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Fetal to Birth

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Abstract

Prior to birth, the brain becomes highly developed, and many early events lay the foundations for later maturation. This chapter begins with a focus on the range of normal brain structure and function, with consideration given to how dynamic changes over time can best be studied. It then explores the extent to which the specification of cortical cell types is constrained during development, followed by a review and discussion of what happens to spatiotemporal patterns of waves of activity during early cortical development and their roles in developmental plasticity. Consideration of the central role of activity in organizing the developing nervous system prompted us to ask how changes in activity prefigure development of pathology. Key conclusions and future directions are summarized at the end of this report.

All theories are wrong but some are useful (after George E. P. Box).

If you torture the data long enough, it will always confess (after Ronald H. Coase).

What Is the Range of Variation of Normal Brain Structure and Function, and How Should We Study the Dynamic Changes Over Time?

We often report averages and jump to general conclusions when we analyze brain structure or function, lumping variation in the numbers of neurons, the sizes of structures, and the numbers of fibers and physiological attributes.

Group photos (top left to bottom right) Nick Spitzer, Terry Sejnowski, Suzana Herculano-Houzel, Yehezkel Ben-Ari, Hannah Monyer, Gordon Fishell, Michael Stryker, Heiko Luhmann, Ileana Hanganu-Opatz, Terry Sejnowski, Michael Stryker and Alain Chédotal, Gordon Fishell and Nick Spitzer, Suzana Herculano-Houzel, Nick Spitzer and Terry Sejnowski, Heiko Luhmann, Ileana Hanganu-Opatz, Gordon Fishell, Yehezkel Ben-Ari, Hannah Monyer, Alain Chédotal, Michael Stryker

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Does the range of the data have implications for function? Is the range during development correlated with outcomes postnatally? Because some of this variability is likely to be relevant, we need to learn the normal range. The first rigorous quantification of the number of neurons in the adult human brain—86 billion—came from analysis of the number of neurons in four adult males (Azevedo et al. 2009).

For example, mutant mice have different defects in their patterns of axon guidance, visualized using whole animal imaging, that are likely to be functionally significant (Belle et al. 2014). However, individual sensory innervation and branching patterns overlap only to a limited extent between the right and left hands in every human embryo tested (Belle et al. 2017), and in this case, the variability in axon guidance may be functionally irrelevant. Variations across species are also highly relevant: mutation of *Robo3* leads to the absence of commissures between bilateral pre-Bötzinger nuclei, and mice, but not humans, die at birth (Jen et al. 2004; Bouvier et al. 2010). In another case, preconceptual and gestational forms of stress lead to larger and smaller brains, respectively, but performance is poor in both cases (Kolb, this volume); furthermore, initially there are unusually large numbers of neurons in the autistic brain. These findings argue that brain size is not directly correlated with ability. In line with this view, there can be twofold differences in the numbers of neurons in a cortical area without an obvious effect on the overall function of the brain.

Is it useful to recommend specific criteria for determination of normality? Detecting the range of variation in a single dimension is not enough. Comparison of numbers across individuals and within individuals (e.g., on both sides of the brain) will be best achieved using multiple criteria. The number and identity of such criteria are likely to differ when assessing different aspects of normality. Particularly useful are measures of brain structure, brain function, and the gain or loss of particular neurons, since some cells are more impactful than others (e.g., hubs versus outliers or pre-Bötzinger neurons regulating respiration). Unfortunately, causality has frequently been inferred from single, inadequately parameterized events. In China, more education has been associated with a lower incidence of Alzheimer disease (AD) (Zhang et al. 1990). In the United States, in the "Nun Study," cognitive reserve was interpreted to be an important determinant in the onset of AD (Snowdon et al. 1996) even when formal education was modest.

It is crucial to inspire investigators to be more aware of the significance of understanding the range of normal variation. We also need to develop new techniques for stereology to accelerate the rate of quantification of anatomical data. Larger sample sizes will make conclusions more robust, and machine learning will now enable accumulation of these larger samples. The Allen Brain Atlas data are based on only six human donors at this point, although one-third of genes are still consistently expressed across 33 gene areas. Interestingly, some aspects of brain development are stochastic: examples include map formation

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in the visual system (Owens et al. 2015) and the ratio of red-to-green cone pigments (Wang et al. 1999).

Light sheet microscopy is an important, recently developed technology for rapid analysis of brain structure; using 3DISCO/iDISCO to clarify the tissue, it allows the range of variation in nervous system architecture to be determined (Belle et al. 2014, 2017; Renier et al. 2014; see Figure 4.1). The standard 3 × 5 cm specimen size is sufficient for analysis of the embryonic and fetal human brain or adult rodent brain. The adult human brain has to be cut into 5 cm slices, imaged, and individual images then stitched together. Resolution is at the level of single axons and spines. One person can process 100 mouse brains per week, so analysis of 3000 lines of mutant mice is feasible. For example, it becomes easy to count the number of neurons expressing a given transcription factor, such as Foxp2, in the whole brain. The ability to see the whole brain allows one to find neurons easily and visualize all their projections in 3D. At present, it is possible to examine three or four markers at one time and it should be possible to do more in human tissues using monoclonal antibodies. This procedure has been successfully applied to birds, reptiles, and other animals. It allows the collection of raw data unhampered by hypotheses or conjectures, thus creating the opportunity for other investigators to analyze the data from their own perspective. One is led to wonder whether serial block face electron microscopy, presently underway in many labs, will be capable of measuring variability that is in the noise? There are now 120 light sheet microscopes

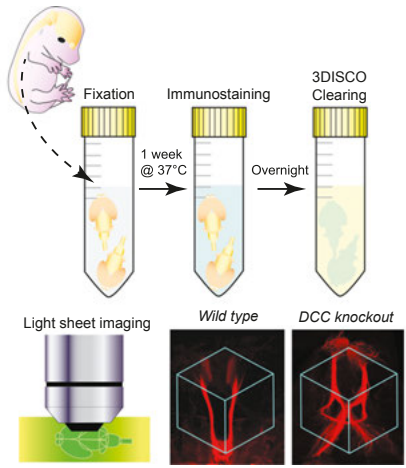

Figure 4.1 A simple procedure combines immunolabeling, solvent-based clearing, and light sheet fluorescence microscopy for rapid high-resolution whole-brain neuroanatomical analysis. This technique allows large-scale screening of axon guidance defects and other developmental disorders in mutant mice (Belle et al. 2014). Reprinted with permission from Alain Chédotal.

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around the world, stitching software has developed rapidly, and data compression facilitates handling large files. Activity-dependent markers such as c-fos and Arc can be used as readouts of function. Among other benefits, these observations provide information about where to interrogate cellular or circuit function.

Although stereology is good for small, well-demarcated samples, the isotropic fractionator ("brain soup") technology (Herculano-Houzel and Lent 2005) is another method that facilitates more accurate counts and higher throughput. Dissection and dissociation of as many as a dozen tissue samples per day, collection of nuclei, and staining for nuclear markers make it simple to count cell numbers. Less training is required and the method is useful for developmental studies. Other methods include recording with new calcium (Ca)indicators and large-scale multiple electrode recording over long periods of time, combined with selective manipulation of distinct neuronal populations that can now be accomplished in freely behaving animals. Currently missing and urgently needed is a generalizable method for tracking gene expression in single neurons during development. All these methods create new ways of collecting, studying, and presenting the data. It is now possible to study multiple parameters in the same brain and in the same neurons over time.

Although we are becoming richer in techniques, conceptualization remains poor. *Phenotypic checkpoints* constitute an example of a useful concept (Ben-Ari and Spitzer 2010). The idea is that developmental expression of a gene generates a transient phenotype, which is necessary for further normal gene expression. If the phenotype is not generated, further development is arrested or proceeds along an abnormal pathway. As hard-headed scientists, we are reluctant to engage in overgeneralization of our findings, and this may account for our hesitancy to formulate developmental rules. One may proceed, however, by elaborating concepts on the basis of simple experiments and then test their validity with more complex studies. Even though knockouts can be uninformative and solid information about phenotype is required, it is possible to identify a phenotype that is consistent and work backward. Increasing evidence supports the relevance of phenotypic checkpoints for development (Donato et al. 2017).

The gap between knowledge and understanding arises in part because we do not know the underlying neurobiology. To study and determine the range of normality, we need to know the range of variation in genes, cells, networks oscillations *combined* with impact on normal function, and/or pathology. To this end, the Free University of Amsterdam operates a brain analysis program linked to euthanasia, wherein ca. 100 brains are processed and scans acquired per year. Over time, the program aims to establish relationships between individual experiences and brain structure. In addition, registries in Scandinavian countries attempt to link birth and life experience to brain outcome.

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How Constrained Is the Specification of Cortical Cell Type during Development?

Determining Cell Types

How do we determine the different cell types? One view is that all that matters is input, intrinsic properties, and output. In the past, cells were often classified by their receptive field (input) alone. In artificial networks, this is not sufficient: one needs to know the projective field (output); that is, where axons project. Another view is to determine how cells cluster by shared properties. As noted by Wolf Singer, "you have to bundle them somehow." For example, parvalbumin (PV) cells from different regions have more in common than PV cells and pyramidal neurons. However, we may not yet have identified all the cells' properties.

Further discussion led to the conclusion that classification is arbitrary and there is an unlimited number of ways to classify. Classification may be fluid; for example, the bursting properties of thalamic neurons depend on the state of the membrane potential. The same problem arises in discussion of the number of cortical areas in the brain, classified by function, functional potential, and other criteria. Classification should serve a purpose, and different purposes will be served by different classifications. It is important to avoid "theological" approaches—getting stuck on a particular view. There is no inherent ground truth. In the retina, one classifies the same cells into the same groups using a variety of measures. Is this unusual or atypical? Perhaps we should start with this as the null hypothesis until it can be shown that it does not work.

The values of classification include having a common language for discussion, getting genetic access for manipulations (although classification should not be made using a single tool), increasing replicability of experiments, and comparing cell types across animals and within animals.

Determining the Origin of Cell Types

How does cell type specification occur? We considered excitatory pyramidal neurons and inhibitory interneurons, remembering that evolution did not need to have names for cell specification. The source of neuronal properties is a key question. Sydney Brenner famously referred to the American plan (in which fates are determined by the environment) and the European plan (in which fates are determined by lineage). We discussed invertebrate neurons (and included neurons in the sensory and motor peripheral vertebrate nervous system, since they seem to share properties with invertebrate neurons) and contrasted them with vertebrate CNS interneurons and pyramidal cells.

Transcription factor codes are established through genes and checkpoints that provide a constraint on cell type (Lieberam et al. 2005). Lineage is predictable and determines cell fate in invertebrates in some cases. Interestingly,

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in flies, cuticle progenitors are equipotential and can assume different fates (Lawrence 1973), potentially determined by positional cues. Overproduction and apoptosis appear to create more options in vertebrates. Moreover interneurons projecting to dendrites versus cell body have different synaptic properties, and properties of synapses depend on innervation. Lineages are not determinate in the retina, spinal cord, and cortex. For interneurons, generally, lineage does not seem to determine fate, but it appears to be more important for pyramidal neurons. It is useful to think of cardinal specification of interneurons as the initial program; definitive properties then result from position and activity. Interestingly, single cell transcriptomes from interneurons reveal that 23 classes fall into four different developmental groups: PV, somatostatin, VIP, and neuroglioform cells. Analysis does not allow identification of specific precursors as the basis for differentiation.

Where Does Deterministic Organization Stop and Environmental Regulation Take Over?

The timing of this switch varies in different parts of the nervous system. Specification is not perfect and some axons project to the wrong place. However, neurons that make these mistakes are usually eliminated. Three classes of postnatal interneurons emerge from the ganglionic eminences, and all initially express Nkx2.1. When Nkx2.1 is lost at a specific point in time, postnatal interneurons diverge into the separate classes. Diversity is seeded by a small number of genes. Satb1 and Sox6 are expressed in the medial but not the central and lateral ganglionic eminences. Surprisingly, a small number of genes specify different cell types, much like the programming of induced pluripotent stem cells (iPSCs). The small number of genes creates an attractor network that draws in other genes (Theunissen et al. 2016). This may be why chimeric neurons are not observed. The intrinsic propensity is then acted on at a later stage to become further specified.

Gord Fishell and colleagues have found that while the position of pyramidal neurons is predictable based on the position of their progenitors, the position of interneurons is not. Positionally constrained pyramidal neurons provide positional information to less-constrained interneurons, allowing the latter to be programmed to position at their settling point. This interaction enables the reorganizations that occur during the later critical periods. In ongoing work (Fishell, unpublished), a bar-coded virus is being used to track interneuronal lineages to determine where they go and what they become. These cells are recovered by unique molecular indicators to determine their relationship to lineage.

Pasko Rakic described the protomap concept, which he developed from his work on the reeler mouse in which neurons do not migrate properly (Rakic et al. 1991). The hypothesis is that neurons are specified at an early stage, thus establishing the relationship of the progenitors to the cortex. Even when they go

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to the wrong layer, these neurons still look like pyramidal cells. Similar observations have been made in macaque monkeys (Herculano-Houzel et al. 2013; Ribeiro et al. 2013). Whether these observations, which concern the formation of the somatosensory cortex, apply to visual, auditory, and other cortical areas remains to be determined.

What is the value of inside-out formation of the cortex? Different cells are located in different layers, and this is very likely to be important for establishing correct connectivity. The protomap hypothesis was controversial because it seemed too deterministic. However the subventricular zone is thick in the human, thinner in the monkey, and thin in the mouse, presumably to reflect the different levels of cortical complexity. If neocortical cells are prevented from migrating and then patched for electrophysiological recordings and dye filling, morphologically they look like neocortical pyramidal cells. Electrophysiologically they can also look normal, but they have altered function. Lineage analysis in cortical progenitors shows that you get a column of neurons above the progenitors and that neuronal birthdate predicts position. One can thus conclude that pyramidal neurons know their position whereas interneurons do not.

Convergence and Stability during Dynamic Changes of the Developing Nervous System: Attractor States and Homeostasis

Neuronal gene expression, cell identity, and circuit structure and function appear to converge on a finite set of phenotypes. The dynamics of gene networks that drive development have much in common with certain types of neural network models that exhibit attractor states (Hopfield 1982). An attractor is a region of the state space of a dynamical system toward which trajectories tend with time. Attractors occur in many dynamical systems with nonlinearly interacting variables. A useful metaphor for an attractor is a valley, and its dynamic behavior is like the flow of water into a basin (Milnor 1985). Attractors may also explain why differentiated cells can be reprogrammed into pluripotency by any 4 of 10 transcription factors (Hochedlinger and Jaenisch 2015). In addition to point attractors, which lead to steady state, dynamical systems can also have limit cycles, driving oscillations that are common during development.

Starting from many different initial states, it is possible to end up in the same attractor state. However, if the initial state is too far away from the basin, the system can end up in a completely different attractor state. This may be a useful metaphor for different stages of development in most cases; however it may also provide the basis for pathological states in cases resulting from a large perturbation early in brain development. For example, chaos is another type of attractor that occurs in nonlinear systems, which happens in the heart during fibrillation.

Maintaining stability of some states while others are changing and converging is another requirement of brain development, and homeostasis is a

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mechanism by which this is achieved. For a one-dimensional variable, such as temperature, the goal of a control signal is to reduce the difference between the current temperature and a set point. The situation is much more difficult when the set point is multidimensional. For example, a neuron may want to maintain the shape of an action potential as well as the firing rate for a given input, while holding constant some of its other properties. There are dozens of ion channels in neurons that interdependently affect these two variables. Moreover, many different combinations of parameters, such as densities and time constants, may lead to the same outcome (Prinz et al. 2004). Thus it is important to measure the correlations between these parameters as well as their means.

Computational modeling offers an increasingly powerful approach to understand how the brain works and it continually evolves in a reciprocal fashion with increasing neuroscience data. Neuromorphic architectures, for example, have brought several advantages for computer design (Liu et al. 2015). During development of the nervous system there is an overproduction of neurons and synapses followed by pruning. Using this approach to structure neural networks has been shown to produce more robust, distributed computer networks than incremental approaches (Navlakha et al. 2015), with application to improvements in the design of routing networks such as the development of airline networks.

What Happens to Spatiotemporal Patterns of Waves of Activity during Early Cortical Development?

Before we focus on humans, we begin with a discussion of rodents at equivalent stages of development, examining the properties of neurons in the somatosensory cortex. Neurons are born at an early stage, and GABA influences proliferation (LoTurco et al. 1995). Since migration is inside out in rodents at the time of birth, the cerebral cortex consists of subplate, layer VI, layer V, cortical plate, and the marginal zone (future layer I) (Figure 4.2). At this stage, the subplate in rodents is only about three cells thick (although much thicker in primates) and much smaller than the cortical plate.

Lesions of the subplate have identified its contributions to cortical development, including formation of thalamocortical projections (Ghosh et al. 1990), descending axonal connections (McConnell et al. 1989), columnar organization (Ghosh and Shatz 1992; Kanold et al. 2003), and maturation of cortical inhibition (Kanold and Shatz 2006).

There is functionally mature thalamic input to the subplate at birth (Hanganu et al. 2002) along with innervation by neuromodulatory systems (NE, 5-HT and ACh). Electrophysiological signatures are evident: subplate neurons can fire at ~20 Hz when activated by cholinergic input (muscarinic receptors, mostly m1 and m5) (Hanganu et al. 2009), while neurons in layers V and VI fire only single spikes upon depolarizing current injection (Luhmann et al. 2000). EEG and 16-site depth silicon electrodes enable recording of the

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thalamic input and intracortical processing *in vivo* in newborn rat barrel cortex (Yang et al. 2009b). The thalamic input onto subplate neurons is glutamatergic, via AMPA and NMDA receptors (Hanganu et al. 2002; Hirsch and Luhmann 2008), and targets a heterogeneous cell population of glutamatergic and to a minor extent GABAergic neurons. Intracortical multielectrode recordings reveal spindle bursts (spindle-shaped discharges with 10–20 Hz bursts on top, which are reminiscent of human delta brushes), along with gamma oscillations at 30–40 Hz (Yang et al. 2009b; Figure 4.2).

Substantial research has elucidated the organization and origin of coordinated patterns of electrical activity in the neonatal brain. Most of the available data originate from sensory cortices. In the visual cortex, spindle bursts (less clear for gamma oscillations) arise from the spontaneous activation of the sensory periphery (retinal waves) (Hanganu et al. 2006). In the somatosensory cortex, in the absence of active whisking, the spindle bursts arise from spontaneous muscle twitches triggered by central pattern generators in the spinal cord (Khazipov et al. 2004; Inacio et al. 2016), brainstem (Blumberg et al. 2013),

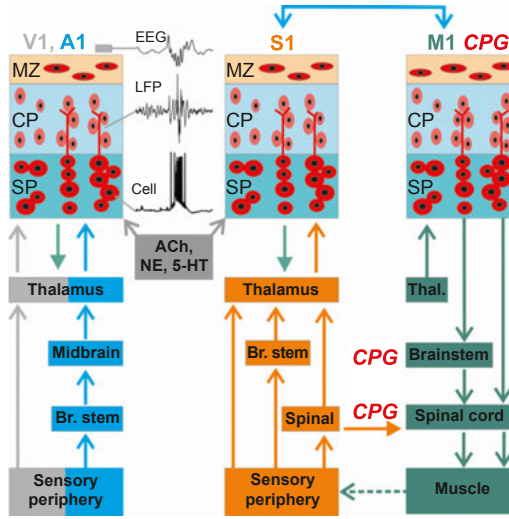

Figure 4.2 Model for the generation of 10–20 Hz spindle burst/delta brush activity in the newborn (P0) rodent and 3rd trimester (preterm) human cerebral cortex. At this stage the cortex consists of the marginal zone (MZ, future layer 1), the cortical plate (CP, future layers 2 to 4), layer 5, layer 6, and the subplate (SP). Thalamocortical input innervates glutamatergic subplate neurons, which also receive neuromodulatory inputs (ACh, NE, 5-HT). Subplate neurons can fire repetitively at 10–20 Hz when activated and transmit this activity to electrically coupled neurons in a local columnar syncytium, thereby generating the spindle burst/delta brush activity. The drive for this cortical activity comes from the sensory periphery (retinal waves, spontaneous activity in the cochlea) or from somatosensory receptors, which are activated following muscle movements. Central pattern generators (CPGs) in the motor cortex, brainstem, and spinal cord trigger these spontaneous movements ("twitches"). Modified after Luhmann et al. (2016).

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or motor cortex (An et al. 2014; Luhmann et al. 2016). Multiple electrodes and voltage-sensitive dyes show that some of these events are local (Yang et al. 2013; Luhmann 2017).

The emerging picture is that subplate cells amplify input from the thalamus (Luhmann et al. 2009) and are connected by gap junctions (probably Cx36) to other immature subplate and layer VI–V cells (Dupont et al. 2006). The temporal pattern of activity is different if recordings are made in different areas (e.g., the prefrontal cortex; Bitzenhofer et al. 2015) and is probably also dependent on the developmental stage. In sensory cortices we can think of this as vertical (columnar) organization above input from the thalamus prior to P5. Among us, there was some debate about the patterns and source of patterns. These early patterns of coordinated activity are necessary to assemble the network. Hardwiring the network from time zero, independent of activity, would require a vast amount of genetic information and is, therefore, less probable.

Different cortical areas show distinct temporal dynamics of coordinated patterns of oscillatory activity. While all cortical areas show discontinuous oscillations, the onset of activity reflects different degrees of maturation. For example, coordinated patterns of oscillatory activity are present already at birth in the sensory cortices and hippocampus (Hanganu-Opatz 2010); in contrast, in the prefrontal cortex (PFC) these rhythms emerge toward the middle to end of the first postnatal week (Brockmann et al. 2011). Major differences can be also detected in the frequency organization of rhythms with rather simple organized spindle bursts and gamma oscillations in the primary sensory cortices (Hanganu-Opatz 2010) and with complex nested gamma spindle bursts (theta–alpha ground rhythm superimposed with fast discharges in beta to low gamma range and high-frequency oscillations) (Cichon et al. 2014; Bitzenhofer et al. 2015; Bitzenhofer et al. 2017). These differences reflect the multiple generators involved: endogeneous drive from the sensory periphery (e.g., retinal waves in the V1) versus theta drive from the hippocampus in PFC (Hanganu et al. 2006; Brockmann et al. 2011). Subcortical modulatory systems (e.g., cholinergic) seem to shape the activity in all cortical areas (Hanganu et al. 2009; Janiesch et al. 2011).

In addition to extrinsic generators, neonatal oscillations can emerge within cortical areas through cross talk across layers. For example, in the prelimbic subdivision of the PFC, different neuronal populations coordinate discontinuous patterns of oscillatory activity at the end of the first postnatal week; this corresponds to the third gestational trimester in humans (Figure 4.3). All layers are in place and the neurons in the upper layers coordinate the emergence of frequency-specific oscillatory rhythms whereas the deeper layers seem to contribute to unspecific activation.

The way in which this compares with preterm human circuitry has been discussed (Khazipov and Luhmann 2006). The cortex looks similar. Spindle bursts/delta brushes in preterms are similar, occur spontaneously, or are triggered by light touch (Milh et al. 2007; Colonnese et al. 2010), but currently

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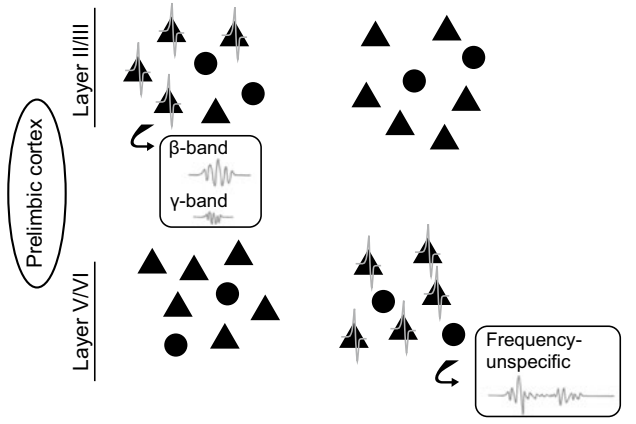

Figure 4.3 Cellular substrate of activity patterns in the neonatal prefrontal cortex. Schematic diagram depicting the contribution of pyramidal neurons in layer II/III of the prelimbic subdivision of the prefrontal cortex to the emergence of fast (beta to low gamma-frequency bands) components of discontinuous network oscillations and the ability of layer V/VI to generate a broad frequency-unspecific activation of the PFC.

there is no evidence for gamma oscillations. Significantly, if this spindle burst activity is blocked in rodent barrel cortex, the barrel field pattern is disturbed (Tolner et al. 2012). One hypothesis is that spindles are signaling the position of coactive muscles to columns of neurons to allow columns to link together, and that this needs to be updated during development as distances change. While initial insights of a functional circuit assembling during development in humans have been obtained for the somatosensory system, sparse knowledge is available for other areas. In line with the rodent data, we hypothesize that driving input in the visual system are the retinal waves; for the auditory system it is likely to be similar activity from the cochlear nuclei (Tritsch et al. 2007; Wang et al. 2015); for the PFC, it is the theta-band hippocampal input (Bitzenhofer et al. 2015).

A different structural and temporal organization of the patterns of coordinated electrical activity is present in the hippocampus. The first signals recorded in the rodent hippocampus are intrinsic Ca currents generated by voltage-gated Ca channels, followed around the time of delivery by synchronized Ca plateaux that interconnect interneurons via connexon-dependent gap junctions. Shortly afterward, the first synapse-driven activities, referred to as giant depolarizing potentials (GDPs), appear (Ben-Ari et al. 1989; Ben-Ari 2014) and have also been observed with some different properties in the neocortex, where they are referred to as early network oscillations (Allene and Cossart 2010). Recording from hippocampal pyramidal neurons at birth and reconstructing them revealed that neurons with no dendrites have no synaptic currents. Neurons with small apical dendrites have only GABA synaptic

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currents, and neurons with long dendrites extending to the distal lacunosum layer have synaptic currents generated by both GABA and glutamate, both in rodents and in primates *in utero* (Tyzio et al. 1999; Khazipov et al. 2001). A similar developmental sequence also occurs in interneurons at an earlier stage, indicating that GABAergic neurons are the first ones to be endowed with active synaptic currents (Hennou et al. 2002). Toward the end of the first postnatal week, discontinuous oscillatory activity in theta-frequency band (4–12 Hz), defined as theta bursts, is present in the hippocampus (Brockmann et al. 2011; Hartung et al. 2016a). This activity evolves into continuous theta oscillations toward the end of the second postnatal week.

There are many important directions for future work: Different frequencies of oscillations are observed at different ages and determining their functional role is an important issue to address. The detailed cellular and molecular mechanisms underlying these transitions remain to be investigated. Changes in the number and identity of glial cells over development will impact neuronal signaling properties. The small number that are present up to P7 control GABA and other neurotransmitter levels that are very important for proliferation and migration. Another important issue needing substantial clarification is the mechanism governing the transition between discontinuous oscillatory activity during neonatal (rodent) or fetal (human) development and continuous rhythms at juvenile age.

Knowledge of the spatiotemporal patterns of waves of activity has clinical importance. Burst activity recorded with EEG in preterms predicts clinical outcome (Benders et al. 2015; Iyer et al. 2015). With so many factors involved, drugs can have disruptive effects in the pregnant mother by interfering with activity patterns and altering apoptosis (Nimmervoll et al. 2013), map formation, and perhaps other features.

What Are the Patterns of Activity and Their Roles in Developmental Plasticity?

What Do We Mean by Activity?

Electrical activity has many forms during the early stages of development. The conclusion from research to date is that there can be substantial variability in the forms of activity across animal models, although Ca entry seems to be a consistent feature. In rodents at early stages of development, GDPs occur spontaneously. They last several hundreds of milliseconds in duration, are triggered by subthreshold depolarization, and involve voltage-gated Ca channels. The GDP frequency is appropriate for activation of the PV gene and likely others as well. It is not clear whether it is permissive or instructive. GDPs are primarily generated in interneurons but seen also in pyramidal cells. The arrival of synaptic inputs is hypothesized to terminate this spontaneous activity.

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In the developing kitten, the visual cortex exhibits distinct forms of plasticity in response to changes in activity. During the first postnatal week, thalamocortical and cortical activity driven by spontaneous waves of activity in the retina plays a crucial role in the formation of high-resolution topographical maps in the lateral geniculate nucleus, primary visual cortex (V1) and superior colliculus, as well as in connections between V1 and superior colliculus.

At the beginning of the "critical period" of V1 in the fourth week of life, visual receptive fields of single cortical cells are not similar when they are driven through the left and right eyes, and simultaneous binocular stimulation is required for them to match, as they do in all normally developing animals after this period. If one eye is deprived of vision and the other eye allowed to see normally during this period, responses to the deprived eye are rapidly reduced, and anatomical connections serving that eye are, more slowly, reduced. The reduction in cortical activity leads to a homeostatic synaptic scaling dependent on TNFα and increases the responses to the open eye, resulting in a change in the balance of responses to stimulation of the two eyes.

Juvenile plasticity can be reactivated in adult V1 by heterochronic transplantation of embryonic PV- or somatostatin-containing inhibitory neurons from the medial ganglionic eminence into postnatal V1 (Southwell et al. 2010). The reactivated plasticity appears in a second critical period, similar to that of the host animal, at the point in time when the transplanted donor cells would have reached the normal critical period had the donor animal survived. The factors which enable these cells to create a second critical period, time-locked to their age, are as yet unknown.

In *Xenopus* embryos, electrical activity—assessed by the ability to generate action potentials—begins at the closure of the neural tube. Neurons are already sensitive to neurotransmitters at this stage and are spontaneously active. Action potentials are 100 ms in duration and Ca dependent initially; they mature into brief 1 ms sodium-dependent spikes over the next few days. They do this even when isolated in culture in minimal medium, indicating that they follow an intrinsic program. Imaging intracellular Ca in neurons in culture, in the intact spinal cord, or in the brain reveals spontaneous transient elevations. This spontaneous activity is generated in response to activation of metabotropic GABA and glutamate receptors (Spitzer and Lamborghini 1976; Gu et al. 1994; Root et al. 2008).

What Are the Roles of Activity?

Altering the frequency of Ca transients in the developing *Xenopus* CNS can change the identity of the transmitter the neuron expresses, both in culture and *in vivo*, where it takes place in response to both artificial perturbations and sustained stimulation by natural sensory stimuli (Gu and Spitzer 1995; Borodinsky et al. 2004; Dulcis et al. 2013). These data suggest that it can be instructive. This transmitter switching involves the loss of one transmitter and

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the gain of another, with corresponding changes in postsynaptic receptors to maintain synaptic function. The most frequently observed switch is between excitatory and inhibitory transmitter or vice versa, switching the sign of the synapse. The transmitter switch causes changes in animal behavior (Spitzer 2017). Transmitter switching involves changes in the levels of transmitters, which is what seems to occur during psychiatric disorders, motivating studies of the role of transmitter switching in depression and schizophrenia. These findings raise the possibility that sustained differences in activity between sleep and wake could change transmitter identity (Levenstein et al. 2017). The relationship between transmitter switching and transmitter coexpression is, however, not yet clear. One can wonder whether GABA projection neurons are precursors to glutamatergic projection neurons.

An important test of the role of activity in development of the nervous system involved the study of the Munc-13 mutant mouse in which vesicular release of neurotransmitters is blocked (Verhage et al. 2000). Strikingly, normally the CNS assembles morphologically in the absence of activity, although it is not clear that all transmitter release is blocked and that all activity was suppressed (e.g., Ca waves).

Putting a cell in a new environment may change its properties in ways that allow it to develop abnormally. The medial ganglionic (MG) eminence makes many cell types, in addition to somatostatin and PV interneurons, but when neurons are removed from the MG, they no longer go through mitosis. While transplantation is often viewed as a challenge to progenitors to differentiate in the new environment, they often no longer divide, dramatically constraining their ability to adopt a new fate. Additionally, when cells are introduced into an environment that produces only a particular type of cells, those are the only cells that differentiate. Study of PV neurons illustrates the importance of intrinsic versus extrinsic cues. These cells are very sensitive to environmental context, which in turn is activity dependent. When animals are raised in the dark, brain-derived neurotrophic factor (BDNF) secretion is reduced, and overexpression of BDNF and other signaling molecules affects terminal maturation. The expression of clock genes may be the only case with completely intrinsic regulation (Kobayashi et al. 2015). It would be interesting to learn what happens if all cells expressed their critical period at the same time.

When we talk about two different critical periods, we may not be discussing the same thing. The visual cortex critical period, which occurs at four weeks postnatally, is sometimes compared to the somatosensory cortex critical period that occurs later. The effect of whisker plucking on the barrel cortex is compared to the effect of eye closure on ocular dominance and the visual cortex. However, whisker plucking constitutes an injury. If we want to compare apples to apples, it would be better to contrast the formation of the visual cortex map with the formation of the somatosensory cortex map. Whether the changes that occur in the visual system during the critical period are accompanied by changes elsewhere in the brain remains to be addressed.

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All of the brain's activity, coupled with development and continuous remodeling, makes it an expensive organ. Although the human brain comprises only 2% of total body weight, the adult and infant brain require 20% and 40% of the total energy budget, respectively (Allman 2000; Herculano-Houzel 2011). The energy budget of the brain is divided between basal metabolism (which in the human brain accounts for ca. 25%), action potentials, synaptic signaling, and, in the developing brain, synaptogenesis. The cost of spontaneous versus evoked activity and the cost of excitation versus inhibition has been analyzed (Attwell and Laughlin 2001; Laughlin and Sejnowski 2003; Buzsáki et al. 2007). There are four times more excitatory cells than inhibitory neurons in the cortex, but the inhibitory cells fire at a faster rate and inhibitory synapses are more reliable, so on average excitatory and inhibitory synaptic drive are balanced. Action potentials vary in their duration, and the thin action potentials of fast-spiking basket cells in the cortex are optimized to reduce the number of sodium ions that need to be pumped out (Hasenstaub et al. 2010).

While connectivity is dominated by thalamic projections to the subplate at birth, this rapidly shifts postnatally. By P5 in mice, there is a large increase of thalamic afferents to somatostatin interneurons in deep cortical layers, which shifts to PV interneurons by P9. As such, rapid and dynamic changes occur in the wiring of the cortex during the early postnatal period. An important future goal will be to examine in close detail the dynamics of wiring shifts in the early cortex. Although we have a lot of descriptive data from development, we lack data related to mechanisms. Inferences from adult physiology and plasticity, while attractive, are dangerous.

Do Changes in Activity Prefigure Development of Pathology? How General Is This Pattern?

A central concept is that insults at early stages cause deviations from normal development. Good examples are afforded by migration disorders. If neurons do not migrate, they display immature properties, retaining the currents that they express at early stages (Ackman et al. 2009). Conditional knockout of *Sox6* leads PV neurons to wind up in layer I. The result is more severe epilepsy than a total knockout of PV neurons: miswiring is worse than no wiring. This is a kind of "neuroarcheology," in which the present arises out of the past.

If, however, extremely strong stimuli are applied later in the adult (e.g., seizures, autism, spinal cord injury), neurons are not able to cope and either commit suicide or activate genes that express immature properties. What is the benefit for neurons to respond by expressing immature properties? Perhaps reopening chromatin, which had been open at an earlier stage, leads to expression of the same genes that had been expressed at the previous stage. This neuronal response has important implications for the treatment of clinical disorders: simply reinstating normal genes and/or proteins in the adult is not

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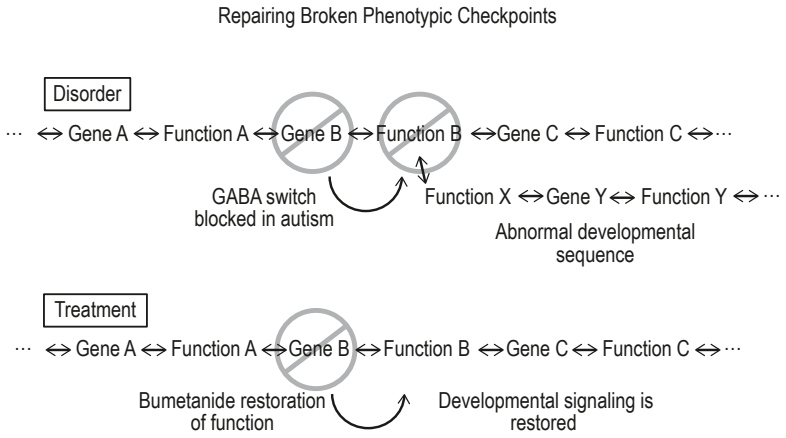

Figure 4.4 Circumvention of arrested phenotypic checkpoints is achievable. The normal developmental sequence is blocked when the GABA switch fails to occur in autism spectrum disorder (top), leading to abnormal development. Restoration of altered gene function with bumetanide bypasses the defect, providing treatment (bottom) that enables normal development.

likely to be effective in restoring function. Reinstatement of gene expression or function at the relevant site and at the relevant developmental stage would be a better approach. The example of bumetanide treatment for high Cl^- in autistic animal models (Tyzio et al. 2014) and patients (Lemonnier et al. 2017) is particularly interesting (Figure 4.4). Intrauterine insults appear to alter normal developmental sequences, disrupting them, with an outcome of misplaced neurons or inadequate connections and immature patterns of current expression. This underscores the difficulty of repairing such defects by inserting the appropriate gene (Manent et al. 2007; Ben-Ari 2008). In a similar case, a major study of vision in children was undertaken by the county government in Cambridgeshire to identify young persons with amblyopia. Appropriate treatments were determined and the burden of the disease was dramatically reduced (Atkinson et al. 2007). Unfortunately, the subsequent change in government resulted in the termination of plans to screen and treat throughout the rest of the United Kingdom.

The situation is even more complex and less well understood in neuropsychiatric disorders with a developmental time course. In schizophrenia, brain structure appears almost normal throughout all stages. Since psychotic episodes are devastating, psychiatrists ask if there is a way to identify vulnerability in advance. Is there any general feature of activity that allows prediction of the disease (Uhlhaas and Singer 2011; Andreou et al. 2014; Leicht et al. 2016)? Blind examination of six mouse multihit models of mental illness has shown differences in the hippocampus in the extent of coupling to the PFC already in neonates (Hartung et al. 2016b). These disorders are likely to be

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the result of multiple hits. For example, a single mutant gene may not be sufficient by itself; inflammation might allow the brain to compensate, but another insult could push the system over the threshold. Accordingly, we need to follow behavior over development to look for changes. A single biomarker may not be sufficient.

An alternative approach would be to look for a series of developmental checkpoints and correlate failure to pass them using a probabilistic model of origin of the disease. This is being done with human autism spectrum disorders: checkpoint analysis has shown that the GABA switch was not happening, but it appears possible to move through the checkpoint by applying bumetanide (Tyzio et al. 2014; Lemonnier et al. 2017; Figure 4.4). Neurons that do not complete their normal developmental sequence retain immature features, as evidenced in genetic disorders such as the double cortex syndrome, in which neurons that have not migrated have immature features (Ackman et al. 2009).

When addressing mental disorders, we need to decide on the strategies to prioritize. Here we can derive a cautionary tale from the history of Nixon's war on cancer. Initially, most money went into chemotherapy, but it was not until recombinant DNA technology was invented that cancer was shown to be a complicated genetic disease. Now immunotherapy is becoming successful (Kosik et al. 2016). Schizophrenia is similarly complicated, and the pathways generating it need to be investigated if we are to gain an understanding of its pathological background.

The use of mouse and other animal models in understanding and treating human disorders has both advantages and disadvantages. Mouse models may be valuable for understanding some aspects of disease etiology. However, they cannot capture the full range of human complexity, since the human brain is not a scaled-up version of the mouse brain (Herculano-Houzel 2009). Multiple animal models of Fragile X syndrome, for example, have responded to mGluR antagonists. However, proximal mouse models have not informed the human disease: clinical trials have either failed or are failing. Mouse models specify early treatment but that opportunity rarely exists with patients, who can only be treated once they search out medical help. Finally, there is the issue of doses and duration of drugs. Many unexpected checkpoints, possibly involved in metabolism, can be expected. In addition, the same mutation can lead to different disorders, implying that other factors must be involved (Guilmatre et al. 2014).

Curing disorders of developmental origin does not seem possible because the multiple insults are early and cannot be treated at the time they are generated using current technology. For instance, attempts to restore migration in a conditional knockout of doublecortin failed when the correction was applied later than a few days postnatally, underscoring the challenge of correcting deviations in the developmental sequence (Manent and LoTurco 2009). We should not, however, give up on animal models; it was necessary to screen 500 drugs before one was discovered for treating strokes. *Treating* disorders of developmental origin seems more feasible, is a more modest approach, and

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seems to work. Combining psychiatric/psychological treatment with biochemical/molecular treatment could result in even better outcomes.

It is useful to reevaluate what it means to be a model. Ideally, each patient should be his/her own control. At the start, as many biomarkers as possible should be obtained to compare against "normal" states; thereafter, machine learning could be used to predict which drugs should be used to treat symptoms. Included here should be the recognition that each disorder is really many disorders. The Adolescent Brain Cognitive Development study—a longitudinal study of 20,000 children, ages 9–19 years—will enable analysis of many aspects of development (Barch et al. 2017). It is now feasible to use iPSCs to generate a patient's neurons and study the genetic disorder. This may assist in developing treatments and is an important avenue to pursue, despite present challenges in implementation.

Another approach is to find a brain closer to ours, such as a nonhuman primate. Currently, the Allen Brain Institute is making physiological recordings from human tissue, and the membrane properties of the neurons are different from those of the mouse. Hideyuki Okano's group in Japan is using marmosets. Macaque monkeys are even closer, and the Institute of Neuroscience in Shanghai is mounting a big effort with macaques as part of the Chinese Brain Initiative.

While the mouse has proven a useful model for CNS development, the limits in the similarities between mouse and humans argue for the need of higher-order models to study human brains. The use of nonhuman primate models as well as *in vitro* models using organoid cultures of human iPSC-derived cells show promise for studying the specification of cell identity as well as the signaling in neural networks. Nonhuman primates provide the requisite neuronal types, but tools will need to be developed to allow for the targeting and manipulation of specific types in a manner akin to what is now possible with mouse genetics. Similarly, the advent of methods to use the neural equivalent of "Yamanaka" factors means that we are well advanced in our efforts to generate specific neuronal types from iPSCs. With these tools in hand, the ability to study brain function using *in vivo* (nonhuman primate) and *in vitro* (iPSC-derived organoids) models will be greatly enhanced, with relatively small advances in opto- and chemogenetic methods.

In conclusion, we do not know enough at this point to be able to determine whether changes in activity will be useful biomarkers. However searches in mice, nonhuman primates, and the use of human registries seem appropriate.

Future Directions

As we look to the future, it is clear that acquiring more data will be important and necessary to derive general principles by which dynamic brain coordination

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is achieved during development. Using the findings achieved to date to construct conceptual frameworks that focus the directions of experimental work should promote the most rapid progress, either by supporting, reorienting, or eliminating particular conceptual schemes. The formulation of computer models, both for data analysis and for testing our understanding of the operation of circuits at different scales, is now possible as never before and should be a component in all research programs. Among the many promising avenues to pursue, we wish to highlight the following:

- Determining the functional role of different frequencies of oscillations observed at different ages is essential.
- Understanding the basis of developmental changes in cortical wiring is crucial.
- Identifying the cellular and molecular mechanisms by which these patterns of activity exert their effects is a critical objective.
- Clinical diagnostics and treatment protocols will likely follow from this knowledge.
- Mice will remain an attractive model for many studies, but nonhuman primates and humans themselves, via registry databases, will be important.

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