Transcription factor DLX6 is selectively expressed in the fetal human central amygdaloid nucleus

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Introduction

Amygdala represents a key component of the limbic system involved in complex patterns of social behavior. Nevertheless, studies on its prenatal development are very scarce in humans. In our previous work we have demonstrated a human specific transient modular organization of this structure during midfetal period (Nikolic & Kostović, Anat Embryol 1986).

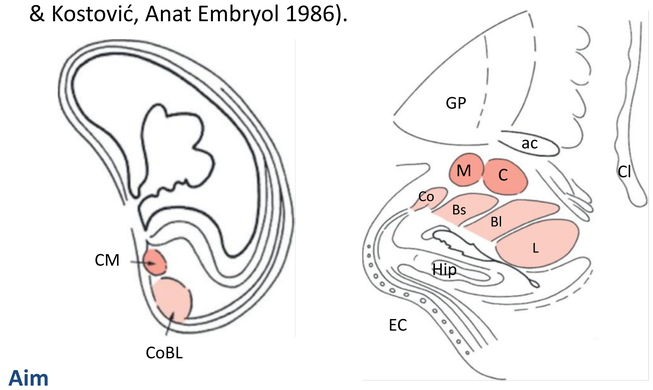

Fig. 1: Development of the human amygdala represents a complex process of anatomical and structural rearranement in assoiciation with reorganizational processes of the developing human brain. The two main divisions, (cortico)basolateral and centromedial, have a diverse developmental origin (pallial and subpallial) and distinct functional roles.
CM-centromedial nucleus, CoBL-corticobasolateral n., M-medial n., C-central n., Co-cortical n., Bs-basal n., Bl-basolateral n., L-lateral, Hip-hippocampus, EC-enthorinnal cortex, GP-globus pallidus, ac-anterior commisure, Cl-claustrum

HJ ten Donkelaar, 2015.

Aim

We aimed to investigate possible indicators of the molecular mechanisms that underlie regional differences in maturational processes between various amygdaloid nuclei. Using publicly available gene expression database of human brain (Kang et al., Nature 2011), we selected several genes which are highly expressed prenatally in human amygdala. One of them is a transcription factor *Dlx6 (Fig. 2)*, previously shown to be required for molecular properties of the striatum, nucleus accumbens, olfactory tubercle and central nucleus of the amygdala in the developing mouse brain (Wang et al., J Comp Neurol. 2011).

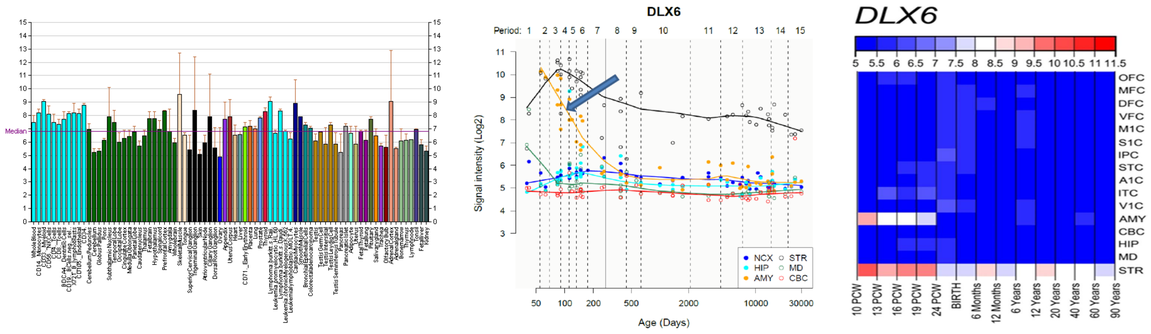

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Fig. 2: Data showing the expression of a homeobox transcription factor gene similar to the Drosophila distal-less gene (DLX – 6). This family has at least six members that encode proteins with roles in forebrain and craniofacial development.
(a) RNA expression pattern of DLX 6 in human tissue (www.biogps.org)
(b) Exon array signal intensity with an arrow pointing to amygdala dataset (Kang et al, 2011)
(c) Heatmap of log2 intensity for DLX6 exon from 10PCW to 90 years of age. It shows a specific DLX6 gene expression in striatum and amygdala.

Materials and methods

To study DLX6 expression we employed immunohistochemistry on fixed-paraffin-embedded sections of postmortem human brains, ranging between 15th and 28th post conception weeks (Fig 3). To confirm our findings we investigated coexpression of DLX6 with NeuN and GFAP respectivly, using a double-immunofluorescence approach (Fig 4).

The procedure for the human autopsy material was approved and controlled by the Internal Review Board of the Ethical Committee at the School of Medicine, University of Zagreb.

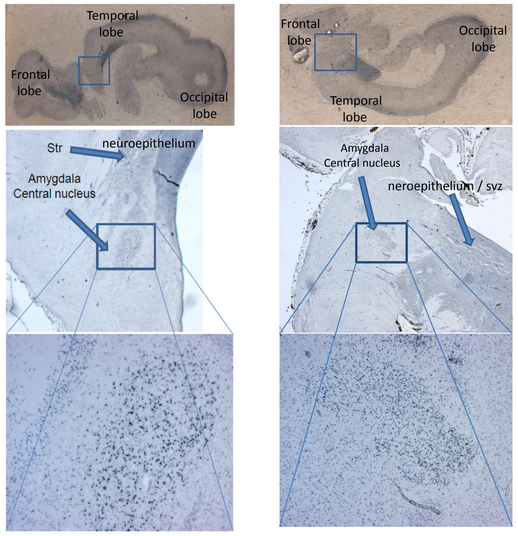

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Fig. 3: Immunohistochemistry on fixed-paraffin-embedded sections of postmortem human brains, 15 (a) and 20 (b) post conception weeks (PCW) old. Expression of DLX6 was visible in the amygdaloid primordium of the youngest specimen examined, in the prospective central nucleus, revealing intense nuclear staining. Using adjacent Nissl preparations, we found that DLX6+ cells correspond well with neurons displaying advanced differentiation of the nuclei (dispersed chromatin).

Results

Our results indicate that DLX6 could be a part of the regulatory molecular program of amygdaloid regionalization, presumably playing an important role in early differentiation of the central amygdaloid nucleus. Since this nucleus represents the main efferent structure, its advanced maturation provides evidence of very early establishment of amygdaloid visceromotor circuitry.

REFERENCES:

1. Nikolic I, Kostovic I. Development of the lateral amygdaloid nucleus in the human fetus: transient presence of discrete cytoarchitectonic units. Anat Embryol (Berl). 1986.
2. Kang et al. Spatio-temporal transcriptome of the human brain. Nature. 2011.
3. Wang et al. *Dlx6* Regulates Molecular Properties of the Striatum and Central Nucleus of the Amygdala. J Comp Neurol. 2011.

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