Review

Neuro-archaeology: pre-symptomatic architecture and signature of neurological disorders

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During brain development cells divide, differentiate and migrate to their assigned targets to form synapses and active cell assemblies. This sequence is controlled both by genetic programs and environmental factors. Alterations of this sequence by mutations or environmental insults leads to the formation of misconnected circuits endowed with a 'pre-symptomatic signature'. I propose here that early- and late-onset neurological disorders as diverse as infantile epilepsies, mental retardation, dyslexia or, in certain conditions, even Huntington's and Alzheimer's disease might be, in part, born at early developmental stages before symptoms appear. The core of this working hypothesis is that imaging or non-invasive recordings might unravel signatures of disorders to come, thereby permitting earlier diagnosis and potential treatment of neurological disorders.

We do not yet understand how genetic mutations lead to neurological disorders and why the delay in onset of phenotypes is so variable (years to decades). At present, the dominant view is that either disrupted proteins are unnecessary at earlier stages (hence the lack of a phenotype) or that time is needed to enable a sufficient accumulation of toxic processes or molecules that leads to a phenotype when a threshold is attained. However, this view does not take into account the fact that the developing brain is not a small adult brain. Developmental neurobiology has taught us that immature neurons and networks express molecules and processes that are not operative in adults. They also follow a crucial developmental sequence that is instrumental in the formation of functional entities. Early insults that retard or accelerate this developmental program and are not necessarily associated with cell loss as in adults might, nevertheless, have an architectural or electrical signature that announces disorders to come well before clinical symptoms appear.

I suggest that many neurological disorders are 'born' *in utero* or at an early developmental stage. Neurons that fail to migrate, to develop and/or to connect properly with their assigned targets establish aberrant connections between structures that would not normally be connected. Misplaced neuronal ensembles remain 'frozen' in an immature excitable state and the pathogenic patterns and oscillations they generate propagate to distal sites and entrain a cascade of events perturbing the construction of functional units. The consequences of these alterations are not expressed immediately but require a maturation period before the phenotype appears. This neuro-archaeology working hypothesis is organized around pre-symptomatic signatures that announce disorders to come by means of structural and/or electrical signals. Experimental and clinical evidence in favour of these pre-symptomatic signatures are already available for developmental disorders associated with cortical malformations including infantile epilepsies, autism, Rett syndrome, dyslexia, certain types of mental retardation and so on. More surprisingly, there are indications that disorders not classically associated with early defective wiring such as Huntington's disease and severe genetic forms of Alzheimer's disease might also include a developmental component. The proposed scheme neither encompasses all neurological disorders (many are clearly triggered later in life after various insults) nor implies that all early insults need a long 'incubation time'. However, it does call for re-examining neurological disorders taking into account the specificity of language and timing of developing networks and the need to incorporate knowledge provided by developmental neurobiology.

The developing brain is talkative but uses a different language to adults

In contrast to one widely held assumption, no developmental stage is fully automated and even undifferentiated cells are not mute. Rather, they are endowed from the earliest developmental stages with functional communication strategies (Figure 1). Contrary to the construction of a machine, for example, the brain is active during its construction. It must generate the appropriate cellular and network patterns that provide the signals needed for development, while avoiding an imbalance between excitation and inhibition that would lead to toxicity or seizures. Undifferentiated cells express voltage- and transmitter-gated channels according to an innate expression timeline [1]. During the proliferation stage, mouse embryonic and undifferentiated progenitors in the sub-ventricular zone possess the machinery to synthesize and respond to γ-aminobutyric acid (GABA) [2–4]. During migration, neurons express functional voltage-gated calcium, GABA and *N*-methyl-D-aspartic acid (NMDA) receptor channels well before synapses are formed, which enables a paracrine modulation of neuronal migration [5,6]. The process of synapse formation starts *in utero*

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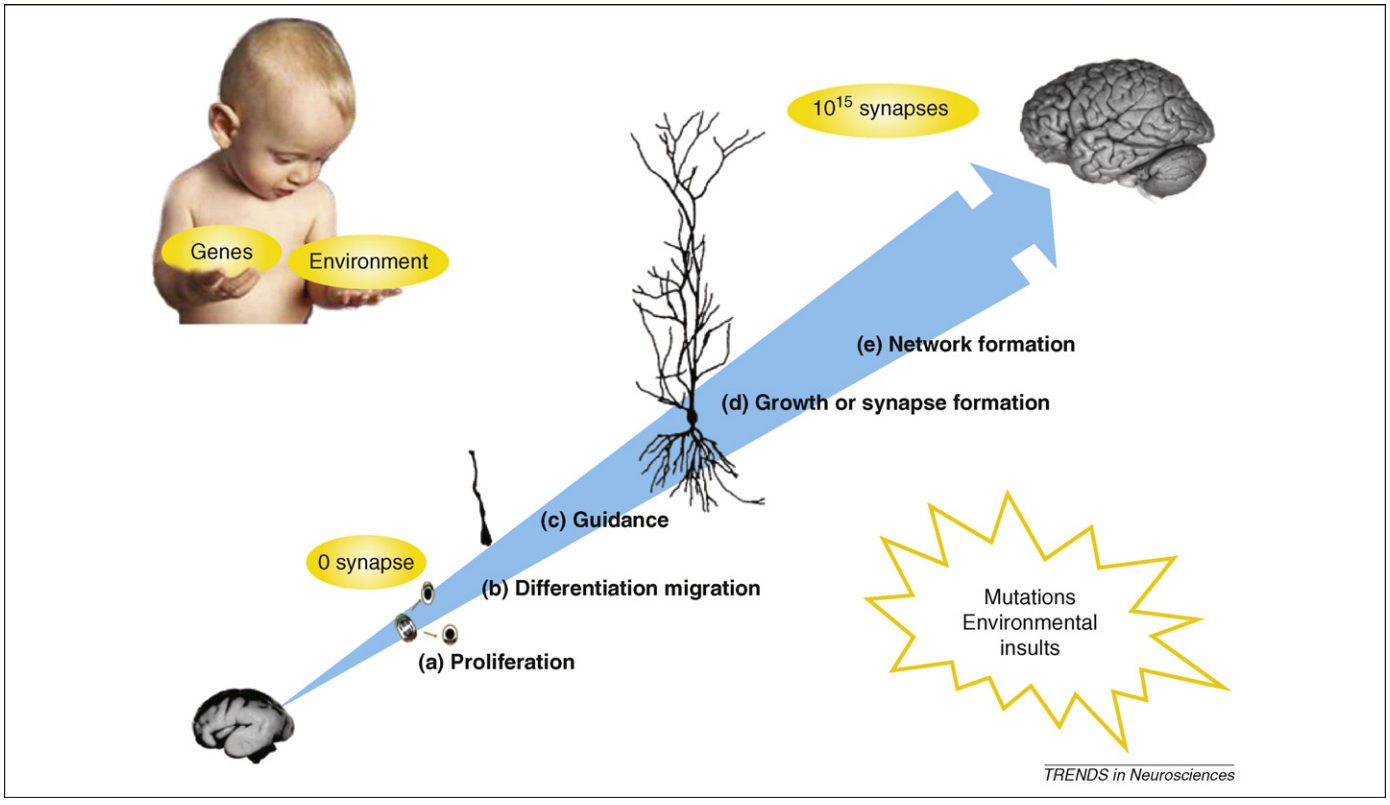

Figure 1. Schematic illustration to depict the impact of the environment and genetic mutations on all developmental stages. **(a)** Proliferation, **(b)** differentiation and migration, **(c)** guidance, **(d)** growth or synapse formation and **(e)** network elaboration are modulated by genetic and environmental factors. Alterations of these steps due to mutations and/or environmental factors can lead to developmental-stage-dependent malformations that will be associated with inappropriate proliferation, migration, guidance, differentiation, growth or synapse formation.

and, in primates by E112, visual cortex neurons possess a dense network of synapses indicating that the system is ready even before visual experience [7,8]. Retinal waves that are generated early [9–11] modulate the construction of the visual system [12,13], and *in utero* enucleation in primates enhances the size of the projection areas in cortex [8,14]. Visual maturation is experience dependent [15–17] with identified inducing factors [18,19]. Activity regulates essential developmental processes including the cell cycle [20], axonal growth [21], phenotype selection [22] and cell outgrowth [11,17,23–26].

Synapse formation follows a programmed sequence, with GABAergic synapses formed before glutamatergic ones in rodents and primates [27,28]. The universal developmental shift in the actions of GABA vividly illustrates the differences between adult and immature brains. GABA (the main inhibitory transmitter in adults) excites immature neurons because of an evolutionarily conserved higher intracellular Cl^- concentration ($[Cl^-]_i$) [26,29–31] (Box 1 Figure I). GABA generates sodium and calcium currents and removes the voltage-dependent Mg^{2+} block from NMDA channels, thereby increasing intracellular Ca^{2+} concentration and exerting trophic actions on developing neurons [32–34]. Delivery in rodents is preceded by an oxytocin-triggered transient excitatory-to-inhibitory shift of the action of GABA that protects neurons from delivery-related anoxic damage [35]. Artificially shifting GABA actions from excitatory to inhibitory increases the formation of GABAergic synapses [36–38].

The data indicating that immature neurons use different ionic and receptor-mediated channels than those used by mature neurons is overwhelming. All receptors and receptor channels investigated (sodium and calcium channels, NMDA and AMPA [α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid] or kainate receptors, acetylcholine, K^+ channels etc), and even extracellular matrix molecules, are different in immature and adult systems and these differences have an important role in development [1,39–47]. Usually, the subunits of immature neurons tend to have slow kinetics (e.g. NMDA receptor subunit shift from NR2B to NR2A) [33,39,48,49], in keeping with the 'clumsy' properties of developing activity. In addition, and perhaps more striking, immature neurons use receptor signals that are subsequently eliminated during development. Thus, vertebrate embryonic neuromuscular junctions express functional GABA, glutamate and glycine receptors, in addition to the acetylcholine receptors that will remain into maturity [50,51]. Spinal cord neurons that are glycinergic in adults express functional GABA receptors first; these are eliminated during development [52]. Similarly, GABAergic synaptic responses in the trapezoid body precede the glycinergic responses of adults [53] (for review, see Ref. [29]). Neuronal activity also plays a crucial part in the selection of the adequate phenotype, reflecting the importance of the interactions between genetic programs and environmental information [51,54,55]. Whether these receptor change-overs are evolutionary remnants or adaptive is, at present, not clear. Nevertheless, this implies that drugs might exert dramatic actions on developing neurons while having little or no effect in the adult.

Developing neurons are highly excitable and have an intrinsic tendency to oscillate [56–58]. *In utero* they

Box 1. Excitatory GABA: does epileptogenesis recapitulate ontogenesis?

Studies on the properties of epileptogenic tissues have provided a general theory regarding the behaviour of neurons in the aftermath of an insult. Under this theory, recurrent seizures produce a quasi-permanent reverse of the inhibitory-to-excitatory shift of GABA actions in humans and animals [121,146,147]. This is due to an intracellular accumulation of chloride that has some resemblance to the developing brain situation because it is mediated by a loss of the chloride exporter KCC2 [148,149]. Studies using the triple *in vitro* chamber with the two interconnected intact hippocampi have shown that the propagation of seizures transforms a naïve network to one that seizes; this effect is also mediated by an accumulation of chloride and an excitatory-to-inhibitory shift of GABA actions [121,147] (Figure I). Neuropathic pain, traumatic lesions of the spinal cord or ischemic lesions [150–152] lead to a similar down-regulation of the transporter. Therefore, epileptogenesis (and other insults) recapitulates ontogenesis leading to a return to immature properties and, similarly, 'freezing' neurons in an immature situation. Together, these features indicate a vicious cycle, with early insults affecting developmental programs and leading to the maintenance of immature patterns and the eventual expression of a phenotype. This raises the possibility that the response of adult neurons to insults includes a rehearsal of early programs.

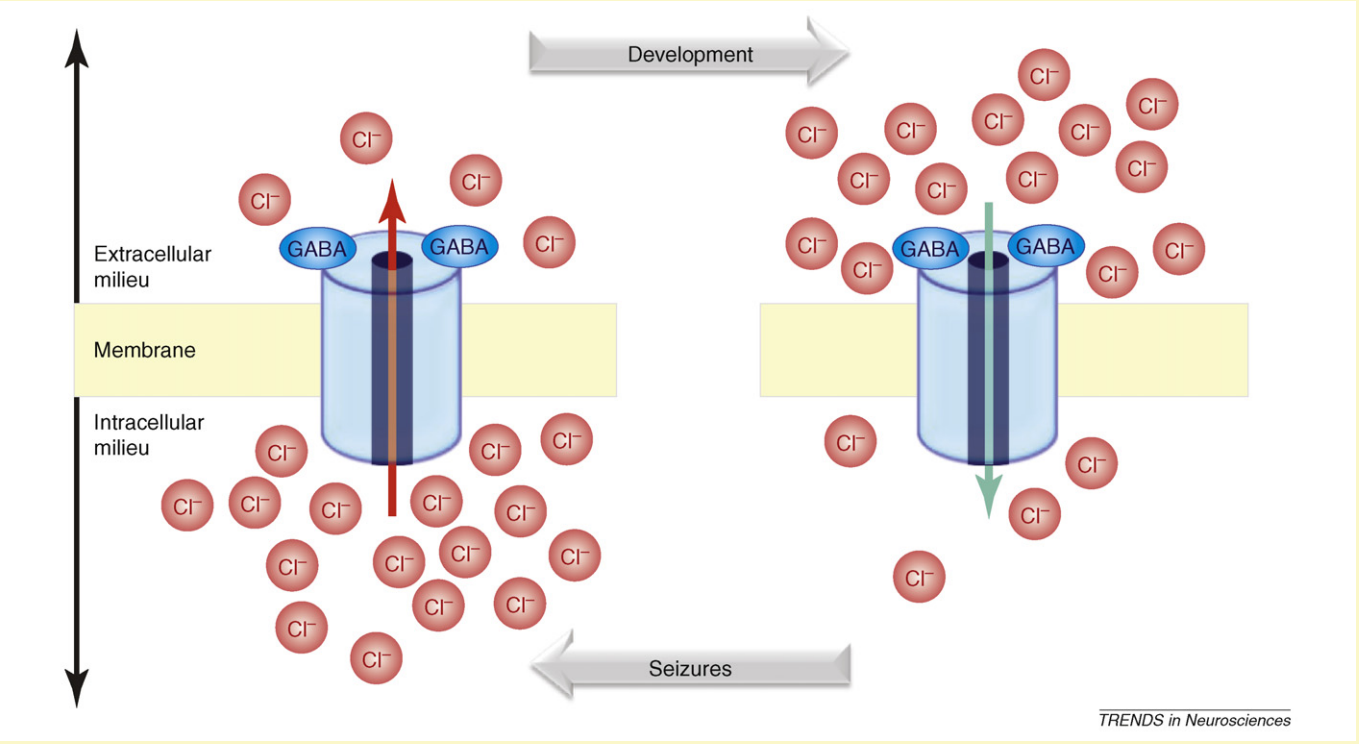

Figure I. Schematic illustration of the effects of seizures on GABA signalling in immature and adult neurons. Immature neurons have a high $[Cl^-]_i$ and an excitatory GABA that shifts to inhibitory GABA with a progressive loss of $[Cl^-]_i$. After recurrent seizures, in both immature and adult neurons, chloride accumulates and GABA excites neurons indicating a return to immature features.

generate intrinsic voltage-dependent calcium currents that do not propagate and then they form small cell assemblies interconnected by gap junctions that generate non-synaptic calcium plateaus around delivery time. The first synapse-driven coherent patterns appear soon after (the so-called giant depolarising potentials [GDPs] [29]) and connect large neuronal populations [56] (Figure 2). Similar coherent patterns are present in macaque neurons *in utero* and in premature infants [28,59]. Extensive investigations also indicate that early signals (e.g. retinal waves) are present before sensory onset [60,61]. Neuronal properties are also rapidly modified to enable important functions to come online [39,40,42,43–45,62]. In summary, neurons have a developmental-stage-dependent expression of electrical signals that provides a read-out of their state and modulates the subsequent operation of the system. These processes are highly susceptible to genetic and environmental effects. Future studies are warranted to determine the mechanisms underlying these developmentally regulated sequences and their functional importance.

The developing and adult brains respond differently to environmental insults

Because several processes including proliferation or migration occur primarily in the developing but not the adult brain, it comes as no surprise that a wide range of agents and procedures produce brain malformations in the former but not the latter. For example, *in utero* administration of the anti-mitotic agent methyl-azoxy-methanol leads to the formation of small heterotopic masses and an aberrant functional bridge between the neocortex and hippocampus [63–66]. Cocaine, alcohol and other drugs of abuse produce severe brain abnormalities *in utero* and brain damage in adults [67,68]. Some of the signals that mediate the cortical and subcortical malformations of prenatal cocaine [69] or alcohol have been identified [70]. *In utero* administration of anti-epileptic drugs at clinically relevant concentrations blocks seizures in adults and leads to cortical malformations in embryos [71], in keeping with their toxicity in foetal human brains [72]. Consistent with the different actions of GABA in young and adult neurons, benzodiazepines excite immature neurons and inhibit adult

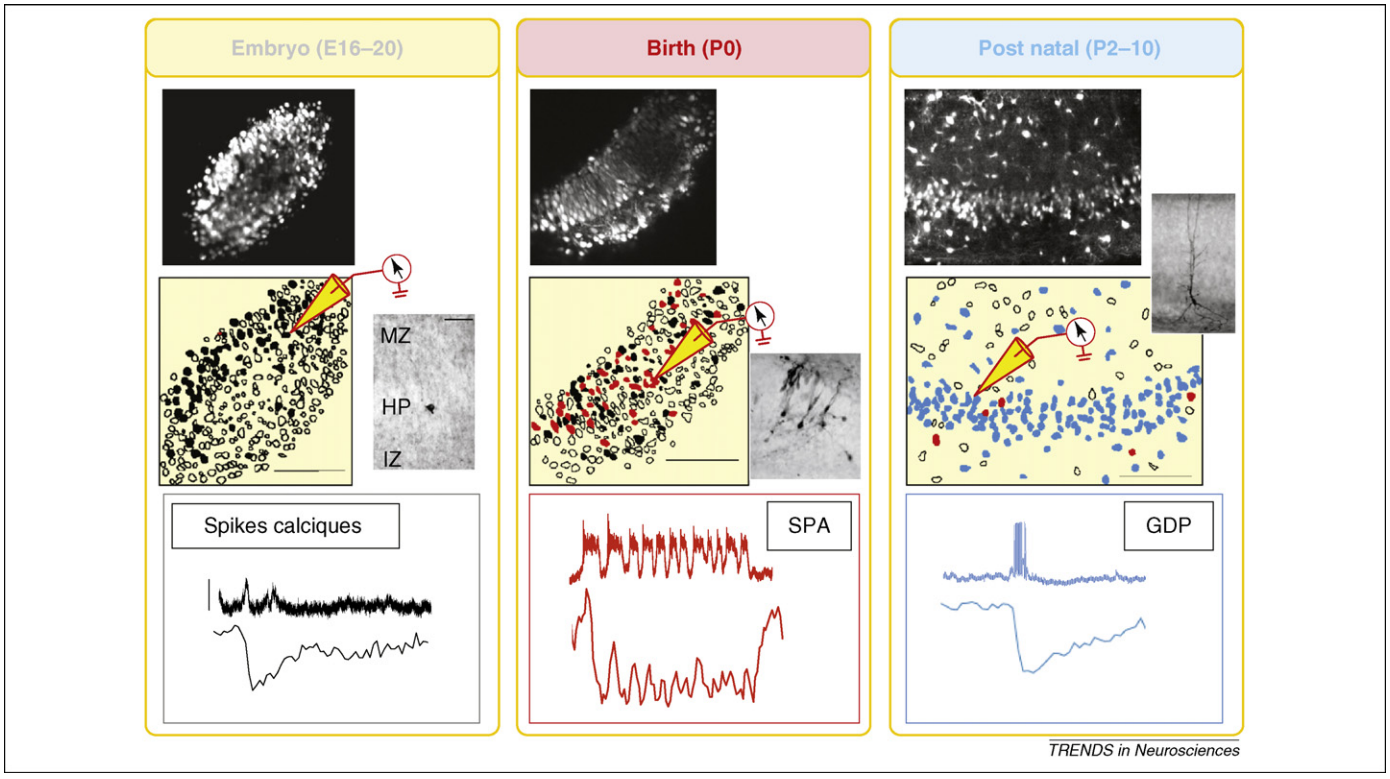

Figure 2. Sequential maturation of brain patterns during development. Hippocampal slices were used (embryonic and post natal). Using dynamic 2 photon imaging, the activity of hundreds of neurons was imaged and the synchronized activities determined using a home-made program (taken, with permission, from Ref. [56]). Note the triphasic sequence with *in utero* intrinsic calcium currents that do not propagate, then a non-synapse-driven synchronised plateaux in cell assemblies (SPAs) (around delivery) and, subsequently, the synapse-driven GDPs that dominate ongoing activity of neonatal neurons.

ones, and cannabis-receptor antagonists promote seizures in pups, but not in adults [73–77].

The way immature neurons respond to seizures is emblematic of these differences. The administration of kainic acid to adults generates a seizure and brain-damage syndrome that mimics temporal-lobe epilepsies and includes a loss of vulnerable neurons, the activation of thousands of genes, the sprouting of glutamatergic fibres and the formation of novel aberrant synapses that generate further seizures. Seizures beget seizures during this cascade [78–80] (Figure 3). Reactive plasticity is also a constituent component of the response of adult neurons to traumatic insults [81,82]. By contrast, although the incidence of epilepsies is higher in immature neurons [83,84], convulsive agents in pups do not produce cell loss. This is in keeping with the high resistance of these neurons to seizures [83,84] and anoxic episodes [83–85]. Nevertheless, these episodes have long-term consequences [86–93] indicating that the effects are most likely to be due to altered developmental programs and long-term modifications of neuronal properties (Figure 3). Therefore, the effects of insults on immature neurons are developmental-stage dependent and differ from the more homogeneous repertoire of reactions of the adult brain. Interestingly, GABA excites immature and adult brains after seizures and other insults, indicating that immature brains remain 'frozen' at an immature stage, whereas adults 'return' to an immature state (Box 1). Priorities for future work should include determination of the differences between the response of adult and immature neurons in animal models of other neurological disorders and the mechanisms linking the developmental and adult forms of neuronal plasticity.

Genetic mutations are talkative during development

Many mutations identified in relation to infantile epilepsies, mental retardation and dyslexia are due to disruption of proteins that are essential for synapse operation, receptor-mediated signalling, transporters, cytoskeleton proteins and other processes [94–96]. It has been suggested that mental retardation unassociated with apparent structural abnormalities has a delayed phenotype because

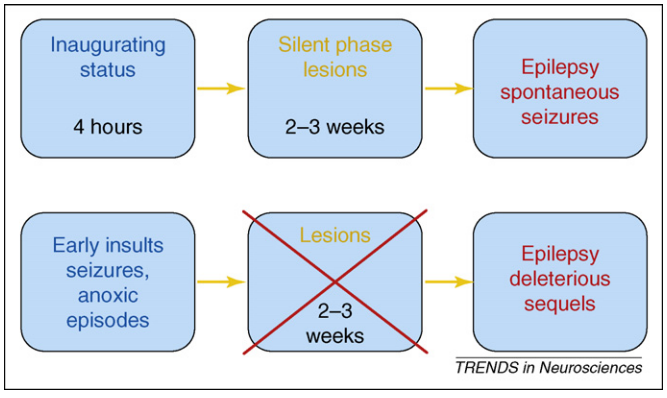

Figure 3. Schematic diagram to illustrate the differences between the response of adult and immature brains to seizures and other insults. Adult brains respond to recurrent seizures by an intermediate phase during which neurons die and others exhibit reactive plasticity with sprouting and formation of novel aberrant synapses that will contribute to the generation of more seizures. By contrast, neonatal neurons do not exhibit cell loss after seizures that nevertheless do lead to long-term neurological sequels.

mutations of proteins that are essential for synaptic activity and plasticity are not needed at earlier developmental stages [97]. This, however, cannot be readily reconciled with the role of early intrinsic and synapse-driven activities in brain maturation [7,28], the presence in primates *in utero* of >7000 glutamatergic synapses on cortical neurons [7,28] and the occurrence of abundant GABA and glutamate synaptic currents and short- and long-term forms of activity-dependent forms of synaptic plasticity [98,99]. The fragile X mental retardation protein (FMRP) that is disrupted in fragile X syndrome binds to the mRNA encoding postsynaptic density protein-95 *in vivo* that regulates neuronal synaptic signalling [100]. Metabotropic glutamate receptors that provide a possible therapeutic avenue to treat this disorder [101,102] modulate neuronal migration, generate oscillations *in utero* [103] and increase message stability in migrating neurons [104]. Knockout of metabotropic-receptor-linked molecules alters spines and morphological arbours and enhances GABAergic signalling in striatal neurons [105]. Particularly interesting observations have been made regarding Rett syndrome, a classical developmental disorder in which children develop normally up to 1 year of age, after which they lose acquired speech and replace purposeful hand use with stereotypes. It has been shown that the methyl cytosine binding protein 2 (MECP2) gene that is mutated in Rett syndrome suppresses the activity of many thousands of genes that will be activated and remain active [106]. This illustrates the complexity of the alterations produced by a mutation of a transcriptional repressor and the multiple biological reactions it entrains.

Therefore, available information supports an extensive role for early activity on brain maturation, resulting in alterations of fundamental developmental programs when this activity is disrupted. It also seems conceptually questionable to define the onset of a disease as the moment it can be diagnosed. Many early disorders such as dyslexia, dyscalculia and sensory malformations are not readily diagnosed at early developmental stages. This does not necessarily imply, however, that earlier signatures are absent. Basic research is instrumental to dissect the chain of events leading from an early mutation to disease. Priorities for future work should include a physiological and morphological determination of the earlier subtle signs produced by animal models of mental retardation and other developmental disorders (see later).

In utero disruption of genes linked to early disorders leads to subtle malformations

Knockout and knock-in strategies have contributed to the study of gene functions but have some inherent limitations owing to compensatory mechanisms and redundancy of essential processes that can result in no phenotype, a lethal one or one that does not mimic the human pathology. Cell-specific and developmental-stage-dependent inactivation of proteins alleviates these limitations and enables identification of the underlying neuronal population in which the proteins are disrupted. A recently developed strategy of suppression by RNA interference (RNAi) *in utero* seems to offer interesting advantages for the study of pre-symptomatic signatures of disorders and the electrical properties of misplaced neuronal assemblies [107,108]. In this approach, constructs are made with short-hairpin (sh)RNAi and transfected together with green fluorescent protein (GFP). Once the transfection has been terminated, embryos are placed back in the mother's womb. After delivery, the brain can be used to investigate the properties of transfected neurons in comparison to adjacent non-transfected neuronal populations. It is, thus, possible to inactivate a signal at a selected developmental stage in cortical neurons that are destined to populate a specific cortical layer and to determine whether and how they impact the networks they were programmed to interact with. This is of the utmost importance because it enables the understanding of the multiple deleterious consequences produced by a single mutation. I illustrate the usefulness of this technique with observations made in relation to two developmental disorders: the double cortex (DCX) and a dyslexia-related mutation.

The DCX mutation

Subcortical band heterotopia or 'double cortex' is characterized by a diffuse subcortical band of grey matter generated by a bi-hemispheric arrest of neuroblast migration during development [94–96,109–111]. Neurons in which DCX proteins have been suppressed fail to reach their targeted areas, thus preventing the normal development of the cortical mantle [95]. These cortical malformations are commonly associated with intractable generalized symptomatic epilepsy (Box 2). In contrast to *DCX* knockouts, which generate hippocampal malformations rather than a double cortex [112,113], *in utero* suppression of the DCX gene in rodents produces an anatomically relevant model of the disorder (Figure 4c). Many neurons form misplaced subcortical band heterotopias within the intermediate zone and the white matter, whereas other neurons migrate to inappropriate neocortical lamina [108]. Despite these alterations, the animals do not exhibit a specific global phenotype (i.e. spontaneous seizures) – probably owing to the limited number of DCX invalidated misplaced neurons produced by the transfection – but animals have a lower threshold for drug-induced seizures (ibid). Most importantly, a delayed expression of the correct transcript during a critical period reduces the size of the misplaced ensemble and the sensitivity to convulsive agents, validating the link between the malformation and the hyperexcitabilty (J.B. Manent *et al.*, unpublished). Recent clinical and experimental studies using this approach have enabled identification of the source of the aberrant patterns and illustrate the links between heterotopia and adjacent cortex (Box 2; Figure 4c).

Disruption of a dyslexia-related gene

Dyslexia is diagnosed as a difficulty with learning to read that occurs in the absence of sensory or neurological impairments and despite an appropriate social, intellectual and educational environment [114,115]. Several observations indicate that dyslexia is highly familial and heritable, resulting from interactions between genetic and environmental factors [114,115]. Observations from the brains of deceased dyslexic patients have linked dyslexia to the presence of polymicrogyria, in keeping with the concept of early developmental origin of the disorder [116]. The *KIAA0319*

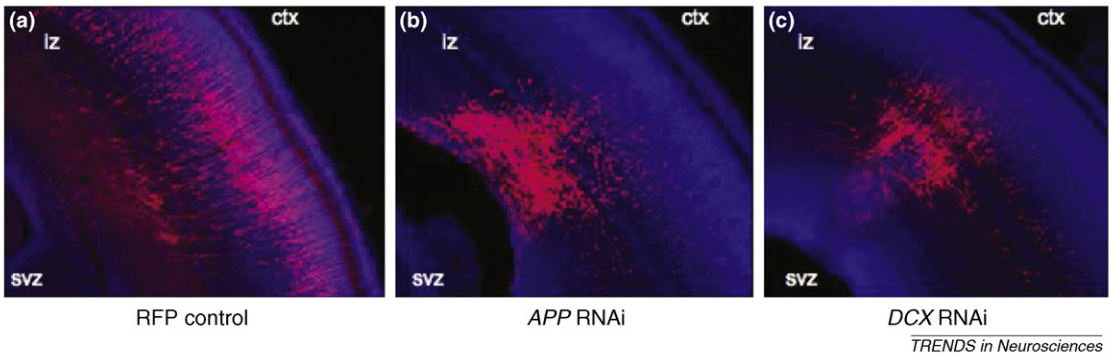

Figure 4. Targeted delivery of shRNA against *DCX* and *APP* genes by *in utero* electroporation. Coronal sections of an E20 rat embryonic cortex show migrating pyramidal neurons electroporated with a red fluorescent protein (RFP) reporter plasmid at E15 **(a)**. However, electroporation of an shRNA directed against *DCX* [108] or *APP* [129] disrupts neuronal migration throughout the cortex **(b,c)** (C. Cardoso *et al.*, unpublished). Abbreviations: CTX, cortex; IZ, intermediate zone; SVZ, sub ventricular zone.

gene that has been associated with dyslexia is strongly expressed in early foetal human brain (57 days post-fertilisation) and has an important role at early developmental stages [117,118]. RNAi of KIAA0319 *in utero* leads to an impairment in radial neuronal migration [118]. Interestingly, other dyslexia-associated genes also influence neuronal migration after RNA inhibition [119,120]. It has been shown that focal microgyria can also disrupt connective architecture illustrating the chain of reaction that might result from this mutation [114–116,121].

Are pre-symptomatic signatures absent from classical late disorders?

One important question arises: is the scenario postulated for early disorders also valid for Alzheimer's, Parkinson's and Huntington's diseases? Is it possible that early developmental malformations precede by decades the manifestation of the disease and, if so, how does the neurodevelopmental insult lead to neurodegeneration with the classical signatures of the disease? Although this terrain is more speculative in view of the overwhelming observations that indicate an adult and/or aging neurodegenerative disorder, clinical and fundamental data indicate that the hypothesis of early 'silent' signals should not be completely discarded.

Standard neuropsychological tests were used to detect evidence of dysfunction in frontal executive systems in pre-symptomatic subjects with known mutation carrier status in the highly penetrant fronto-temporal dementia and parkinsonism linked to chromosome 17 [122,123]. Executive dysfunction was reported many decades before the predicted onset of dementia. This cognitive dysfunction did not correlate with the age of the subjects, indicating that it does not represent a progressive process but, rather, a premorbid state. This indicates a potentially important neurodevelopmental component to a dementing condition that has been predominantly considered to be a disease of aging. Along similar lines, it was reported that knockout of a transcription factor FoxA2 [124], which is crucial for dopamine neuron specification and survival in mice, causes a late-onset, asymmetric degenerative condition affecting motor systems in a manner very similar to Parkinson's disease. It might be hypothesized that loss of FoxA2 affects proper target innervations leading to loss-of-target-derived trophic support. This scheme involves an early genetic signal that leads via a cascade of events to a late onset of the disorder. As such, this seems to provide a more relevant model of Parkinson's disease than other models that lack the progressive expression of the degeneration. It will be important to determine whether and how the developmental sequence of targets of dopaminergic neurons are affected by the early invalidation.

Fascinating observations have also been obtained on Huntington's disease [125,126]. Cortical morphology was examined using magnetic resonance imaging (MRI) scans

Box 2. Heavy impact of misplaced neurons on adjacent cortex

In patients suffering from pharmaco-resistant epilepsies, the important challenge is to define the epileptogenic zone accurately because surgical removal of that area can alleviate the occurrence of seizures. In children with malformations of cortical development (MCD) [153], including DCX mutations, intrinsic epileptogenesis is characteristic of focal cortical dysplasia with typical rhythmic paroxysmal activities [154–157]. Using a non-invasive electroencephalographic functional-MRI technique, it was recently shown [158,159] that the blood oxygen-level-dependent changes in response to seizures varied with the type of MCD, supporting the notion that defects occurring at different times of embryogenesis lead to divergent consequences. Thus, in focal and band heterotopia, interictal and ictal events are generated within the lesion (displaced) area, whereas, in nodular heterotopia, the spikes appear in the heterotopic neurons and the seizures are generated by the overlying cortex rather than the displaced masses. It was concluded that, although the nodular grey matter heterotopia might have the cellular substrate to produce interictal events, seizures (ictal events) are generated primarily by the abnormal overlying cortex. Therefore, the insult caused by inadequate migration is not restricted to the misplaced cells, and programmed targets also take part in the generation of abnormal activity.

Using *in utero* DCX suppression coupled with imaging and electrical recording techniques in slices, we recently found that $DCX^{-}GFP^{+}$ labelled heterotopic neurons that fail to migrate develop axonal aberrant projections to cortical and sub-cortical structures (J. Ackman *et al.*, unpublished). Heterotopic neurons retain immature electrical properties; overlying cortical neurons exhibit a dramatic increase of glutamatergic currents and together generate coherent synchronized oscillations. These observations illustrate the important information that can be obtained with a combined *in utero* deletion of a protein and physiological studies. The observation that a few thousand misplaced neurons can produce such profound changes illustrates the importance of the cascade of events that occurs after developmental malformations [160].

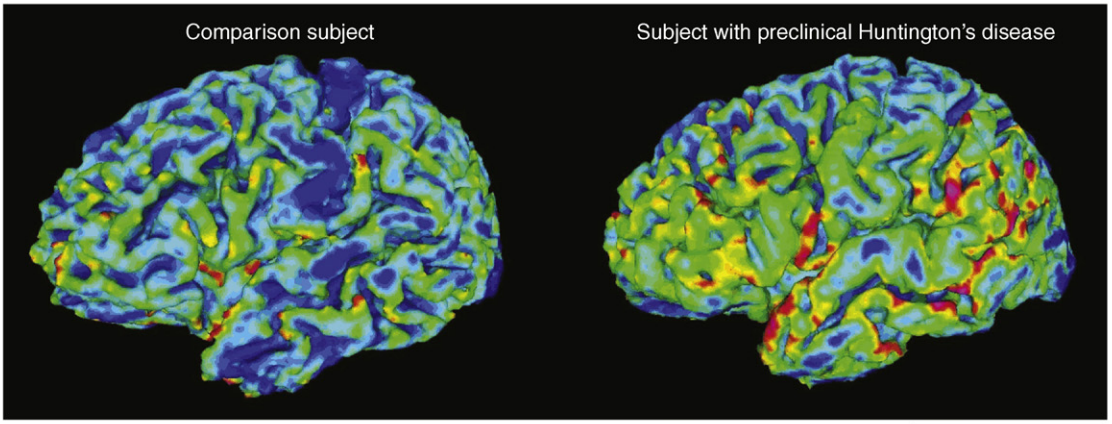

Figure 5. Three-dimensional images of the cortical surface in the brain of a healthy comparison subject and an age- and sex-matched subject with preclinical Huntington's disease. The colours represent cortical thickness in hues of blue and thicker cortex is represented in yellow and red. Taken, with permission, from Ref. [125].

of subjects with known gene expansion for Huntington's disease who had not yet shown clinical signs of the disease (Figure 5). Subjects with preclinical Huntington's disease showed altered cortex morphology with enlargement of gyral crowns and abnormally thin sulci. It was concluded that '...abnormal neural development may be an important process in the patho-etiology of Huntington's disease'. In a more recent study, called 'Predict HD', a closer scrutiny of cortex showed thinning and thickening of different areas over time, notably the cingulated cortex, which are linked to both depression and cognition. These changes were correlated with the probability of onset. The striatum, putamen and caudate were smaller, from as early a time as could be imaged. It was indicated that using structural MRI to track markers of change might reduce the numbers of subjects needed for clinical trials, and similar examination of children might reveal a neurodevelopmental component in Huntington's disease. In support of this, corticostriatal synaptic function is altered in pre-symptomatic Huntington's-disease-gene-expressing mice [127]. We lack, however, detailed information as to the electrical and/or morphological properties of misplaced neuronal ensembles and their connections to comprehend how they affect brain development.

Evidence in favour of early developmental alterations of classical late disorders has also been obtained for Alzheimer's disease with the *in utero* RNAi approach. The β amyloid precursor protein (APP) is a type I transmembrane glycoprotein that is post-translationally cleaved and its large extracellular domain released. Over 20 mutations of the APP have been linked to familial Alzheimer's disease [128]. *In utero* electroporation of shRNA constructs to acutely knock-down APP in cortical progenitors resulted in cells that failed to migrate and remained tightly packed below the cortical plate, in contrast to cells electroporated with either a GFP or an inactive APP control shRNA plasmid [129]. In contrast to knock-down of APP, which inhibited cortical-plate entry, overexpression of APP caused accelerated migration of cells past the cortical plate (also see Ref. [130]). Co-electroporation of full-length human APP rescued the phenotype when performed before delivery, strongly reinforcing the developmental role of the protein. These observations challenge neither the well-known degenerative manifestations that occur with aging nor the important role that the protein has in adult networks leading to neurodegeneration when disrupted. However, they do indicate that, at least in these genetic cases, an early developmental affliction might precede the disorder to come and include a signature of signals that progressively mature and possibly enhance or facilitate the ease with which aging triggers degeneration. It is not unreasonable to suggest that aberrantly established synaptic connections during brain development might exhibit a different reactive plasticity to adverse conditions, which eventually facilitates neuronal damage. Studies aimed at identifying pre-symptomatic imaging and/or cognitive disorders in patients identified as potentially susceptible will provide important information [131]. Future priorities should include clinical and imaging investigations of familial at-risk patients to search for architectural alterations, in addition to fundamental research on the physiological properties of misplaced neurons after early APP inactivation.

Time and space dependence of the effects of mutations: a 'programmed response to insults'?

Things are, however, more complex because genes have divergent roles at various developmental stages and within different brain structures. Thus, a mutation of the sushi SRPX2 gene leads to perisylvian polymigrogyria indicating an early developmental function, but another mutation of the same gene leads to a syndrome with verbal dyspraxia but no apparent brain malformation [132,133]. It is possible that, like other sushi domain proteins (the drosophila hikaru genki), SRPX2 also plays a part in synapse operation. If a given protein has different roles at different developmental stages, then the same protein could have different roles in different brain structures, contributing to divergent phenotypes according to their localisation. Thus, different $GABA_B$-receptor subunits might be expressed depending on the presence or absence of sushi domains [134].

Another point to consider is the complex relation between innate and acquired early insults and the existence

of a programmed succession of phenotypes. Thus, infantile convulsions with paroxysmal dyskinesias [135] include an early, transient appearance of benign infantile seizures that are followed years later by paroxysmal dyskinesia. Cytomegalovirus infections (CMV) can lead to polymicrogyria much like those produced by hereditary mutations [136]. The syndrome of Aicardi-Goutières of progressive encephalopathies is due to either mutations of ribonuclease H2 subunits or to CMV [137,138], indicating a common mechanism between genetic and environmental insults [104,139]. These observations speak in favour of complex relationships between genetic and environmental factors that act in series and raise the possibility of a developmental-stage-dependent programmed response of the developing brain to insults.

In conclusion, the extensive work performed to identify neurological-disorder-linked mutations stands in striking contrast to the information available on the maturation of neuronal activity and morphological connections and its relation to neurological disorders (>20 000 versus a few hundred citations in PubMed). A strategy based on the identification of additional mutations and intracellular signalling cascades will not suffice because it does not take into account the electrical and morphological behaviour of neurons and networks that fail to behave as programmed and their downstream effects on further gene expression. It is difficult to imagine that an organ as complex and plastic as the brain could suffer a disruption of essential proteins at an early stage without an electrical and/or architectural trace of this event and without alterations of developmental programs. This 'developmental hypothesis' is not unprecedented. Adverse conditions during gestation, foetal growth retardations and prematurity have important impacts on later health, including an increased incidence of attention deficit disorders, schizophrenia and depression [140–143], all of which support a developmental hypothesis for neurological disorders [142]. It has been suggested that these disorders result from alterations of the set points of neuroendocrine systems and the hypothalamo–pituitary–endocrine axis [142,144,145], rather than a neurodegenerative mechanism.

The novelty of the developmental hypothesis presented here perhaps stems from its effort to include these seemingly diverse domains in a coherent general model that encompasses specific phenotypes and aetiologies and reconciles genetic and environmental factors with developmental neurobiology. Indeed, the prism through which these issues are presently discussed (early programmed versus late environmental and genetic versus acquired) seems to be due more to technical limitations and paucity of information than to genuine conceptual justification. With the development of sophisticated imaging tools we will be able to detect microscopic heterotopic masses, record their activity with non-invasive techniques and elaborate novel classifications of disorders based on individual signatures; much like, and potentially better than, individual genetic maps suggested to provide a passport of sensitivity to disorders. Understanding how neurons fail to behave as programmed and react electrically to atypical environments will open novel therapeutic avenues focusing on the unique properties of those misplaced neurons that remained frozen with immature features. As for other developmental insults, an early identification of the malformation will facilitate the discovery of correctives based on brain plasticity that operate preferentially during windows of opportunity that might correspond to the classical critical periods. A better comprehension of the links between migration or other developmental malformations and the disease phenotype is necessary. It seems *a priori* unlikely that the location of a cortical malformation directly and exclusively explains a phenotype. However, studies on epilepsies have amply demonstrated how a focal insult can entrain a wide range of brain structures by the propagation of aberrant patterns and generate a highly complex phenotype. Is it possible that misplaced neurons disturb preferentially during development an ensemble of structures they were programmed to operate with, leading to a coordinated manifestation of complex phenotypes?

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