Phenotypic checkpoints regulate neuronal development

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Nervous system development proceeds by sequential gene expression mediated by cascades of transcription factors in parallel with sequences of patterned network activity driven by receptors and ion channels. These sequences are cell type- and developmental stage-dependent and modulated by paracrine actions of substances released by neurons and glia. How and to what extent these sequences interact to enable neuronal network development is not understood. Recent evidence demonstrates that CNS development requires intermediate stages of differentiation providing functional feedback that influences gene expression. We suggest that embryonic neuronal functions constitute a series of phenotypic checkpoint signatures; neurons failing to express these functions are delayed or developmentally arrested. Such checkpoints are likely to be a general feature of neuronal development and constitute presymptomatic signatures of neurological disorders when they go awry.

Constructing the nervous system: the scale of the problem

The complexity of the nervous system makes the developmental assembly of this structure unusually challenging. Neuronal phenotypes are specified and synaptic connections are formed with prodigious specificity. An argument can be made that the brain begins simply and that complexity is built up gradually. However, 80% of 20 000 mouse genes are expressed in the adult nervous system [1]. With 10^{11} neurons making 10^{15} synapses, this number of genes is insufficient to program the development of the nervous system on a single-gene-to-single-component basis. How is such a complex program regulated during development? Cascades of transcription factors play an important role [2,3]. However, there is significant potential for disruption of neuronal development by mistakes in transcriptional machinery or perturbations of gene expression. Indeed, there are a vast number of genetically- or environmentally-driven developmental disorders, with adverse societal and financial impact.

Fortunately, developing neurons are not mute during development. They express cell- and developmental stage-specific sequences of voltage-gated and transmitter receptor-linked ion-channel currents that provide read-outs of their state of differentiation Often, owing to the expression of different channel subunits, immature currents are more 'sloppy' than adult ones and their long synaptic durations account for the relatively slow kinetics that enable calcium influx at early developmental stages [4–8]. Immature networks also follow a specific developmental sequence initially characterized by intrinsic, synapse-independent voltage-gated calcium currents, followed by large calcium plateaus in small neuronal populations connected by gap junctions. Subsequently, primitive spontaneous synapse-driven patterns appear, such as the so-called giant depolarizing potentials (GDPs) in the hippocampus and neocortex that suppress the large calcium plateaus [9–11] (Figure 1) and retinal waves in the visual system [12–17]. These simple patterns of activity then disappear as the networks become capable of generating more diversified behaviorally relevant patterns. Interestingly, these primitive patterns are essential for the correct construction of cortical ensembles but are generated at a time when sensory systems are not yet working. Thus, retinal waves are generated well before vision is functional and operate to enable adjacent neurons to fire together and make synaptic connections with adjacent targets [12–18]. Although the timing of these patterns differs between different animal species and brain structures, the sequences appear identical in postnatal rodents and *in utero* primates, suggesting that they have been preserved throughout evolution. Here we suggest that these developmental sequences constitute a series of checkpoints controlling the appropriate progression of genetic programs.

The general role of phenotypic checkpoints

The construction of a building requires repeated inspection and determination of the extent to which the architectural and engineering programs have been respected, before subsequent stages of construction can be initiated. Similarly, advancement in the educational system and in the workplace depends on evaluations of performance. We suggest that the expression of embryonic neuronal functions at different developmental stages satisfies a similar requirement. With this view in mind, failure to realize a given step in development – such as migration from one position to another – delays or arrests the developmental sequence of ion currents and/or other signaling messengers at the stage at which the failure has occurred. The genetic program is affected because the genes and functional

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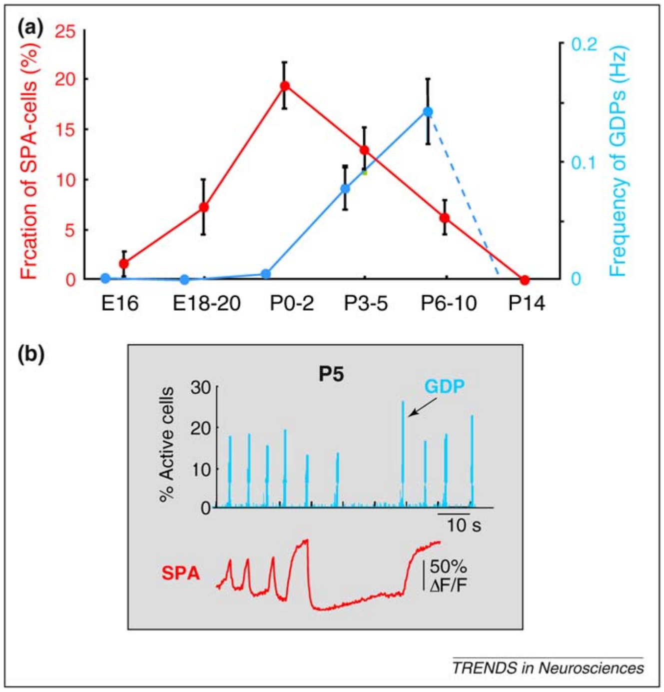

Figure 1. Network activity maturation. **(a)** The first coordinated pattern of activity generated *in utero* in rodent cortical structures consists of large plateau elevations of intracellular calcium triggered in small populations of neurons interconnected by gap junctions (synchronous plateaus in cell assemblies, SPAs). SPAs are generated by intrinsic non-synaptic, voltage-gated calcium currents. Later, synapse-driven giant depolarizing potentials (GDPs) are generated synchronously by large populations of neurons until the GABA depolarizing-to-hyperpolarizing shift has occurred (around the end of the first postnatal week in rodents). **(b)** GDPs suppress SPAs (negative deflections measuring calcium elevation with Fura-2). When they are both present during the postnatal period, GDPs arrest SPAs that are synchronized with the end of GDPs. Moreover, blocking GDPs with receptor antagonists reinstates SPAs at an age when they have normally disappeared (not shown). ΔF/F, change in fluorescence over baseline fluorescence. Reproduced, with permission, from Ref. [9].

feedback act in series, and the affected neurons generate electrical activity corresponding to stage A instead of stage B. Thus checkpoints provide punctuated control of the implementation of the genetic program (Figure 2). These phenotypic feedback loops can provide developing biological systems with sufficient flexibility to accommodate perturbations to programs of gene expression and enable responses to changing environments in which the nervous system develops. They enable integration of genetic and environmental messages and provide a degree of plasticity in the construction of networks. The power of this mechanism for enabling environmental input and the ubiquity of these findings suggest that phenotypic checkpoints are a general feature of neuronal development.

Remarkably, major aspects of development such as proliferation, migration, and differentiation are not fully programmed genetically, but instead rely on phenotypic checkpoints: times and places during development at which functional validation appropriate to the stage of the cells enables the process to go forward normally, take an alternative route, or become arrested. In the following we marshal evidence for the role of phenotypic checkpoints during development and following disruption of normal developmental processes.

Phenotypic checkpoints in embryonic development

Proliferation checkpoint

Neural progenitors in the ventricular and subventricular zones of the developing brain undergo mitotic divisions that give rise to neuroblasts that express transmitters. Genes regulating proliferation have been identified [19–21], but electrical activity modulates this process. GABA and glutamate are secreted at early stages of development, and paracrine actions of both GABA and glutamate depolarize the progenitors, generate elevations of intracellular calcium and inhibit DNA synthesis in the ventricular zone [22,23]. Release of GABA from neuroblasts also activates $GABA_A$ receptors and suppresses proliferation in the subventricular zone [24–26]. On the other hand, GABA induces proliferation of postnatal rat immature cerebellar granule cells through depolarization and activation of calcium channels [27]. Release of glutamate from the glia that ensheath proliferating cells expressing NMDA-type glutamate receptors is crucial for neuroblast survival [28]. Serotonin increases proliferation of neuronal progenitors [29]. Expression of serotonin receptors (5-HT1A, 5-HT2), $GABA_A$ receptors and AMPA-class glutamate receptors enables functional feedback that regulates the number of neurons generated and constitutes a phenotypic checkpoint signature.

Migration checkpoint

As neurons are generated they commence migration along stereotyped pathways to their permanent locations in the brain. Although genes regulating this process have been identified [30–32], the speed and extent of both radial and tangential migration are regulated by paracrine action of neurotransmitters. The activation of glutamate receptors – produced by non-vesicular release that predominates at this stage – increases migration of cerebellar granule neurons via elevations of intracellular calcium [33]. Furthermore, activation of glutamate or $GABA_A$ receptors promotes migration of hippocampal and cortical neurons

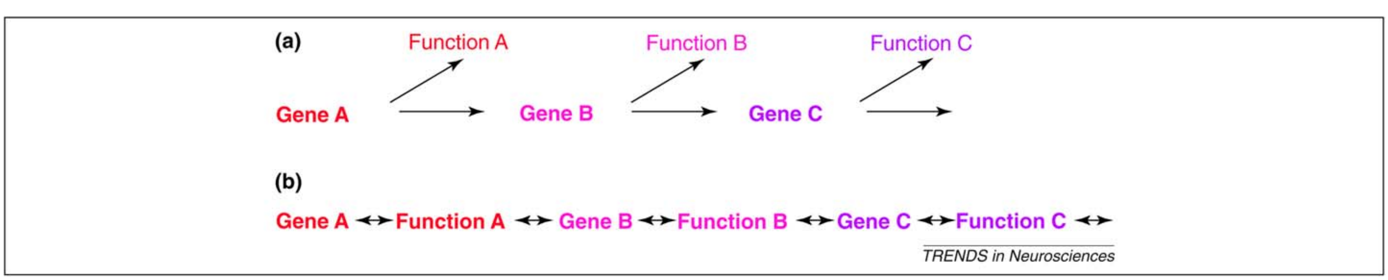

Figure 2. Phenotypic checkpoint signaling. **(a)** The classical view of brain development involves serial expression of genes that lead to embryonic functions. **(b)** Phenotypic checkpoint signaling integrates embryonic functions in the process of gene expression by feedback and feedforward signaling.

[34–36]. By contrast, activation of $GABA_A$ receptors decreases migration of neuronal precursors from the subventricular zone to the olfactory bulb [37] and can act as the stop signal for migration [38]. Both depletion and excess of serotonin can reduce interneuron migration in different systems [29,39,40]. The expression and activation of receptor-mediated and voltage-gated currents well before synapses have been formed is required for appropriate migration and attests that the process is not an automated one independent of the influence of the external milieu. Thus, the activation of transmitter signaling provides a phenotypic checkpoint for migration.

Axon guidance checkpoint

Growth cones at the tips of axons navigate through the embryonic nervous system to reach targets with which synapses will be formed, usually interacting with a series of intermediate targets *en route*. Growth cones appear to express receptors appropriate for the recognition of each intermediate target, thus avoiding errors in axon guidance. For example, commissural axons grow toward the midline and then leave it again on the opposite side and normally never recross; on the contralateral side they turn anteriorly or posteriorly to reach other targets [41–43]. Their growth cones are initially attracted by Netrin-1 protein secreted by cells at the midline, for which they express high levels of DCC receptor; these receptors stimulate calcium influx that drives growth-cone turning [44–46]. Commissural neurons are unaffected by the midline repellent protein, Slit, for which they express a low level of Robo receptor. However, after they cross the midline they become insensitive to Netrin-1 and are repelled by Slit [47] (Figure 3) as well as other repellant molecules [48]. The switch from attraction to repulsion results from insensitivity to Netrin-1 through physical interaction between DCC and activated Robo, and repulsion by Slit due to increased levels of Robo. The sequential expression of DCC and unblocking of Robo constitute a checkpoint signature, driving midline crossing. Without this crucial step, later guidance steps are blocked. Growth cones exhibit local protein synthesis [49,50] that can introduce expression of new classes of receptor [51]. Thus, growth cones are likely to move from one phenotypic checkpoint to the next during axonal pathfinding.

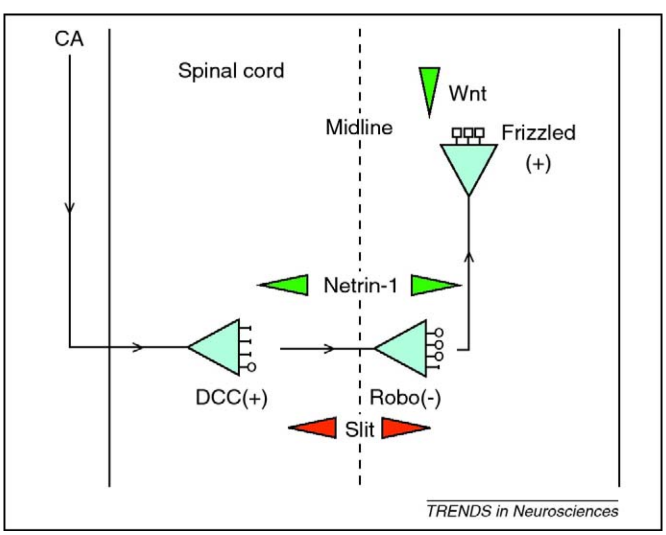

Figure 3. Axon guidance checkpoint. Commissural axons (CA) cross the midline of the spinal cord to ascend on the opposite side. Their growth cones are initially attracted by a gradient of Netrin-1 secreted by midline cells, which binds to DCC receptors. After crossing the midline, growth cones are repelled by a gradient of Slit secreted by midline cells, which binds to Robo receptors. Insensitivity to Netrin-1 is mediated by interaction of DCC with activated Robo that constitutes a checkpoint for midline crossing. Attraction of growth cones is subsequently mediated by a gradient of Wnt binding to Frizzled receptors.

Neurotransmitter and receptor specification checkpoints

Neurons communicate by release of neurotransmitter molecules that bind to receptor proteins on other neurons and target cells. Specifying the correct transmitter in a population of neurons, from the 100 or so that have been identified, is essential for network activity. The mechanism by which appropriate transmitter specification is achieved in different classes of neurons involves a partnership between gene expression and electrical activity [52]. Calcium spikes are generated in embryonic amphibian spinal neurons with cell type-specific frequencies, and increasing or decreasing spike frequencies prior to synapse formation changes transmitter specification. Suppressing calcium spiking increases the number of neurons expressing excitatory transmitters, whereas enhancing calcium spiking increases the number of neurons expressing inhibitory transmitters, typically by 30–50% [53,54]. Altering sensory input to postembryonic neurons once synapses have formed changes the specification of transmitter selectively within the activated circuit to a similar extent [55].

This phenotypic checkpoint involves the expression of developmentally transient calcium spikes, triggered by calcium-dependent action potentials resulting from large voltage-gated calcium currents largely unopposed by small voltage-gated potassium currents. Changes in transmitter receptor expression occur postsynaptically to match changes in transmitter expression [55,56] (Figure 4), demonstrating another phenotypic checkpoint. Thus, unlike previously described checkpoints that occur prior to synapse formation, transmitter and receptor specification checkpoints at this stage can be responsive to synaptic input.

GABA/chloride signaling checkpoint

The levels of intracellular chloride are elevated at early developmental stages in a wide range of animal species and brain structures, suggesting that this has been preserved during evolution [4,57,58]. The initially depolarizing and excitatory actions of GABA are the result of developmental expression of a chloride importer (NKCC1) prior to a chloride exporter (KCC2) [59]. Activation of GABA (or glycine) receptors generates sodium and calcium currents and activates NMDA receptors by removing their voltage-dependent magnesium block, leading to a large calcium influx observed only in immature neurons [11]. GABA depolarization regulates early aspects of development including proliferation, migration (see above), neurite outgrowth [60,61] and the formation of GABAergic and glutamatergic synapses [62–64]. Because GABAergic neurons and synapses mature before glutamatergic ones, GABA also provides the first source of neuronal activity [65]. The abrupt maternally-triggered reduction

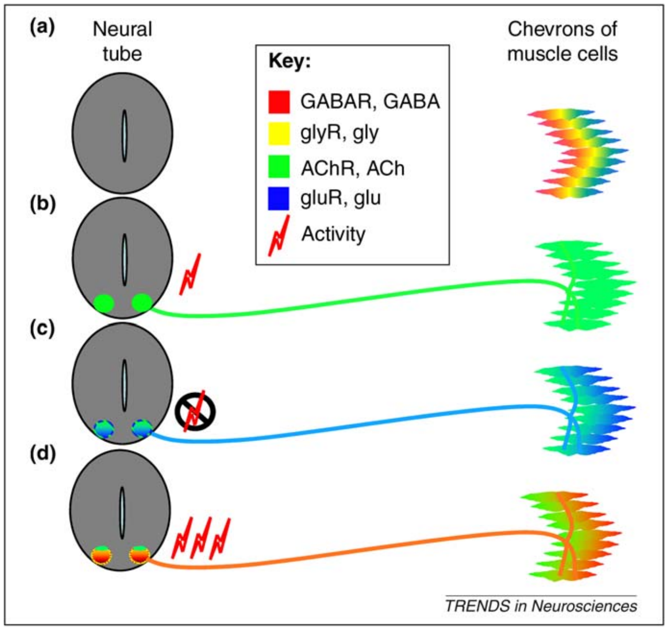

Figure 4. Neurotransmitter receptor selection checkpoint. Activity-dependent transmitter specification acts as a checkpoint for selection of receptors at the embryonic neuromuscular junction. **(a)** Prior to axon outgrowth from the amphibian neural tube (*Xenopus*), trunk muscle cells express a range of different transmitter receptors. **(b)** During normal development, spontaneous neuronal calcium spike activity contributes to the expression of acetylcholine in motor neurons (localized within the neural tube), whereas acetylcholine receptors are stabilized on muscle cells and other classes of receptors disappear. **(c)** When calcium spike activity is suppressed, motor neurons express glutamate in addition to acetylcholine, and muscle cells express glutamate (NMDA and AMPA) receptors in addition to acetylcholine receptors. **(d)** When calcium spike activity is enhanced, motor neurons express GABA and glycine in addition to acetylcholine, and muscle cells express $GABA_A$ receptors and glycine receptors in addition to acetylcholine receptors. Reproduced, with permission, from Ref. [56].

of embryonic intracellular chloride during parturition, via enhanced oxytocin levels, together with the corresponding hyperpolarizing action of GABA that protects embryonic neurons from anoxic insults, illustrate the important biological function of the polarity of GABA's effects [66]. If this conversion in polarity of GABA signals is accelerated or prevented, subsequent stages of development are altered [61–64]. Thus the switch from depolarization to hyperpolarization is an important phenotypic checkpoint during development.

Phenotypic checkpoints during development in the adult nervous system

Are phenotypic checkpoints operative in an adult environment where neurogenesis is known to take place? Or are they restricted to brain maturation? This issue is complicated by the fact that neurogenesis is restricted to a small number of brain regions [67–70], hampering general conclusions as to the roles of checkpoints in the adult nervous system in general. Nevertheless, proliferation of neural progenitor cells generating dentate granule cells in the adult hippocampus is modulated by NMDA [71] and serotonin signaling [72,73]. Voluntary wheel-running by rodents or seizure activity increase the proliferation of these cells, consistent with a role for physiological activity in generating new neurons [74,75]. The development of adult granule cell dendrites and synapse formation is controlled by tonic GABAergic depolarization, and reducing chloride accumulation by the suppression of NKCC1 or expression of KCC2 blocks these processes [76,77]. Thus, proliferation and integration checkpoints operate in an adult environment for the correct assembly of networks, suggesting that they cannot be fabricated *de novo* without following the developmental sequence. However, compared to neuronal development at embryonic stages, neural development in the adult brain is significantly prolonged; acceleration of the speed of development during adult neurogenesis resulting from increased activity (e.g. seizures) or genetic defects (e.g. mutations in the expression of important developmental genes, such as disrupted-in-schizophrenia-1 (*DISC1*), can lead to deficits in neuronal development.

Checkpoint mechanisms

Activity-dependent regulation of transcription factors provides a mechanism for neurotransmitter specification checkpoints. Spontaneous calcium spike activity in the hindbrain of developing amphibian larvae modulates the specification of serotonergic neurons by controlling the number of neurons expressing the LIM homeobox transcription factor 1b (Lmx1b). Activity acts downstream of the Nkx2.2 homeobox transcription factor, but upstream of Lmx1b, leading to regulation of the serotonergic phenotype [54]. Manipulations of activity and targeted alteration of Lmx1b expression demonstrate that these changes in the number of serotonergic neurons change larval swimming behavior [54]. Spontaneous calcium spike activity in the spinal cord of amphibian larvae regulates transcription of

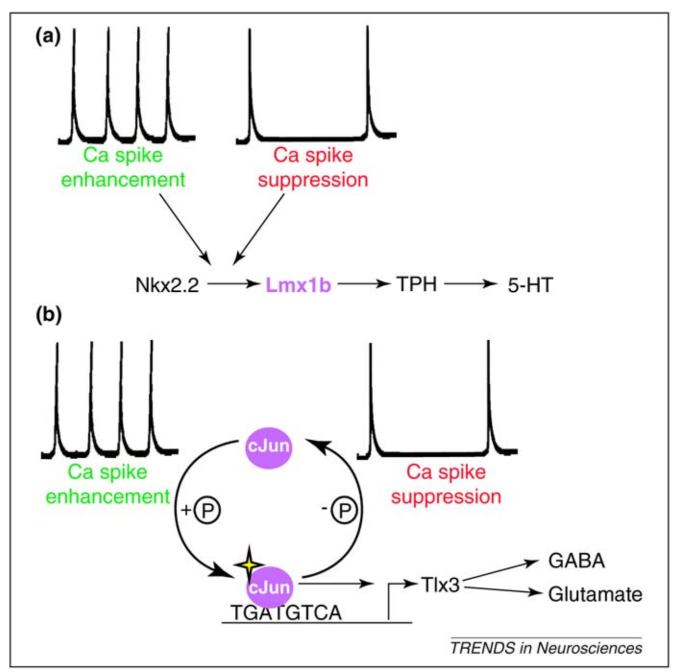

Figure 5. Checkpoint mechanisms. The intersection of calcium spike activity and gene expression determines neurotransmitter specification in the brain and spinal cord. **(a)** Calcium spike enhancement decreases and spike suppression increases the number of neurons expressing the LIM homeobox transcription factor 1b (Lmx1b), leading to changes in the number of neurons making tryptophan hydroxylase (TPH) and serotonin (5-HT). The number of neurons expressing the Nkx2.2 homeobox transcription factor is not affected. **(b)** Calcium spike enhancement and suppression modulate phosphorylation of the cJun transcription factor, which regulates transcription of the *tlx3* homeobox gene through the cAMP response element (TGATGTCA) in the *tlx3* promoter. Tlx3 determines the glutamatergic fate over the GABAergic fate in the dorsal spinal cord. Reproduced, with permission, from Refs [54,78].

the GABAergic/glutamatergic selection gene *tlx3* through a variant cAMP response element (CRE) in its promoter [78]. Calcium signals through phosphorylation of the cJun transcription factor, which binds to this CRE site and modulates transcription, thereby integrating activity-dependent and intrinsic neurotransmitter specification. This mechanism provides a way for early activity to regulate genetic pathways at crucial decision points, switching the phenotype of developing neurons (Figure 5).

Epigenetic imprinting that links environmental factors to the coding of genetic programs is highly suited to implement phenotypic checkpoints generally, because epigenetic modifications reversibly regulate gene expression preferentially during brain maturation. Molecular modifications to the structure of histone proteins and DNA (chromatin) regulate the transcription of genes without altering their nucleotide sequence. DNA methylation and histone deacetylation are two major epigenetic modifications that contribute to the stability of gene expression [79–81]. Environmental stimuli such as maternal care and social interactions, as well as drugs, activate epigenetic mechanisms in post-mitotic neurons during development that result in alterations of neuronal phenotype with long-term behavioral consequences [82–85]. This DNA methylation and chromatin patterning is programmed during early development and appears to be highest at early stages [86,87] although it can also impact memory processes in adults [88]. At later stages epigenetic control is less reversible, preventing potentially dangerous phenotypic checkpoint signaling. DNA methylation patterns and epigenetic factors differ in the chimpanzee and human cortex [89] and distinguish brain regions, providing a mechanism for region-specific functional specialization [90].

Epigenetic phenotype specification provides a developmental mechanism that enables rapid, efficient and quasi-permanent alterations of phenotypes during development. The Aicardi–Goutière syndrome is interesting in this context because a genetic mutation and an environmental insult – cytomegalovirus infection during gestation – converge on the same signaling cascade to generate polymicrogyria via a programmed succession of phenotypes common to both insults [91,92]. This syndrome illustrates the convergence of genes and environment that impact brain development via signaling cascades.

Checkpoints signal branch points in genetic programs

The roles of biological checkpoints have been extensively characterized in cell division where the failure of these feedback controls can lead to cell death or extensive proliferation [93–95]. When neurons are misplaced or misconnected for genetic or environmental reasons they can signal this situation by arresting some of their developmental sequences. Evidence has been obtained with intra-uterine short-interfering RNA (siRNA) knockdown of the expression of a variety of proteins associated with major neurological disorders including Rett syndrome, lissencephaly, dyslexia, and certain forms of mental retardation [4,96,97]. Although neurons with mutations in the genes encoding these proteins fail to migrate, they develop, arborize and form synapses with adjacent neurons, suggesting that cell death is not solely a consequence of checkpoints and that some aspects of differentiation are intrinsic to neurons. However, they generate misplaced

Box 1. When does checkpoint-control end? Re-examining the concept of critical periods

Cell proliferation, migration and differentiation are almost entirely restricted to brain maturation. Does this imply that phenotypic checkpoint signaling is terminated once the principal developmental sequences have run their course, the genetic program has been implemented, and networks are operational? Specific answers to this question are lacking, but experiments aimed at repairing the consequences of genetic mutations provide conceptual hints. *In utero* RNAi-mediated knockdown of doublecortin (DCX) –as with many other proteins involved in neurological disorders – appears to produce an arrest in neuronal maturation at the developmental stage at which its expression has been stopped. These neurons are misplaced and are characterized by immature and aberrant electrical features [98,99]. A similar return to immature features has been observed following a variety of insults, suggesting recapitulation of an immature state [105–107]. Interestingly, attempts to repair the phenotype by reintroduction of the correct gene – under tamoxifen control to enable the desired temporal expression of the gene – produces a partial rescue [108]. However, this rescue only occurs when the gene is induced during early postnatal life (P0–5), and not at later points in postnatal development (>P10) (Figure I), suggesting that the mechanisms required for repair are no longer accessible after a certain timepoint in development. Thus, there is a window of opportunity for genetic repair during which plasticity is available. Interestingly, this window broadly corresponds to the so-called 'critical period' during which the wiring of networks changes in response to environmental stimuli. We suggest that the extensive wiring plasticity at this age corresponds to a window of checkpoints that closes with permanent wiring. This conclusion could have significant implications for gene therapy of neurological disorders and for our understanding of whether and how immature neuronal features can be repaired by genetic manipulations after insults such as seizures in adults.

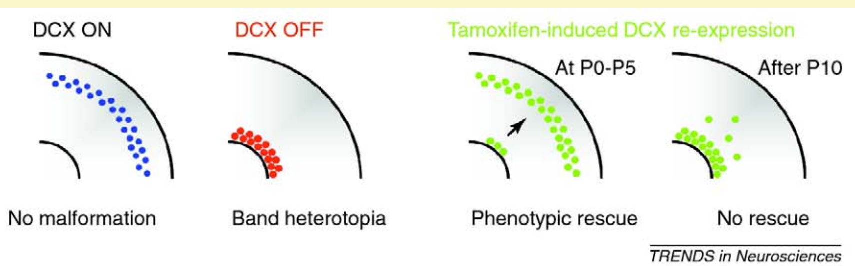

Figure I. *In utero* knockdown of DCX leads to the formation of a double cortex (band heterotopia) with neurons that fail to migrate to their assigned layer. Delayed re-expression of DCX (using conditional gene expression under the temporal control of tamoxifen) – produces a partial phenotypical rescue only when this is done during early postnatal life (i.e. before P10). Reproduced, with permission, from Ref. [108].

ensembles that impinge on the construction and function of adjacent cortical units. Interestingly, following knockdown of doublecortin (DCX), neurons are 'frozen' in an immature state with voltage-gated currents and oscillatory features that normally disappear when it is expressed [98,99] (Box 1).

Early malformations can occur years or decades before the manifestation of clinical syndromes with which they are typically associated, suggesting that they provide early electrical signatures of disorders to come: the neuro-archeology concept [4]. In Huntington's disease, brain malformations are revealed by brain imaging and behavioral tests well before the onset of clinical manifestations [100,101]. Other observations suggest that immature neurons are more resistant than adult neurons to insults that nevertheless produce severe long-lasting sequelae, indicating that mechanisms other than cell loss are involved, such as neuro-immune interactions [102–104]. We suggest that early insults, whether they are genetic or environmental, are 'programmatic' (i.e. mediated by alterations of developmental programs resulting from mismatches at phenotypic checkpoints). In this model, the electrical signature of neurons provides a stage-dependent readout attesting to whether or not they have achieved their program adequately [4].

Conclusions

The functional feedback and feedforward provided by phenotypic checkpoints serves several purposes. Phenotypic checkpoints can play a role in specifying the 'what' and 'where' of development: what genes are turned on or off, what cytoskeletal components are post-translationally modified, and where these events take place. Activity-dependent transmitter specification provides an example of such regulation. Phenotypic checkpoints can specify 'how much' through positive and negative feedback loops. Neurotransmitters regulate the extent of proliferation and migration of developing neurons. Phenotypic checkpoints can specify 'when', somewhat like the function of a clock, turning gene expression or other processes on or off at particular times. Conversion of GABA signaling from depolarization to hyperpolarization provides a timing signal for subsequent stages of development. Thus, checkpoints provide both plasticity and precision for the assembly of the nervous system. Current evidence suggests that phenotypic checkpoint signaling terminates at the conclusion of development (Box 1).

What is the relationship of phenotypic checkpoints to the homeostatic behavior of neurons and networks? Homeostasis is instrumental in the operation of adult networks; it maintains a balance across a wide range of crucial functions (e.g. pH, temperature) and prevents excessive excitation or inhibition, limits the density of synapses on neurons, and controls a large variety of signaling cascades through feedback loops. However, homeostasis does not operate on developmental processes in which genetic programs and the environment converge to generate networks of cells. Developing neurons are generally in a progressive state and not in homeostasis as they execute their programs. Thus, blocking expression of a given phenotype in immature neurons often fails to promote the appearance of a phenotype that compensates for the missing one, and instead leads to sustained expression of the previous phenotype (Figure 1). Blockade of GDPs leads to reappearance of synchronous plateaus in cell assemblies (SPAs), the earlier phenotype. Neurological disorders involving aberrant neuronal migration do not develop because homeostasis has been disrupted, but instead because misplaced neurons create aberrant connections and generate patterns of activity that perturb normal network operation. However, as neurons begin to mature, homeostasis becomes important and is involved, for example, in neurotransmitter specification. Suppression of activity leads to an increase in the number of neurons expressing excitatory transmitters and a decrease in the number of neurons expressing inhibitory transmitters. Enhancing activity produces the opposite result. Thus, phenotypic checkpoints enable both progressive and homeostatic neuronal development.

In contrast to the construction of an inanimate machine, the brain is active at the earliest stages of its assembly and this activity is cell-, network- and developmental stage-dependent. We suggest that this activity is an online signature of the genetic program, providing checkpoints that condition the activity of previous elements and the acquisition of subsequent elements in the program. We have outlined issues that seem ready for further investigation (Box 2); these may be expected to clarify both the mechanisms and the impact of phenotypic checkpoints. Such checkpoints supply crucial feedback information for error correction and integrate environmental information through alterations in activity that adapt and fine-tune the realization of the program. They also appear to resolve the nature versus nurture debate, because both operate in series in this scheme.

Box 2. Outstanding questions

- What is the mechanism by which neuronal functions influence the expression of genes at phenotypic checkpoints? Is there a common checkpoint cascade of signals for different elements or do different signals control the expression of different signaling proteins/processes?
- Does sensory information during critical periods act through phenotypic checkpoints to eliminate depolarization by GABA, alter NMDA and AMPA receptor expression levels, and suppress primitive patterns of network activity?
- Can checkpoints be bypassed by convergent genetic pathways for aspects of development that are so basic that functional validation is unnecessary?
- Following checkpoint failure, do aberrant networks impact the operation of adjacent normal circuits? How are immature features integrated with the behaviorally relevant physiological patterns that the latter generate?
- Does inappropriate activation of phenotypic checkpoints lead to neurological disorders?
- What are the mechanisms involved in the delay between an early checkpoint failure and the manifestation of disease?
- Does the development of other organs, such as the heart or the lungs, involve phenotypic checkpoints?

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