Rapid eye movement sleep deprivation revives a form of developmentally regulated synaptic plasticity in the visual cortex of post-critical period rats

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Abstract

The critical period for observing a developmentally regulated form of synaptic plasticity in the visual cortex of young rats normally ends at about postnatal day 30. This developmentally regulated form of in vitro long-term potentiation (LTP) can be reliably induced in layers II–III by aiming high frequency, theta burst stimulation (TBS) at the white matter situated directly below visual cortex (LTPWM-III). Previous work has demonstrated that suppression of sensory activation of visual cortex, achieved by rearing young rats in total darkness from birth, delays termination of the critical period for inducing LTPWM-III. Subsequent data also demonstrated that when rapid eye movement sleep (REMS) is suppressed, thereby reducing REMS cortical activation, just prior to the end of the critical period, termination of this developmental phase is delayed, and LTPWM-III can still be reliably produced in the usual post-critical period. Here, we report that for approximately 3 weeks immediately following the usual end of the critical period, suppression of REMS disrupts the maturational processes that close the critical period, and LTPWM-III is readily induced in brain slices taken from these somewhat older animals. Insofar as in vitro LTP is a model for the cellular and molecular changes that underlie developmental synaptic plasticity, these results suggest that mechanisms of synaptic plasticity, which participate in brain development and perhaps also in learning and memory processes, remain susceptible to the effects of REMS deprivation during the general period of adolescence in the rat.

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During a restricted postnatal phase of brain development, synaptic connectivity in cortex exhibits a high level of plasticity as synapses are formed and retracted, a process strongly driven by sensory activity. The final adult conformation of synaptic connectivity in visual cortex is achieved, in part, through activity-dependent mechanisms [9]. It is argued that a Hebbian form of homosynaptic, long-term potentiation (LTP) corresponds to activity-dependent plasticity. This form of synaptic plasticity is thought to be a model of at least some of the cellular mechanisms underlying the maturing network of synaptic connections in the visual system [8,10].

A specific, in vitro, form of homosynaptic LTP in visual cortex has been shown to be regulated in young rats by age and experience [11] as well as by rapid eye movement sleep (REMS) [27]. This developmentally regulated form of LTP is reliably produced in brain slices taken from rats younger than postnatal day (PN) 30 by direction of theta burst stimulation (TBS) at the white matter (WM) just under visual cortical layers II–III, and is therefore referred to as LTPWM-III [11,20]. It has been shown that this developmental form of LTP is still producible in rats older than PN30 if they are raised from birth in the dark (dark-rearing) [11], supporting the argument that dark-rearing delays the processes responsible for the usual termination of the so-called “critical period” (CP) for this form of plasticity (LTPWM-III) in visual cortex [11]. This period of brain maturation is often referred to as a “sensitive period” of brain development [15] insofar as its termination depends as much upon the degree of previous stimulation by both REMS-related [27] and sensory activity [11] as upon exact chronological age. Accordingly, we use the terms “critical period” and “sensitive period” interchangeably throughout.

Recently, we reported that REMS deprivation (REMSD), when started prior to the usual, age-defined end of the CP, also extends the developmental end-point for producing LTPWM-III [27]. We demonstrated that 7 days of REMSD is at least as effective in extending the CP for LTPWM-III as several weeks...
of dark-rearing started before eye-opening [27]. In this study, we explored whether REMSD has to be instituted before the age-determined end of the CP so as to “extend it”, or, alternatively, can REMSD also invoke LTPWM-III when applied after the age-determined end of the CP. In other words, are the mechanisms that close off developmentally regulated synaptic plasticity fully established in visual cortex at the accepted, age-defined end of the CP? To answer this question, we investigated whether suppressing REMS shortly after the end of the CP (PN35) would affect induction of LTPWM-III. We report here the finding of an “interim” phase of developmentally synaptic plasticity, lasting approximately 3 weeks beyond the typical end of the CP (PN35–60). During this phase, which is roughly equivalent to adolescence in the rat, REMSD appears to enable production of LTPWM-III. We also determined that a 4-day period of REMSD was as effective as 7 days in reviving LTPWM-III in the post-CP phase of brain development. Other work had previously demonstrated that REMSD does not allow for production of this form of LTP in normal adults (older than PN60) [26].

Male long Evans hooded rats were used in this study to avoid possible confounds from uncontrolled hormonal influences during the adolescent phase. Animals were obtained at specific ages from timed-pregnant dams, or neonates were purchased at weights that put them within the targeted age-range (Harlan, Illinois, USA). All procedures were carried out in conformity with guidelines of the NIH and with approval of the local Internal Review Board for Care and Use of Animals. Every effort was made to minimize animal suffering. The minimum number of animals necessary to obtain statistically reliable data was studied.

Animals were obtained in two age groups. Animals in the younger of the two (interim-aged) groups were between PN42 and PN60 at the time of in vitro testing (mean = 48.8, 5.1 S.D., n = 23). The adult group was between PN61–68 (mean = 64.8, 2.1 S.D., n = 10) at testing. The mean ages of the animals in the two groups (interim-aged and adults) at the time of the in vitro studies were significantly different (F = 5.13, d.f. = 1, p < 0.031). In the first study, REMSD was carried out on both the adolescent (REMSD7, n = 17) and adult groups for 7–10 days by the multiple-small-pedestals-over-water method. Seven-centimeter diameter pedestal-tops allowed the two sets of post-critical period animals to obtain variable amounts of non-REMS before the loss of postural control as REMS emerged ended nascent REMS bouts. Selectivity of the pedestals for reducing REMS was confirmed behaviorally [27]. On the day of the in vitro experiments, animals were removed from the pedestals and quickly sacrificed under isoflurane anesthesia. Brain slices from visual cortex were prepared (see below). A second cohort of interim-aged rats experienced REMSD for periods of only 1–4 days (REMSD4, n = 6) after which they were utilized for in vitro studies, as described below. The average age of the REMSD4 group rats (PN42–50) was not significantly different from the REMSD7 group of interim-aged animals (F = 1.7, ns).

Trunk blood was collected at sacrifice, spun down at 10°C (15,000 rpm) for 15 min, stored at ~20°C, and later analyzed for corticosterone levels. The corticosterone assay used the ICN (Costa Mesa, CA) double-antibody kit with the following modifications: primary antiserum solution is used at half strength. The lowest standard solution (25 ng) is serially diluted three times to yield standards at 12.5, 6.25 and 3.12 ng, respectively. Serum samples are diluted 1:50 instead of 1:200 and are run in duplicate. Results are reported in μg/dl.

Immediately after removal of the animals from the cages that housed the pedestals, they were decapitated under isoflurane anesthesia and the brains rapidly excised. Brain slices were obtained from tissue blocks containing the postlateral gyr that were sectioned on a vibratome into 400 μm thick slices in the coronal plane. Trimmed tissue slices containing primary visual cortex (corresponding to Bregma ~5.8 to ~7.8 mm in the Paxinos & Watson atlas, [19]) were transferred to a slice-interface chamber (Fine Science Tools, Foster City, CA). The tissue slices were maintained in an oxygenated, humidified atmosphere and superfused with 35°C (+1) ACSF at a rate of 1–2 ml/min for at least 1 h before the start of electrophysiological recording (see below).

The remaining portions of the brains were rapidly frozen on dry ice and stored at ~80°C. The hypothalamus was dissected from the frozen brain for radioimmunoassay of corticotropin-releasing factor (CRF) using previously published methods [27]. Neuropeptide concentrations were given as pg/mg protein. The differences in mean expression of CRF were compared between Normals, a group of unmanipulated animals that resided in home cages throughout the experiment, and the REMSD7 and REMSD4 rats by one-way ANOVA.

Electrophysiological recording followed previously reported procedures [27] and are briefly described here. A glass-pipette recording electrode filled with 1 M NaCl or ACSF (impedance 1–2 MΩ) was placed in visual cortical layers II-III. Extracellular field potentials were evoked by 10–150 μA square wave stimuli of 0.2 ms duration. After a full input/output curve was obtained, stimulation intensity was set at 50% of the maximum response. We attempted to record a stable 20 min baseline with a single-stimulation intensity delivered every 30 s (30–75 V). After a stable 20 min baseline was recorded, five consecutive episodes (0.1 Hz) of TBS were delivered through a concentric, bipolar stimulating electrode (outside diameter, 200 μm, #16-60-3; Frederick Haer, Brunswick, ME, USA). The evoked field potentials were analyzed for the maximum negativity below baseline of the digitized field potential, as a measure of the evoked, population, excitatory post-synaptic current [1]. LTP was considered successfully induced if the post-TBS response was greater than 120% of the mean baseline amplitude.

No difference was found between the groups in the number of days spent on pedestals (REMSD7 group 8.2 days, 1.2 S.D.; adults, 7.6 days, 1.8 S.D.; F = 1.21, d.f. = 1, ns). TBS directed at the WM-interface of the adult group failed to produce LTP in layers II-III in 9 of 10 attempts (10% success, Table 1). In the nine cases in which TBS failed to induce LTP, the response to post-tetanus stimulations was initially depressed, then gradually approached but never entirely returned to baseline levels, as seen in Fig. 1A. In contrast, in the interim-aged group, TBSs of the WM-interface produced LTP in 21 of 23 animals (91% success; see Fig. 1B). The higher likelihood of produc-
Effects of age on inducibility of long-term potentiation (LTP) in visual cortical layers II–III after theta burst stimulation (TBS) directed at the underlying white matter

<table>
<thead>
<tr>
<th>Age-group</th>
<th>Interim-aged rats (PN42–60)</th>
<th>Adult rats (PN61–68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBS to WM produces LTP</td>
<td>Failure</td>
<td>2</td>
</tr>
<tr>
<td>Success</td>
<td>21</td>
<td>1</td>
</tr>
</tbody>
</table>

The number of successful versus failed attempts to produce LTP are indicated according to the two age groups. *Interim-aged rats are significantly more likely to show layers II–III LTP after stimulation of the WM (p < 0.0001).*

During the accepted, age-defined CP for induction of the LTPWM-III in the interim-aged REMS-deprived group was statistically significant (continuity corrected Chi-square = 17.24, d.f. = 1, p < 0.0001, Table 1). Ages of the two interim-aged animals that failed to show LTP after REMSD were P59 and P52. Both of these animals were in the REMSD7 group.

In brain slices from visual cortex taken from the interim-aged animals that resided on multiple small-platforms-over-water for fewer than 4 days, TBS induced LTPWM-III in the interim-aged REMS-deprived for 4 or fewer days. Of these six animals, one was REMS-deprived for 1 day, one for 2 days, and the remaining four for 4 days. Inasmuch as no overall differences in amplitude or general shape of responses were observed, we have plotted the data for both REMSD4 and the REMSD7 animals together in Fig. 1B. Serum levels of corticosterone measured in untreated rats (Normals) were compared with those in the REMSD4 and REMSD7 animals as well as in the adult rats. Serum levels of corticosterone in trunk blood taken after sacrifice were not statistically different among any of the four groups (Table 2, upper panel, F = 0.05; p, ns). Table 2 displays the means, standard errors, and numbers of animals in each group. CRF levels measured in hypothalamic tissue taken from Normals were also compared to the REMS-deprived interim-aged and adult rats. Levels of CRF did not differ significantly among the three groups (Table 2, lower panel, F = 0.99; ns). During the accepted, age-defined CP for induction of the developmentally regulated form of synaptic plasticity in visual cortex, visually driven cortical activity and REMS cortical activity together determine the functional connectivity underlying maturation of neural networks by controlling molecular mechanisms that consolidate developmentally regulated synaptic plasticity. Ultimately, these processes bring about the end of the CP of special sensitivity to exogenous and endogenous activity in the visual system. The data in this study indicate, however, that for about 3 weeks after the usual end of the conventional CP, REMSD apparently persists in effecting the molecular and cellular changes necessary for the LTPWM-III that is usually seen in younger animals [11,20,27]. Shorter durations of REMS deprivation also appear to reliably produce this effect in 4 or fewer days. Taken together, the evidence indicates a post-CP, “interim” phase of visual cortical development corresponding generally to adolescence in the rat, when plasticity mechanisms that regulate brain maturation are not fully consolidated. Inasmuch as REMS-deprived rats are awake in the light more than controls, the current data indicate that these plastic, developmental processes still require normal amounts of REMS to fully establish and finalize adequate synaptic connectivity in visual cortex for normal visual system maturation.

We hypothesize that in normal animals, despite the functional close of the CP, the putatively inhibitory mechanisms thought to regulate its termination have not yet fully matured (see below and [22]). Our data also indicate that REMSD-enforced changes in neuronal activity and neurochemical milieu appear to be sufficient to uncover this latent synaptic plasticity. Following the “interim” phase (i.e. >PN60), however, the mechanisms responsible for the “developmental” type of plasticity become resistant to the effects of removing REMS-related activation [26]. Inasmuch as in vitro LTP models the molecular mechanisms that underlie developmental synaptic plasticity and also learning and memory, our results suggest that alterations in REMS amounts continue to affect brain development and perhaps memory and learning during rat adolescence.

Enforcement of REMS deprivation for 4 days or fewer during this interim phase affects brain development at a time when manipulations of sensory input (either monocular patching or complete darkness) have little detectable effect on synaptic plasticity [12,17]. Even after extension of the CP by dark-rearing, very brief exposure to light rapidly initiates a cascade of developmental events that leads to the end of the sensitive period for production of these forms of synaptic plasticity [11]. The data also suggest that REMS may be a necessary requirement for bringing about complete termination of the CP of synaptic plasticity. The number of successful versus failed attempts to produce LTP after REMSD for 4 or fewer days.

Table 2

<table>
<thead>
<tr>
<th>Stress measures</th>
<th>Normals</th>
<th>Interim rats (REMSD7)</th>
<th>Interim rats (REMSD4)</th>
<th>Adult rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosterone (µg/dll)</td>
<td>166.13</td>
<td>165.30</td>
<td>161.51</td>
<td>156.13</td>
</tr>
<tr>
<td>S.E.</td>
<td>12.2</td>
<td>19.8</td>
<td>22.7</td>
<td>22.9</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>13</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>CRF (pg/ml)</td>
<td>309.2</td>
<td>290.3</td>
<td>414.0</td>
<td></td>
</tr>
<tr>
<td>S.E.</td>
<td>41.2</td>
<td>52.8</td>
<td>93.7</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

Measurements of corticosterone levels in trunk blood and corticotropin-releasing factor (CRF) content in hippocampal tissue. No significant differences exist between the compared groups (ANOVA). Hippocampal tissue unavailable from the REMSD4 animals. REMSD7 = rapid eye movement sleep deprivation for 7–10 continuous days. REMSD4 + REMSD for 4 or fewer days.
plasticity in visual cortex, perhaps because of a unique contribution to the final consolidation of CP processes. The mechanisms supporting such an effect remain to be determined. One possible explanation is that the enforced waking resulting from REMSD likely leads to extended periods of cellular activity that, either by exhausting available rate-limiting enzymes or by downregulating target receptors, reduces monoaminergic tone in visual cortex. At an immature stage of development, if monoamine expression is required to terminate the CP (for which there is some evidence [13]) insufficiency of monoaminergic tone in cortex could account for the extension or revival of the neurochemical milieu necessary for production of LTPWM-III. It is the case that norepinephrine (NE) is necessary for the ocular dominance shift during development of the cortical visual system [2,11,13], which only occurs during the CP. Serotonin (5-HT) is also strongly implicated as a mechanism that underlies CP plasticity [5].

Additional effects of REMSD may be considered as well. Interference with the usual REMS state-enforced shutdown of both neurotransmitter systems causes them to continue to operate unabated. Overexpression of NE and 5-HT at this stage of development may maintain cortical plasticity mechanisms and preclude normal development of layer IV inhibitory mechanisms that are thought to be involved in termination of the CP (c.f., [22]). Serotonin reuptake, which inhibits increased synaptic availability of 5-HT, has been linked to disruption of the neural anatomy in developing somatosensory cortex [28]. Another consequence of REMSD is maintenance of cholinergic activity in basal forebrain sites at lower waking levels that are typically higher during REMS [14]. Higher levels of acetylcholine...
associated with REMS may be a threshold requirement for develop-
ment of the inhibitory mechanisms necessary for terminating
the sensitive period of visual cortex development [1,22]. Finally,
its must be acknowledged that, though REMSD results in more
waking light experience, retnial activity alone is insufficient to
end the plasticity processes. Similarly, the dark-rearing literature
(e.g., [11,17]) suggests that the same is true for REMS activa-
tion effects. Previous work in REMS- and monocularly-deprived
kittens indicates additive effects on consolidation of visual sys-
tem synaptic plasticity from these two sources of activation
[25].

Our data do not directly speak to which specific mechanisms
are responsible for LTPWM-III in REMS-deprived, adolescent
rats, but the foregoing discussion suggests that REMSD may have
its effects on LTPWM-III production by blocking the inhibitory
mechanisms (thought to be responsible for termination of the
CP) that close the developmental “inhibitory gate” controlling
relay of activity to the superficial layers of visual cortex during
brain maturation [1,22]. One candidate mechanism is mediated
through neurotrophin expression. Expression of brain-derived
neurotrophin factor (BDNF), a member of the nerve growth
factor family of neurotropins, appears to play a key role in
development of the putatively GABAergic inhibitory system and
may be the penultimate step in the process of maturation of this
inhibitory system in visual cortex [5,7,22]. Visual activity modu-
lates expression of BDNF mRNA’s. For example, BDNF mRNA
expression in visual cortex is reduced in rats dark-reared from
birth [23] and its expression can be restored by exposure to light
[23]. Exogenous intracortical infusions of BDNF during the CP
of kittens, but not in adults, expand ocular dominance columns
in visual cortex [6]. GABAergic transmission in visual cortex
slices taken from dark-reared CP rats show a threefold reduction
in GABAergic input compared to the levels seen in light-reared
animals [16]. Development of GABAergic mechanisms in visual
cortex appears to be dependent on BDNF availability. Studies
in this laboratory are currently directed at determining whether
REMSD mediates LTPWM-III through its effects on BDNF and
GABAergic mechanisms [24].

Our initial studies relied upon a 7–10-day period of REMSD,
in part, because this period of REMSD was effective in altering
synaptic plasticity in a monocular deprivation model of kitten
visual cortical development [18,25]. This long period of REMSD
was utilized in our early LTP studies so as to incontroversially
demonstrate extension of the CP for LTPWM-III [27]. Shorter
periods of REMSD may not have unambiguously demonstrated
extension of the CP over biological variability. In the present
study, however, effects of shorter periods of REMSD (4 days
or less) could be determined, and are equally effective as 7-day
periods at enabling LTPWM-III in the interim-phase rats in terms
of response amplitude, degree of potentiation and reliability (see
Fig. 1).

It was beyond the scope of this study to probe whether the
synaptic plasticity mechanisms affected by REMSD are regu-
lated by the same mechanisms before and after the end of the
CP. Continuing work in this laboratory is aimed at determin-
ing whether such differences exist. The fact remains that the
developmental form of synaptic plasticity evoked in the interim
period after REMSD is obtained by the same parameters as
during the CP and are not achievable in the adult rat (also see
[26]).

Finally, the corticosterone and CRF data in this study largely
rule out stress effects as responsible for our results, particu-
larly when taken in conjunction with previous data from postnatal
REMS-deprived rats that showed no differences in several other
stress-related hypothalamic neuroendocrines between REMS
and control animals [27]. This reduces concern that stress is
responsible for the ability to unmask LTP after the usual end
of the CP. Serum from trunk blood at sacrifice may not directly
reflect the cumulative effects of stress on synaptic plasticity.
However, observed CRF/corticosterone levels were not signifi-
cantly different in any of the groups in this study. This indicates
that the pedestal experience did not have a preconditioning
effect that could predispose pedestal-deprived rats to exagger-
ated stress-axis responses.

The results of this study underscore the importance of REMS
in normal synaptic development and lend further support to a
functional role of REMS in brain maturation [21]. Insofar as
REMSD in adolescent rats disrupts the usual course of matura-
tion of the visual cortex, the effects of sleep deprivation, and
particularly, REMSD on brain development in teen-aged chil-
dren may require reevaluation [4].

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