A developmental ontology for the mammalian brain based on the prosomeric model

Luis Puelles[1], Megan Harrison[2], George Paxinos[3], Charles Watson[4]

[1]Department of Human Anatomy, University of Murcia, Spain

[2]Fremantle Hospital, Fremantle WA 6160

[3]Neuroscience Research Australia, Sydney, Australia and University of New South Wales, Sydney, Australia

[4]Faculty of Health Sciences, Curtin University, Perth, Australia and Neuroscience Research Australia, Sydney, Australia

Corresponding author: Watson, C. (c.watson@curtin.edu.au)

ABSTRACT

In the past, attempts to create a hierarchical classification of brain structures (an ontology) have been limited by lack of adequate data on developmental processes. Recent studies on gene expression during brain development have demonstrated the true morphologic inter-relationships of different parts of the brain. A developmental ontology takes into account the progressive rostrocaudal and dorsoventral differentiation of the neural tube, and the radial migration of derivatives from progenitor areas, using fate-mapping and other experimental techniques. We have used the prosomeric model of brain development to build a hierarchical classification of brain structures based chiefly on gene expression. Because genomic control of neural morphogenesis is remarkably conservative, this ontology should prove essentially valid for all vertebrates, aiding terminological unification.

KEYWORDS
ontology; gene expression; neuromeres; forebrain; midbrain; hindbrain

This article describes a developmental ontology which takes into account the progressive differentiation of the neural tube and the radial migration of derivatives from progenitor areas, using fate-mapping and other experimental techniques. We have used this model to build hierarchical classification of brain structures based chiefly on gene expression during development.

What is ontology?

The concept of ontology (see Glossary) was borrowed from the realm of philosophy by information scientists, who now use it as a way to represent an existing domain of knowledge in the form of a hierarchical taxonomy [1]. The availability of a brain ontology is vital for the field of neuroinformatics. There have been a number of attempts to create a brain ontology, the most notable of which are NeuroNames [2, 3, 4], the Biomedical Information Research Network (BIRN) [5], and the Brain Architecture Management System (BAMS) [6, 7]. However, these ontologies are largely based on traditional topographic classification of parts of the adult brain, whereas the discovery of gene targeting in mice [8] has revealed details of gene expression, lineage mapping, and causal inductive mechanisms during development, leading to a new form of hierarchical classification based on ontogeny.

Conventional ontologies have the weight of tradition, but they do not include fundamental ontogenetic data such as neuromeric developmental units, genoarchitectonic evidence for natural boundaries between brain parts, or their inner subdivisions (as opposed to many arbitrary classical divisions unrelated to causal mechanisms). Therefore, they have little power to encapsulate emergent understanding of brain development and structural evolution. In contrast, a developmental ontology connects adult neuroanatomy with the tradition of comparative embryology; it contemplates developmental structural units shared among vertebrates, as revealed by fate mapping studies, and is consistent with evolutionarily conserved gene patterns. Because of this, a developmental ontology has the capacity to stimulate insights about causation. Our proposal of a developmental ontology for the adult mouse brain is a simplified version of the extended version designed by L. Puelles for the Allen Developing Mouse Brain Atlas (Allen Institute for Brain Science. ©2009, http://developingmouse.brain-map.org). The new ontology is consistent with the most recent version of the prosomeric model [9], a known paradigm for structural and molecular analysis of vertebrate brains (Fig.1). Note that this essay centers on the ontology and does not aim to explore the prosomeric model itself.

Comparing traditional and developmental ontologies

All adult brain ontologies start with the recognition of three basic elements - forebrain, midbrain, and hindbrain. Even at this level, the developmental ontology is distinctive, in that it includes the isthmus within the hindbrain, rather than in the midbrain, as found in traditional ontologies. The importance of transgenic fate-

mapping to the understanding of such delimitation has been emphasized previously ([10], [11], 12]).

There are many novel features at the next hierarchical level of the developmental ontology. In the forebrain, three components are recognized, the telencephalon, the hypothalamus (including the eye vesicle), and the diencephalon (Fig. 1). The telencephalon and hypothalamus constitute the secondary prosencephalon [12] (Fig. 1c), which can be separated from the diencephalon proper on the basis of gene expression patterns [13, 14, 15]. This is further supported by experimental evidence showing that the holoprosencephalic malformation syndrome selectively affects the telencephalon and the hypothalamus [16], and the Otx2-/-; Emx1+/- double mutant mouse selectively loses the diencephalon, but retains the hypothalamus/telencephalon complex [17, 18]. In traditional ontologies, the hypothalamus was incorporated within the diencephalon, because it was incorrectly assumed to be the equivalent of the basal and floor plates of the thalamus [48]. Recent fate maps and molecular findings [14, 15] indicate that the hypothalamus is topologically rostral to the thalamus and other parts of the diencephalon, and the telencephalic and eye vesicles represent bilateral alar evaginations. The neural tube ends rostrally at the terminal wall (between the mamillary body and the anterior commissure) with a distinct bow-like specialized median domain named the acroterminal area across the tuberal (basal) and chiasmatic (alar) hypothalamus and the preoptic area {16}. Though it does not evaginate, the preoptic area belongs to the telencephalic subpallium on the basis of shared molecular profiles [19]. This classification is further supported by the finding that it contributes tangentially migrating neurons to the telencephalic pallium, like the other parts of the subpallium, but not to the hypothalamus [20, 21, 22, 23].

In the hindbrain, the developmental ontology recognizes twelve segments (the isthmus and eleven rhombomeres) (Fig. 1d,e), principally on the basis of Fgf8 [10, 24] and Hox gene expression [25], whereas the traditional ontology divides the hindbrain into pons and medulla oblongata - classic gross divisions that are not consistent with the rhombomeric subdivisions [12]. In the developmental ontology, the cerebellum is separated from the basilar pons, and properly classified as a dorsal outgrowth of the isthmus and the first rhombomere, a result of several fate-mapping studies [26, 27, 28] (Fig. 1d,e).

At the third level of the developmental ontologic hierarchy, the telencephalon is divided into pallium and subpallium, as it is in traditional ontologies (Fig. 2a). But as noted above, the 'new' subpallium includes the preoptic area [19], parallel to three other distinct radial subpallial domains, identified in medio-lateral order as diagonal domain (old substantia innominata, covered superficially by the diagonal band formation), pallidum, and striatum (Fig. 2a). Each of these domains extends radially from the ependyma to the pial surface and has diverse stratified derivatives. The striatum contacts the pallium across the pallio-subpallial boundary [29]. The four subpallial domains are elongated along the septoamygdaloid axis, and they form at their ends composites known as the subpallial amygdala and the subpallial septum [29] (Fig. 3) The hypothalamus is divided into terminal (rostral) and peduncular (caudal) neuromeric portions, respectively continuous dorsally with the preoptic area (unevaginated telencephalon) and the evaginated telencephalic hemisphere [16] (hypothalamic prosomeres hp2 and hp1; Fig. 2a) . At this third level, the diencephalon is divided caudo-rostrally into three diencephalic prosomeres - the pretectum

(prosomere 1), the thalamus (prosomere 2) and the prethalamus (prosomere 3), including corresponding tegmental components [14, 16, 30, 31, 32] (Fig. 2a). Conventional ontologies assign the diencephalic tegmental territory to either midbrain or posterior hypothalamus. The diencephalic tegmentum contains rostral parts of the ventral tegmental area and substantia nigra, which are actually isthmo-meso-diencephalic plurisegmental entities [11, 16, 33, 34, 35].

Subsequent hierarchical levels of subdivision of the forebrain, midbrain and hindbrain are based on the well-known dorso-ventral regionalization of the neural tube into roof plate, alar plate, basal plate, and floor plate, introduced by His [36]. These longitudinal zones can now be more precisely defined by their differential molecular profiles. The longitudinal extent of the floor plate, ending at the mamillary floor, is marked by by genes such as Shh, Ntn1, and Lmx1b, though these markers are not unique to this zone [15]. The basal plate of the forebrain, midbrain and hindbrain is defined by the presence of either Nkx2.1 or Nkx6.1 [37]. At early developmental stages, expression of Pax genes (Pax6 in the forebrain; Pax 3 and Pax7 in the midbrain and hindbrain) broadly characterizes the alar plate [38, 39]. Genes of the Zic family are expressed at the dorsal part of the alar plate, next to the roof plate [40]. Consistent with a specific set of gene expression patterns (e.g., Nkx2.2, Ptc1, Gsh1), the developmental ontology includes a distinct liminal band at the ventral rim of the alar plate. The liminal region is defined as the junction of basal and alar plates.

This broad-brush picture of the developmental ontology is based upon the redefinition of brain organization suggested by the prosomeric model, which attempts to connect developmental discoveries with adult neuroanatomy across the whole brain. This picture needs more detailed explanation in three areas:

- the issue of the now obsolete columnar forebrain axis of Herrick [41]
- the definition of neuromeres
- the organization of the pallial and subpallial telencephalic territories

Herrick and the 'columnar' forebrain axis

Twentieth century ideas on forebrain subdivisions were dominated by the columnar model of the forebrain put forward by Herrick [41]. Contradicting the earlier analysis of His [42], Herrick proposed that the brainstem axis simply extends rostrally as a straight line into the forebrain, so that the thalamus/hypothalamus complex (then called the diencephalon) stands in direct axial continuity with the telencephalon rostrally and the midbrain caudally. This concept was not based on developmental findings, and it disregarded the ostensible cephalic flexure, present in all vertebrates. It was based instead on an ill-fated *ad hoc* attempt to extrapolate brainstem functional columns into the forebrain. The columnar hypothalamus was held to be exclusively basal (and connected directly with the midbrain tegmentum), matched with an exclusively alar thalamus. The columnar concept of forebrain organization has not stood the test of developmental gene expression patterns and related causal mechanisms, whereas these recent discoveries have strongly endorsed the earlier axial conception of His, in which the telencephalon is dorsal to the hypothalamus, and the diencephalon proper lies caudal to it [16, 42, 43, 44, 45, 56] (Fig. 2b).

This fundamental error has had a major impact in the interpretation of the mutual topographic relationships of the telencephalon, eyes, hypothalamus, and diencephalon by many authors [47, 48]. In the updated revised model of the forebrain that underpins our developmental ontology, the term diencephalon accordingly includes

only the prethalamus, thalamus, and pretectum, each with a piece of basal tegmentum, and excludes the hypothalamus (Fig. 2b).

Within the developmental ontology, the hypothalamus is divided first into a rostral part (terminal hypothalamus) and a caudal part (peduncular hypothalamus) [16] (Fig. 2a). The intrahypothalamic boundary separating these parts runs parallel to the subsequent course of the fornix tract; the boundary lies just rostral to the fornix and the neighboring medial forebrain bundle and lateral forebrain bundle (cerebral peduncle) (Fig. 2a). This boundary also separates the preoptic area from the diagonal region in the evaginated telencephalon, and also divides the mamillary and retromamillary areas one from another [16] (Fig. 2a). The evaginated eyes and surrounding supraoptic and suprachiasmatic/anterior-hypothalamic areas are all alar derivatives of the terminal hypothalamus, whereas the tuberal region, the attached neurohypophysis, and the mamillary bodies are corresponding basal derivatives (see [16]). The peduncular hypothalamus (marked by its role as 'bed' of the medial and lateral forebrain bundles and the fornix) contains the main part of the deep paraventricular nucleus and the radially migrated entopeduncular complex as its principal alar derivatives, and includes retrotuberal and retromamillary formations in its basal subregion. The basal subregion contains the subthalamic nucleus (a migrated retromamillary derivative), which was previously not considered to be a part of the hypothalamus [16, 49] (Fig. 2a).

Neuromeres

Periodic transverse outpouchings in the neural tube wall were first recognized over a century ago ([50], [51], [12]). Orr [51] called them neuromeres (prosomeres in the

prosencephalon, mesomeres in the midbrain, rhombomeres in the hindbrain), thus viewing them as neural segments within the general plan of head segmentation (Fig. 1d). The neuromere concept fell into disuse with the rise in popularity of Herrick's columnar paradigm, basically because brain segments did not offer at that time a recognizable functional significance. However, interest in neuromeres has returned in the molecular era because distinct molecular profiles characterize these developmental units and their fate-mapped derivatives, thus dispensing with the old myth that neuromeres were transient early phenomena. The molecular segmental scenario provides an opportunity for causal explanations of brain structure, an endeavour that completely collapsed with the old columnar theory. Moreover, physiologists have identified various examples of brainstem functional circuitry that relate to neuromeric compartments [52, 53]. In a similar way, functionally distinct prethalamic, thalamic, pretectal, and midbrain circuits are now recognized to be neuromerically organized, after their interpretation as longitudinal zones was abandoned. Any developmental ontology is therefore obliged to take account of these transverse components of the neuraxis.

The boundary between diencephalon and midbrain is marked by the caudal boundary of the forebrain expression of *Pax6* [54]. The boundary between midbrain and hindbrain is sharply defined by the interface of the expression territories of *Otx2* and *Gbx2* [55, 56]. The midbrain proper can be subdivided into rostral and caudal mesomeres (m1 and m2; Fig.1) [11, 57]. The existence of a thin m2 component of the midbrain, separating the isthmus from the inferior colliculus, was previously recognized by early embryologists [58]. Gene mapping studies support the existence of m2, defined by co-expression of *Otx2* and *Pax2* [59]. Its alar subregion has been called the pre-isthmic domain (which includes the nucleus sagulum, the cuneiform

gray, and subbrachial nucleus) and its basal structures include the retrorubral A8 catecholaminergic cells and the rostralmost part of the dorsal raphe nucleus [11, 12, 59] (Fig. 4).

The developing hindbrain is overtly segmented in its central part (r2-r6). Its rostral (isthmus, r1) and caudal (r7-r11) parts are not overtly segmented, but are differentially coded molecularly into cryptic neuromeric compartments [12, 25, 60, 61, 62] (Fig. 1d,e). The rostral hindbrain is influenced by the isthmic organizer [63, 64, 65] and can be divided into the isthmus and rhombomere 1, which contribute to the formation of the cerebellum in the same general way as the hypothalamus builds the telencephalon [65, 66, 67] (Fig. 4). The isthmus itself is defined by the early expression of *Fgf8* [24]. The remainder of the hindbrain is marked by the diversified expression of *Hox* genes and ephrins [67, 68, 69, 70, 71, 72].

Rhombomeres r2 to r6 are usually recognized as overt bulges separated by constrictions in the embryonic hindbrain [51, 67, 68, 72]. However, the boundaries of cryptorhombomeres r7 to r11 were distinguished in the chick on the basis of fate mapping and differential *Hox* gene expression, and the same subdivisions exist in the mouse [73]. In fact, the series of rhombomeres seems to be conserved among all vertebrates [74, 75].

These segments in the hindbrain are molecularly distinct developmental units [25, 68, 76, 77]. For example we can now recognize 11 neuromeric parts of the trigeminal sensory column across r1-r11. The vestibular column can be similarly subdivided segmentally across r1-r9. Raphe nuclei have been recently analysed in the rhombomeric context, identifying some 45 separable cell groups across the whole hindbrain [78]. Differential histogenetic behaviour due to regional changes in the

molecular identity of both progenitors and derived neurons correlates with the development of differentiated transverse blocks of hindbrain structure [25, 61, 67, 72]. Because of shared DV patterning, many rhombomeres contain similarly placed nuclei, and because early observable boundaries largely become invisible as development proceeds, such serial nuclei form plurineuromeric sensory columns, but the unique molecular identities and consequent differential hodologic or functional properties of individual segments often persist [52]. Fate-mapping has shown that the neuromeric developmental units are still separated by cryptic boundaries as the hindbrain matures [61, 67, 72] and therefore represent necessary conceptual levels for structural classification in any modern ontology.

Three major tangential migrations cause significant alterations to the anatomy of the hindbrain region. Pontine neurons migrate from the caudal rhombic lip (r6-r7) to the ventral surface of r3 and r4, where they form the pontine nuclei [72, 73, 78, 79, 80, 81, 82]. More caudal rhombic lip derivatives (r8-r11) migrate via an extramural tangential stream into the lateral reticular nucleus and external cuneate nucleus, and via an intramural stream into the inferior olive [61, 82, 83, 84]. The mammalian facial motor nucleus migrates from the medial part of r4 to reach its familiar superficial position in r6 [18, 85]. Recently, the interpeduncular nucleus has been shown to form by tangential migratory convergence of diverse alar and basal cell populations at the isthmic and r1 midline [86, 87].

The new developmentally based hindbrain ontology purposefully avoids using the classic subdivision into pons and medulla. The term 'pons' is particularly misleading

as a regional descriptor. The 'pons' of the human brain mistakenly refers to a region that apparently extends from the midbrain to the caudal border of the sixth rhombomere (caudal end of the facial nucleus). In fact, the basilar pontine formation arises from the rhombic lip in r6 and r7, and migrates to the ventral margin of r3 and r4 [28, 73, 82, 88]. In the mouse, the crossed pontocerebellar fibers of the middle cerebellar peduncle grow across r2 into the r1 entrance into the cerebellum, thus encircling the trigeminal root; this gives the impression that r2 also forms part of the 'pons'. The term 'pons' as a regional descriptor of a zone ventral to the cerebellum reaching from the midbrain to the medulla must therefore be abandoned, but the r3-r4 region can be called 'pontine hindbrain.' The word 'pons' *sensu stricto* can be properly applied to the nuclei and crossing fibres of the basilar pontine formation. Note that there is a substantial prepontine hindbrain, often misidentified as a caudal midbrain domain, which includes isthmus and rhombomeres 1 and 2. The trapezoid body and neighbouring trapezoid and superior olivary and periolivary nuclei of the auditory pathway, as well as the nucleus abducens, are strictly retropontine, being associated with r5. The pyramidal decussation lies in r11.

Pallium and subpallium

The telencephalic hemisphere clearly is not an axial vesicle, since there are two of them. It is formed by an idiosyncratic patterning mechanism that generates pallial and subpallial regions already at neural plate stages [89, 90, 91, 92]. The traditional columnar misconception that the longitudinal axis of the brain ends in the telencephalon has resulted in the widespread assumption that the embryonic pallium lies dorsal to the embryonic subpallium; in reality the pallium is topologically caudal to the subpallium, as shown conclusively by neural plate fate maps [16, 90, 91, 93].

The rostral end of the brain axial dimension is identified by landmarks in the roof, the floor, and the alar/basal boundary. The rostral end of the roof plate has been fate-mapped to the locus of the anterior commissure [16, 90]; the floor plate ends at the mamillary pouch [16]; the alar/basal boundary is continuous from left to right just under the suprachiasmatic nucleus [16]. These three points are equally rostralmost and can be traced molecularly from early neural plate stages onwards. The median wall interconnecting them builds the terminal wall of the neural tube. The telencephalon sits alongside the dorsal alar subregion of this wall, with the preoptic area extending into the bed of the anterior commissure. Differential gene expression defines the telencephalic pallio-subpallial boundary [93, 94, 95] (Fig. 2a). The presence of specific regional molecular profiles has profound histogenetic and functional consequences, with the subpallium becoming the almost exclusive source of inhibitory (GABAergic) telencephalic neurons [96], and the pallium becoming the main source of excitatory (glutamatergic) neurons [97, 98]. The GABAergic subpallial neurons that migrate tangentially into the developing pallium express *Dlx*, *Lhx6*, and various other genes [96].

The amniote pallium can be divided on anatomical and molecular grounds into septal, medial, dorsal, lateral, ventral, and amygdaloid zones [98]. In mammals the medial pallium forms hippocampal and parahippocampal structures; the dorsal pallium forms the neocortical mantle, and the lateral and ventral pallium are held to form the olfactory cortical structures with attached pallial claustroamygdaloid nuclei [98, 99]. The intimate relationship of the mammalian claustrum with the insular cortex is still not well understood. Non-mammalian vertebrates show the same pallial domains, but mammals are the only animals that have a fully developed neocortex [9, 100].

Further developmental ontologic subdivisions of each of the pallial and subpallial zones can be recognized [101]. The medial pallium differentiates into the diverse components of the hippocampal formation - dentate gyrus, CA fields, subiculum, presubiculum, parasubiculum, entorhinal cortex, and retrosplenial cortex [102]. The dorsal pallium includes the typical central group of neocortical areas (orbitofrontal, parietal, occipital, and temporal) and the mesocortical areas at its periphery (cingulate, perirhinal, periorbital, and insular cortex) [103]. The lateral pallium and ventral pallium each contribute to the olfactory areas, the insular claustrum, and the amygdala [99].

The subpallium is adjacent to the ventral pallium along the pallio-subpallial boundary. It is organized bilaterally as several bands of differentially specified radial histogenetic territories, stretching along the septoamygdaloid axis (Fig. 3). Each subpallial band has septal, paraseptal, central, and amygdaloid differentiated portions, wherein the major derivatives are the central ones (striatum, pallidum, diagonal-basal domain, and preoptic area; Fig. 3). The best-known paraseptal element is the striatal nucleus accumbens, which is complemented by analogous paraseptal parts within the pallidal, diagonal and preoptic zones. The concept of the extended amygdala [104] antedated the realization that some amygdaloid nuclei share properties with subpallial sectors beyond the apparent limits of the traditional amygdala, extending supra- and infracapsularly all the way to the septum. The present ontology is consistent with this view, with its medial (diagonal) and lateral (pallidal) portions of the stria terminalis complex and central amygdalod striatal elements [29]. The other major components of the diagonal-basal domain are the substantia innominata, the basal magnocellular nucleus, and the nuclei of the diagonal band. Ontological representation of these

regions is complicated by the fact that some classic anatomic entities (such as the septum and the amygdala) are made up of developmentally distinct sectors deriving respectively from the pallium, striatum, pallidum, and diagonal area, encompassing as well tangentially migrated cells derived from the alar hypothalamus and the preoptic area [21, 29, 97, 98].

Conclusion

A new brain ontology based on modern developmental criteria and models provides a more accurate and complete view of the natural morphologic inter-relationships of different parts of the brain. Neuronal populations have been classified on the basis of progressive rostrocaudal and dorsoventral differentiation of the neural tube. Radially migrated (stratified) derivatives can be traced from progenitor areas based on fate-mapping, gene mapping, and other experimental evidence. Overall, this demonstrates a consistent relationship between embryonic patterns and adult structure, which is expressed in the form of a developmental ontology. Because genomic control of neural morphogenesis is remarkably conservative, this ontology should prove to be essentially valid for all vertebrates, aiding terminological unification. The new ontology will undoubtedly be contested, and many details are still subject to revision that will be based on further discoveries (Box 1– The future of brain ontology).

Box 1 The future of brain ontology

- Is the new ontology flexible enough to incorporate future discoveries in gene expression during development?
- How will future changes to the ontology be moderated and approved for inclusion?
- Who will host the current ontology and its future derivatives?
- What are the barriers to extending the ontology to include all vertebrate classes?
- In cases where a neuron group migrates from its place of origin to another brain region, should it be classified according to the region of origin, the final location, or both?

GLOSSARY

Diencephalon - the caudal subdivision of the forebrain, that joins the midbrain to the secondary prosencephalon; it contains three major alar domains (pretectum, thalamus and prethalamus), as well as the corresponding tegmental regions.

Evo-devo – an approach to the analysis of brain structure based on the merging of concepts drawn from evolution and embryonic development

Hodology - the study of connections within the central nervous system ('odos' is Greek for a road)

Neuromeres - transverse unitary subdivisions of the neural tube that share a common dorsoventral structure (floor, basal, alar and roof plates), but have each differential molecular identities and fates; they make up the secondary prosencephalon, diencephalon (prosomeres), the midbrain (mesomeres), and the hindbrain (rhombomeres)

Ontogeny – Greek for the genesis of being: the process of development

Ontology - a formal conceptualization of the structure of a knowledge base, usually in the form of a hierarchical classification

Pallium - major subdivision of the telencephalon, usually visualized as covering and surrounding the subpallium; in mammals it gives rise to the cerebral cortex and a number of claustroamygdaloid pallial nuclei

Prosencephalon - Greek for forebrain: the part of the brain that appears at the rostral end of the neural tube

Secondary prosencephalon - the rostral major subdivision of the developing forebrain which separates from the diencephalon caudally (early in development both are encompassed within the primary prosencephalon); the secondary prosencephalon includes the telencephalon, the eye and the hypothalamus

Subpallium - a major subdivision of the telencephalon usually visualized topographically as lying under the pallium, at the brain 'base'; it generates the so-called basal ganglia, including the striatum, pallidum, diagonal-basal area, and preoptic area

Tagma - a meaningful higher level unit of biological structure, composed of segments which share a general character (e.g., the *Drosophila* thorax tagma as opposed to the abdominal tagma)

Telencephalon - a dorsal subdivision of the secondary prosencephalon that forms the pallium and subpallium

Topography - a system for describing and representing position relative to external references.

Topology - a system for describing relative position of the components of a structure irrespective of external references and any non-disruptive deformations; topology attends exclusively to the invariant neighbourhood relationships between the components

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FIGURE LEGENDS

Figure 1. A series of diagrams of lateral views of the developing mouse brain. The top diagram (A) shows the neural primordium (NP), which is a hollow tube with no subdivisions. In diagram B, the rostral (left) part of the neural tube shows the appearance of the forebrain (F), midbrain (M), and hindbrain vesicles (H), with the developing spinal cord (SpC) on the right. In diagram C, the forebrain vesicle has two divisions, the secondary prosencephalon (SP) and the diencephalon (D), and the hindbrain is divided into four regions – the prepontine hindbrain (PPH), the pontine hindbrain (PH), the pomtpmedullary hindbrain (PMH), and the medullary hindbrain (MH). In diagram D from the top, more subdivisions appear in the forebrain (caudal secondary prosencephalon – CSP or hp1; rostral secondary prosencephalon – RSP or hp2; prosomeres 1-3 of the diencephalon – p1, p2, and p3), midbrain (mesomere 1 – m1; mesomere 2 – m2), and hindbrain (isthmus – is; rhombomeres 1 to 11 – r1 to r11). In diagram E, some parts of the forebrain have become further differentiated: the caudal prosencephalon has formed the main part of the telencephalon; the rostral secondary prosencephalon has formed the preoptic telencephalon (POTel), the terminal hypothalamus (THy), and the peducular hypothalamus (PedHy)); and prosomeres 1 to 3 have formed the pretectum (Pt), thalamus (Th), and prethalamus (PTh), respectively. In this diagram, the diencephalon and midbrain are further subdivided by the alar/basal boundary, which bounds distinct tegmental regions (prethalamic tegmentum – PThTg; thalamic tegmentum – ThTg; pretectal tegmentum – PtTg; midbrain tegmentum – MTg; preisthmic tegmentum – PIsTg). The dorsal part of the midbrain is divided into the main midbrain tectum (MTt) and smaller preisthmic tectum (PIsTt). This figure was created by L Puelles for the Allen Brain

Institute (Allen Institute for Brain Science. ©2009, http://developingmouse.brain-map.org).

Figure 2

2a. A diagram of a lateral view of developing mouse brain at a stage later than the last element in Figure 1. The telencephalon is now divided into pallium and subpallial regions (striatum, pallidum, diagonal domain (Dg), and preoptic area). The septal roofplate (gray shading) extends from the telencephalic roof to the developing anterior commissure (ac). Within the terminal hypothalamus, the eye vesicle, the neurohypophysis, and the mamillary bodies (M) are differentiating. Within the peduncular hypothalamus the subthalamic nucleus (STh) is developing. The red line represents the alar/basal boundary, also in the midbrain and diencephalon. In the diencephalon this molecular boundary is for a short distance pulled to the diencephalic roof as the zona limitans (ZLi), which largely separates p2 and p3 at alar plate levels. The gray area above the cephalic flexure represents the most rostral area of Shh expression in the floor plate. The diagram shows that the developing substantia nigra extends rostrally from midbrain into the diencephalon. Other abbreviations are as in Figure 1.

2b. This is another version of the diagram 2a, to show the alar/basal boundary from spinal cord to rostral hypothalamus. The basal plate is colored green and the alar plate is colored pink.

Figure 3. A diagram to show the main subdivisions of the subpallium examined in a coronal plane. On the left are represented the four main histogenetic domains of the

subpallium are represented – striatum (Striat), pallidum (Pall), diagonal domain (Diag), and preoptic area (Preopt). These extend radially from the ventricle to the pial surface. On the right are shown.the secondary subdivisions of these domains along the septoamygdaloid axis. Each subpallial histogenetic domain shows diversely differentiated septal (Sept), paraseptal (Parasept), central, and amygdaloid regions (Amygd). This concept recapitulates and expands the idea of extended subpallial amygdala.