Cortical Development, Electroencephalographic Rhythms, and the Sleep/Wake Cycle

Supplemental Information

Supplemental Discussion

Development of the Rodent Cortex

The development of the rat brain has been subdivided into four stages (1). In the fetal period cell division produces 94-97% of all brain cells. The first postnatal phase (P0-P10) is characterized by explosive growth: at P0 the brain is only 15% of its final size, but by P10 most growth of cells, and especially of axons and dendrites, has been completed (2-4). In the third phase (P11-P20) the rate of growth is much reduced, the extracellular space decreases significantly, blood vessels grow, mature astrocytes and oligodendrocytes are easily found, and myelinization starts (5,6). After weaning (usually at P21 in mice and rats) growth is very slow (fourth phase).

Cortical neurons originate from the ventricular zone. This germinal zone first gives rise to the preplate, located just below the pial surface, which comprises loosely packed polymorphic postmitotic neurons. Neurons forming cortical layers II to VI are generated by subsequent rounds of cell divisions, and their radial migration splits the preplate into two zones, the marginal zone just below the pial surface (layer I), and the subplate, below the cortical plate. Morphologically, subplate neurons are highly differentiated, show relative mature electrophysiological properties, and are strongly connected with thalamus and multiple cortical areas (7). Thus, although they are a transient cell population (in mice and rats they appear at E11-E15 and most disappear by P21-P30), they serve as a crucial hub station for the development of cortical columns and the maturation of cortical inhibition (7). They also promote the entrance of thalamic axons into layer IV, and indeed thalamic axons wait in the subplate before the invasion of the cortical plate, which in the rat occurs at E19-E20, about 2 days before birth (P0, equivalent to E21-E22). By P2 these axons have gone through layers V-VI and reached presumptive layer IV, and by P8 the cortex reaches its final mature lamination (8).

Preplate neurons contain some of the earliest functional synapses including electrical synapses, and thalamic stimulation in rodents activates subplate neurons by E16, while cortical plate activation starts at E21 (7). In the rodent cortex between P3 and P5 a significant proportion of excitatory glutamatergic synapses (30-60% depending on species and cortical area) are silent, i.e. they contain NMDA-type glutamate receptors but not AMPA-type receptors, but by P8-P11 silent synapses have almost disappeared (9,10). Early electron microscopy experiments demonstrated a large increase in the number of synapses in rat cortex between P10 and P15 (11,12). More recent two-photon experiments in layer IV of mouse barrel cortex found a 3-fold increase in connectivity at P9 relative to P4, and a ~250-fold increase in spines (most cortical spines contain synapses) between P8 and P13 (13). Intriguingly, the induction of many immediate early genes whose expression tracks neuronal activation starts in the cortex at P14 (14). Local neuronal coupling via gap junctions is also extensive in rat cortex during the first two postnatal weeks, but disappears almost completely by P16 (15). GABA A receptors signaling also undergoes a profound evolution in cortex and hippocampus, with GABA A synaptic responses switching from being depolarizing and often excitatory at birth to becoming hyperpolarizing by the end of the second postnatal week in rats (16). This change occurs in association with the downregulation of the Na-K-2Cl cotransporter isoform 1 (NKCC1), which imports chloride ions into the cell, and the upregulation of KCC2, which in mature neurons maintains low intracellular levels of chloride ions by actively extruding them (17,18).

The second week also marks a major switch in the rules of synaptic plasticity. For instance, in layer IV of rat somatosensory cortex both long-term potentiation (LTP) and long-term depression (LTD) of thalamocortical synapses can be induced easily until P7, while after P8 plasticity is reduced, especially LTP, perhaps due to a developmental decline in NMDA current duration (19). In mouse barrel cortex the rules for spike timing-dependent plasticity in layer IV to layers II/III synapses change at P13, switching from LTP exclusively, independent of spiking order, to LTP or LTD depending on whether the presynaptic neuron spikes before or after the postsynaptic neuron, respectively (20). In the same synapses whisker deprivation also induces LTD in the deprived column only after P14, consistent with a strong bias toward LTP in the first two weeks (20). P12 is

also the earliest time when spreading depression can be induced in rat cortex (21). In the hippocampus, by contrast, high-frequency stimulation is unable to induce LTP before P12 (22-25). Thus, the second week marks a pivotal point in the transition from immature to mature cortex.

In vitro patterns. A large number of oscillations have also been described in vitro in many brain areas, from cortex to spinal cord (17,26-28), and like the patterns seen in vivo they tend to include long periods of silence interrupted by transient bouts of activity. However, the relationship between the many patterns seen in vitro and the fewer oscillations described in vivo remains unclear (29). Interestingly, the events in vitro seem to follow a similar developmental time course in all brain regions, with intrinsic patterns dependent on voltage-gated calcium currents appearing first, followed by synapse-driven network patterns. In slices of rat somatosensory cortex, for instance, intrinsically-driven activities include calcium spikes already evident during embryonic development, followed by calcium plateaus that are present between P0 and P5 (30). Synapse-driven patterns include early network oscillations (ENOs) driven by NMDA and AMPA receptors, which also occur during the first postnatal week, and giant depolarizing potentials, which mainly depend on GABAergic transmission and become prominent by the end of the first postnatal week (30).

Oscillations in disgranular cortex. One study described three types of oscillations in medial dysgranular cortex of frontal, parietal and occipital lobes during the first two postnatal weeks: cortical sharp potentials, gamma bursts (similar to those already described), and SATs (31), the latter also similar to those described in premature human infants (32). All these patterns occur in all behavioral states, although the first two tend to cluster in active sleep, while the third type occurs more often in active sleep at P5-P7, and in quiet sleep later.

Hippocampal rhythms: sharp waves and theta bursts. Simultaneous recordings in cortex and hippocampus, under urethane anesthesia, show that sharp waves with strong multiunit activity are present in CA1 already at birth, and discontinuous

oscillations with main frequency in the theta band (~7 Hz), called hippocampal theta bursts, start at P1 (33). These two hippocampal rhythms differ in their dependence on GABAergic neurotransmission, since the lesion of GABAergic neurons in CA1 during the first postnatal week affects the generation of sharp waves but not that of theta bursts (34). By the end of the first week, ripples and gamma oscillations superimpose on sharp waves and theta bursts, respectively, and hippocampal activity switches from discontinuous to continuous two days earlier than in prefrontal cortex, at P8-P10 (33). Early hippocampal theta bursts are important to drive gamma activity in the prefrontal cortex, and are believed to promote the coupling between prelimbic cortex and hippocampus. It has been suggested that during development this coupling may occur in a task-independent manner during sleep (33).

The onset of hippocampal theta activity does not appear to be consistent across studies, ranging from P1 in anesthetized pups (33) to P8 in unanesthetized pups (35). However, despite many similarities (e.g. (36)), anesthesia and sleep are different states (37,38), and thus it may not be necessarily the case that network oscillations observed under urethane or other anesthetics mimic exactly those present during sleep (e.g. (39)). An early study (40) that recorded freely moving rats from 2 to 23 days of age identified short trains of theta during voluntary movements and active sleep starting at P8-P9 in the dentate gyrus; at P10 theta trains became larger and longer, and a clear separation was observed between theta activity during voluntary movements and active sleep, and low-amplitude irregular activity during quiet wake and NREM sleep. A similar evolution of theta activity was found in the CA1 region, but it lagged ~ 1 day behind that of the dentate gyrus (40).

More recent hippocampal recordings performed between P1 and P12 without anesthesia also found that most cells in CA1 and dentate gyrus are sleep active, and among them, the majority fire more during active sleep than during quiet sleep (35). All neurons firing during active sleep increase their activity during twitches, and a subset of them shows bursts of activity after twitching, suggesting that the latter can provide sensory feedback not only to the cortex but also to the hippocampus. Twitch-related bursts of hippocampal unit activity are coupled with gamma activity as early as P2, and with theta activity starting at P8, when the theta rhythm first emerges (35). During quiet

sleep, by contrast, hippocampal population bursts occur in association with sharp waves, high amplitude CA1 population events that are the first pattern of endogenous activity recorded in infant mammals (35,41-43). In adult rats sharp waves are associated with ripples, but these high frequency oscillations (140-200 Hz) are missing in infant rats, appearing for the first time during the second week in recordings without anesthesia (42,44). In neonatal rats sharp waves occur in both wake and sleep (42) but most of them are preceded by startles (41). This temporal relationship is transitory because startles disappear by the third week of age, while sharp waves persist in adults during quiet wake, consummatory behaviors, and slow wave sleep (41). Thus, the second postnatal week marks major changes also in hippocampal activity, with the appearance of theta bursts and the association of ripples with sharp waves.

Supplemental References

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