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Differential developmental strategies by Sonic hedgehog in thalamus and hypothalamus

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ABSTRACT

The traditional concept of diencephalon (thalamus plus hypothalamus) and with it the entire traditional subdivision of the developing neural tube are being challenged by novel insights obtained by mapping the expression of key developmental genes. A model in which the hypothalamus is placed in the most rostral portion of the neural tube, followed caudally by a diencephalon formed by prethalamus, thalamus and pretectum has been proposed. The adult thalamus and hypothalamus are quite unlike each other in connectivity and functions. Here we review work on the role of the secreted morphogen protein Sonic hedgehog (Shh) in the developing diencephalon and hypothalamic region to show how different these two regions are also from this point of view. Shh from the prechordal plate (PCP) induces and patterns the hypothalamus but there is no evidence that this role is fulfilled by a morphogen gradient. Later, the hypothalamic primordium itself expresses Shh and a large part of the hypothalamus belongs to the Shh lineage, including the ventral domains. Neural Shh is necessary to complete the specification (lateral hypothalamus), differentiation and growth of the hypothalamus. Although Gli2A is the major effector of Shh in this region, hypothalamic specification also depends on the suppression of Gli3R by Shh secreted by the PCP as well as the neuroepithelium. The thalamus is patterned by an Shh morphogen gradient originated in the ZLI following similar mechanisms to those in the spinal cord. The thalamus itself does not belong to the Shh lineage. Gli2A is necessary for appropriate growth and specification of the thalamic nuclei, to the exception of the medial and intralaminar groups (limbic-related), whose development depends on Gli3R. Beyond specification and patterning, the scarce data available about cell sorting and aggregation in these two regions shows key differences between them as well. In summary, not only expression patterns but also developmental mechanisms support a separation of the traditional thalamus and hypothalamus into different prosomeric domains.

1. Does thalamus plus hypothalamus equal diencephalon?

1.1. A new diencephalon based on a new view of development

Traditionally, "diencephalon" was the name of a region of the brain deemed to be precisely "between" telencephalon and midbrain, hence its name of *interbrain* (Swanson, 2012) or *Zwischenhirn* for German neuroanatomists. This traditional diencephalon comprises two large, complex and essential regions of the brain, the thalamus and the hypothalamus. This is a current nomenclature, taught to medical students, zoology students and budding neuroscientists alike. But "terms cannot be used outside a theory" (Jacobson, 1993), and the theory behind "diencephalon = thalamus + hypothalamus" has been called into question increasingly often in the last 20 years. This is the old theory by Herrick (1910), one of the founding fathers of Neuroanatomy, called sometimes "columnar theory" mainly by its detractors (see below). Herrick proposed that the neural tube, primordium of the central nervous system, ends rostrally in the telencephalon (the "endbrain", appropriately), which is followed caudally by the diencephalon (interbrain), then the mesencephalon (midbrain), the hindbrain and the spinal cord. The dorsal half of the neural tube at diencephalic levels would develop, according to this theory, into the thalamus and the ventral into the hypothalamus. The thalamus, according to external reference points, seems undoubtedly dorsal to the hypothalamus in the adult brain so that the traditional nomenclature seemed more a statement of the obvious than a theory. However, modern investigations of embryonic development with genetic markers have shown that the adult (or postnatal) nomenclature does not overlap with the presumptive embryonic regions (Puelles and Rubenstein, 2003). According to this notion, the hypothalamus, with dorsal and ventral portions,

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together with the telencephalon, are part of the most rostral portion of the neural tube, and the telencephalon is a sort of dorsal extension from it. Caudal to it, the prethalamus (the traditional ventral thalamus), then the thalamus (the traditional dorsal thalamus) and then the pretectum: together, these three structures form the new diencephalon, which does not include the hypothalamus anymore. The neural tube has to fold more or less like an "S" in order to fit neatly into the "new head" of the vertebrates, formed by repurposed neural crest derivatives (Gans and Northcutt, 1983; Jandzik et al., 2015). After the folding, having in mind external spatial references, prethalamus and thalamus seem to end up on top of the hypothalamus, and so originates the unfortunate confusion that gave rise to the traditional concept of diencephalon. In this view, the hypothalamus would be by itself an independent part of the forebrain, newly defined as telencephalon plus hypothalamus plus diencephalon (Puelles et al., 2012a, 2012b).

1.2. The diencephalon: A longitudinal continuum including the hypothalamus?

The longitudinal representation of the early brain (neural tube) and the novel nomenclature that springs from it offer conceptual advantages and also disadvantages. Emphasizing the morphological changes (epithelial folding, differential proliferation...) that transform the embryonic neural tube into the brain as an adult organ is certainly enlightening. After all, we do not imagine the embryonic kidney, pancreas, liver or heart as tiny, adult-shaped organs. Much on the contrary, we are well aware of the complex changes and interactions that turn early endodermal buds into functioning postnatal viscera like the pancreas and the liver (see for instance McCracken and Wells, 2012; Shin and Monga, 2013). For the same reason, we would not expect that such an intricate body part as the brain started developing essentially as a miniature adult brain (a sort of "brain preformationism").

On the other hand, interrupting the continuity of hypothalamus and midbrain that has usually been taken for granted in the traditional nomenclature has its problems also. Some accepted assumptions which depend on the physical continuity of the ventral midbrain with the hypothalamus do not fit well into the new neural tube geometry, among them the concept of the lateral hypothalamus as the rostral part of the reticular formation, or modern ideas about the development of the axonal framework of the brain (Croizier et al., 2014). Other major models of brain function, like the adult hypothalamus as a behavioral control column (Swanson, 1992, 2000) would in principle not be affected by the new nomenclature.

1.3. Toward one single nomenclature valid for embryo and postnatal brains?

Since very few early embryos ever go to see a doctor, clinicians and Medicine in general use the traditional brain nomenclature based on postnatal neuroanatomy. Modern neuroscience, to a high degree powered by a desire to understand the human brain and its diseases, has therefore until recently accepted this practice without question, as reflected by modern authoritative accounts of brain structure (see for instance Swanson, 2012). The strong push of molecular biology, however, as well as the increasing realization that many brain diseases (and other) have their origins in developmental problems are making the developmental nomenclature (not yet adopted by all scientists) and the "new" diencephalon that comes with it increasingly better known (Allen-Institute-for-Brain-Science, 2009; Thompson et al., 2014), to the point that collisions start to occur between authors and reviewers differentially oriented and trained. If the proposed new understanding of the neural tube is valid or not is not yet clear. As a matter of fact, using the same genetic markers other authors reach different conclusions, and there is often a certain confusion between classical and novel assumptions (see for instance Shimogori et al., 2010). Can we have a single description of the brain valid for evolution, development and clinic? If and when this will happen, and if it would be useful or desirable, is still open.

2. Sonic hedgehog underlines differences between the developing thalamus and hypothalamus

Remarkably, as expression patterns suggest a new understanding of the neural tube and its subdivisions, current analysis of developmental mechanisms suggests that thalamus and hypothalamus are indeed better understood as separate entities. Perhaps the best example is that of morphogen protein Sonic hedgehog (Shh), one of several secreted factors crucial for the specification of the forebrain. During forebrain development, Shh is secreted first by the axial mesoderm (notochord and prechordal plate or PCP), and it then induces expression of its own gene in the overlying ventral neural tube. Shh secretion by the PCP is required for hypothalamus specification, and, later, most of the hypothalamic primordium expresses Shh. In this way, large areas of the adult hypothalamus are of Shh lineage. Shh from the *zona limitans intra-thalamica* or rather *inter-thalamica* (ZLI) specifies and patterns the thalamus following the morphogen gradient model, which has been worked out in detail for the spinal cord. This is not the case at all in the hypothalamus. Additionally, the thalamus itself does not belong to the Shh lineage. In this way, developmental mechanisms show clear signs of essential differences between thalamus and hypothalamus which are reflected in the quite different connectivity and functions of these regions in the adult. The role of Shh in the development of these regions has been often reviewed, see for instance (Epstein, 2012; Blaess et al., 2014).

2.1. Shh and Gli factors

A very short reminder of some key facts relative to Shh function seems in order before we proceed. The Shh gradient is detected by a receptor complex in the cell membrane formed by transmembrane protein Patched and some coreceptors; as a result, Patched is inactivated and protein Smoothened is derepressed (Stone et al., 1996; Taipale et al., 2002; Izzi et al., 2011). Activation of Smoothened translates into regulation of the activity of the Gli transcription factors Gli1, Gli2, and Gli3 (Hui et al., 1994; Lee et al., 1997; Ruiz i Altaba, 1998) which can work as either transcriptional activators or repressors, their exact function depending on body organ, region of the brain or animal species. In mouse neural tube, Gli2 is the required Shh-dependent activator (Mo et al., 1997; Ding et al., 1998; Matise et al., 1998; Motoyama et al., 1998), Gli1 is an activator dispensable for neural development (Park et al., 2000; Bai and Joyner, 2001) and Gli3 has a "full" activator form (Gli3A) and a "truncated" repressor form (Gli3R). (In Fishes as well as Amphibians the roles of Gli2 and Gli1 are reversed Ruiz i Altaba, 1998; Mullor et al., 2001; Karlstrom et al., 2003). In the mouse, cells competent to respond to Shh express Gli2 and Gli3, which, upon Shh signaling become functionally activated and activate transcription of the *Gli1* gene (Dai et al., 1999; Sasaki et al., 1999; Park et al., 2000).

The formation of Gli3R is repressed by Shh (Bai and Joyner, 2001; Bai et al., 2002, 2004; Nguyen et al., 2005; Stamataki et al., 2005; Tyurina et al., 2005), so that the Shh gradient creates in competent tissues an opposite gradient of Gli3R (Persson et al., 2002; Bai et al., 2004). This translates in the developing neural tube into two opposite gradients of transcriptional regulators which compete to either activate or repress Shh targets (Jacob and Briscoe, 2003; Ruiz i Altaba et al., 2007).