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Timing of developmental sequences in different brain structures: physiological and pathological implications

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Abstract

The developing brain is not a small adult brain. Voltage- and transmitter-gated currents, like network-driven patterns, follow a developmental sequence. Studies initially performed in cortical structures and subsequently in subcortical structures have unravelled a developmental sequence of events in which intrinsic voltage-gated calcium currents are followed by nonsynaptic calcium plateaux and synapse-driven giant depolarising potentials, orchestrated by depolarizing actions of GABA and long-lasting NMDA receptor-mediated currents. The function of these early patterns is to enable heterogeneous neurons to fire and wire together rather than to code specific modalities. However, at some stage, behaviourally relevant activities must replace these immature patterns, implying the presence of programmed stop signals. Here, we show that the developing striatum follows a developmental sequence in which immature patterns are silenced precisely when the pup starts locomotion. This is mediated by a loss of the long-lasting NMDA-NR2C/D receptor-mediated current and the expression of a voltage-gated K^+ current. At the same time, the descending inputs to the spinal cord become fully functional, accompanying a GABA/glycine polarity shift and ending the expression of developmental patterns. Therefore, although the timetable of development differs in different brain structures, the g sequence is quite similar, relying first on nonsynaptic events and then on synaptic oscillations that entrain large neuronal populations. In keeping with the 'neuroarcheology' theory, genetic mutations or environmental insults that perturb these developmental sequences constitute early signatures of developmental disorders. Birth dating developmental disorders thus provides important indicators of the event that triggers the pathological cascade leading ultimately to disease.

Introduction

Developing neurons and networks are active from the earliest developmental stages. Immature neurons progressively acquire the capacity to generate behaviourally relevant patterns. Voltage- and transmitter-gated currents follow a developmental sequence shifting progressively from long kinetic properties to faster currents required for the generation of time-locked activities. These changes are mediated by alterations of subunit compositions and are cell fate- and developmental stage-dependent, respecting the developmental gradients of brain structures. The long-lasting kinetics of immature currents enable the generation of coherent activities by cell assemblies endowed with few functional synapses. Their function is to enable neurons to fire together in spite of an extreme neuronal heterogeneity. In addition to intrinsic cell-autonomous sequences, the developmental programme includes network-driven patterns and coordinated maturation of different brain structures to generate a coherent behaviour. Thus, within the same population, earlier born neurons will already have operative synapses at a time when adjacent later born neurons are endowed with few or no functional synapses. The differences between the earliest and latest born neurons can exceed several months in the human brain, reflecting the degree of heterogeneity. Networks are also cell fate- and developmental stage-dependent; 'senior' older neurons connect and create networks with other 'senior neurons' (Deguchi *et al.*, 2011; Picardo *et al.*, 2011). Finally, development of activity in brain structures follows a developmental gradient, occurring earlier in 'older' structures than 'younger' structures – the brain stem or the spinal chord before the neocortex. In addition to these requirements, the connections between different brain structures must also mature in a timely fashion to enable neuronal ensembles to generate coherent patterns at appropriate times.

Several examples of these shifts have been extensively investigated. For example, *N*-methyl-D-aspartate (NMDA) currents that are instrumental for synaptic plasticity have a much longer time course in immature neurons because of specific subunits that are expressed early (Pollard *et al.*, 1993; Monyer *et al.*, 1994). Another example is the developmental shift in the polarity of gamma-aminobutyric acid (GABA) signals due to the higher intracellular chloride [$(Cl^-)_i$] levels of immature neurons (Ben-Ari *et al.*, 1989). The depolarization produced by GABA in developing neurons can generate action potentials either directly or by activating other intrinsic voltage-gated currents (Valeeva *et al.*, 10). It also removes the voltage-dependent Mg^{2+} block of NMDA channels, enabling GABA- and NMDA-generated currents

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to act coherently to produce large calcium influx (Leinekugel *et al.*, 1997). Immature networks generate a smaller repertoire of patterns than adults, and which are very different from those generated later (Ben-Ari *et al.*, 1989, 2007; Crepel *et al.*, 2007). Determining these developmental sequences is important because neuronal activity propagates early on from the periphery to the centres in sensory and motor systems in human and experimental animals, suggesting that centripetal activity helps to program the connectivity of central neuronal ensembles (Galli & Maffei, 1988; Meister *et al.*, 1991; Pizzorusso & Maffei, 1996; Hensch *et al.*, 1998; Milh *et al.*, 2007; Colonnese & Khazipov, 2010). These early patterns have their own functions and are better suited to developmental processes than the operation of adult networks.

These observations have important clinical implications as alterations of these sequences by environmental or genetic factors early in life lead to malformed or misconnected neuronal ensembles associated with lifelong outcomes. Birth dating of many neurological or psychiatric disorders reveals that they are induced by insults *in utero* or during early postnatal periods. Determining how these developmental sequences are generated and altered by insults is therefore of great importance.

Here we review these sequences and their links to developmental malformations. Specifically, we discuss their similarities and differences among different brain structures. We also reasoned that if a given brain structure is instrumental for the generation of a behaviour required at a specified age, it must have completed its developmental sequence for this behaviour to be produced at the appropriate age. This implies that the sequence has a beginning and a well-determined programmed end that takes into account cell-autonomous and network maturation. The corticostriatal system is particularly suitable in this regard, as developmental patterns must be silenced by the end of the first week after birth to enable target-coordinated motor behaviour. Finally, we briefly discuss the pathological and therapeutic implications of modifications of these sequences.

A general model – examples from hippocampus and neocortex

The developmental sequence of $GABA_A$ currents

Extensive studies of the embryonic and postnatal maturation of cellular and network events have been performed in the hippocampus. Using single-channel recordings of GABA and NMDA currents, large-scale dynamic network imaging coupled with targeted recordings from identified neurons and morphofunctional techniques, they have provided a detailed description of the patterns of developing networks and identified their generators. They revealed a number of features that were subsequently confirmed in many other brain structures, suggesting that this feature has been conserved throughout evolution (Ben-Ari *et al.*, 1989; Ben Ari *et al*., 2007; Crepel *et al.*, 2007).

GABA depolarizes and often excites immature neurons

GABA depolarizes and often excites immature neurons because of a higher intracellular concentration of intracellular chloride. The GABA polarity shift has now been confirmed in a wide range of brain structures and neuronal types. Using dual single GABA and NMDA channel recordings to measure the resting membrane potential (V_{rest}) and the driving force for GABA (DF_{GABA}), immature neurons were consistently found to have higher intracellular concentrations of intracellular chloride ($[Cl^-]_i$) than adult neurons (Tyzio *et al.*, 2003; Ben Ari *et al.*, 2007). There is a progressive decline in DF_{GABA} from embryonic stages to the second post-natal week (Fig. 1, right). Shortly before and after delivery, there is an abrupt reduction of DF_{GABA} to levels that are never again encountered (Tyzio *et al.*, 2006). This shift is coordinated by the hormone oxytocin, which triggers labour and reduces $[Cl^-]_i$. It exerts a neuroprotective and anaesthetic action on the newborn's brain (Tyzio *et al.*, 2006; Mazzuca *et al.*, 2011). Therefore, modulation of $[Cl^-]_i$ is an important means by which activity is regulated at early developmental stages.

Parallel investigations revealed that depolarizing GABA can generate sodium and calcium spikes in some neurons. In others, the depolarization produced by GABA is insufficient to reach spike threshold, but the slow kinetics of GABAergic postsynaptic currents (PSCs) are sufficient to trigger the noninactivating sodium current (I_{NaP}), which boosts depolarization to spike threshold (Valeeva *et al.*, 2010) (Fig. 2A). In addition, the depolarization removes the voltage-dependent block of NMDA channels, generating large calcium influxes that have trophic actions (Leinekugel *et al.*, 1997). The shift in polarity is brain structure-dependent (it occurs earlier in older structures) but is also neuronal fate- and age-dependent and even sex-dependent (Ben Ari *et al.*, 2007). Therefore, the developmental sequence of GABA polarity is a signature of neuronal maturation along many other parameters that progressively mature.

GABAergic synapses are operative before glutamatergic synapses

The first synaptic currents that hippocampal pyramidal neurons or interneurons generate are GABAergic ($GABA_A$ currents), with glutamatergic PSCs being generated later. A morphofunctional study revealed that in the postnatal rodent and in the *utero* primate hippocampus, pyramidal neurons are initially silent and possess an axon but no apical dendrites. At later stages, they have GABAergic but not glutamatergic PSCs and a small apical dendrite, and only at a later stage do they generate GABA and glutamatergic excitatory postsynaptic currents (EPSCs). At that stage they have basal and apical dendrites that reach the upper molecular layers (Tyzio *et al.*, 1999; Khazipov *et al.*, 2001). Clearly, the formation of glutamatergic but not GABAergic synapses is conditioned by the degree of development of the postsynaptic target. GABAergic interneurons follow the same sequence as glutamatergic neurons, but at an earlier age. Thus, almost 80% of interneurons already generate GABAergic and glutamatergic PSCs *in utero* at a time when most pyramidal neurons are silent (Hennou *et al.*, 2002). Therefore, at least in the rodent and *in utero* primate hippocampus (the only brain structure where this detailed analysis has been performed), $GABA_A$ signalling precedes glutamatergic signalling, i.e. very immature neurons 'speak' GABA first (Ben Ari *et al.*, 2004). The situation differs in neocortex where abundant glutamatergic activity is observed at early stages (Fig. 2B), although a detailed morphofunctional study of specific neuronal types has yet to be performed.

Intracellular chloride dynamics and the polarity of GABA actions

Two chloride cotransporters, NKCC1 and KCC2, the former importing and the latter exporting chloride, play a particularly important role in this sequence. In a large number of brain structures and animal species including humans, the membrane-bound KCC2 matures somewhat later than the chloride importer and operates more efficiently in adult than in immature neurons (Rivera *et al.*, 1999). Cytoplasmic KCC2 is present at earlier stages, acting to modulate the formation of spines. However, the diuretic and NKCC1 antagonist Bumetanide reduces the driving force of GABA in adult slices, suggesting that this chloride importer is functional in adult neurons (Tyzio *et al.*, 2008). Early expression of KCC2 (Fig. 1, right) and shift

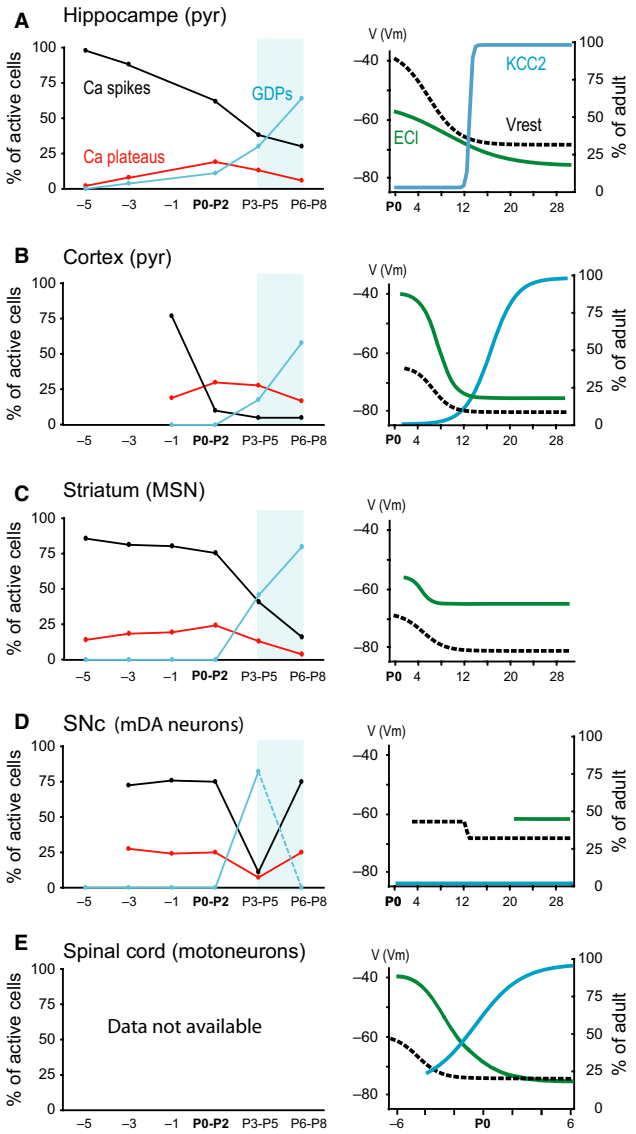

FIG. 1. Developmental profiles of immature activities and chloride equilibrium potential in the pyramidal neurons of the hippocampus (rat) (A), sensorimotor cortex (rat) (B), in the ouput medium spiny neurons (MSN) of the striatum (mouse) (C), in the dopaminergic neurons of the substantia nigra compacta (mDA neurons, mouse) (D) and in the motoneurons of the spinal cord (rat) (E). Left – graphs indicate the mean fraction of cells evoking at least one intrinsically driven Ca^{2+} spike (black), one intrinsically driven Ca^{2+} plateau (red) and synapse-driven synchronous Ca^{2+} spikes (GDPs, blue) relative to the number of active cells at successive age groups between embryonic to first postnatal stages. The phase of synapse-driven synchronous activities is indicated in light blue. All diagrams are aligned at P0 (rat – E21 = P0; C57Bl6 mouse – E19 = P0). Hippocampus and cortex – −5 = E16, −3 = E18, −1 = E20. Striatum and SNc – −5 = E14, −3 = E16, −1 = E18. Right – schematic summary of the perinatal development of the chloride equilibrium potential (E_{Cl}; left *y*-axis) and the resting membrane potential (V_{rest}; left *y*-axis) in the output neurons of the different structures. The hyperpolarizing shift is mainly due to the increased expression of KCC2 (right *y*-axis, normalized to adult, 100%). Data obtained from Rivera *et al.* (1999), Washio *et al.* (1999), Dzhala *et al.* (2005), Crepel *et al.* (2007), Tepper & Lee (2007), Delpy *et al.* (2008), Rheims *et al.* (2008) and Dehorter *et al.* (2011).

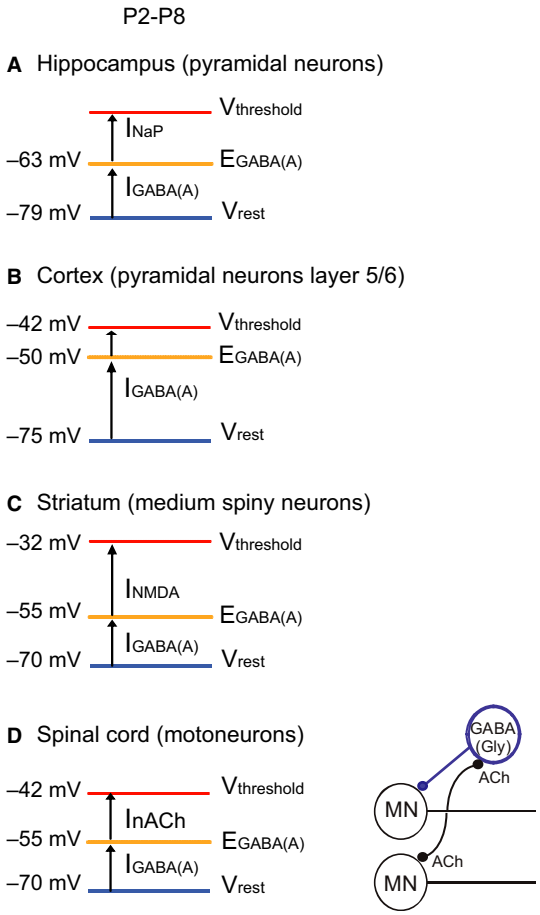

FIG. 2. Different ways by which GABA excites immature neurons. (A, B) The $GABA_A$–I_{NaP} relationship. $GABA_A$-induced depolarization activates the tetrodotoxin- and phenitoin-sensitive, subthreshold, persistent sodium current (I_{NaP}), which in turn triggers action potentials. (C) The $GABA_A$–NMDA reloationship. $GABA_A$-induced depolarization removes the Mg^{2+} block and activates the NMDA channel. (D) The $GABA_A$–nACh relationship. $GABA_A$-induced depolarization; right – functional coupling between spontaneous dorsal and ventral root activities in the neonatal spinal cord. The network generating spontaneous activity activates GABAergic interneurons responsible for primary afferent depolarizations (PADs). Action potentials that are triggered by PADs reaching firing threshold propagate both antidromically and orthodromically. The orthodromic volley generates EPSPs in motoneurons and/or premotor interneurons. Modified from Bos *et al.* (2011).

of GABA actions from depolarizing to hyperpolarizing impacts neuronal maturation and promotes GABAergic synapse formation (Chudotvorova *et al.*, 2005; Kriegstein, 2005; Wang & Kriegstein, 2008). In contrast, invalidating KCC2 generates *in utero* seizures and aberrant patterns, in keeping with important roles that the cotransporter exerts at an early stage that are not restricted to control of chloride fluxes (Li *et al.*, 2007; Pellegrino *et al.*, 2011). Whatever the underlying mechanism, immature neurons remove chloride much less efficiently than adults, indicating that ongoing activity will lead to higher levels of intracellular chloride (Nardou *et al.*, 2011). In fact, even brief synaptic stimuli alter the driving force for GABA, particularly in immature neurons. This is mediated by an important plasticity of the chloride exporter KCC2, which is readily internalized by enhanced neuronal activity (Fiumelli & Woodin, 2007; Balena & Woodin, 2008; Lee *et al.*, 2010; Nardou *et al.*, 2011). This type of plasticity is restricted to GABAergic (and glycinergic) synapses as it shifts the polarity of the actions of GABA or glycine, and not only the amplitude of the currents as in more traditional forms of plasticity (long-term depression and long-term potentiation). Interestingly, two types of KCC2 have now been identified. The fact that one is primarily expressed in immature neurons raises the possibility that KCC2, like other systems, follows a developmental sequence (Blaesse *et al.*,

2009). Therefore, chloride cotransporters follow a developmental sequence and are 'sloppier' at an early stage.

The developmental sequence of brain patterns

Hippocampal networks follow a triphasic sequence that was recently determined in detail using dynamic multiphoton imaging and identifying the activity of hundreds of neurons in slices (Crepel *et al.*, 2007) and was confirmed in the sensorimotor cerebral cortex (Allène *et al.*, 2008) (Fig. 1A and B, left).

Intrinsic calcium currents

Embryonic pyramidal neurons, like interneurons, first generate intrinsic voltage-gated currents. At this early stage, neurons do not have functional synapses nor do they fire together. Recordings from several thousand neurons show no synchronies or synchronized patterns. These events are clearly due to voltage-gated calcium currents with no involvement of transmitter-gated synaptic currents. At that early stage, neurons generate 'sloppy' sodium currents with no overshoots or long-lasting durations, in keeping with their inability to generate time-locked events.

Synchronized calcium plateaus in small cell assemblies interconnected by gap junctions

These events, the first recorded synchronized currents, are intrinsic nonsynaptic voltage-dependent currents associated with large plateaus and high-frequency calcium oscillations. They are particularly frequent among small ensembles of GABAergic interneurons (Crepel *et al.*, 2007). Calcium plateaus are resistant to applications of GABA and glutamate receptor antagonists but are readily suppressed by blockers of voltage-gated calcium currents. Although blockers of gap junctions are not highly specific, there are indications that calcium plateaus are indeed generated by gap junctions, most likely enabling the generation of calcium oscillations required for the activation of genetic cascades and the generation of peptides specific to various types of interneurons.

Giant depolarising potentials (GDPs)

An early study of neuronal activity revealed the presence of a polysynaptic pattern dominating the activity generated by immature hippocampal slices until the second postnatal week (Ben-Ari *et al.*, 1989). The background activity between these recurrent bursts was much reduced and progressively augmented until the failure of these events. This pattern, referred to as GDPs, was observed in other brain structures and considered to be the first synapse-driven pattern of developing networks (Ben-Ari *et al.*, 1989; Ben Ari *et al.*, 2007). GDPs are generated by depolarizing GABAergic currents and glutamatergic currents and as such are modulated/blocked by a wide range of molecules acting on voltage- and transmitter-gated currents (Canepari *et al.*, 1999; Kasyanov *et al.*, 2004; Mohajerani & Cherubini, 2006). GDPs are synchronized events that engage large numbers of neurons before disappearing by the end of the second postnatal week (Leinekugel *et al.*, 1998). GDPs are orchestrated by a subset of GABAergic hippocamposeptal interneurons identified as Hub neuronal generators (Bonifazi *et al.*, 2009). These neurons are endowed with unique features, including early maturation, specific morphofunctional features and a widely arborized axonal arbour (Bonifazi *et al.*, 2009; Picardo *et al.*, 2011). Hub neurons often discharge before other GABAergic or glutamatergic neurons during generation of GDPs. None of the pyramidal neurons orchestrated GDPs, confirming the crucial role of GABAergic signals in their synchronization. Therefore, specific subtypes of GABAergic neurons are programmed to coordinate the earlier synaptic patterns that the developing network will generate.

The developmental sequence in the basal ganglia – intrinsic then input-driven

The basal ganglia are a set of interconnected nuclei that play a role in the automatic execution of learned motor plans. They are part of corticobasal ganglia-thalamocortical loops. The striatum or caudate putamen is the major entry structure, receiving information from nearly all cortical areas. The basal ganglia is highly suited to identification of developmental sequences as adult striatal neurons are mostly silent to enable incoming information from the cortex to activate relevant loops involved in motor coordination. Therefore, these neurons are expected to behave like other neurons, with synchronized activities at an early stage, and then shift to a pattern compatible with targeted motor behaviour with specific timing. The integrative function of the striatum, composed almost exclusively of GABAergic neurons, depends on the activity of cortical and dopaminergic afferents. Recent studies have determined the developmental activities of the striatum and midbrain dopaminergic neurons, as well as the maturation of corticostriatal synapses (Dehorter *et al.*, 2011; Ferrari *et al.*, 2012).

The developing striatal network generates a sequence of immature patterns reminiscent of that recorded in the motor cortex

There are striking similarities between the developmental activities of cortical and striatal networks, but also subtle differences due to the particularities of the structure of the striatum. The striatal network does not contain glutamatergic neurons and their principal neurons, the dominant GABAergic medium spiny neurons (MSNs), are highly hyperpolarized at rest and require strong coincident excitatory glutamatergic inputs to fire, due to the expression of diverse voltage-gated K^+ currents (Wilson & Kawaguchi, 1996; Blackwell *et al.*, 2003). These adult features are absent in embryonic MSNs and develop slowly after birth, thus allowing spontaneous immature patterns to emerge before being switched off.

Like neocortical or hippocampal neurons, MSNs first generate nonsynaptic, intrinsic, voltage-gated Ca^{2+} spikes and Ca^{2+} plateaus via activation of L- and N-type Ca^{2+} channels (Dehorter *et al.*, 2011) (Figs 1C and 3A). Because embryonic and young postnatal MSNs have a V_{rest} closer to $V_{threshold}$ than the adult and a high membrane resistance (Fig. 3C), spontaneous activation of Na^+ and Ca^{2+} channels may occur. The intrinsic nature and time coherence of Ca^{2+} plateaus between MSNs organized in clusters (Fig. 3B), together with their link to long-lasting or recurrent membrane depolarization, is reminiscent of cortical calcium plateaus.

The first synapse-driven pattern appears in the striatum at the end of the first postnatal week (P5–P7) and subsequently disappears. The majority of the active striatal neurons (MSNs and interneurons) are engaged in this highly synchronized activity, consisting of synchronized bursts of 2–3 Na^+ spikes that lead to synchronized Ca^{2+} spikes. Striatal GDPs, like hippocampal GDPs, are mainly driven by NMDA channels (Figs 2C and 3E) (Dehorter *et al.*, 2011). MSNs generate a large and transient NR2C/D-mediated component in the cortically evoked excitatory postsynaptic potentials (EPSPs) that is blocked by a preferential antagonist of NR2C/D-containing NMDA channels. This suggests that a transient NR2C/D-mediated current

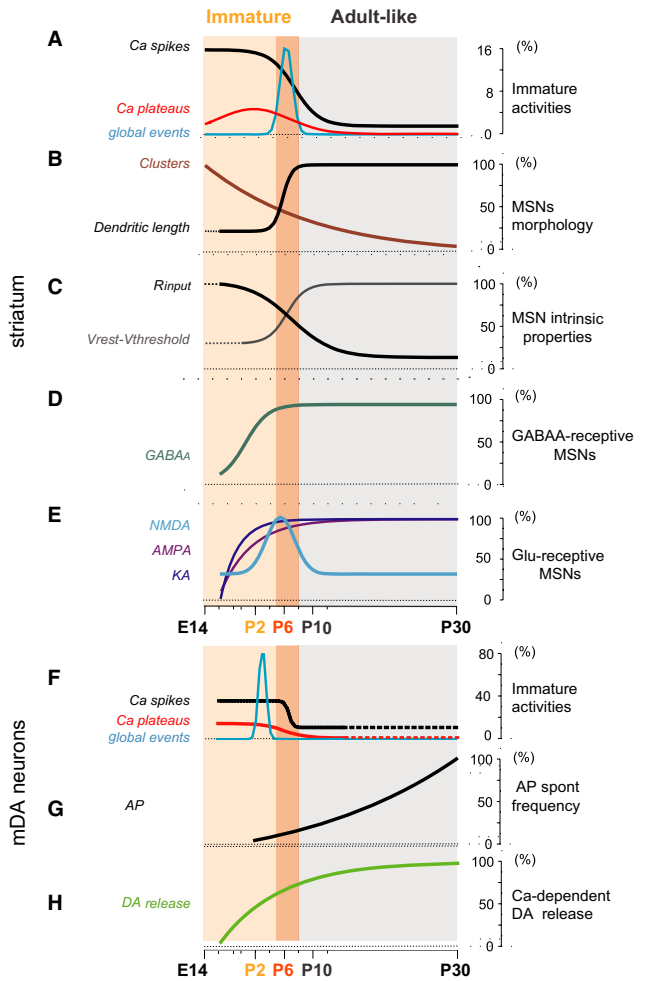

FIG. 3. Parallel development of the intrinsic and synaptic properties of medium spiny neurons (MSNs) of the striatum (A–E) and of midbrain dopaminergic (mDA) neurons (F–H). Immature (light orange) and adult-like (grey) phases are separated by a transitory immature period (orange). From Dehorter *et al.* (2011) and Ferrari *et al.* (2012).

plays a major role in the generation of GDPs (Dehorter *et al.*, 2011). Before P2 and after P10 cortically evoked EPSPs is mostly mediated by AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors. GABAergic synapses depolarize MSNs to a potential closer to the action potential threshold in immature than adult MSNs, but other depolarizing currents are required to reach spike threshold (Fig. 2C).

Therefore, the immature striatum generates GDPs during the transient period of long-lasting synapse-driven patterns of activity with large NMDA receptor-driven currents, a general feature of many developing brain structures (Pollard *et al.*, 1993; Monyer *et al.*, 1994; Nansen *et al.*, 2000; Logan *et al.*, 2007; Dravid *et al.*, 2008). Together with voltage-gated Ca^{2+} currents, they trigger the large calcium fluxes needed for a wide range of essential developmental functions, including neuronal growth, synapse formation and the formation of neuronal ensembles (Ben-Ari, 2001; Spitzer, 2006). Clearly, GDPs are mainly driven by corticostriatal synapses at a time when cortical neurons also generate GDPs (Fig. 1B and C, left). Then, in the middle of the second postnatal week, MSNs shift to an adult-like pattern characterized by little activity *in vitro* (Carrillo-Reid *et al.*, 2008). This rather abrupt shift is due to a major alteration of intrinsic and extrinsic currents of MSNs, including an abrupt loss of NR2C/D and an increased expression of IK_{IR} (Belleau & Warren, 2000) leading to reduction of input resistance, hyperpolarizing shift of V_{rest} and increased driving force of GABA (Figs. 3A-E). As a result, MSN activity shifts from an immature low-threshold activation state to a high-threshold adult state with a low activity profile during resting conditions.

Midbrain dopaminergic neurons

Rodent midbrain dopaminergic (mDA) neurons contact striatal neurons a few days before birth. If the nigrostriatal pathway is functional at an embryonic stage, the release of dopamine may impact the development of the striatal network. Using immature nigrostriatal slices (Ammari *et al.*, 2009) from tyrosine hydroxylase (TH) GFP mice, a recent study has shown that mDA neurons spontaneously generate propagating Na^+ spikes and release DA upon stimulation around birth (P0). Before E18, mDA neurons generate nonsynapse-driven, voltage-gated Ca^{2+}spikes and Ca^{2+}plateaus similar to those recorded in cortical and striatal networks, via N- and L-type Ca^{2+} channels (Ferrari *et al.*, 2012). Immediately after birth, they generate ongoing synchronized synapse-driven patterns (Figs 1D and 3F). These, however, are not frequent *in vitro* because subthalamic nucleus-derived glutamatergic inputs, like GABAergic inputs from the substantia nigra reticulata, pallidum and striatum, are lacking in slices. Interestingly, there is an abrupt change in the generation of sodium spikes immediately after birth (Fig. 3G) (Trent *et al.*, 1991; Ferrari *et al.*, 2012). Direct measures of evoked dopamine overflow in the striatum revealed that mouse mDA neurons release dopamine upon stimulation in a Ca^{2+}-dependent manner (Fig. 3H). Therefore, the transmitter release machinery including Ca^{2+} influx through presynaptic voltage-gated Ca^{2+} channels and Na^+ action potentials are operative shortly before birth, suggesting that they are in a position to modulate the development of striatal networks.

The developmental sequence in the spinal cord – intrinsic then input-driven

An overview of the developmental sequence of the spinal cord is difficult, as the major discoveries have been made in *Xenopus* sp., chick and rodents. However, some striking similarities emerge from the comparison between the development of the spinal cord and that of cortical and striatal networks.

Maturation of inhibitory synaptic transmission

In contrast to the adult hippocampus, neocortex and basal ganglia, inhibitory synaptic transmission in the adult spinal cord relies on both GABA and glycine. However, the developmental sequence is nearly identical, with GABAergic signalling appearing first. GABA-immunoreactive cells are detected in the ventral horn at E11.5 and E13.5 at cervical and lumbar levels, respectively (Ma *et al.*, 1992; Allain *et al.*, 2004) and the first synaptic activity, at E12.5, is composed exclusively of GABAergic postsynaptic events (Scain *et al.*, 2010). Glycinergic postsynaptic currents appear 1 day later (E13.5). There is a switch from GABA to glycine in the pharmacological nature of mIPSCs during perinatal development (Gao & van den Pol, 2001; Sadlaoud *et al.*, 2010). Therefore, GABA provides the universal programmed transmitter-gated system even in neurons that are later inhibited by glycine (also see Jonas *et al.*, 1998).

In parallel with cortical neurons, GABA and glycine depolarize and even excite (trigger action potentials) immature spinal cord neurons *in*

vitro (Wu *et al.*, 1992; Gao & Ziskind-Conhaim, 1995; Nishimaru *et al.*, 1996; Gao *et al.*, 1998; Delpy *et al.*, 2008) (Figs 1E and 2D). Interestingly, blockade of $GABA_A$ receptors by injection of gabazine or bicuculline *in ovo* blocks embryonic movements, suggesting that GABAergic currents are likely to be excitatory in the living embryo (Wilhelm & Wenner, 2008). The switch from depolarizing to hyperpolarizing GABA/glycine occurs in lumbar motoneurons shortly after birth in both the rat (Takahashi, 1984; Jean-Xavier *et al.*, 2006; Stil *et al.*, 2009) and the mouse (Stil *et al.*, 2009, 2011). The expression of KCC2 increases significantly while that of NKCC1 decreases during this time window (Stil *et al.*, 2009, 2011).

Relative contributions of neurotransmitters to spontaneous activity

The neuropharmacological control of spontaneous activity changes during development (Hanson & Landmesser, 2003; Ren & Greer, 2003; Myers *et al.*, 2005). At the earliest stages, acetylcholine and GABA/glycine play a key role, and stimulation of motoneurons elicits episodes of activity that propagate through the lumbar spinal cord, suggesting that motoneurons make excitatory connections on each other and on glycine/GABAergic interneurons via nicotinic receptors (Fig. 2D). As in cortical networks, electrical transmission is important because blockade of gap junction coupling by carbenoxolone abolishes the rhythmic episodes. Later, glutamatergic neurotransmission (acting via non-NMDA receptors) becomes necessary, whereas blocking cholinergic transmission has little effect. There is a switch in the contribution to spontaneous activity by chloride-mediated conductances from excitatory to inhibitory during late gestation; this switch occurs at about the same time as the transition from cholinergic to glutamatergic transmission (Fig. 2D).

Calcium oscillations

As in the cortex and the striatum, spinal cord neurons become excitable prior to synapse formation, and generate spontaneous calcium transients, as shown in *Xenopus* embryos ('calcium spikes'; Gu *et al.*, 1994). Spontaneous network activity is also accompanied by Ca^{2+} oscillations (Wenner & O'Donovan, 2001; Wang *et al.*, 2009). Calcium currents are particularly robust in the very first postnatal days and underlie stimulation-induced tetrodotoxin-resistant spikes (Walton & Fulton, 1986). Similar spikes can be triggered only when potassium conductances are reduced at the end of the first postnatal week. T-type calcium channels, responsible for the low-voltage-activated current, are present in spinal motoneurons at embryonic ages (E11 in rats) and are essential, in addition to an Na^+ persistent current, to initiate spontaneous bursts and associated transients (Wang *et al.*, 2009). The current density rapidly decreases with age, and functionally mature mouse motoneurons (P9–16) do not express this current (Carlin *et al.*, 2000). In contrast, the high-voltage-activated calcium current increases during development (Gao *et al.*, 1998).

NMDA and non-NMDA receptors are transiently expressed at high levels throughout the spinal grey matter early during development in rodents as well as in humans at late fetal ages (Kalb & Fox, 1997; Akesson *et al.*, 2000), and decrease postnatally. The composition of the different NMDA, kainate and AMPA receptors changes with age in rat, the highest levels of expression being observed during the two-first postnatal weeks for all subunits (Stegenga & Kalb, 2001). Non-NMDA receptors expressed by neonatal rat motoneurons show greater sensitivity to agonists and allow Ca^{2+} entry (Jakowec *et al.*, 1995; Metzger *et al.*, 2000) as shown in cultured *Xenopus* spinal neurons (Gleason & Spitzer, 1998; Rohrbough & Spitzer, 1999).

Spontaneous activity modulates important developmental functions

The frequency of Ca^{2+} transients specifies the neurotransmitter phenotypes of *Xenopus* spinal neurons (Borodinsky *et al.*, 2004; Spitzer & Borodinsky, 2008). *In ovo*, early motor axon path finding is highly dependent on the normal pattern of bursting activity. A modest decrease in episode frequency results in dorsal–ventral path finding errors by lumbar motoneurons, and in the downregulation of several molecules required to successfully execute this guidance decision (Hanson & Landmesser, 2004). Increasing the episode frequency strongly perturbs the path finding process by which motoneurons fasciculate into pool-specific fascicles at the limb base and then selectively grow to muscle targets (Hanson & Landmesser, 2006). Resumption of normal frequency allows axons to correct the antero-posterior path finding errors by altering their trajectories distally, indicating the dynamic nature of this process and its continued sensitivity to patterned activity.

In addition, spontaneous activities and associated muscle twitches contribute to an activity-dependent synaptic plasticity and thereby to the refinement of synaptic connections, as shown for the nociceptive withdrawal reflexes (Petersson *et al.*, 2003; Schouenborg, 2004). This reflex system undergoes a profound functional adaptation, with erroneous connections depressed/eliminated and adequate connections strengthened. Interestingly, tactile sensory feedback due to spontaneous movements controls the formation of functional nociceptive networks.

An abundant literature illustrates the role of early patterns in the formation of sensory systems (Shatz & Stryker, 1988; Pizzorusso & Maffei, 1996; Katz & Crowley, 2002; Stellwagen & Shatz, 2002; Cang *et al.*, 2005; Colonnese & Khazipov, 2010). Together, these observations suggest several commonalities between the spinal cord, basal ganglia and neocortex that follow a similar sequence with large neuronal ensembles engaged in synchronized activities. At early stages they are quite independent of external inputs. More coordinated activities then become possible when these structures become interconnected and the upper centres start controlling behaviourally relevant patterns.

Spontaneous activity in the immature spinal cord

Immature vertebrates exhibit spontaneous movements, as shown in the rat fetus (E16–20; Narayanan *et al.*, 1971) (Fig. 1E). Motility extends gradually in rostrocaudal and proximodistal directions. Movement of the different limbs occurs synchronously, i.e. at nearly the same instant (Kleven *et al.*, 2004). Spinal cord transection, in which the spinal cord is isolated from the brain, prevents neither spontaneous movements of the hind limbs (Robinson *et al.*, 2000) nor the frequency of movements (Waldenstrom *et al.*, 2009), demonstrating that these patterns are generated within the spinal cord (Landmesser & Odonovan, 1984; Nakayama *et al.*, 1999; Vinay *et al.*, 2002; Yvert *et al.*, 2004; Bos *et al.*, 2011). This is observed as early as E12–13 in mice, when many lumbar motoneurons are still migrating and extending their peripheral projections (Hanson & Landmesser, 2003). This spontaneous activity follows marked developmental changes (Nakayama *et al.*, 1999; Yvert *et al.*, 2004) with only cervical and thoracic ventral roots exhibiting rhythmic activity at the earliest stages in rats (E13.5). The activity then spreads to lumbar segments. Spontaneous movements in the tail are sporadic at the beginning of the postnatal period, attain a peak at P5–P11

and then decline gradually in occurrence when locomotion starts (Fig. 1E) (Waldenstrom *et al.*, 2009).

Relevance to the development of locomotion

Maturation of the striatum and locomotion onset

The development of the striatal network, and the chronology of MSN silencing in particular, parallels the development of quadruped motion in pups. Pups only crawl and rotate during the first postnatal week with their abdomen stuck to the floor whereas they begin to spontaneously move with the ventral surface of their body held above the floor during the middle of the second postnatal week (Dehorter *et al.*, 2011). There is thus a perfect coincidence between the timing of the abrupt shift of MSN intrinsic and corticostriatal inputs and the onset of locomotion, suggesting that the developmental sequence includes a stop signal adequately timed to enable striatal neurons to generate quadruped movements. The alterations of NMDA currents, shortening of cortico-striatal EPSCs and associated expression of IK_{IR} are some of the important elements of this programmed sequence. Therefore, a stop signal in the basal ganglia blocks developmental activities at the appropriate stage for coordinated quadruped locomotion. The thalamo-cortical loops, sensorimotor cortex and ventral networks of the spinal cord that play a crucial role in movement coordination follow a similar parallel development timescale (Fig. 4) (Gianino *et al.*, 1999; Vinay *et al.*, 2002; Allene *et al.*, 2008; Evrard & Ropert, 2009; Dehorter *et al.*, 2011). The maturation of intrinsic programmes occurs in parallel with that of functionally related units to generate locomotion at the appropriate age (Grillner *et al.*, 2005) (see below).

Modulation of spinal cord networks by the brain

Although the presence of the brain is not required for spontaneous activity in the spinal cord, the gradual arrival of descending pathways affects the excitability of spinal networks. In the rat, projections from the brainstem nuclei start to reach the lumbar cord a few days before birth, and the number of descending axons then increases gradually until the end of the second postnatal week when most descending pathways are fully developed (leong *et al.*, 1984). The first corticospinal projections can be detected only at the end of the first postnatal week in the rat (Donatelle, 1977). Spinal cord sections, removing all supraspinal influences, or inhibition of serotonin synthesis during the perinatal period markedly affects the maturation of electrical properties (Pflieger *et al.*, 2002) and prevents the maturation of inhibitory synaptic transmission (Jean-Xavier *et al.*, 2006; Sadlaoud *et al.*, 2010). The full maturation of descending inputs during the second postnatal week corresponds to the disappearance of spontaneous activity in the spinal cord and the appearance of weight-bearing locomotion (Fig. 4). Therefore, fully mature descending pathways constitute a prerequisite for the emergence of locomotion.

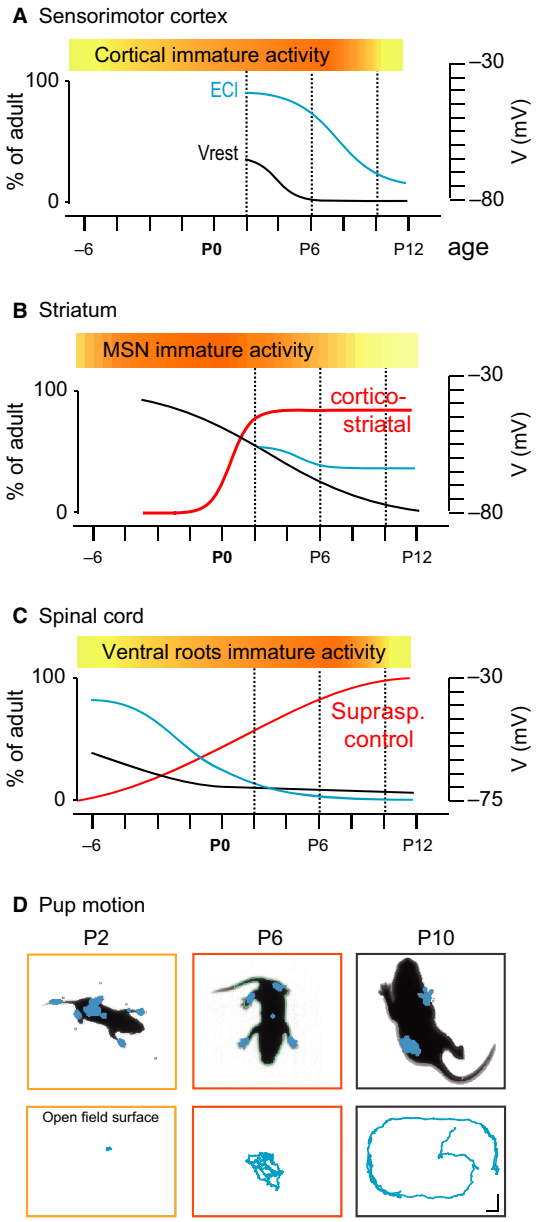

FIG. 4. Sensorimotor cortex, striatum and spinal cord output neurons enter an adult-like phase before pups begin quadruped locomotion. (A–C) The development of corticostriatal and supraspinal control (left *y*-axis) are indicated in parallel with the developmental profile of ECl and V_{rest} (right *y*-axis). (D) Photos of representative postnatal mice showing the number and extent of pressure points (in blue) at P2, P6 and P10 (top). Body motion of the same pups in the open field (scale bars, 3 cm) during a 1-min test (bottom). Reproduced from Dehorter *et al.* (2011).

Clinical and pathological implications – birth dating brain disorders

Developing neurons thus have their own agenda in many brain structures, generating specific patterns that are not observed later. As ionic currents of immature neurons differ from their adult counterparts, drugs developed to treat adult neurons may exert different actions on immature neurons, calling for the development of therapies adapted to immature neurons (see also below).

A conceptual model has recently been proposed to accommodate these observations. It has been suggested that early neuronal activity operates as a checkpoint controlling the correct implementation of the genetic programme (Ben-Ari & Spitzer, 2010). Neurons and networks operate with feedback controls that act as homeostatic devices and serve as useful sources of information during the implementation of the programme. In this theory, activity and the events associated with it – molecular, biochemical and genetic activation – act as quality control, providing useful information on the developmental stage and the adequacy of the programme implementation. Using the sRNAi *in utero* approach, neuronal migration can be slowed by invalidating the double cortin (DCX) gene. We observed that neurons that have not correctly migrated express immature currents as if they have remained

'frozen' in an immature state (Ackman *et al.*, 2009). The suggested model is that a neuron meant to shift from current A to B when it has performed a given developmental step will remain with current A if this step fails. Interestingly, this situation has been repeatedly observed after seizures and brain lesions, trauma and spinal chord lesions. Indeed, in each of these situations, chloride accumulates in the intracellular medium and GABA excites neurons (Cohen *et al.*, 2002, 2003; Khalilov *et al.*, 2003, 2005; Boulenguez *et al.*, 2010). Other immature parameters have been observed, most notably immature NMDA receptor-channel currents. Clearly, selective antagonists that block immature but not adult currents may provide useful therapeutic strategies in many drug-resistant developmental disorders. The administration of such drugs may silence these immature patterns without altering or blocking adult networks.

These observations imply that an insult occurring at an early developmental stage will disrupt the normal sequence, leading to a clinical syndrome with a variable delay – the onset of the disease does not coincide with this initial insult (Ben-Ari, 2008). Studies on a variety of disorders, including monogenic disorders such as Huntington disease, have shown important brain malformations well before the first clinical signs (Nopoulos *et al.*, 2010). After a series of modifications, gene therapy will probably not be efficient. Interestingly, conditional introduction of the correct DCX gene after invalidation partially corrects the deficiency a few days after birth but not later (Manent *et al.*, 2009). In summary, better understanding of developmental neurobiology, its rules and failures will heavily impact our understanding of the operation of adult networks in health and disease.

Conclusions

The results of the last few decades have provided a general model of developmental sequences with invariably local differences due to the different roles of each functional unit. The common features include the early appearance of calcium intrinsic signalling and nonsynapse-driven events prior to the onset of synaptic currents. Immature neurons first communicate using voltage-gated currents and this is in keeping with the early evolution of these currents. The timing of the GABA shift from depolarizing to hyperpolarizing is sex-, brain structure- and neuronal type-specific, again stressing the importance of the fate and birth date of individual neurons. Collectively, this suggests that greater focus should be given to the fate and birth dates of neurons and networks. A wide range of evidence suggests that synchronized patterns first emerge within a given brain structure before external patterns of activity arise to influence their properties and outcome. An important missing element in this scheme is the mechanism that controls the time at which local patterns start to be modulated and coordinated with external sources. The emergence of coordinated targeted movements and mobility takes place quite abruptly around P8–P10. This coincides with a reduction of corticostriatal EPSCs and ongoing activity due to the loss of the long-lasting NR2C/D subunits, and at the same time the expression of mature descending inputs to the spinal cord. The striatum, like the neocortex and the spinal cord, have previously followed a quite similar developmental sequence and are now ready to operate coherently with their inputs and outputs to generate the required behaviour at the appropriate time. An error or delay in this convergence would be catastrophic. Thus, a striatum or a spinal cord generating high-frequency spontaneous GDPs would fully incapacitate coordinated movements at a time when these must be generated. The animal would be either akinetic – like Parkinson patients – or following erratic noncoordinated movement. Similarly, long-lasting retinal waves persisting after eye opening would lead to blindness. Although we do not know at present how these distal networks are organized in time, it is reasonable to assume that the youngest neuronal population conditions the timing of this sequence – the target neurons of descending pathways in the spinal cord must have finished their developmental sequence like the striatal neurons to enable time-locked corticostriatal synaptic currents. A recent study provides some clues, showing that thalamocortical inputs initially control the generation of immature cortical patterns prior to the maturation of corticocortical horizontal connections (Minlebaev *et al.*, 2011). If this scheme is confirmed and its mechanisms understood, it will be easier to better delineate how developmental disorders are produced. We anticipate that incorrect implementation of the programme either locally or in the connections with distal structures will lead to abnormalities in wiring and/or migration that can later develop into pathology. Clearly, developmental neurobiology will provide better clues to the birth dates of disorders and possibly enable preventive interventions to delay/prevent expression of the disease.

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Abbreviations

$[Cl^-]_i$, intracellular concentration of chloride; EPSCs/IPSCs, excitatory/inhibitory postsynaptic currents; GABA, gamma-aminobutyric acid; GDPs, giant depolarizing potentials – the first synapse-driven network pattern; KCC2, a chloride cotransporter that exports two molecules of chloride and imports two molecules of potassium; mDA, midbrain dopaminergic; MSNs, medium spiny neurons – the major neuronal type of the striatum; NKCC1, a chloride cotransporter that imports two molecules of chloride and exports one molecule of sodium and one of potassium; NMDA, *N*-methyl-D-aspartate; PSCs, postsynaptic currents; $V_{rest,}$ resting membrane potential; $V_{threshold,}$ minimal voltage at which action potentials are generated.

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