Forebrain gene expression domains and the evolving prosomeric model

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The prosomeric model attributes morphological meaning to gene expression patterns and other data in the forebrain. It divides this territory into the same transverse segments (prosomeres) and longitudinal zones in all vertebrates. The axis and longitudinal zones of this model are widely accepted but controversy subsists about the number of prosomeres and their nature as segments. We describe difficulties encountered in establishing continuity between prosomeric limits postulated in the hypothalamus and intra- telencephalic limits. Such difficulties throw doubt on the inter- segmental nature of these limits. We sketch a simplified model, in which the secondary prosencephalon (telencephalon plus hypothalamus) is a complex protosegment not subdivided into prosomeres, which exhibits patterning singularities. By contrast, we continue to postulate that prosomeres p1–p3 (i.e. the pretectum, thalamus and prethalamus) are the caudal forebrain.

Topographic comparison of diverse gene expression patterns in the forebrain requires a comprehensive interpretive paradigm. Scientists working on gene expression patterns rely on a particular brain model, although many select this pragmatically, by authority or apparent convenience, and few have a rationale for their choice. It is, therefore, important to explain and discuss the properties of given models. For the past ten years, we have been developing the prosomeric model for the vertebrate forebrain [1–6]. At its core, this model recognizes the bent longitudinal axis of the forebrain relative to midbrain and hindbrain, and defines the primary anteroposterior (AP) and dorsoventral (DV) divisions – transverse neuromeres and longitudinal zones, respectively (Box 1). The model contemplates explicitly the existence of additional subdivisions of the main AP and DV zones (Figs 1,2) and recognizes the optic and telencephalic vesicles as specialized neural fields with patterning properties that are in part independent from the rest of the neural tube. It should be noted that the prosomeric model is primarily a morphological instrument (paradigm) that emphasizes evolutionarily conserved topological and molecular expression relationships in the neural tube. It is neither a hypothesis nor a theory of the development of brain parts. Such a model is useful for furthering progress as it provides a conceptual framework within which preconceived assumptions and deduced predictions can be tested.

The prosomeric model has been useful for classifying topologically numerous novel molecular expression patterns and associated histogenetic data. Molecularly specified domains of the neural wall can be delimited across species, and postulated gene functions can be tested subsequently in terms of regional and cellular fates. The topological definition of DV and AP subdivisions within prosomeres, each one capable in principle of producing particular types of neurons or glia, facilitates interpretation of observations in embryonic and adult brains in terms of the sequence of DV and AP patterning mechanisms that organize the neural tube (Figs 1,2). This paradigm has indeed helped causal thinking in terms of AP and DV patterning processes in the complex forebrain of vertebrates.

The model was supported importantly by its capacity to provide morphological meaning to numerous gene expression patterns; many expression domains were found to respect a subset of the boundaries postulated in the model [3–8]. The prosomeric model was conceived to be useful for all vertebrates, as was duly corroborated in several comparative morphological studies in organisms as diverse as lampreys and humans [9–11]. Knowledge about gene expression patterns in non-mammalian vertebrates has expanded considerably in recent years. Genes homologous to those with established molecular boundaries in the mouse forebrain have been mapped in the chick [12], Xenopus [13] and zebrafish or medaka [14–19]. Comparable expression of some of these genes was also located in the forebrain of agnathans [20,21]. In this field, the anatomically detailed prosomeric model seems helpful for interspecies comparisons. This approach is facilitating an emerging, novel perspective on the evolutionary origin of the vertebrate neural ‘Bauplan’ [22–24].

The prosomeric model seems now widely known and some of its novel premises, including the redefinition of the axial dimension of the forebrain and the proposed longitudinal zones, with their prechordal and epichordal parts, have been widely accepted. By contrast, the number, limits and nature as neural compartments of the prosomeres remain partially controversial [25,26] (Box 1). Here, we propose a simplification of the model, eliminating some of the controversial aspects without losing the

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Segmentation
For animal forms having bilateral symmetry and a length axis, segmentation involves patterning of the form into distinct and complete transverse parts (segments) aligned serially along the longitudinal (anterior-posterior, or AP) axis. ‘Distinct’ implies that there are detectable intersegmental boundaries but does not preconceive their nature or how we visualize them; during development, boundaries can change their status from non-overt to overt, and then change again to a hidden status, thus requiring experimental demonstration at given stages. ‘Complete’ implies that only a complete cross-sectional (transverse) part of the form can be a segment.

Metamery
A set of segments of a form have the topological property of metamery if their identifying structural pattern is conserved; that is, if there are intersegmental boundaries but does not preconceive their nature or how we visualize them; during development, boundaries can change their status from non-overt to overt, and then change again to a hidden status, thus requiring experimental demonstration at given stages. ‘Complete’ implies that only a complete cross-sectional (transverse) part of the form can be a segment.

Neural segment
We apply the general principle of segmentation (above) to the vertebrate neural tube. Neural segments are distinct and complete transverse parts of the neural tube, itself clearly a bilaterally symmetric and AP-elongated form. Transverse neural subdivisions are already apparent as molecularly distinct parts of the neural plate (protosegments), and these areas can have a characteristic fate [58]. After closure of the neural tube, complete transverse subdivisions known as neuromeres transiently appear as serial bulges separated by transverse constrictions of the neural wall [59]. This owes to differential proliferative rates, in addition to various secondarily acquired properties of the neuroepithelial cells at the boundaries (i.e. clonal restriction, gap-junctional isolation and differential expression of cell adhesion and matrix molecules). Bulging varies across species and therefore is not strictly required for neural segment definition. By contrast, differential molecular specification along the AP dimension is evolutionarily conserved and required for definition purposes, because it leads to the segmental identity of the bound-aries. Thus requiring experimental demonstration at given stages. ‘Complete’ implies that only a complete cross-sectional (transverse) part of the form can be a segment.

Neuromere
All distinct neural segments postulated by us share a fundamental doro-ventral (DV) structural pattern composed of four longitudinal zones (roof, alar plate, basal plate and floor). We therefore see them as generally metamere; hence, our term ‘neuromere’ refers to the common pattern of fundamental DV zones, whereas our term ‘neur-al segment’ is less restrictive and does not require this or any shared pattern, just distinct and complete transverse boundaries. Particular regions of the neural tube can be conceived as ‘tagmata’, in which local sets of segments share regional characters. This leads to the classic distinction of forebrain, midbrain, hindbrain and spinal neuromeres, called prosomeres, mesomeres, rhombomeres and myelomeres, respectively [59]. Depending on the ‘fundamental structure’ selected for comparisons, prosomeres and rhombomeres might or might not be mutually metameric. Note that there exist alternative concepts of neuromeres, in which metamery is postulated as a necessary condition for any neural segment. This combines with a definition of segments according to their boundary properties, rather than their internal structure, often disregarding dorsoventral completeness [26,62]. This viewpoint accepts only a limited number of ‘transient brain segments’ and leaves other transverse brain parts as morphological non-entities waiting for a concept. This is of limited value for most applied (e.g. causal, comparative or functional) purposes.

Compartment
A compartment is a self-contained developmental unit in terms of cell populations. The primary precursors at the inception of the compart-ment generate a spatially restricted polyclone of derivatives. Bound-aries are, thus, clonal restriction limits, which are supposed to be absent inside the compartment, where cells should freely intermix. In the 1990s, it was conjectured that brain segments or neuromeres might actually be compartments, at least with regard to neuroepithelial cells [57]. Some authors have even hypothesized that, if clonal restriction is not detected, ‘other segments’ do not exist. However, it remains con-ceivable that further research might eliminate false negative results, or that clonal restriction might after all be a necessary criterion for intersegmental boundaries. It has not yet been proven that the clonal restriction boundaries separating adjacent rhombomeres in the hindbrain satisfy the property of completeness (they are absent across the floor plate); restriction might be absent in some distinct complete transverse limits and is not uniquely present throughout development. Indeed, a multiplicity of transverse clonal boundaries is established progressively over time, even inside the segments [63].

Segmental or neuromeric subdivisions
Segmental or neuromeric subdivisions are non-complete parts distin-guished within segments or neuromeres; they can be variously disposed relative to the axial dimension of the segment.

Longitudinal zones
Longitudinal zones result from comparable dorsoventral patterning across the neural primordium [64].

Zonal divisions
Zonal divisions are subdivisions that form locally along the DV dimension of a primary longitudinal zone. Advancing DV patterning generates such subdivisions owing to step-like recruiting of differential gene expression patterns in localized groups of neural precursors at various distances from locally efficient signal sources, leading to the generation of specific neuronal or glial subtypes in a spatially graded fashion [65]. These derivatives usually adopt a stereotyped position in the corresponding mantle layer, unless they migrate elsewhere.
within the hypothalamus continued into the telencephalic roof, to establish the required segmental property of completeness (Box 1), met with various difficulties.

A fate map of the late neural plate in the chick [28] showed the subpallium and septum to lie topologically rostral to the telencephalic pallium, agreeing with fate-mapping data at the closed neural tube stage [29]. In principle, the pallium–subpallium boundary seemed a good candidate for continuing one of the interrupted transverse hypothalamic limits [27,30]. However, we were...
between prosomeres requires that these be transverse and schematically as observed in sections (topological patterning singularities, represented semi-underlying rostral diencephalon. That might not be directly related to any boundaries in the underlying hypothalamus. The prethalamus (ventral thalamus) from making contact with the basal ganglia; the overall AP and DV dimensions of the neural tube (i.e. see also Fig. 11 in Ref. [12]). The resulting curved intra-telencephalic boundaries (Fig. 3; see also Fig. 11 in Ref. [12]) appear to be unrelated to the overall AP and DV dimensions of the neural tube (Fig. 3). Furthermore, induction of 'ventral' molecular properties seem to interrupt potential continuities. Moreover, genes expressed in the optoeminential domain, such as Sim-1 or Otp, show a long intra-telencephalic spike continuing along the terminal sulcus into the pallial amygdala [31,32] (Fig. 3); this feature blocks the rostral boundary of the prethalamus (ventral thalamus) from making contact with the pallium—subpallium limit. In addition, special patterning mechanisms that subdivide the telencephalon seem to generate criss-crossing roof signals originating either rostrally at the anterior neural ridge (the end of the roof plate [24]) or caudally at the cortical hem [33–36]. The modiﬁcations of ‘ventral’ properties in the telencephalon (i.e. Shh and Nkx-2.1 expression in the basal ganglia; Fig. 3) take place in rostral areas via inductive processes that lack continuity with the axial mesendoderm [37] and differ in part mechanistically from ventral patterning of the hypothalamus [38]. Thus, the telencephalon is a derivative of the alar plate that, during its evagination, secondarily becomes patterned into several pallial and subpallial subdivisions with boundaries that might not be directly related to any boundaries in the underlying rostral diencephalon. Figure 3 displays these topological patterning singularities, represented semi-schematically as observed in sections (Figs 1,2).

Because the definition of intersegmental boundaries between prosomeres requires that these be transverse and complete from roof to floor of the neural tube, our analysis inclined us more and more to the conclusion that observable hypothalamic boundaries could be secondary ones that develop inside the hypothalamus owing to various local influences and, thus, that do not reveal formal segmental elements of the forebrain.

The modified model
Our present proposal, which also includes convenient terminological simplifications, reduces the number of postulated prosomeres to the three caudal diencephalic ones, p1–p3. These contain in their alar regions the pretectum (p1), the thalamus (previously known as the dorsal thalamus) plus the habenula or epithalamus (p2), and the prethalamus (previously known as the ventral thalamus) plus the eminentia thalami (p3) (Figs 1–3). The present proposal represents just two major changes upon our initial model [2–4]. First, the eminentia thalami – previously considered a dorsal part of p4 – was recognized as a domain dorsal to the prethalamus, occupying there a position analogous to that of the habenula relative to the thalamus, traversed longitudinally by the stria medullaris (sm in Fig. 3); the dorsal position of the eminentia thalami relative to the prethalamus is supported by diverse gene markers expressed in it, such as Tbr-1 [12,27], PLZF [39], R-cadherin [40,41], Ebf3 [42] and Lhx2 [43]. The entire alar p3, including the eminentia thalami, expresses the gene Arx [44], whereas the eminentia is excluded from the p3 domain expressing Dlx genes (Figs 1a,b, 3). There are mouse and chick data suggesting that some neurons might migrate tangentially out of the eminentia thalami into the adjoining amygdala [12,32,45]. Second, present changes affect the old p4–p6 prosomeres, which are reinterpreted as secondary subdivisions.
restricted to the hypothalamus within the secondary prosencephalon. The secondary prosencephalon is the entire prechondal (rostral-most) portion of the neural tube. Seeking terminological continuity, we divide the secondary prosencephalon into telencephalon and rostral diencephalon or hypothalamus [6] (Fig. 3). We define rostral and caudal parts of the hypothalamus (RHy and CHy, respectively, in Figs 2,3). The longitudinal or DV zones of this large domain remain unchanged, so that alar and basal parts of the classic hypothalamus can be distinguished.

The rostral hypothalamus correlates topographically with early expression of Six3, which extends from the septum to neurohypophysis [46]; this was the best evidence so far for defining molecularly the old p6 prosomere; however, analysis of the Six3 or Hex1 knockout mice [47,48] revealed a loss of the whole secondary prosencephalon which, thus, seems to behave as a single

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histogenetic field. The alar portion of the rostral hypothalamus contains the preoptic region plus the rostral optoeminential (anterior hypothalamic) and suprapallial DV subdivisions (Fig. 3); the corresponding basal portion, characteristically Shh- and Nkx2.1-positive at early stages, contains the ventromedial and dorso medial hypothalamic nuclei, which later partially downregulate Nkx2.1 expression (Figs 1e, 2b); the rostral hypothalamic floor plate includes the median eminence, the neurohypophysis and the arcuate nucleus.

The caudal hypothalamus (the subthalamic or peduncular region in some literature) is aligned with the superficially coursing telencephalic peduncle (yellow arrow in Fig. 3; ped in Figs 1c,f, 2c, 3). Underneath it, the fornix tract (fx and orange arrow in Fig. 3) also traverses dorsoventrally the caudal hypothalamus, approaching the mammillary body. The alar part of the caudal hypothalamus contains the supraopto-paraventricular (caudal optoeminential) area and the posterior entopeduncular area (origin of the migrated entopeduncular nucleus; PEP in Fig. 1b,c). The basal plate is represented by the posterior hypothalamic area, in addition to the mammillary and retromammillary regions (often, part of the posterior hypothalamus is prolonged into the p3 tegmentum, but we characterize here the latter as ‘Forel fields’ (FF in Fig. 3)). The subthalamic nucleus arises from the retromammillary region and migrates to the deep surface of the peduncle (Figs 1b,c,f, 2a,b, 3).

The boundary between caudal hypothalamus and p3 is well defined by the expression of several genes. In the alar plate, it coincides with the sharp caudal limit of the Sim-1, Otp and Brn-2 genes, which are expressed in the optoeminential area, and with the sharp rostral limit of Arx, Dlx and Pax6 transcripts in the prethalamus (Figs 1a–d, 2c, 3). In the mouse, the dorsal end of this limit surrounds the eminencia thalami along the stria terminalis into the vicinity of the amygdala, apparently to end at the fissura chooroidea in the caudomedial wall of the telencephalon [31]. In the basal plate, the posterior hypothalamus of our model shows a distinct boundary with the Forel fields [i.e. in expression of Otp [49] (outlined in yellow in Fig. 1a; Fig. 3)]. There are specialized radial glia at this boundary [50]. Observations in frogs [7,51] reveal a marked cell-poor gap at this boundary, analogous to the aspect of the zona limitans intrathalamica (p2–p3 limit). Although studies aiming to detect clonal restriction properties of chicken interprosomeric boundaries failed to identify this boundary [28,29], this forebrain area might not have been sampled sufficiently. We therefore expect that additional studies might clarify whether or not there is a boundary of clonal expansion within the neuroepithelium at this limit, although we do not believe that clonal restriction uniquely defines intersegmental limits (Box 1). Figdor and Stern [25] did not separate p3 from the hypothalamus, thus postulating their large D1 neuromere (D2 corresponds to p2 and D3–D4 seem to represent secondary AP subdivisions in p1, in so far as they are described only in the alar plate [25,26]; compare with p1 in Fig. 3). Figdor and Stern and Larsen et al. [25,26] reported p3 cell clones that partially extended into the adjacent telencephalon as evidence of lack of a p3–p4 clonal restriction boundary. These conclusions need to be re-examined considering that p3 includes an eminential portion that partially evaginates beyond the hemispheric sulcus and thereby encroaches upon the amygdala (the hemispheric sulcus accordingly is not the rostral boundary of p3; Fig. 3).

Concluding remarks

Comparison of the final schema of Larsen et al. [26] with our model suggests fundamental agreement with our p1–p3 prosomeres and a non-segmented secondary prosencephalon; we distinctly converge in our views on fundamental forebrain subdivisions. The proposal to reduce terminological confusion by calling the classically defined dorsal thalamus simply ‘thalamus’ and the classically defined ventral thalamus ‘prethalamus’ seems apropos at this point in time [52]. Finally, the present model should be a useful template for future fate-mapping experiments, gene expression analyses, gene and cellular function investigations and cross-species anatomical comparisons, all of which will contribute towards establishing more definitive information about the topological organization of the prosencephalon.

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