showed greater activation in P3b. This may be due to the later maturation of the frontal cortex than the more posterior sites. The children may have used strategies and/or brain areas different from those of the young adults to prepare for stimuli and responses.

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[P1.15]

Development of focused attention in visual search experiments

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Visual search implicates the use of focused attention in order to discriminate a target between distractors which share some visual attributes. In pop-out type of experiments the search for the target occurs in a parallel form, and requires less attentional resources. In fact pop-out search can be considered as a parallel search based on a single attribute. Present research tries to define how parallel and sequential search evolves with age in children.

The sample consists of 69 subjects aged between 6 and 16 years of age, and a mean equal to 9.8. The group was composed by 38 girls and 31 boys. Visual display with 2, 4 and 6 items were presented. Distractors differ in one attribute from target in pop-out while in visual search they differ in two attributes.

The results show that the difference in reaction times between ages is minimal in parallel search and increases in serial search. Paradoxically, the slope for the attentional sequential search defining the time for scanning one object (RTs. vs. number of distractors) was less pronounced in children than in adolescents. However, there is a greater difference when comparing the ages with the number of hits. At middle ages (10 years old) the number of hits increases dramatically.

We conclude that young children have more difficulty in processing information presented when focused attention is required than adolescents, and they show much more errors. Maturation of this function as shown by visual search should occur around 10 years, while pop-out seems to occur earlier.

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[P1.16]

ADF/Cofilin is necessary for neuritogenesis in the developing mammalian brain

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Keywords: Neuritogenesis; Actin; Cytoskeleton; Cofilin

During brain development, initially spherical neurons undergo drastic morphological changes to become the complex, highly branched units of neuronal networks. Studies in cultured neurons and invertebrates have identified signalling proteins that modulate the actin cytoskeleton during neuronal development. However, the physiological role of many of these proteins during neuronal development in mammals and their precise effects on the neuronal cytoskeleton remain to be addressed. The F-actin severing and depolymerizing, ADF/cofilin proteins (AC proteins) have been implicated in regulating various phases of neuronal development. In this study, we analyzed the functional consequences of brain-specific genetic ablation of ADF and cofilin during in vivo development and in regulating actin dynamics during neurite formation. The ablation of AC proteins resulted in several abnormalities in the developing brain including a striking decrease in cortical mass with corresponding increases in ventricle volume and hydrocephaly, anomalous cortical ectopias, and a severe reduction in axonal tract formation. In line with these findings, AC knockout neurons showed a failure of neuritogenesis. Cytoskeletal aberrations including increased F-actin, absence of radial actin bundles and filopodia, and irregular looping microtubules in AC KO neurons underlie the failure of neurite initiation. Furthermore, AC KO neurons displayed a complete immobilization of F-actin retrograde flow, indicating that AC proteins are the primary driving force underpinning actin turnover. The exogenous expression of cofilin fully restores neuritogenesis in AC KO neurons, whereas ADF only partially rescues neurites. Using specific activity blocking mutants, we found that although both the F-actin depolymerizing and severing activities of cofilin are necessary for optimal neurite initiation, the severing activity is of greater consequence for neuritogenesis. Taken together, these data suggest that AC proteins regulate neuritogenesis and neural patterning during cortical development.

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[P1.17]

Cortico-cerebral histogenesis in the opossum Monodelphis domestica: Generation of a hexalaminar neocortex in the absence of a basal proliferative compartment

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Keywords: Monodelphis domestica; Cortico-cerebral neuronogenesis; Basal precursor; Tbr2

The Metatherian Monodelphis domestica, commonly known as South-American short-tailed opossum, is an appealing animal model for developmental studies on cortico-cerebral development. Given its phylogenetic position, it can help tracing evolutionary origins of key traits peculiar to the eutherian central nervous system. The capability of its pup to regenerate damaged cortico-spinal connections makes it an ideal substrate for regenerative studies. Recent sequencing of its genome and ex-utero accessibility of its developing cerebral cortex further enhance its experimental interest. However, at the moment, a comprehensive cellular and molecular characterization of its cortical development is missing.

A variety of approaches and techniques was used to study opossum cortico-cerebral development. Neocortical lamination was investigated by combined BrdU pulse-chase analysis and time-course profiling of selected laminar markers. Generation of cortical projection neurons was assayed by systematic and integrated immunoprofiling of pallial precursors for M-phase/S-phase markers as well as for apical/basal markers. GABAergic neuronogenesis was studied by BrdU/IdU pulse-chase profiling of telencephalic explants and living brains.

It resulted from this study that opossum projection neurons and interneurons are generated by pallial and subpallial precursors, respectively, similarly to Rodents. A six-layered cortex with a eutherian-like molecular profile is laid down, according to the inside-out rule. However, neocortical projection neurons are generated by apical neural precursors and almost no basal progenitors may be found in the neuronogenic neopallial primordium. In the opossum neocortex, Tbr2, i.e. the hallmark of Eutherian basal progenitors, is transiently expressed by postmitotic progenies of apical precursors, prior to the activation of more mature neuronal markers.
The neocortical developmental program predates the Eutherian–Methatherian branching. However in Metatherians, differently from Eutherians, a basal proliferative compartment is not needed for the formation of a six-layered neuronal blueprint.

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[P1.18]

Inhibition of gliogenesis and promotion of neuronogenesis by patterned overexpression of Emx2 and Foxg1

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Keywords: Cortico-cerebral precursor; Emx2; Foxg1; Gliogenesis

Neural stem cells (NSCs) give rise to all cell types forming the cortex, neurons, astrocytes and oligodendrocytes. The transition from the former to the latter ones takes place via lineage-restricted progenitors and is mastered by large sets of genes, among which some implicated in CNS pattern formation. Aim of this study was to disentangle the kinetic and histogenetic roles exerted by two of these genes, Emx2 and Foxg1, in cortico-cerebral precursors.

An integrated in vitro assay design was set up for this analysis. Early embryonic cortical progenitors were trasduced with lentiviral vectors driving overexpression of Emx2 and Foxg1 in NSCs or neuronal progenitors (NPs) and were kept under pro-proliferative or pro-differentiative culture conditions for different times. Cells belonging to different neurogenic and gliogenic compartments were labeled by spectrally distinguishable fluoroproteins, driven by cell-type-specific promoters, as well as by cell-type-specific antibodies. They were subsequently scored, via multiplex cytofluorometry and immuno-cytofluorescence. Finally, these conditional gain-of-function assays were complemented by similar (immuno)fluorocytometric profiling of neural cultures constitutively loss-of-function for each of these two genes.

A detailed picture of Emx2 and Foxg1 activities in cortico-cerebral histogenesis resulted from this study. We found that Foxg1 inhibits gliogenic commitment of neural stem cells. Moreover, it augments their neuronal output, by inhibiting them and neuronally committed progenitors from leaving cell cycle. Finally, the same gene, when over expressed in neuronal progenitors, further stimulates neurite outgrowth which subsequently occurs in their post-mitotic progenies. Conversely, Emx2, while early promoting neural stem cell self-renewal, later strongly pushes these cells to differentiate, along both the neurogenic and the gliogenic lineages.

Patterned overexpression of Foxg1 and/or Emx2 may be exploited to substantially ameliorate the absolute and relative neuronal outputs obtainable from neural cultures, for purposes of cell-based brain repair.

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[P1.19]

Noggin elicits retinal fate in Xenopus animal cap embryonic stem cells

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Keywords: Noggin; Embryonic stem cells; Neural differentiation; Retina

Noggin, a small secreted protein, can induce ectoderm to form neural tissue. The animal cap tissue of the blastula stage Xenopus embryo consists of a few layers of ectodermal cells, which are pluripotent and have been used as an indicator of tissue differentiation to determine factors causing inductive events. We exploited the potential of noggin to drive multipotent Xenopus animal cap embryonic stem (ACES) cells toward retinal cell fates. Using RT-PCR, in situ hybridization and immunohistochemistry, we found that different doses of noggin have different effects on the expression, in cultured ACES cells, of terminal differentiation markers of specific retinal cell types. ACES cells expressing high doses of noggin efficiently induce the expression of retinal differentiation markers, and, following in vivo transplant, form a retina both in the presumptive eye field and in ectopic posterior regions. By contrast, ACES cells expressing low doses of noggin induce retina formation only in a low percentage of cases. The eyes originating from the transplants in the eye field region are functionally equivalent to normal eyes, as revealed by electrophysiology and c-fos expression in response to light.

Our studies show that in Xenopus embryos, appropriate doses of a single secreted molecule, Noggin, can drive ACES cells toward retinal cell differentiation without additional cues. This makes Xenopus ACES cells a model system to direct differentiation of stem cells toward retinal fates and encourages further studies on the role of Noggin in the retinal differentiation of mammalian stem cells for promoting retinal regeneration.

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[P1.20]

Activation of Fezf2 by toll-like receptor 4 (TLR4) signalling in neural progenitor cells

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Keywords: Fezf2; TLR4 signalling; Neural progenitor cell

Neural progenitor cells (NPCs) have been extensively studied for their potential to protect and/or replace cells lost in neurological disorders. Fezf2, a transcription factor required for the development of populations of subcerebral projection neurons (Molynieux et al., 2005), was recently shown to be regulated by the immune signalling pathway activated by the toll-like receptor (TLR4) in mammary cells (Sugimoto et al., 2006). In this study we tested whether Fezf2 also responds to TLR4 activation in neural progenitor cells.

The expression of TLR4 signalling components in NT2 cells was analysed using reverse transcriptase PCR, western blotting and confocal microscopy. The functional response to TLR4 signalling was tested using the TLR4 agonist lipopolysaccharide (LPS; 0.02 – 10 μg/mL). Translocation of NFκB to the nucleus, and IRF3 phosphorylation were analysed by western blot. Induction of IFNβ and TNFα were determined by RT-PCR. HMGB1 response (nuclear export) was