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Pallial expression of *Enc1* RNA in postnatal mouse telencephalon

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Abstract

We analysed the pallial expression pattern of *Enc1* (a member of the *kelch* family of genes) in postnatal mice (P1–P10). At early developmental stages this gene plays a role in the histogenesis of cortical structures [M.C. Hernández, P.J. Andrés-Barquin, S. Martínez, A. Bulfone, J.L.R. Rubenstein, M.A. Israel, *Enc1*: novel mammalian *kelch*-related gene specifically expressed in the nervous system encodes an actino-binding protein, J. Neurosci. 17 (1997) 3038–3051]. A restricted expression of *Enc1* was found in the mouse pallium, notably within claustroamygdaloid derivatives of the lateral pallium and in some cortical layers in the lateral, dorsal and medial pallium sectors, with distinct regional differences. The strongest cortical expression was found in isocortical layer II and in the piriform cortex, anterior olfactory area and olfactory bulb mitral cells. The lowest signal occurred in the retrosplenial cortex. The subgranular layers V/VI were also positive, particularly layer V, with clearcut areal differences. The hippocampal CA3/CA4 areas and the dentate gyrus were strongly positive. The dorsolateral (core) portion of the claustrum and dorsal endopiriform nucleus were moderately positive, as were the amygdaloid lateral and basolateral nuclei.

Keywords: Cerebral cortex; Cortical areas; Lateral pallium; Dorsal pallium; Medial pallium; Claustrum; Amygdala

1. Introduction

The major part of the mammalian pallium develops as a layered neuronal structure, the cerebral cortex (subdivided into differentiated iso- and allocortical sectors [2,10], and these into a number of areas), but the pallium encloses also a number of underlying claustroamygdaloid nuclear derivatives [8,3,5] (we leave aside here the tangential migration of subpallial GABAergic neurons into the pallium). Every cortical area and pallial nucleus is characterized by its own cytoarchitecture, connections and function. The development of this complexity is controlled by genetic patterning acting already upon the proliferating pallial progenitors [9,6], and is modulated later in development by added epigenetic effects (i.e., migration of immature neurons into specific layers, development of thalamic or cortical afferent inputs [7,11]). Gene markers that appear restricted to specific areas, layers or nuclei within the pallium are of interest in studies of the patterning mechanisms that lead to pallial differentiation in mammals. Preliminary data indicated a parcellated pallial expression of the gene *Enc1* in mouse cerebral cortex, motivating us to analyse in detail its expression at postnatal stages.

The *Enc1* gene (Ectoderm and Neural-Cortex 1) is a member of the *kelch* family, and encodes a cytoplasmic protein that interacts with actin [14,4]. This product is present in mouse telencephalon already at early stages of development, where

Abbreviations: Acb, accumbens nucleus; Aco, anterior cortical amygdaloid nucleus; AO, anterior olfactory nucleus; BLA, basolateral amygdaloid nucleus, anterior part; Bol, olfactory bulb; BLP, basolateral amygdaloid nucleus, posterior part; BST, bed nucleus of the stria terminalis; CA1–4, fields 1–4 of Ammon's horn; cc, corpus callosum; Cig, cingulate cortex; Cl, claustrum; CPu, caudate-putamen; DG, dentate gyrus; DP, dorsal peduncular cortex; Ent, entorhinal cortex; EP, endopiriform nucleus; FL, cortical sensory representation of the forelimb; Fr, frontal cortex; GP, globus pallidus; HL, cortical sensory representation of the hindlimb; IG, indusium griseum; IL, infralimbic cortex; Ins, insular cortex; La, lateral amygdaloid nucleus; LOT, nucleus of the lateral olfactory tract; LSD, lateral septal nucleus, dorsal part; LSI, lateral septal nucleus, intermediate part; LSV, lateral septal nucleus, ventral part; Occ, occipital cortex; Par, parietal cortex; Pir, piriform cortex; Po, posterior thalamic nuclear group; Prh, perirhinal cortex; RSA, retrosplenial agranular cortex; RSG, retrosplenial granular cortex; SI, innominate substance; Su, subiculum; Te, temporal cortex; TT, taenia tecta; Tu, olfactory tubercle; VMH, ventromedial hypothalamic nucleus; VPM, ventral posteromedial thalamic nucleus; ZID, zona incerta, dorsal part

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it was suggested to play a role in the development of cortical structures [4].

2. Materials and methods

The animals were treated according to the regulations and laws of the European Union (86/609/EEC) and the Spanish Government (Royal Decree 223/1998) for care and handling of animals in research. Mice sacrificed at postnatal days 1, 2, 4 and 10 (P1–P10) were used in the present study. The animals were anesthetized by cold and perfused transcardially with 4% paraformaldehyde in standard phosphate-buffered saline solution (PBS). The brains were postfixed overnight in buffered 4% paraformaldehyde. Afterwards, they were embedded in 4% agarose and 120 μm thick vibratome sections were obtained. For the *Enc1* riboprobe preparation, we digested a 2390 bp fragment subcloned in plasmid p2X-clone10. Digestion was achieved with XhoI, and antisense riboprobe was synthetized with T7 polymerase (Roche), in presence of digoxigenin-11-UTP. Purification of the probes was achieved using Quick Spin Columns (Roche). The hybridization of the floating thick sections followed the protocol of Shimamura [12].

3. Results

3.1. Enc1 expression pattern in mouse cerebral cortex at P4

Enc1 is widely expressed in the pallium, as well as in the striatum. The horizontal section illustrated in Fig. 1 shows intense signal in layers II/III of the isocortex changing abruptly into low signal at the boundary of temporal cortex with the entorhinal cortex (Fig. 1). Deeper in the isocortex, layer V stands out by its strong signal, showing distinct regional boundaries between cingulate, frontal, parietal and temporal cortical sectors (Fig. 1). The rest of the isocortical primordium diffusely displays moderate *Enc1* expression. The entorhinal cortex shows moderate to strong signal in its innermost layer, which continues into the subiculum and hippocampus. The latter has the strongest expression in the CA3/CA4 fields and in the dentate gyrus (Fig. 1). The superficial layers of the pre/parasubiculum are selectively strongly positive (Fig. 1).

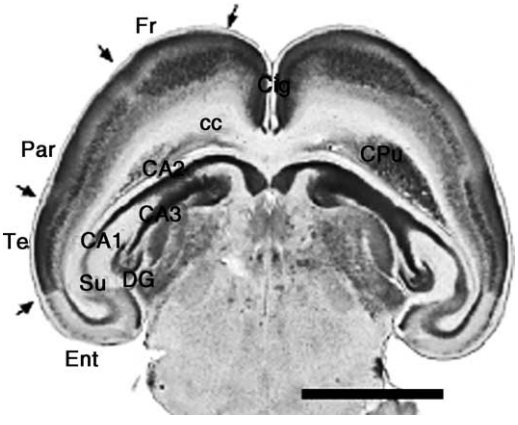

Fig. 1. Horizontal section through the telencephalon of a P4 mouse, hybridized for *Enc1*. Intense signal can be observed in layers II/III and V of the isocortex, with clearcut regional differences, particularly in layer V. The boundary between temporal and entorhinal cortex is also distinct, as are the diverse hippocampal subregions. Note moderate expression in the caudoputamen. Bar = 2 mm.

3.2. Enc1 expression pattern in mouse cerebral cortex at P10

In general, the background *Enc1* signal level has decreased relative to that observed in P4 brains (Fig. 2), and signal has practically disappeared in the striatum, except in the rostral part of nucleus accumbens, islands of Calleja and the olfactory tuberculum (Fig. 2B and C). Intense signal remains in layers II/III in most of the isocortex, though it decreases considerably in medial parts of the cortex. The piriform cortex, anterior olfactory nucleus and olfactory bulb mitral cell layer are also strongly positive (Fig. 2A–F). In frontal cortex, moderate *Enc1* expression is still detected in layer V, which presents subtle areal differences probably related to primary or secondary subdivisions of the motor cortex. Layer VI has a weaker signal, and the subplate is also positive (Fig. 2A–D).

In the parietal and temporal cortex, layer V is thinner than in frontal cortex, whereas layer VI tends to be more developed and subdivided into superficial and deep parts (Fig. 2B–F). The *Enc1* expression in the subplate is as intense as in layer V. Both layer IV and layer I are negative for *Enc1*, as in the frontal cortex. We also found subtle differences in *Enc1* expression possibly corresponding to the cortical sensory representation of the fore- and hindlimb. In the occipital cortex, the primary and secondary visual areas can be distinguished (not shown). The *Enc1* domain presents in this region a distinct signal in layer V, which is thinner than in parietal cortex (Fig. 2E,F). In the cingular cortex, the *Enc1* expression appears strongly in a broad layer V, as well as in layer VI, which has a weaker signal. The transition into the retrosplenial cortex, is accompanied by a marked decrement in the expression level in both supragranular and infragranular layers (Fig. 2C–F).

In the olfactory cortex, the *Enc1* signal is intense in layers II and III (Fig. 2B–F). The hippocampal cortex keeps at P10 the expression pattern described at P4 (Fig. 2D–F).

Finally, we examined the transitional mesocortical areas. On one hand, the infralimbic cortex, indusium griseum, dorsal peduncular cortex and taenia tecta had an *Enc1* expression similar to that in the cingular cortex, but with less development of each layer (Fig. 2). On the other hand, the insular, perirhinal and entorhinal cortex showed *Enc1* transcripts in layer II, as well as in a deep layer apparently representing fused layers V/VI (Fig. 2).