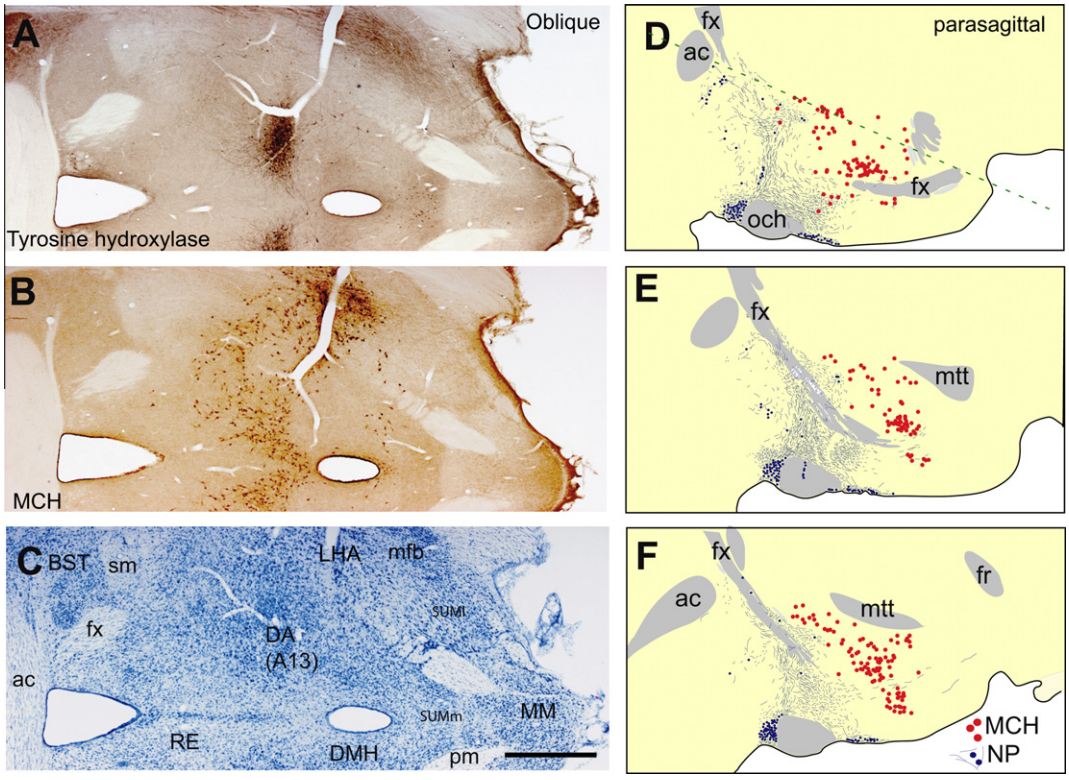

Fig. 10. MCH in *Rattus norvegicus*: oblique and parasagittal planes. (A–C) Adjacent frozen sections passing through the hypothalamus and cut in the parafornical (oblique) plane of section (the angle is shown in (D) by the dashed line). Figures (A) and (B) illustrate immunohistochemical stainings using the peroxidase anti-peroxidase method with antibodies to tyrosine hydroxylase (A) or MCH (B). The section in (C) is stained with toluidine blue for cytoarchitectonic purpose. Note the distribution of MCH perikarya in the dorsomedial hypothalamus/zona incerta, around the dopaminergic cell group (A13) and in the LHA that also contain tyrosine hydroxylase-labeled axons. (D–F) MCH perikarya (large dots) and vasopressin/ocytocin-containing neurons and axons (small dots and lines; detected by an anti-neurophysin 1 and 2) are plotted on line drawings of parasagittal sections of the hypothalamus arranged from medial (D) to lateral (F). Note the distribution of magnocellular elements rostral and ventral to the fornix, while MCH cell bodies are mostly dorsal to the fornix and rostral/ventral to the mtt. Abbreviations: ac: anterior commissure; BST: bed nucleus of the stria terminalis; DA: dopaminergic cell group of the zona incerta (A13); DMH: dorsomedial nucleus of the hypothalamus; fr: fasciculus retroflexus; fx: fornix; LHA: lateral hypothalamic area; mfb: medial forebrain bundle; MM: mammillary bodies; mtt: mammillothalamic tract; NP: neurophysin; och: optic chiasm; pm: principal mammillary tract; RE: reuniens nucleus of the thalamus; sm: stria medularis; SUMl, m: supramammillary nucleus, lateral or medial parts. Scale bar (*C*) = 1 mm in (A–C).

3.1.2. MCH neuron distribution in the caudal hypothalamus of other mammalian species

Very few studies have analyzed with enough precision the distribution of MCH perikarya in other species than in the rat. Nevertheless, some examples exist in the literature that can be used to clearly illustrate the diversity in MCH distribution among mammals.

In mouse, distribution of MCH cell bodies is similar to that observed in rat, with a high number of neurons in the dorso-lateral hypothalamus (Fig. 11). However, a comparative study showed the absence of some periventricular MCH perikarya in mice as compared to rat (i.e. in the posterior periventricular nucleus – L.W. Swanson's nomenclature in Swanson (1998)) (Croizier et al., 2010). In mouse, CART is also expressed in MCH neurons, but only in 45% of them, compared to 66% in rat (Croizier et al., 2010).

At first sight in sheep (Tillet et al., 1996), MCH perikarya exhibit a distribution similar to the rat pattern (Fig. 12). A more careful examination shows that neurons are however located very ventrally in the LHA; rostral-most perikarya are observed close to the supraoptic nucleus over the optic chiasm, while they are close to the hypothalamic paraventricular nucleus in rat. At more caudal levels, MCH cell bodies are abundant at the angle of the cerebral peduncle and the optic tract. Finally, caudal-most neurons are plotted in the supramammillary region and even in the ventral tegmental area, while MCH neurons are absent from these structures in the rat or mouse. Neurons may also react differently in sheep than in rat with regard to nutritional challenges (Chaillou et al., 2003).

In cat (Torterolo et al., 2006), the distribution of MCH neurons was compared to that of Hcrt cell bodies (Fig. 13). The majority of MCH perikarya are located in the perifornical regions, with Hcrt cells being slightly more dorsal and medial. In this species, MCH neurons are not abundant in regions close to the cerebral peduncle.

Finally, in the human hypothalamus, the distribution of MCH perikarya is very nicely illustrated by Krolemski et al. (2010). These neurons are observed mostly in the posterior hypothalamic nucleus dorsal to the mammillary body, and in perifornical regions of the LHA (Bresson et al., 1989; Krolemski et al., 2010; Takahashi et al., 1995) (Fig. 14). However, a comparison with Hcrt neurons shows that unlike in rat, MCH cell bodies are more medial than Hcrt neurons (Krolemski et al., 2010). As in other species, these cells are caudal to the magnocellular neurosecretory neurons (Fig. 14).

Therefore, summarizing these observations on a sketch portrait of the caudal hypothalamus (using fiber tracts and the ventricular surface as reference points) reveals important interspecific differences in the MCH distribution pattern among mammals (Fig. 15).

3.2. Projections

MCH projections have been studied with some details only in rat, but by and large it is generally assumed that a diffuse distribution is common in mammals.

In the rat, our group observed that most spinal projections originate from MCH neurons that do not express CART/NK3 (Cvetkovic et al., 2004). These projections form a clear tract in the caudal dor-

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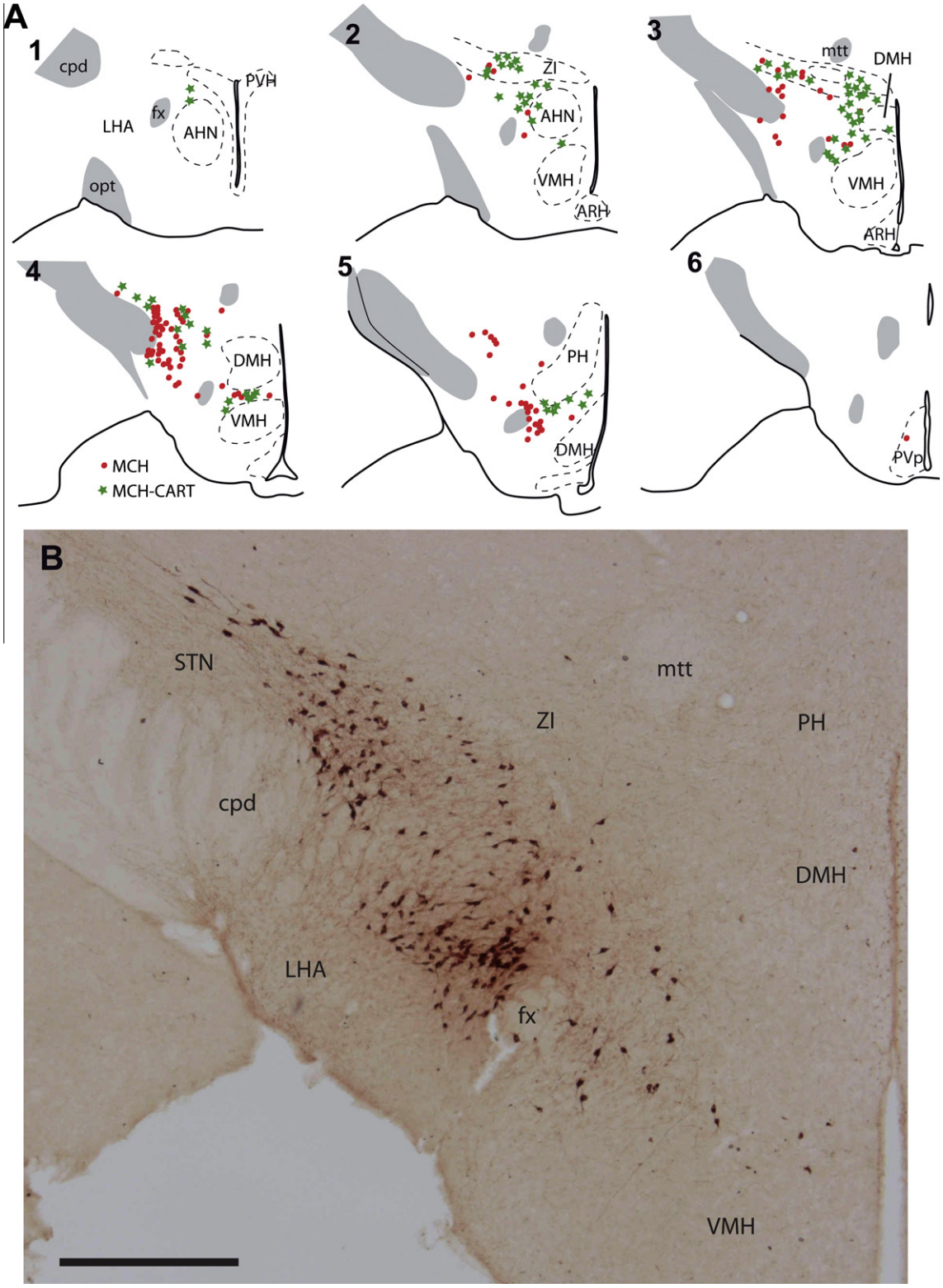

Fig. 11. MCH in *Mus musculus*; transverse plane. (A) Distribution of MCH/CART (stars) and MCH-only (dots) neurons in the dorsal hypothalamus plotted on a series of line drawings made from transverse sections passing through the posterior hypothalamus and arranged from rostral (1) to caudal (6). Reproduced from Croizier et al. (2010). (B) Photomicrograph to illustrate the distribution of MCH neurons. Neurons were labeled using the peroxidase anti-peroxidase method. Note the dense condensations of cell bodies in the dorsal lateral hypothalamus and perifornical region. The ventral LHA as well as the posterior hypothalamic nucleus are devoid of MCH perikarya. Some are in the dorsal shell of the ventromedial hypothalamic nucleus. Abbreviations: AHN: anterior hypothalamic nucleus; ARH: arcuate nucleus of the hypothalamus; cpd: cerebral peduncle; DMH: dorsomedial nucleus of the hypothalamus; fx: fornix; LHA: lateral hypothalamic area; mtt: mammillothalamic tract; opt: optic tract; PVH: paraventricular nucleus of the hypothalamus; PH: posterior hypothalamic nucleus: PVp: posterior periventricular nucleus of the hypothalamus; STN: subthalamic nucleus; VMH: ventromedial nucleus of the hypothalamus; ZI: zona incerta. Scale bar (*B*) = 400 μm.

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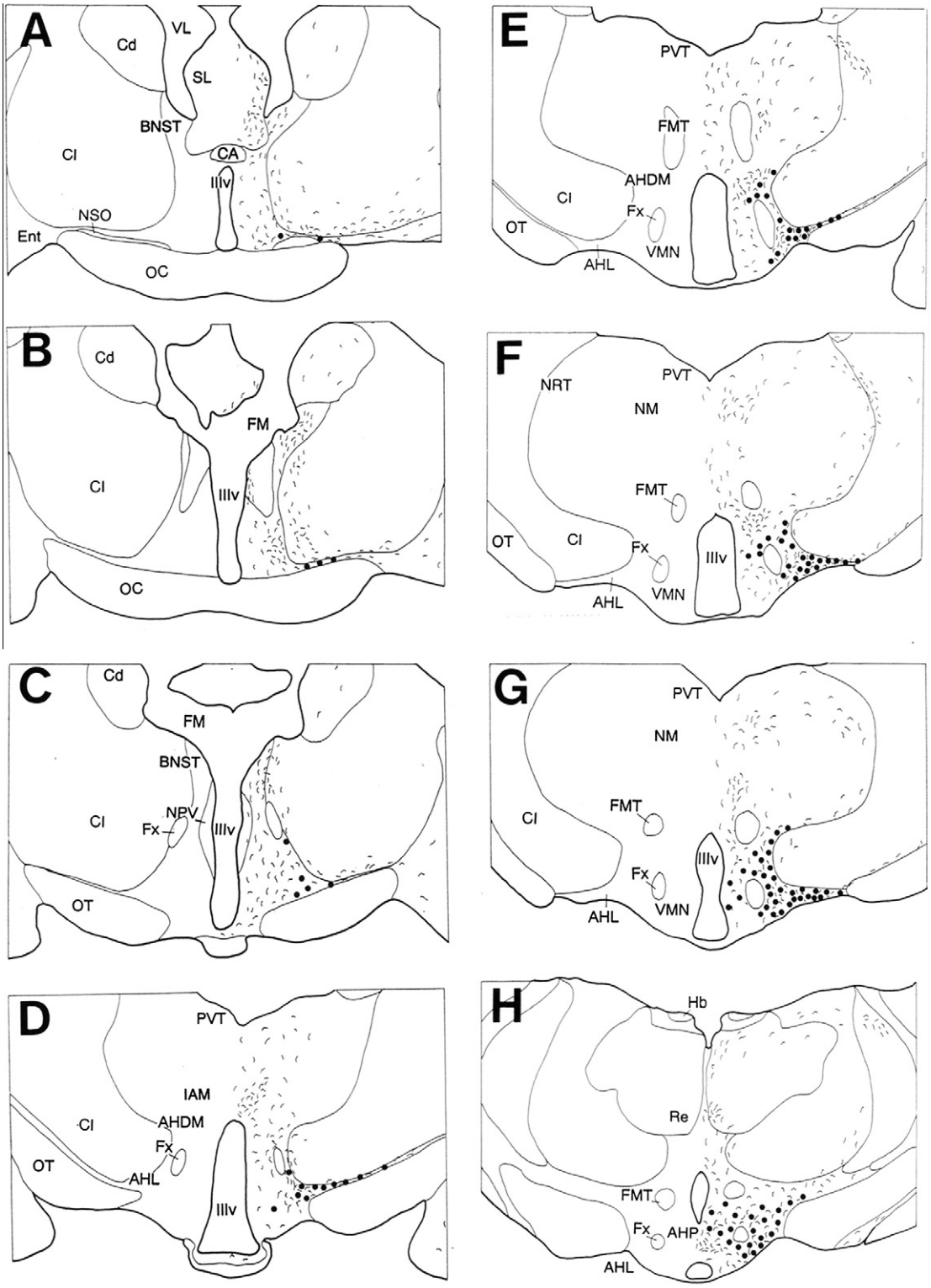

Fig. 12. MCH in *Ovis aries* (sheep): transverse plane. Distribution of MCH perikarya in the sheep hypothalamus. Reproduced with permission from Tillet et al. (1996). Note the very abundant MCH neurons in the LHA, especially in its ventrolateral component. Abbreviations: AHDM: area hypothalamica dorsomedialis; AHL: area hypothalamica lateralis; AHP: area hypothalamica posterioris; ATV: area tegmentalis ventralis; BNST: bed nucleus of the stria terminalis; CA: commissural anterioris; Cd: caudate nucleus; CI: capsula interna; CMm: medial mammillary nucleus; CP: commissura posterioris; Ent: nucleus entopeduncularis; Fx: fornix; FM: foramen of Monro; FMT: fasciculus mamillothalamicus; Hb: habenular nucleus; IAM: nucleus interaneromedialis; IIIV: third ventricle; NM: nucleus medialis (thalamus); NPV: nucleus paraventricularis hypothalami; NRT: nucleus reticularis thalami; NSO: nucleus supraopticus; OC: optic chiasma; OT: optic tract; PedCer: cerebral peduncle; PVT: nucleus paraventricularis thalami; Re: nucleus reuniens; SL: septum laterale; VL: ventriculus lateralis; VMN nucleus ventromedialis hypothalami.

so-lateral hypothalamus, adjacent to the subthalamic nucleus (Figs. 16 and 17B). This tract reaches the mesencephalon through the posterior ZI, just lateral to the medial lemniscus. Some axons then arch dorsally to reach the tectum, or take a ventral route to-

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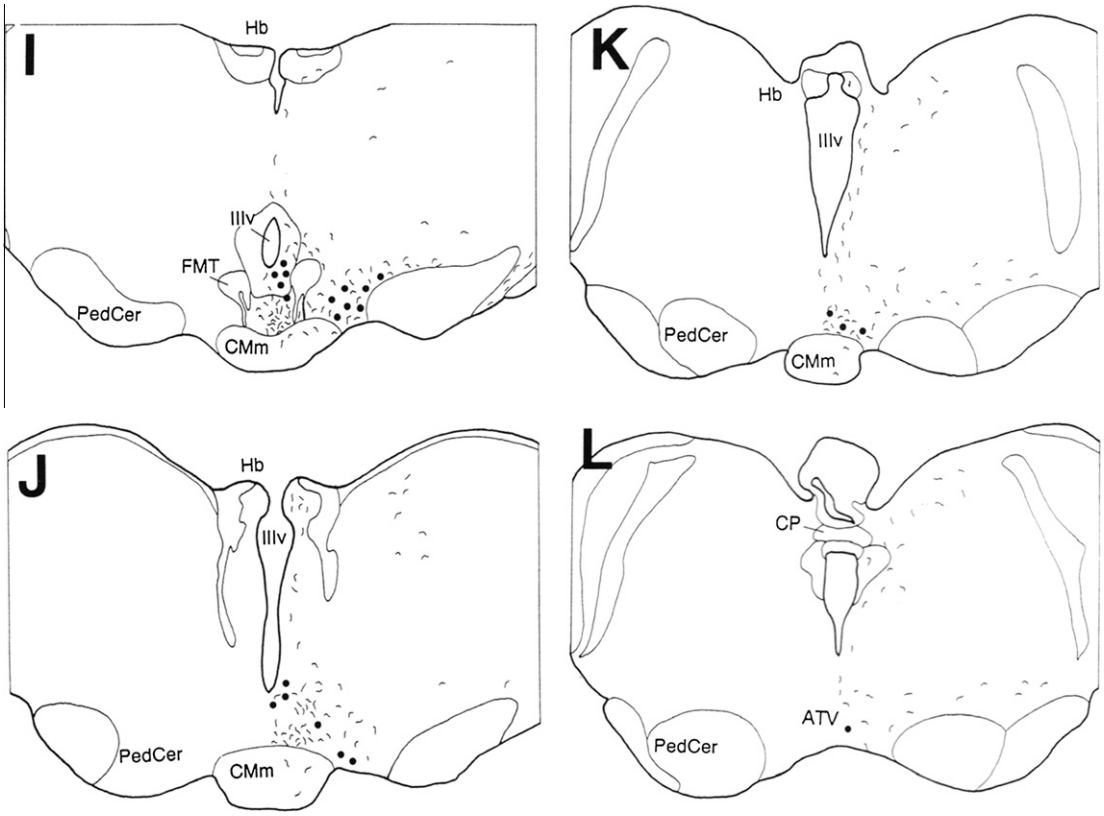

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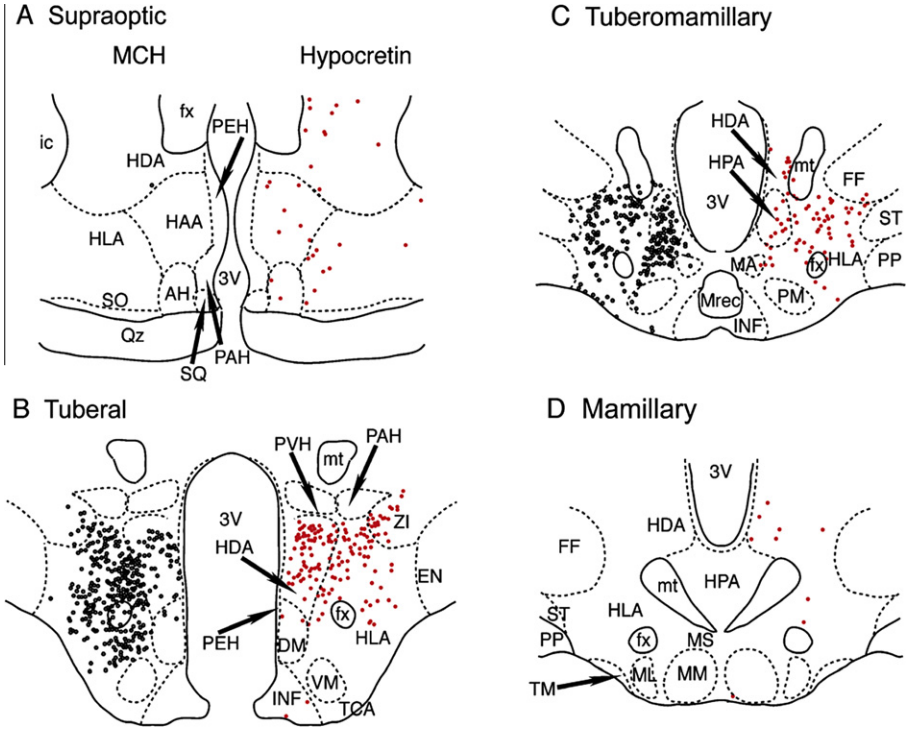

Fig. 13. MCH in *Felis silvestris catus* (cat): transverse plane. Distribution of MCH (left side) and Hcrt (right side) perikarya in the cat hypothalamus. Reproduced with permission from Torterolo et al. (2006). MCH cell bodies are particularly abundant in the perifornical region, but still mostly laterally to Hcrt producing cells. Abbreviations: AH: anterior hypothalamus; DM: dorsomedial hypothalamus; EN: entopeduncular nucleus; fx: fornix; FF: fields of Forel; HAA: anterior hypothalamic area; HDA: dorsal hypothalamic area; HLA: lateral hypothalamic area; HPA: posterior hypothalamic area; ic: internal capsule; INF: infundibular nucleus; MA: anterior mammillary nucleus; MM: medial mammillary nucleus; ML: lateral mammillary nucleus; M. Rec: mammillary recess of the third ventricle; MS: supramammillary nucleus; mt: mammillothalamic tract; PAH: paraventricular nucleus; PEH: periventricular complex; PM: premammillary nucleus; PP: pes pedunculi; PVH: parvocellular nucleus; Qz: optic chiasm; SQ: suprachiasmatic nucleus; SO: supraoptic nucleus; ST: subthalamic nucleus; TCA area of the tuber cinereum; TM: tuberomammillary nucleus; VM: ventromedial nucleus; ZI: zona incerta; 3 V: third ventricle.

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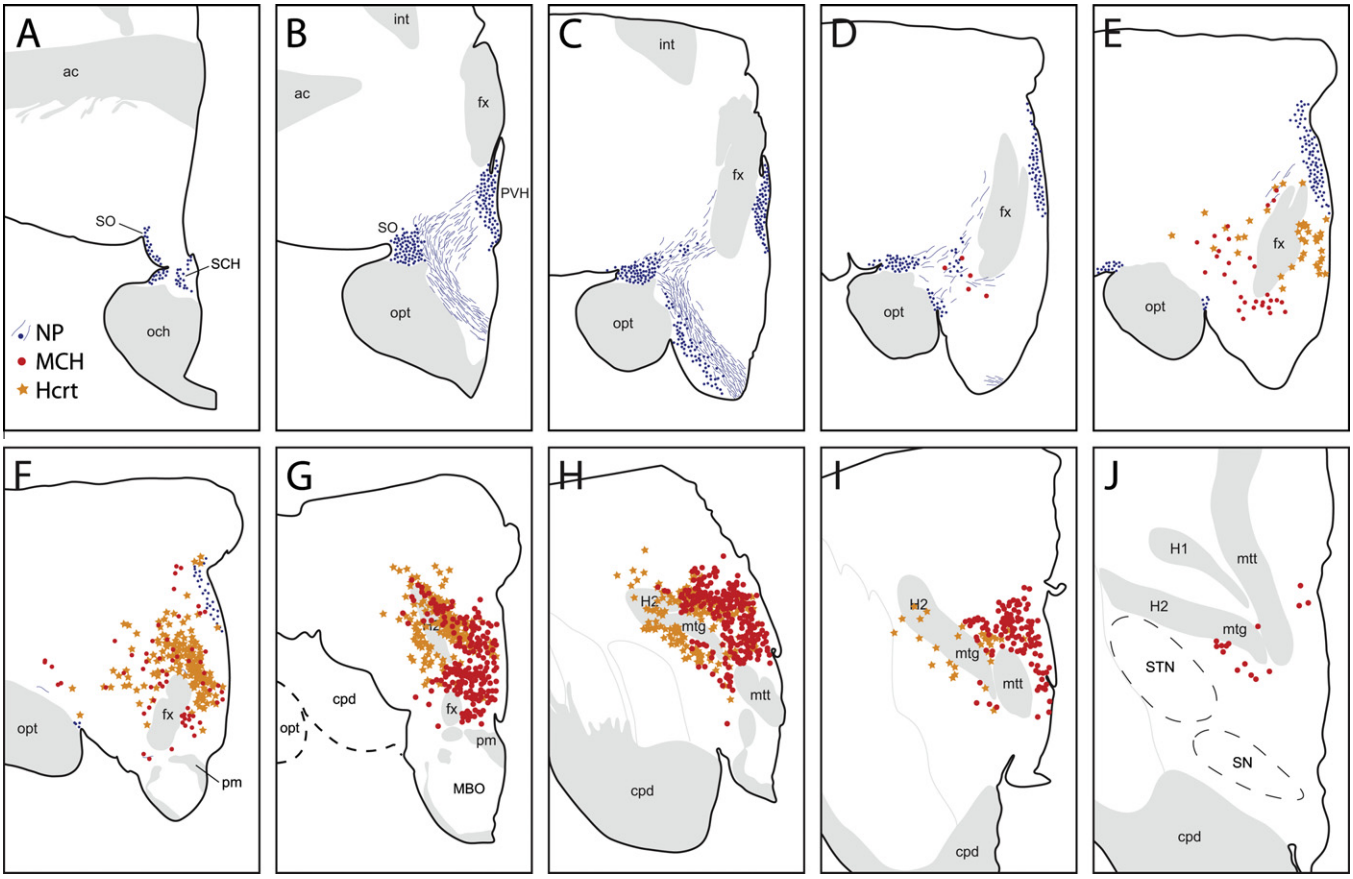

Fig. 14. MCH in *Homo sapiens*: transverse plane. Series of line drawings of coronal sections passing through the hypothalamus, organized from rostral (A) to caudal (J), and illustrating the distribution of magnocellular neurons and fibers (small dots and lines; detected by an anti-neurophysin 1 and 2), of MCH (large dots) and Hcrt (stars) perikarya. Note that MCH cell bodies are massively detected in the cytoarchitectonic posterior hypothalamic nucleus, medial to the largest condensation of Hcrt neurons and adjacent to the third ventricle. Abbreviations: ac: anterior commissure; cpd: cerebral peduncle; fx: fornix; H1: Thalamic fasciculus; H2: Lenticular fasciculus; int: internal capsule; MBO: mammillary body; mtg: mammillotegmental tract; mtt: mammillothalamic tract; och: optic chiasm; opt: optic tract; pm: principal mammillary tract; PVH: paraventricular hypothalamic nucleus; SCH: suprachiasmatic nucleus; SN: substantia nigra; SO: supraoptic nucleus; STN: subthalamic nucleus.

ward the spinal cord. Caudally, their diameter becomes very thin and they are difficult to follow until they reach the spinal cord. Nevertheless, the initial tract from the dorsal hypothalamus is very clear and can be best illustrated on parasagittal sections (Figs. 16 and 17B).

MCH/CART axons also run caudally, but they take a more medial and dorsal pathway in the tegmentum. Cvetkovic et al. (2004) showed that these descending MCH/CART projections end massively in the dorsal part of the paragigantocellular nucleus, and very few are found caudal to this nucleus. In the rat, MCH/CART axons form the largest contingent of the ascending MCH outputs. A large proportion of these axons follows the mfb to reach the septal region and enter the cortical mantle in the frontal lobes, while some others take the fimbria to reach the hippocampus. Observations of horizontal or oblique parafornical sections allow to see that many axons take a more lateral route (Fig. 16). They join the cerebral peduncle directly from the lateral hypothalamus and continue through ventral component of the internal capsule to reach lateral cortical fields (Figs. 16 and 17A). These axons provide inputs to the globus pallidus and the claustrum, while those taking a medial route innervate the medial septal/diagonal band nuclei.

MCH projections are therefore diffusely observed throughout the brain, but they are not homogeneously distributed: for example, ascending MCH axons are seen through the rat cortical mantle (with a higher density in olfactory, prefrontal, cingular, insular, entorhinal and occipital fields). The dorsal striatum is weakly innervated but projections in the ventral striatum are clear (accumbens nucleus, fundus striatum) and a dense input to pallidal regions is also noted. Little information exists concerning the specific targets of MCH projections.

A short analysis of MCH projections in rat and mouse recently pointed out interspecific differences: the proportion of MCH/CART vs. MCH-only inputs to the cortical mantle diverges, as well as the pattern of projections within the pallidum, and in the arcuate nucleus (Croizier et al., 2010). We shall not pursue beyond this very short description of MCH pathways and organization, but additional and detailed comparative studies will become necessary in the near future because interspecific differences in responses of MCH neurons to experimental conditions are beginning to emerge in the literature (Chung et al., 2011).

4. Phylogenesis and development

The anatomy of the MCH system is very diverse in the caudal hypothalamus of vertebrates. Neurons can be periventricular or lateral, large or small, densely packed or scattered over large areas, exhibiting diffuse or dense and specific projection patterns. This diversity clearly contrasts with the stability observed for neuroendocrine magnocellular systems in the anterior hypothalamus. A supraoptic nucleus differentiates in amniotes, and the peptidergic nature of magnocellular neurons evolves with, for example, differentiation of vasopressin and ocytocin in mammals. Nevertheless, the anatomy of the magnocellular systems is preserved, and in

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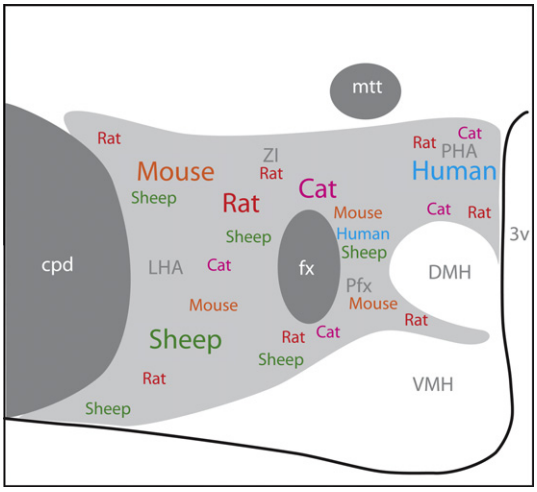

Fig. 15. Relative distributions of rat, mouse, sheep, cat and human MCH neurons: The overall distribution area (in light grey) of MCH neurons is schematized on a sketch portrait of the hypothalamus. To realize this sketch portrait, all major landmarks (fiber tracts such as cerebral peduncle, fornix, mammillothalamic tract, and the third ventricle) are figured, as well as the relative place of major cytoarchitectonic structures. In mammals, MCH cell bodies are observed in the LHA, including the perifornical region, the rostromedial zona incerta, the posterior hypothalamic nucleus, and the capsule of the ventromedial nucleus. They mostly avoid the dorsomedial and ventromedial nuclei. However, in human MCH neurons are mostly found in the posterior hypothalamic nucleus but avoid regions close to the cerebral peduncle; in cat they are mostly in the perifornical region; in mouse they are abundant in the latero-dorsal LHA, but absent in the posterior hypothalamic nucleus; in sheep they are observed in the whole LHA, but especially in its ventro-lateral component; finally in the rat, they are very abundant in the dorsolateral LHA, and show the widest distribution among studied mammals. Abbreviations: cpd: cerebral peduncle; DMH: dorsomedial nucleus fx: fornix; mtt: mammillothalamic tract; LHA: lateral hypothalamic area; PH: posterior hypothalamic nucleus; Pfx: perifornical region of the LHA; VMH: ventromedial nucleus; ZI: zona incerta 3v: third ventricle.

all species, neurons are large, densely packed, and project with a strikingly similar pattern in the neurohypophysis. The functions of magnocellular neurons are vitally important for individuals and species, and the conserved anatomy of magnocellular systems is concordant with the fact that neurosecretion through projections in the neurohypophysis is a very efficient means by which these functions are ensured.

By contrast, the MCH system is very plastic. The conserved structure of the MCH peptide pleads in favor of important roles, but it is involved in distinct functions and processes, some of which are clade specific (for example its action on skin color in fishes). Differences observed in the anatomy of caudal hypothalamic MCH neurons also suggest that the means by which they act can be species specific. Distinct functions and means of action are, in essence, adapted to the expression of specific responses, corresponding to specific behavioral repertories. As behavioral expression involves the whole forebrain, these differences might therefore reflect differences in the forebrain development and evolution among vertebrates.

4.1. MCH expression in the posterior diencephalon is a conserved trend

The presence of MCH neurons in the posterior hypothalamus, caudal to the optic chiasm, is a common feature observed in all vertebrates. No exception has been reported from cyclostomes to mammals. Furthermore, in some clades as teleosteans or sauropsids, MCH is not observed in other cell populations, suggesting that the posterior hypothalamus corresponds to the ancestral source of MCH (Baker, 1991; Baker and Kawauchi, 1997; Griffond and Baker, 2002).

This conserved pattern of distribution probably results from conserved developmental mechanisms. Recently it was shown in rat and mouse embryos that the secreted protein sonic hedgehog (SHH) plays an essential role in the differentiation of the MCH phenotype (Croizier et al., 2011; Szabó et al., 2009). This protein is a key factor for early CNS morphogenesis and patterning (Chiang et al., 1996; Roelink et al., 1995; Rohr et al., 2001). It is expressed in the basal plate through the caudal neural tube and rostrally in a strip of tissue passing through the caudal hypothalamus and reaching the optic chiasm. This early expression pattern of SHH is illustrated in Fig. 18A. As the embryo develops, SHH expression extends rostrally in the preoptic region and in the zona limitans intrathalamica (zli), dorsal to the posterior hypothalamus (Diez-Roux et al., 2011; Marin et al., 2002; Puelles and Rubenstein, 2003; Roelink et al., 1995; Shimogori et al., 2010) (Fig. 18B). In the embryo, MCH neurons appear along the SHH band of tissue ventral (rostral) to the zli, in a territory characterized by the expression of other SHH dependent genes such as *Nkx* family members *Nkx2.1* and *Nkx2.2*, as well as *Lim* homeodomain transcription factors *Lhx9* and *Lhx6* (Croizier et al., 2011; Shimogori et al., 2010). For a more complete analysis of functions involving these proteins, see Shimogori et al. (2010) and Diez-Roux et al. (2011). Broadly, *Nkx* genes may engage progenitors into specific differentiation paths, and *Lhx* genes could play a role in the differentiation of specific phenotypes. Interestingly, in the mouse posterior hypothalamic tissue, *Lhx9* is specifically expressed in Hcrt neurons and Lhx6 has been reported in MCH neurons (Croizier et al., 2011; Shimogori et al., 2010). The expression domain of these proteins is well conserved through the vertebrate kingdom (for example Medina et al., 2005; Rubenstein et al., 1994). The caudal hypothalamus as an ancestral source of MCH would then correspond to the region genetically programmed in all vertebrate embryos to produce these neurons and Hcrt neurons which are co-localized in mammals (as well as in fish – Huesa et al. (2005)). The plasticity observed in MCH neuron distribution through the vertebrate phylogeny might also in part be related to SHH expression in the embryo. Literature data strongly suggest that SHH could be a powerful motor for forebrain evolution through its expression in signaling centers. Telencephalic ventral midline and zli are such centers expressing SHH (Marin et al., 2002; Vieira and Martinez, 2006). The role of this protein in hypothalamic evolution is well illustrated in a model of fish, *Astyanax mexicanus*, in which cave-living and surface-dwelling forms have been described. Menuet et al. (2007) have shown an enlarged expression of SHH in the cavefish-living compared to the surface-dwelling forms. This increase in forebrain SHH expression was correlated to a larger hypothalamus and ventral forebrain with expanded NKX2.1 and LHX6 expression domains. Among the phenotypic differences between the two fishes, authors also signal the loss of eyes, loss of pigmentation, increased body fat, increased aggressive behaviors and a developed feeding apparatus in the cavefish.

4.2. Mediolateral differentiation of the MCH system

MCH neurons are associated with the LHA in mammals. This structure is made of cells radially migrating away from the ventricular surface, especially from the dorsal hypothalamic periventricular germinal layer (Altman and Bayer, 1986). The supraoptic nucleus is apparently formed by a similar migratory stream (Fig. 19A). In lampreys or anurans, all magnocellular neurons are periventricular. In mammals the SON is made following an active migration of magnocellular cells, relying on guidance molecules (Xu and Fan, 2008). A similar process could be involved in the migration of MCH neurons (Fig. 19B). However, MCH neurons settle following a lateral to medial (outside-in) gradient. First neurons differentiate in the lateral mantle layer, while later generated cells

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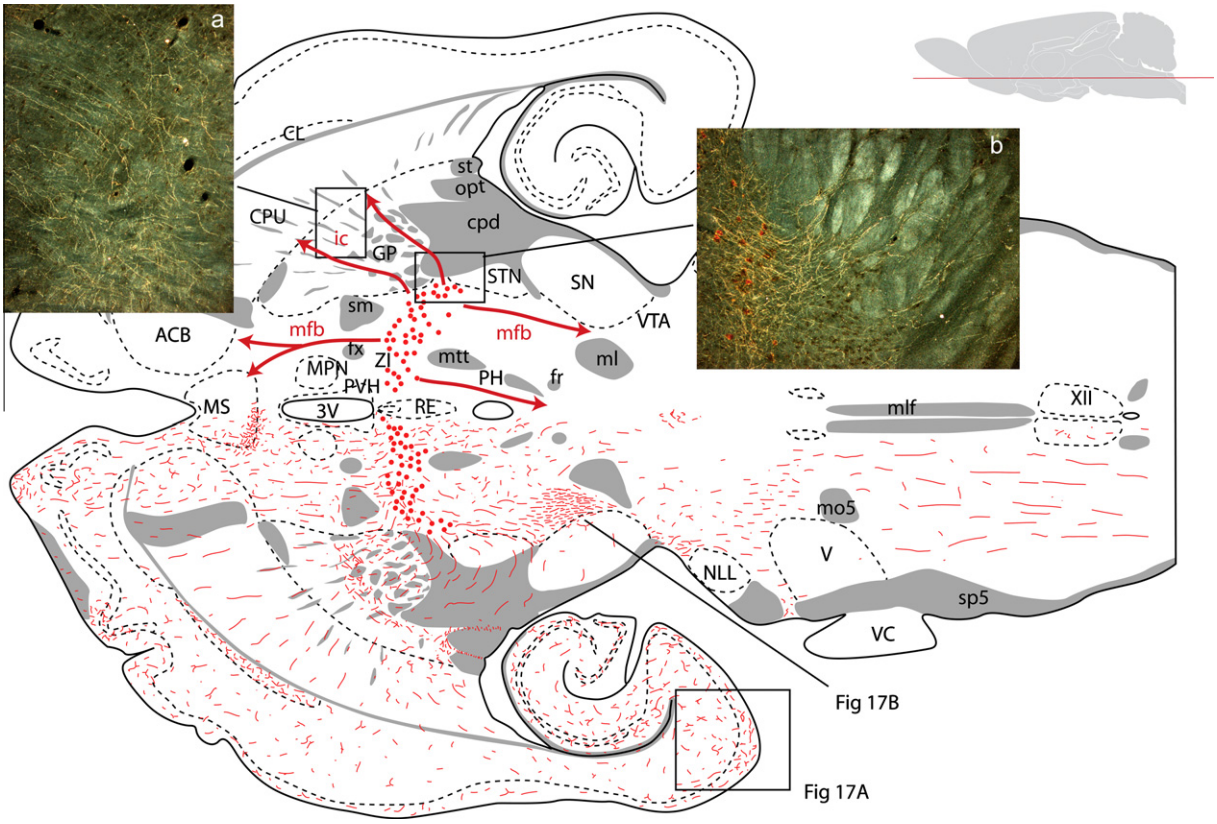

Fig. 16. MCH pathways in the rat: horizontal plane. Distribution of MCH cell bodies (dots) and axons (short lines – bottom half of the drawing) is schematized on a line drawing of a horizontal section passing through the dorsal hypothalamus of a rat brain. Note the distribution of MCH perikarya in the dorsal hypothalamus/zona incerta region. Main pathways taken by MCH axons in the diencephalon/telencephalon, are schematized by large arrows (top half of the drawing). Axons follow the mfb, but many also take a more lateral route, entering the cerebral peduncle and then the ventral internal capsule (framed pictures a and b, darkfield photomicrographs of MCH axons), providing an input to the globus pallidus and reaching the claustrum and lateral cortical fields. MCH projections in the entorhinal cortex are illustrated in Fig. 17A. Some descending axons form a dense bundle close to the substancia nigra; they are illustrated in Fig. 17B. Abbreviations: Ac: anterior commissure; ACB: accumbens nucleus; CL: claustrum; cpd: cerebral peduncle; CPU: caudoputamen nucleus; fr: fasciculus retroflexus; fx: fornix; GP: globus pallidus; ic: internal capsule; mfb: medial forebrain bundle; ml: medial lemniscus; mlf: medial longitudinal fasciculus; mo5: motor root of the trigeminal nerve: MPN: medial preoptic nucleus; MS: medial septal nucleus; mtt: mammillothalamic tract; NLL: nucleus of the lateral lemniscus; opt: optic tract; PH: posterior hypothalamic nucleus; PVH: paraventricular hypothalamic nucleus; RE: reuniens nucleus of the thalamus; sm: stria medularis; SN: substantia nigra; sp5: spinal tract of the trigeminal nerve; st: stria terminalis; STN: subthalamic nucleus; VC: ventral cochlear nucleus; VTA: ventral tegmental area; V: motor nucleus of the trigeminal nerve, XII: hypoglossal motor nucleus; 3v: third ventricle.

are observed between these first generated neurons and the ventricular surface. Lateral neurons are therefore generated earlier than medially located cells. We have seen in rat and mouse that differences in MCH distribution were correlated to a distinct profile in the peak of MCH neuron genesis (Croizier et al., 2010). MCH neurons could thus follow, at least in part, a passive migration. Differences in the lateral or medial distribution of MCH neurons could then result from a delayed or accelerated MCH neurogenesis depending on species. However, more data in MCH genesis in other species are needed.

If MCH cell bodies are very abundant in the LHA of most mammals, this is not true in other tetrapods. In batrachians, neurons have not colonized the LHA. Overall, the reptile and bird hypothalami are well developed. A clear cytoarchitectonic lateral zone exists, although MCH is little expressed in LHA neurons in those species. The reason why the MCH system retains a primitive periventricular arrangement, as for example in *P. muralis*, is unknown. We can only conclude that a lateral hypothalamic MCH system is less developed in those species as compared to mammals.

Baker and Bird (2002) had proposed an interesting scheme for the evolution of the MCH system in fishes (see above) with the differentiation of the NLT containing neuroendocrine MCH neurons. The appearance of a lateral group of MCH neurons, although quite ventral in the hypothalamus is a convergent evolutionary trend with the tetrapod LHA. In the goldfish, Hcrt neurons are found in the NLT, along with MCH neurons (Huesa et al., 2005). These Hcrt neurons are not magnocellular and do not project to the neurohypophysis. Co-expression of MCH and Hcrt in two separate but co-distributed cell populations suggests that the NLT shares at least neurochemical characteristics with the mammalian tuberal LHA. Fiber tracts interconnecting telencephalon and brainstem which passe through the LHA in mammals, travel far dorsal to the NLT in fish. This nucleus is close to the infundibulum, on the path of magnocellular projections en route to the neurohypophysis and MCH axons from neurons in this nucleus follow the same pathway.

5. An hypothesis about the evolution of MCH neurons

The great diversity in shape and distribution pattern of MCH perikarya in the vertebrate posterior hypothalamus raises the question of whether or not these neurons are homologous. Their general topological distribution in the dorsal region of the posterior hypothalamus agrees with such a hypothesis. The expression of developmental genes associated to the differentiation of the MCH phenotype is conserved through the vertebrate phylogeny. In species in which a second gene encodes a preproMCH precursor, neurons expressing one or the other of these genes are co-distributed. Finally, in mammals as in fishes, MCH- and Hcrt-producing cells

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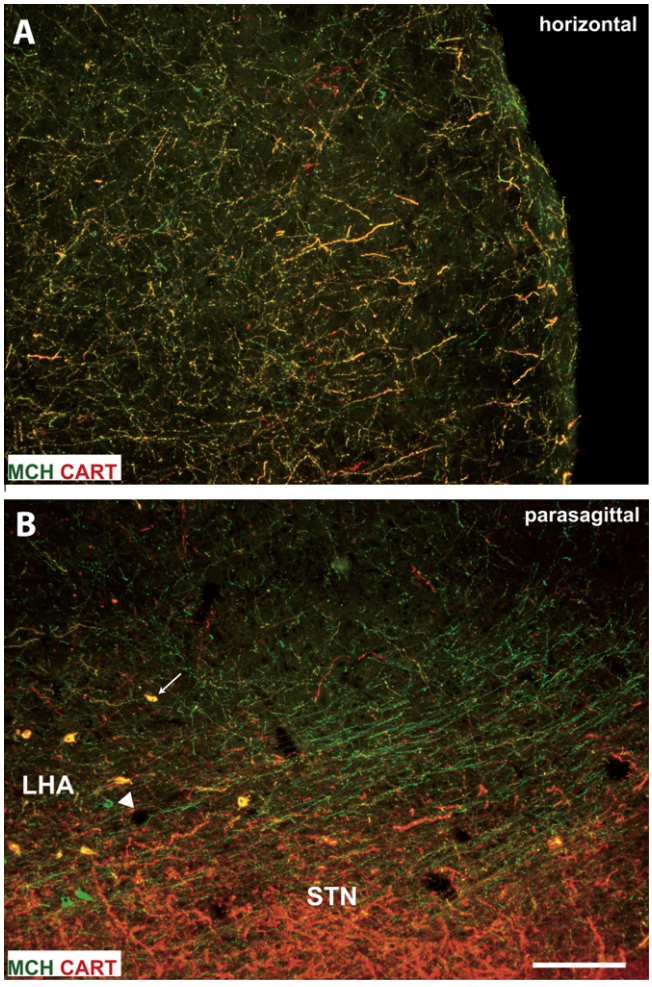

Fig. 17. MCH pathways in the rat. (A) In the cerebral cortex, most MCH axons are also labeled by a CART antiserum (dual immunofluorescence: MCH = green; CART = red; MCH + CART = yellow). (B) Some of the descending axons form a dense bundle when leaving the hypothalamus (Fig. 16). These axons that are not labeled by the CART antibody, continue caudally to innervate the spinal cord (picture taken from a sagittal section as to illustrate a longer segment of these axons). Dual immunofluorescence: MCH = green; CART = red; MCH + CART = yellow. Arrow points a MCH/CART perikaryon, arrowhead shows a MCH-only neuron. Scale bar (*B*) = 200 μm.

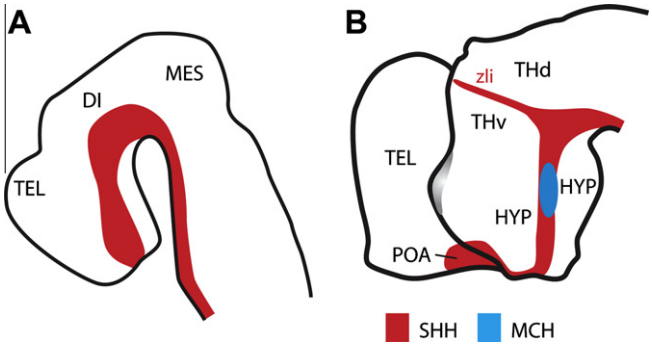

Fig. 18. Schematic distribution of SHH in the ventral prosencephalon in a young rodent embryo just after the anterior neuropore closure (A) or in an older embryo as the telencephalon increases in size (B). Adapted from Croizier et al. (2011), Diez-Roux et al. (2011), Marin et al. (2002), Roelink et al. (1995) and Shimogori et al. (2010). Abbreviations: DI: diencephalon; HYP: hypothalamus; MES: mesencephalon; TEL: telencephalon; THd, v: dorsal and ventral thalamus; zli: zona limitans intrathalamica.

are co-distributed. Therefore, MCH neurons in the caudal hypothalamus of all vertebrates are very probably homologous. Their

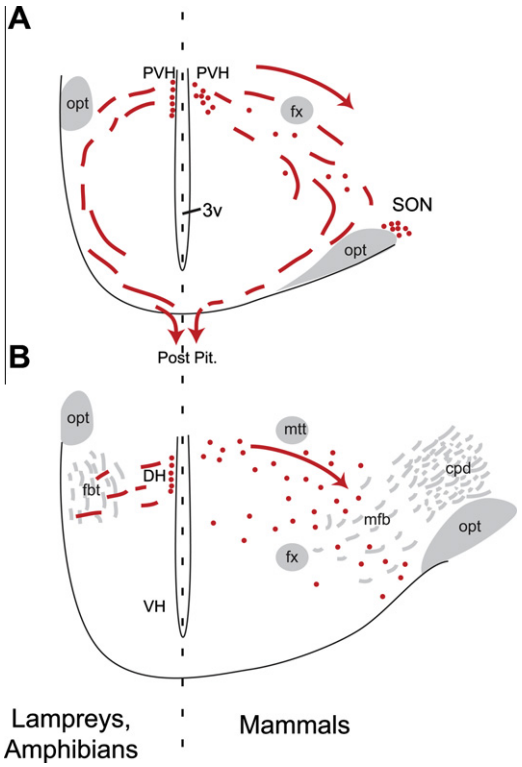

Fig. 19. Schematic illustration of the radial migration of magnocellular neurons to form the supraoptic nucleus (A), or of MCH neurons in the LHA (B) in mammals compared to their periventricular distributions in lampreys or amphibians. Abbreviations: cpd: cerebral peduncle; DH: dorsal hypothalamus; fbt: fasciculus basalis telencephali; fx: fornix; mfb: medial forebrain bundle; mtt: mammillothalamic tract; opt: optic tract; Post Pit: posterior pituitary; PVH: paraventricular nucleus of the hypothalamus; SON: supraoptic nucleus; VH: ventral hypothalamus; 3v: third ventricle.

diversity in shape and distribution patterns results from evolutionary processes that need to be discovered. SHH may play a significant role in these processes. The expression domain of this protein extends in the prosencephalon from lampreys to gnatostomes, and its expanse in expression is accompanied by telencephalic and hypothalamic development along the phylogenetic scale (Rétaux and Kano, 2010). Finally, in rat and mouse embryos, a clear correlation exists between the development of MCH subpopulations and telencephalic neurogenesis (Croizier et al., 2011). Because SHH is essential to forebrain morphogenesis as well as to MCH phenotype differentiation, it is tempting to hypothesize that significant interspecific differences in the anatomy of the MCH system is correlated to fundamental aspects of the forebrain evolution.

The forebrain of fishes has followed a very distinct evolutionary direction as compared to that of tetrapods. The telencephalon of ray-finned fishes undergoes pallial eversion. Consequently, structures analogous to the medial pallium of tetrapods are in a very lateral position in the ray-finned fish telencephalon (Northcutt, 2008). This may influence the organization of descending tracts toward the brainstem. Pallial and sub-pallial connections with the diencephalon are complex and involve structures as the posterior tuberculum and the migrated polyglomerular nuclei. These structures which may not have analogs in tetrapods, relay sensory inputs to the pallium, while the thalamus projects only in subpallial nuclei. MCH neurons sit outside these structures. A post-chiasmatic region characterized by MCH and Hcrt neurons sits outside tracts connecting the brainstem with the telencephalon. On the other hand, the NLT sits within the path of magnocellular projections en route toward the neurohypophysis. As evolution pursued

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its selective work, MCH cell bodies differentiating along this tract may have acquired a neurosecretory magnocellular morphotype and projection pattern, and then, in holosteans and teleosteans, the peptide itself acquired its characteristic role on melanophores (the hypothesis of Baker and Bird (2002)).

The evolutionary transition to tetrapody, and then from anamniotes to amniotes occurs in animals showing an evaginated telencephalon. The increase in telencephalic size and complexity goes along development of inputs from the thalamus to the pallium and of sensory processing in the pallium (Aboitiz, 2011; Butler, 2008; Puelles, 2001). The enlargement of the telencephalon corresponds also to the enlargement of structures connected with it as well as of the corresponding tracts (Aboitiz, 2011; Puelles, 2001; Reiner et al., 2005; Striedter, 1997; Yamamoto and Vernier, 2011). Broadly in amniotes, we observe an increased complexity in the MCH system organization, with abundant perikarya in the lateral hypothalamic zone and abundant MCH projections in the telencephalon including the pallium in rodents through the medial forebrain bundle as well as the cerebral peduncle/internal capsule. It is important to recall here that the telencephalon exerts a powerful influence on the diencephalon during development and attracts axons from the thalamus, ventral mesencephalon, but also axons from MCH neurons in rodents (Croizier et al., 2011). If development may sometime reflect evolutionary processes (Striedter, 1997), it is tempting to hypothesize that an increase in telencephalic size during evolution has impacted MCH anatomical organization as well.

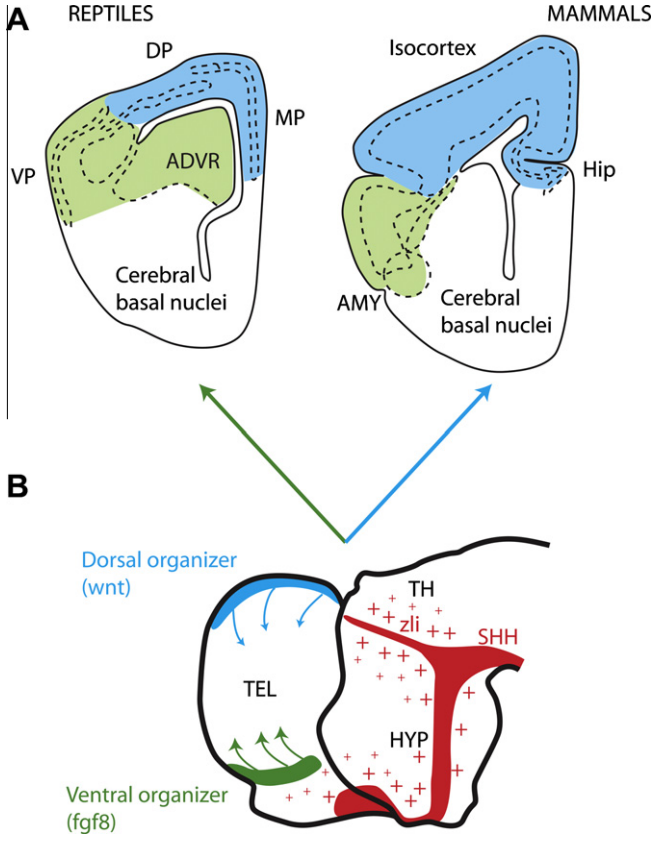

Fig. 20. Recent hypothesis of the development of the sauropsidian and mammalian prosencephalon. Adapted from Aboitiz (2011) and Aboitiz et al. (2002). (A) Differences in the morphological organization of the sauropsidian and mammalian pallium. A dominant action of the dorsal organizing center in mammals is involved in the expansion of the isocortex, while in sauropsids a large ADVR is the result of a dominant action of the ventral organizer. See text for additional informations. (B) Schematic illustration of organizing centers in the prosencephalon: the SHH expression domain in the diencephalon, including the zli, and in midline of the ventral telencephalon plays a key role in the differentiation of the thalamus, hypothalamus and ventral telencephalon. A dorsal (expressing Wnt) and a ventral (expressing Fgf8) organizing centers in the telencephalon drive the development of the pallium. Abbreviations: ADVR: anterior dorsal ventricular ridge; AMY: amygdala; DP: dorsal pallium; Hip: hippocampus; HYP: hypothalamus; MP: medial pallium; TEL: telencephalon; TH: thalamus; VP: ventral pallium; zli: zona limitans intrathalamica.

The MCH system seems less developed in sauropsids than in mammals. The telencephalon has evolved independently in both clades (Aboitiz et al., 2002; Bruce and Neary, 1995c; Puelles, 2001; Striedter, 1997). Divergent evolution could again explain this difference between the mammalian and sauropsidian MCH systems. Evidence exists that the sauropsidian pallium development might have been under the influence of ventralizing factors. Sauropsids have a large nuclear "amygdala-like" ventral pallium (the ADVR) but a small "allo-isocortical-like" pallium (Fig. 20A). The mammalian pallium was dwarfed by dorsalizing influences, and mammals have a prominent dorsal pallium including the whole isocortex (Fig. 20A). The evolution of the pallium is tied up with evolution of the thalamus and midbrain. However, cell proliferation and nuclear organization of the thalamus is strongly dependant of the zli (Kiecker and Lumsden, 2004; Kitamura et al., 1997; Vieira and Martinez, 2006) (Fig. 20B). This organizing center also influences the development of tissue in the ventral thalamic side, which includes the ZI in mammals. Increase in thalamic expansion under the control of the zli, could also influence dorsal hypothalamic/ventral thalamic structures, including MCH neurons that are found in the ZI in many mammals. The coordination between pallial and thalamic evolutions is not well understood. Puelles (2001) suggested that perhaps diffusible factors could affect both structures. Recently, it has been exposed that SHH in the zli affect WNT expression in the pallial dorsal organizing center (Rash and Grove, 2011), influencing the dorsal pallium development and the diencephalon-telencephalon junction. In the fish *A. mexicanus* (see above) the expanded SHH expression induces an earlier expression of FGF8 in the ventral organizing center, and an enlarged ventral telencephalon (Pottin et al., 2011). These two examples illustrate that SHH in the diencephalon affects ventral and dorsal organizing centers in the telencephalon. As expression of MCH in the rodent embryo is SHH-dependent and as MCH neurons express SHH-dependent transcription factors as NKX2.1 and NKX2.2 (Croizier et al., 2011), it may be assumed that processes coordinating diencephalic and telencephalic growths affect MCH neurons proliferation/differentiation as well.

Connectional records show that the ventral hypothalamus of lizards develops strong bidirectional anatomical links with the ADVR (ventral pallium), similar to the anatomical links between ventromedial hypothalamic nucleus (VMH) and amygdala in mammals through the stria terminalis (Bruce and Neary, 1995a, 1995b) (Fig. 21). By contrast, the lizard dorsal hypothalamus is connected to the anterior septum (which receives projections from the medial pallium that is homologous to the mammalian hippocampus) and the dorsal pallium which shows homologies with the prefrontal cortex (Bruce and Neary, 1995c; Font et al., 1997a, 1997b, 1998; Lanuza et al., 2002). Again, this is reminiscent of medial prefrontal and septal inputs to the mammalian LHA through the medial forebrain bundle (Risold, 2004; Risold and Swanson, 1997). Divergences between the anatomy of saurospsidian and mammalian MCH systems reflect therefore divergences in forebrain morphological organization.

6. Conclusions

Postchiasmatic MCH neurons have evolved from a common pool of cells in the dorsal and caudal periventricular hypothalamus. In both fishes and tetrapods, convergent evolutionary processes

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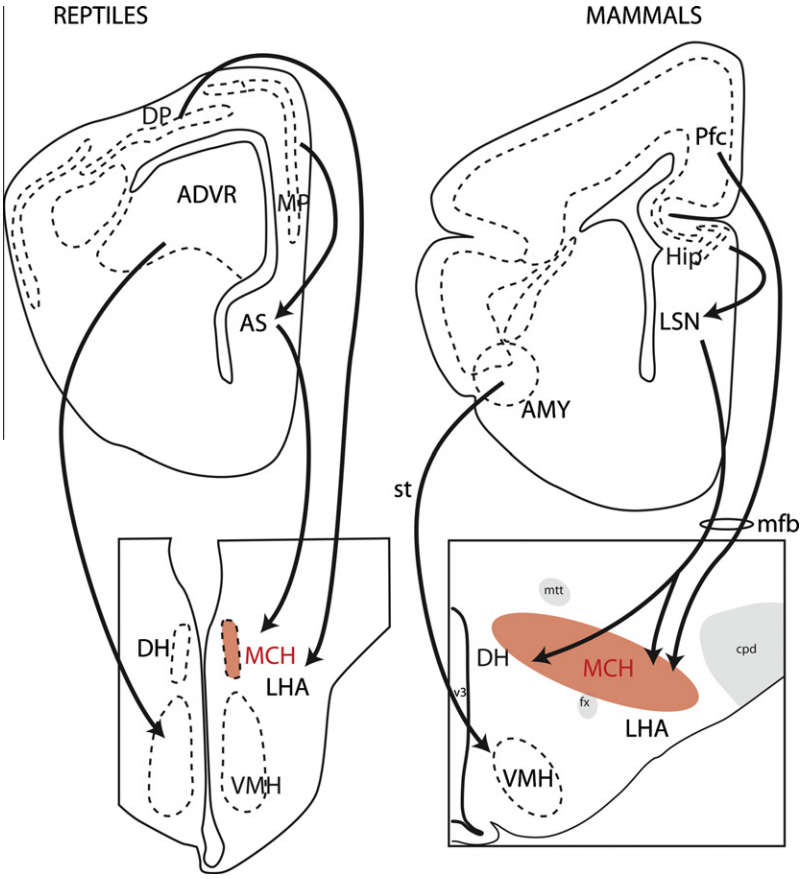

Fig. 21. Schematic comparison of projections from the telencephalon to the dorsal and ventral hypothalamus in lizards and mammals (see text for details). Adapted from Aboitiz et al. (2002) and Bruce and Neary (1995a, 1995b). Abbreviations: ADVR: anterior dorsal ventricular ridge; AMY: amygdala; AS: anterior septal nucleus; cpd: cerebral peduncle; DH: dorsal hypothalamus; DP: dorsal pallium; fx: fornix; Hip: hippocampus; LHA: lateral hypothalamic area; LSN: lateral septal nucleus; mfb: medial forebrain bundle; MP: medial pallium; mtt: mammillothalamic tract; Pfc: prefrontal cortex; st: stria terminalis; VMH: ventromedial hypothalamic nucleus; 3v: third ventricle.

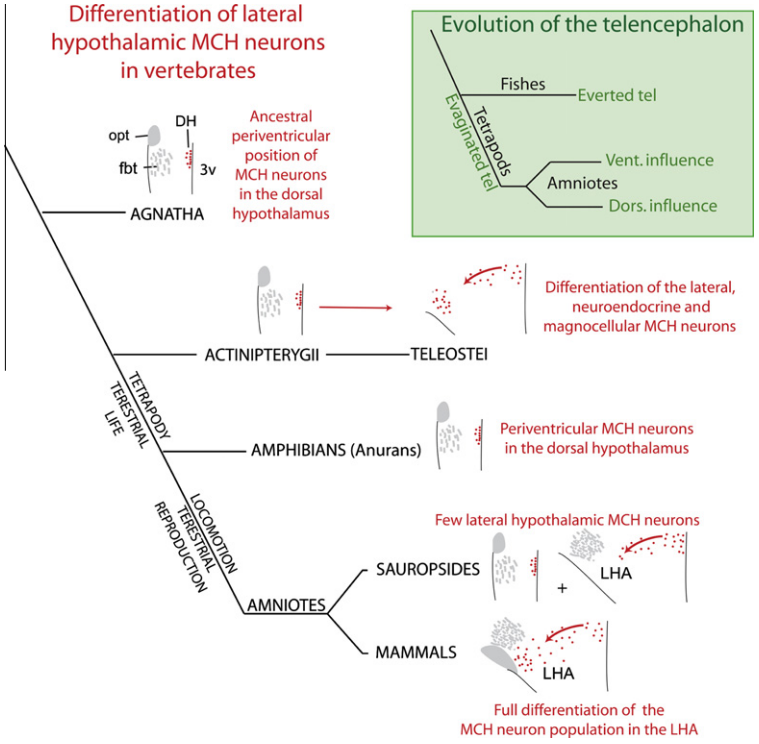

Fig. 22. Cladogram illustrating the evolution of the MCH system in vertebrates (see text for details). Abbreviations: DH: dorsal hypothalamus; fbt: fasciculus basalis telencephali; LHA: lateral hypothalamic area; opt: optic tract; 3v: third ventricle.

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Table 1
Used antibodies.

Antibodies	Type	Antigens	Origins	Dilution
sMCH (rabbit)	Polyclonal	Salmon MCH	Labo Histologie EA3922	1/1000
Orx A (goat)	Polyclonal	Human Orx A	Santa Cruz Biotechnology, Inc.	1/1000
CART (mouse)	Monoclonal	Rat CART	Dr. Clausen*-Danemark	1/1000
TH (rabbit)	Polyclonal	TH from rat pheochromocytome	Institut Jacques Boy	1/1000
AVT (rabbit)	Polyclonal	Synthetic peptide	S. Bläsher*	1/1000
Neurophysin (rabbit)	Polyclonal	Synthetic peptide	Labo Histologie EA3922	1/1000

have resulted in the differentiation of lateral groups of neurons (Fig. 22). However, the shape, distribution and projection patterns of these neurons vary and are correlated to the differentiation of the telencephalon and mfb. In fishes, a causal relationship between differentiation of an everted telencephalon and of neuroendocrine MCH neurons (instead of amniote-like interneurons) appears likely. Tetrapody and terrestrial life occur in animals with bilobed brains. Increased pallial size in sauropsids and mammals are associated with an increased behavioral repertory and greater ability for locomotion. In addition, structures connected with the pallium also appear larger. These structures include the mfb and LHA, as well as the whole posterior hypothalamus and the dopaminergic ventral tegmentum. Few comparative anatomical studies performed in amniotes suggest that the MCH system reach its greatest extent in mammals, because MCH neurons are very abundant in the LHA and project throughout the cerebral cortex. On the contrary, the MCH system seems to retain a more primitive periventricular position in some sauropsids. Sauropsidian and mammalian forebrains probably evolved from that of a common batrachian-like ancestor, but followed distinct fates (Aboitiz et al., 2002; Reiner et al., 2005). Anatomical and developmental studies clearly suggest strong links between MCH and telencephalon in mammals (Croizier et al., 2011), therefore suggesting putative links between the evolution of the MCH system in the posterior hypothalamus and the telencephalon in amniotes.

MCH plays a role in feeding in fishes as in mammals (Berman et al., 2009; Bittencourt, 2011; Griffond and Baker, 2002). However, the MCH system appears to be extremely plastic, and, as the brain changes and behavioral repertories increase, MCH is involved in new functions or behaviors, such as control of skin color in fishes (Baker and Bird, 2002), or REM sleep in mammals (Peyron et al., 2009). Obvious divergences in the distribution of MCH neurons in mammals, may therefore suggest that they are also involved in species specific functions.

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Appendix A

A.1. Material and methods

A.1.1. Sample collections

Five *L. fluviatilis*, five trouts *Oncorhynchus mykiss*, seven *R. temporaria* were obtained from local suppliers (Doubs or Gironde France) in spring. Brains were dissected and fixed 24 h in PFA4%, dehydrated and wax embedded. Seven male rats three male mice were obtained from Janvier (France) and perfused with the same fixative, as amply described (Brischoux et al., 2001, 2002; Croizier et al., 2010). Brains were frozen and serially cut in series of free floating sections that were 30 μm thick. The lizard (*P. muralis*) and human material belong to a frozen or paraffin embedded histological collection obtained during the 1980s (Bresson et al., 1989) and 1990s (Cardot et al., 1994).

A.1.2. Immunohistochemistry

Immunohistochemical procedures were performed as already extensively described (Brischoux et al., 2001, 2002; Croizier et al., 2010). The used primary antibodies, their suppliers, and dilutions can be found in Table 1. The specificity of the salmon MCH-antiserum (AS) was verified by liquid phase inhibition, dot blot, and immuno-affinity as well as by immunohistochemistry/*in situ* hybridization double labeling in the rat. The labeling provided by these AS was revealed through the standard peroxidase anti-peroxidase procedure (Dako, France) or indirect immunofluorescence procedures using secondary donkey anti-mouse IgG conjugated to Alexa Fluor-555 (1:800, Invitrogen), a goat anti-rabbit IgG conjugated Alexa Fluor-488 (1:800 or 1:1000, Invitrogen), Cy3-conjugated donkey anti-rabbit secondary antibodies (1:400, Jackson ImmunoResearch Laboratories, PA, USA), or a FITC-conjugated goat anti-mouse (1:400, Jackson ImmunoResearch Laboratories, PA, USA) for 1 h at room temperature.

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