ACTA PÆDIATRICA
NURTURING THE CHILD

Acta Pædiatrica ISSN 0803-5253

ACTA PAEDIATRICA PERSPECTIVES

Neuropaediatric and neuroarchaeology: understanding development to correct brain disorders

Yehezkel Ben-Ari (yehezkel.ben-ari@inserm.fr)

INMED, INSERM, Campus scientifique de luminy route de luminy, Marseille, Cedex

DOI:10.1111/apa.12161

The construction of the brain, with its 10^{15} synapses, follows a tight genetic programme. But at the same time, the neurons and networks follow a developmental sequence, from immature activities to adult ones. In essence, all voltage-gated and synapse-driven currents differ in young and adult neurons, and primitive networks generate patterns that have little to do with those that are relevant to adult behaviour. It is also clear that activity modulates the construction of functional units and neuronal ensembles, raising the fundamental question of the relationships and interactions between the genetic programme and activity. It has been suggested (1) that genes and activity have a checkpoint type of relationship, the latter confirming the validity of the former.

In addition, there can be numerous errors in the formation of neuronal entities, and these are associated with severe neurological sequels. This raises the highly relevant clinical issue of the fate of neuronal ensembles that do not perform as they should do and end up misplaced and/or misconnected. How do these aberrant networks mature and how do they generate disorders? How do misplaced and/or misconnected neurons behave and malfunction to the extent that they exert adverse effects on the operation of the cortex? It has, for example, been suggested that these neurons remain 'frozen' in an immature state (1,2), paving the way for interesting therapeutic advances. Of course, many other therapeutic advances have been suggested, including gene and stem cell therapy, and it is very helpful to debate these issues with experts specializing in various relevant neurological areas.

I recently organized an international meeting in Valescure, France, where I brought together some of the best experts in their fields, who seldom meet, to discuss brain development and how to correct neurological and psychiatric disorders. There was a particular emphasis on the links between activity and genes in generating functional networks and how these can lead to neurological disorders when they do not operate correctly.

HOW SIGNAL DEPRIVATION AFFECTS DEVELOPMENT

Introductory talks by Nick Spitzerand and myself emphasized the importance of links between genes and environment/activity and the difficulty of separating them as they act in series, not in parallel. At all developmental stages, cells and the differentiated neurons generate activities that are also altered progressively, providing a signature of their state of development. The main issue here is to realize that concentrating all our efforts on identifying more mutations involved in brain disorders will not be sufficient. We need to develop a better understanding of how networks operate when they are deprived of important signals and, even more importantly, when and how the developmental programme is altered. Indeed, neurons and networks are, by definition, very flexible and plastic in that they are modified by experience and activity and do not remain idle when something goes wrong! They respond to the situation and adapt themselves by modifying their currents and connections, and this modification provides a sort of signature that confirms that something has gone wrong. This signature can remain infraclinical, with no obvious signs or can result in large or small clinical manifestations. This, in turn, may be conditioned by other events, including what is often called a second hit or another insult that is normally innocuous but becomes more severe if the terrain on which it occurs is receptive for that I have suggested the concept of neuroarchaeology with presymptomatic electrical or architectural signatures of disorders to come to illustrate that idea. This is readily accepted by clinicians who realize that disease can be a late manifestation of an earlier inaugurating event. It has received strong support in recent publications, and this

support was further confirmed during the meeting. It implies that an insult, for example a mutation, will activate a cascade of events that will ultimately lead to a clinical syndrome after a long delay. When the disease manifests itself, it is not the sole direct consequence of the mutation. The alterations produced by the programme are crucial. The interruption of the sequence that the mutation has produced leads to the misplaced neurons and connections that finally generate the clinical symptoms. These must be the target of the treatment, not the mutation. In other words, even if the introduction of the correct gene were possible, this would not necessarily provide a 'cure'. It is like an exploding wheel leading to a car accident. Replacing the wheel may not necessary tackle all the damage caused by the explosion, even if superficially it appears to sort out the main problem.

Several observations illustrate this point. For example, studies using *in utero* short hairpin RNA invalidation (SHRNA) of genes and proteins involved in brain disorders mimic the disease, at least in its morphological and biochemical manifestations. They have been used to test the validity of the concept by recording misplaced and misconnected neurons following a large range of invalidation, including filamin, SRPX2, double cortex or tuberous sclerosis. All of them show that these neurons remain immature, with immature currents.

Alfonso Represa analysed the extensive evidence obtained by his group on recurrent excitatory networks in various genetic mutations associated with seizures, notably the double cortex.

Periventricular nodular heterotopia – the most common form of brain malformation in adults – is caused by migration defects and the presence of ectopic nodules. Carlos Cardoso demonstrated that these are due to disruption of the radial glia scaffold in animal models that are similar to post-mortem human material. Again, these cells have immature features.

Pierre Szepetowski provided a good illustration of the epileptiform activity generated by early *in utero* invalidation of the Srpx2 protein involved in epileptic activities in the speech cortex. This demonstrated how very early malformations lead to delayed severe sequels.

Nael Burnashev and colleagues showed, along similar lines, how in tuberous sclerosis, neurons in the tubers in rodents and human have NMDA receptor-mediated currents with subunits that are normally present in immature but not adult neurons. This vividly illustrates the concept of neuroarchaeology (2) and the persistence of immature currents in adult misplaced or misconnected neurons. In this situation, using drugs to selectively block the long-lasting currents also blocks the seizures. Constance Hammond, working on the PINK1 genetic model of Parkinson's provided an astonishing illustration of the long delays between intrauterine malformations and neurological sequels. Abnormal electrical activity in the striatal networks, with gigantic bursts appeared several months before the first clinical signs in mice.

Another illuminating example was provided by the study on the alterations of GABA during brain development and in various pathological conditions. Indeed, intracellular chloride is elevated in immature neurons leading to excitatory actions of GABA in many neurons (3). A vivid illustration of the GABA polarity developmental sequence is shown by the oxytocin-mediated abrupt and dramatic reduction in intracellular chloride that occurs during delivery (4). This shift is restricted to a couple of hours before and after delivery and exerts a major neuroprotective and analgesic action on the brain during a highly vulnerable stage. Interestingly, intracellular chloride levels are again elevated in epilepsies, where recurrent seizures lead to an increase of the activity of a chloride importer NKCC1 and an internalization of the main chloride exporter – KCC2 – leading to reduced efficacy of chloride removal (3). These shifts shown in Figure 1 that illustrate the common features between immature and adult pathological conditions have been extended to many other brain disorders including spinal chord lesions, brain trauma and various other insults suggesting that the regulation of intracellular chloride is an important signature of the types of changes occurring in pathological conditions. These observations have also lead to considerable interest in developing drugs that re-establish the correct intracellular levels of chloride. One of these strategies is the use of a diuretic bumetanide known to selectively block KCC1 the main chloride importer and to reduce intracellular chloride thereby also reinforcing GABAergic inhibition. Experimental and clinical trials using bumetanide have been initiated in epilepsies. Very promising results have been obtained in autism. Administration of a diuretic that reduces intracellular chloride and re-establishes the correct polarity of GABA in a randomized double-blind study was shown to reduce the severity of the syndrome (5). The author of this meeting report also illustrated the long pathway from basic research to clinical applications in autism, where the absence of treatment remains a major issue, considering its high incidence and the impact and burden this condition has on everyday life.

The conclusion from these presentations is that neurons that are not correctly placed are 'forced' to wrongly establish local connections with neurons that they are not meant to interact with, leading to aberrant connections and aberrant electrical manifestations. This in turn leads to seizures and other clinical manifestations. It also paves the way to novel therapeutic perspectives, because immature currents can be blocked by selective antagonists of immature currents without affecting adjacent adult ones. There are many examples of therapies to block aberrant activities generated by misplaced neurons, including some currently being investigated in experimental conditions and others that will provide the focus for future clinical trials.

EARLY EVENTS AND THEIR IMPACT ON FUTURE DISORDERS

The importance of early events on future disorders was abundantly illustrated during the meeting, including a strong discussion on the importance of embryonic and early maturation and the consequences of prematurity on subsequent neuropsychiatric problems. Daniela Prayer

Acta Paediatrica Perspectives

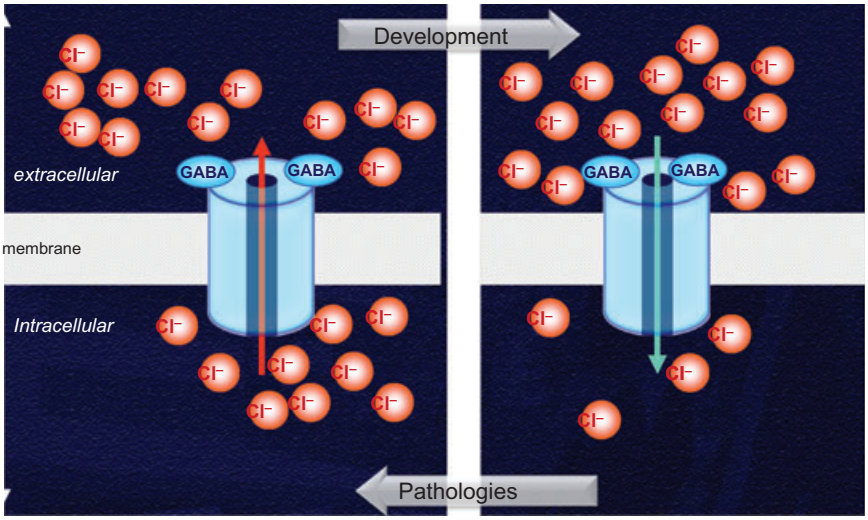

Figure 1 Intracellular chloride is elevated in immature neurons but also in various pathological conditions. The shift in the chloride gradient will lead to different actions of GABA, predominantly depolarizing and occasionally excitatory in immature and pathological neurons but predominantly inhibitory in naive adult neurons. This shift that illustrates the links between immature neurons and pathological ones has also important therapeutic implications.

used spectacular *in utero* images to illustrate the complexity of intrauterine life and its importance in prognosis and early detection of disorders. Pasko Rakic developed the importance of neuronal migration in relation to subsequent pathological issues – a domain that turned out to be central to the meeting. Eric Courchesne then illustrated the importance of early insults in relation to autism, where early subtle malformations play a central role. Hugo Lagercrantz vividly illustrated the crucial issue of how prematurity is associated with delayed complications stemming from the altered environment and communication.

Greg Holmes analysed the alterations that follow early life seizures and how the brain reacts with persistent cognitive and electrophysiological alterations. Few seizures early in life can have late devastating consequences, including in the operation of place cells and other essential neuronal constituents.

A *lasting legacy of DNA memories* was brought to the meeting by Dietmar Spengler, who illustrated the long-term sequels of stress during infancy and how early stress-dependent DNA memories arise from the coupling of neuronal activity and epigenetic machinery. In addition, these early insults can alter specific neuronal populations, leading to highly specific sequels in the behaviours in which these neurons are particularly engaged.

The acquisition and exploitation of adaptive skills was then analysed by Pico Caroni, who has been studying a circuit-based mechanism of basket cells. When these cells are invalidated early on, they will impact heavily on the operation of networks in which they play central roles. In addition, they mature at different stages and are invalidated accordingly.

Rosa Cossart used a large repertoire of elegant imaging and genetic tools and preparations to analyse the maturation of cortical interneurons and how these are specified from the earliest age to perform their tasks and control the operation of brain networks. GABAergic interneurons subtypes mature early and their invalidation will impact heavily on how the network matures.

Intrinsic voltage-gated currents also have their sequence. Neurofibromatosis type 1 is associated cognitive impairments, and these were analysed by Yppe Elgersma, who notably demonstrated that HCN currents are selectively reduced in parvalbumin-positive interneurons. This illustrates the complexity of the developmental sequence and the convergence of voltage and synapse-driven currents.

In addition, some neurons are only expressed transiently to facilitate and enable some connections to be made in a timely and specific manner. This is the case with the subplate transient networks that play a role in the integration of thalamo-cortical circuits, as reported by Zoltan Molnar and Guillermina Lopez-Bendito. They illustrated how thalamo-cortical activity is intrinsically regulated and crucial for the formation of adequate networks.

Heiko Luhmann reported his team's extensive analysis of the synchronized network activity in the developing cortex and how this impacts on programmed cell death.

IDENTIFYING MUTATIONS AND KEY PLAYERS

A number of studies illustrated the importance of identifying mutations to gain some idea of the key players. Chris Walsh showed the importance of somatic and point mutations, which are not expressed in blood and can only be expressed in subsets of cells in developmental disorders. Jamel Chelly developed the role of mutations of cytoskeletal genes in migration disorders and how these can help us to understand brain development. P (first name not supplied) Patric Bolton developed the case of tuberous sclerosis and

notably how cognitive defects follow recurrent spasms. Tony Winshaw-Boris discussed the alterations that take place in reeler mice, notably the dysregulation of glutamatergic signals due to decrease in reelin content. Gord Fishell examined the consequences of mutations of Mef2c and other signals in the formation of cortical basket cells and how this may lead to various psychiatric conditions. The vast repertoire of genes controlling the development of telencephalic interneurons was analysed by John Rubenstein, with particular regard to the development of the basal ganglia and possibly in relation to brain disorders.

REPLACING MISSING NEURONS WITH GRAFTED IMMATURE CELLS

Of course, one way out to solve these issues is to replace missing neurons by grafting immature cells from various sources and hope that these will replace the missing elements.

Theo Palmer discussed the issue of cellular reprogramming to identify and study neuron specific features of Parkinson's disease. Mohamed Jaber also discussed how grafts can be used to rescue networks after insults, showing how grafted cells send axons to their targets, notably from the insulted cortex to the spinal chord. The therapeutic usefulness of these tools is conditioned by a better understanding of the specific mechanisms of cortical neurons that Pierre Vanderhaegen developed at the meeting. Vania Broccoliadded to the debate by claiming that reprogramming dopaminergic cells is a promising therapy for Parkinson's disease. However, clearly this field is at a very premature situation notably because the crucial problems of the early malformations that will complicate the effects of grafts.

WHAT WE CAN LEARN ABOUT NONGENETIC INHERITANCE FROM OTHER DISCIPLINES

Last but not least, our special guest speaker came from a very different world, namely biology, ecology and evolution, where all sorts of animals are examined to detect and understand how these societies operate and how they evolve. Indeed, neuroscientists seem to disregard evolution and Darwinism as an important topic; yet, there are many common issues, at least conceptually. Evolution does not follow a preprogrammed plan – it reacts to the external conditions that organisms are facing. Nobody can predict the dominant colour of butterfly wings in 2050 or the animal species that will still be in existence in a couple of centuries. Animal behaviour provides us with many clues about how they adapt to ever-changing conditions. Etienne Danchin's spectacular talk covered nongenetic inheritance and the biological information that can be transmitted by genetic and nongenetic mechanisms. For example, female fish that are placed in certain conditions where they can view the performance of male fish, and select those they will accept, are heavily conditioned to favour those that other females are chasing. At least some of these features are transmitted across generations. The speaker called for a revival of the exclusively geno-centric framework, by adopting a broader view that included nongenetic inheritance into an extended theory of evolution.

BETTER UNDERSTANDING OF HOW EARLY INSULTS AFFECT BRAIN CIRCUITS IS NEEDED

These presentations were associated by lively, and at time tough, debates, which I think is an important feature of scientific meetings. Most notable was the discussion on genocentric dominating science, with delegates debating whether, and how, this could help from a diagnostic and, more questionably, therapeutic perspective. My personal view, as author of this review and organizer of the meeting, is that the time has come to focus strongly on how neurons and networks are active during brain development in health and disease rather than put our limited resources in identifying more mutations. We need to explore how mutations or environmental insults modify the programme, leading to a sort of freezing of the neuron at the developmental stage at which it was blocked and providing a signature of the disease. We also need to develop therapeutic tools that rely on drugs that selectively block immature, but not adult, currents. This possibility is already feasible in many circumstances and will be advocated more and more as proof of the neuroarchaeology concept grows.

My conviction is that we need to develop a better understanding of how insults occur early on and how they affect brain circuits, if we want to treat developmental disorders. Chasing novel mutations and investing primarily in projects that rely on big numbers and large amounts of data – such as the genome projects and the proteome, connectome, metabolomeor 'blue brain' – will at best illustrate our incapacity to innovate, based on novel concepts. Techniques help science but cannot generate concepts. Or, as Einstein used to say, 'innovation is thinking aside' (of highways).

References

1. Ben-Ari Y, Spitzer NC. Phenotypic checkpoints regulate neuronal development. *Trends Neurosci* 2010; 33: 485–92.
2. Ben-Ari Y. Neuro-archaeology: pre-symptomatic architecture and signature of neurological disorders. *Trends Neurosci* 2008; 31: 626–36.
3. Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev* 2007; 87: 1215–84.
4. Tyzio R, Cossart R, Khalilov I, Minlebaev M, Hübner CA, Represa A, et al. Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science* 2006; 314: 1788–92.
5. Lemonnier E, Degrez C, Phelep M, Tyzio R, Josse F, Grandgeorge M, et al. A randomised controlled trial of bumetanide in the treatment of autism in children. *Transl Psychiatry* 2012; 2: e202.