Monocular Visual Deprivation Suppresses Excitability in Adult Human Visual Cortex

Astrid Rosenstand Lou1,2,3, Kristoffer Hougaard Madsen1,4, Olaf Bjarne Paulson1,5, Hanne Olsen Julian2, Jan Ulrik Prause6, Hartwig Roman Siebner1 and Troels Wesenberg Kjaer7

Materials and Methods

Participants
Nineteen participants (13 women, median age 23.2 years, range 19–27 years) took part in the study. All participants were healthy without any known disease of the eye or brain. All participants had normal vision (self-reported) without correction. They were all right eye dominant. All patients gave their informed verbal and written consent. The study was performed in accordance with the Declaration of Helsinki (1964) and approved by the ethical committee of the Capital Region of Copenhagen (Copenhagen and Frederiksberg Municipalities; KF-01-131/03).

Introduction

Monocular visual deprivation (MD)-induced shift in ocular dominance (OD) is a classical example of experience-dependent cortical plasticity (Wiesel and Hubel 1963). This effect is most prominent during a short critical period immediately after birth. Yet, recent animal studies have shown that OD plasticity can also be induced later in life by a 5 to 7-day period of MD (He et al. 2006; Chen and Bear 2007; Fischer et al. 2007; Sato and Stryker 2008). In adult animals, 2 phases of OD plasticity have been identified in the visual cortex: An initial rapid homosynaptic long-term depression (LTD) which produces a weakening of the responses evoked through inputs from the deprived eye (Heynen et al. 2003; Smith et al. 2009). Three days or more after the onset of MD (Kirkwood et al. 1996) and strengthens the cortical response from the nondeprived eye, which is thought to drive the shift in OD (Smith et al. 2009).

Unilateral impairment of vision is commonly encountered in clinical ophthalmology. This includes diseases affecting one eye, pregeniculate lesions of the optic nerve, as well as protective patching of one eye as part of a therapeutic intervention (Doshi and Rodriguez 2007). Therefore, research that sheds light on plastic changes in the adult human visual cortex following MD is not only of basic neuroscientific interest but also of substantial clinical relevance. In humans, cortical plasticity can be studied noninvasively with transcranial magnetic stimulation (TMS) (Pascual-Leone et al. 2005). Inspired by the work on visual cortical plasticity in adult animals, we used TMS in the present study to examine how MD of the right dominant eye alters the excitability of visual cortex in the adult human brain. We focused on the initial phase of deprivation-induced plasticity which is primarily driven by the loss of afferent input from the blindfolded eye (Antonini and Stryker 1993). Taking into account the duration of this initial phase in rodents (Smith et al. 2009), we limited the period of visual deprivation to 2 days. Before, during, and after eye patching, we applied paired-pulse TMS to the right and left occipital cortex at different stimulus intensities to evoke phosphenes. Stimulus–response curves were constructed which describe the relationship between the intensity of TMS (i.e., stimulus) and the intensity of TMS-induced phosphenes (i.e., response). For each stimulus–response curve, we estimated the TMS intensity which induced phosphenes that were perceived by the participants as having half of the maximal phosphene intensity (stimulus intensity, SI50%). The SI50% was used as a psychophysical marker for experience-dependent changes in visual cortex excitability.
eye. The control experiment involved the same measurements at identical schedule but without any manipulation of the normal visual input (Fig. 1). The order of experimental conditions was counterbalanced across subjects. The time line of the experimental procedures is illustrated in Figure 1. Each experiment began with an eye dominance test, to ensure that all participants were right eye dominant. This was followed by paired-pulse TMS of the visual cortex to determine the baseline level of visual cortex excitability. In the interventional session, the right eye was completely covered by a nontransparent patch for a period of 48 h. During this period, repeated TMS measurements of phosphene induction were done to trace changes in visual cortex excitability as a consequence of MD (Fig. 1). After removal of the patch the eye dominance test was repeated and additional TMS measurements of phosphene induction were done during the next 3 h. Immediately after the end of MD, functional magnetic resonance imaging (fMRI) of the visual system was performed using alternating right and left half-field checkerboard stimulation. The results of the fMRI measurements will be reported separately.

Eye Dominance Test

Eye dominance was determined for each participant using a variant of the Porta test (Wade 1998). Participants were asked to extend both arms forward and form a ring with both first and second fingers. Both eyes were open initially. Then they had to fixate on an object within the ring and close each eye alternately. Participants reported which eye closure caused the largest alignment change. This eye was recorded as the dominant eye.

Phosphene Measurements

Phosphenes were elicited with double-pulse TMS in a semidark room without windows. Participants were blindfolded and lights were turned off, except for a dim orientation light. Illumination conditions were kept constant within and across experimental sessions. In the intervals between the experimental sessions, the light was turned on. Double-pulse TMS was delivered every 10 s (range: 8-12 s) through a double 70-mm coil connected to a Magstim Rapid stimulator with maximal field strength of 2.2 T (Magstim Co. Ltd.). The biphasic stimulator had the same intensity and were separated by an interval of 50 ms. Paired-pulse TMS was used to increase the efficacy of TMS to induce phosphenes. The specific paired-pulse protocol was chosen on the basis of previous experiments (Boroojerdi et al. 2001; Brigihina et al. 2002). TMS intensity is expressed as percentage of maximal stimulator output (MSO).

At the beginning of each experiment, we determined the optimal site for evoking phosphenes over the left and right occipital cortex. The center of the TMS coil was initially placed tangentially on the scalp 3 cm rostral and 2 cm lateral from the inion on each hemisphere (Gothe et al. 2002). The handle of the 8-shaped coil pointed laterally. During the first phase of the biphasic stimulus, the current in the center of the coil flowed toward the handle. Stimulus intensity was initially set at 65% of MSO. If TMS only evoked weak or no reported phosphenes, stimulus intensity was increased in steps of 5% until reported phosphenes of medium intensity were reliably evoked by paired-pulse TMS. The coil was then moved slightly in all directions to identify the stimulation site where double-pulse TMS elicited the strongest reported phosphene. These coil positions were marked on the left and the right occipital cortex, with a wax pen on the participants scalp to ensure constant stimulation condition during the experiment.

The ability of TMS to elicit phosphenes was repeatedly assessed at baseline, 45, 90, and 135 min as well as 24 and 48 h after onset of MD and 45, 90, 135, and 180 min after the end of MD (Figs 1 and 2). At each time point, TMS was given at a range of intensities (30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, and 80% of MSO) over the left and the right occipital cortex. At each intensity level, 10 double pulses were consecutively applied every 4-6 s. Participants were asked to report the occurrence and intensity of TMS-induced phosphenes. The reported intensity of the phosphenes was a combination of brightness and size. Double-pulse TMS started at an intensity of 65% of MSO. In half of the participants, the intensity was first gradually reduced in steps of 5% until the participants were unable to detect phosphenes in 10 of 10 trials. Thereafter, TMS intensity was set to 60% of MSO and increased in 5% steps until the participant reported maximal phosphenes in 10 of 10 trials. In the other half of the participants, ascending stimulation preceded descending stimulation. The order of descending and ascending measurements was counterbalanced across subjects while keeping the order constant within each subject to avoid a systematic bias in phosphene estimation.

For each intensity level and hemisphere, participants scored the occurrence and intensity of the perceived phosphene after each of the 10 double-pulse stimuli. When they experienced no phosphene, they gave a score of 0. If they noticed a phosphene, a score of 1 was given for a phosphene of weak intensity, a score of 2 for a phosphene of medium intensity, and a score of 3 for an induced phosphene of strong intensity. The minimal total score for a given stimulus intensity was zero indicating that none of the 10 double-pulse stimuli elicited a phosphene. A maximal score of 30 could be reached when all 10 double-pulse stimuli evoked phosphenes of strong intensity.

Participants were also required to describe in detail the shape of the reported phosphene and its location in the visual field. At each intensity level, participants were asked to draw the location and shape of the strongest reported phosphene that had been induced by 1 of the 10 double pulses on a sheet of paper with a schematic representation of the entire visual field (Fig. 2A). To test the correctness of the participants, we introduced occasionally catch trials (approximately every 10th trial) by shifting the coil 3 cm laterally and 4-cm cranial from the optimal stimulation site and applying double-pulse TMS over this ineffective control site.

Data Analysis

The perception of phosphenes varied across individuals, especially the ability to perceive phosphenes and to describe their intensity. This variability may in part be due to interindividual variations in the topography and morphology of the stimulated cortical surface (Meyer et al. 1991; Marg and Rudiak 1994; Ray et al. 1998). Some subjects found it particularly difficult to decide whether they perceived a weak phosphene or not when TMS was given at stimulus intensities close to phosphene threshold. This prompted us to use a measure of cortical excitability that took into account the whole stimulus-response curve of the reported phosphene measurements instead of estimating the phosphene threshold. For each TMS measurement, individual phosphene scores were plotted as a function of TMS intensity (Fig. 2A). Based on the stimulus-response curve, we then calculated the TMS intensity that was required to produce a phosphene score of 15, which corresponds to 50% of the maximal possible phosphene score (Fig. 2B). We refer to this TMS intensity as SI50% throughout the manuscript.

![Figure 1. Experimental design. All participants underwent 2 experiments in a counterbalanced order, an interventional experiment that involved MD and a control experiment. In the interventional experiment, the right dominant eye was visually deprived for 48 h. The ability of TMS to evoke phosphenes was assessed before, during, and after MD. Double-pulse TMS was applied to the left and right occipital cortex at a range of stimulus intensities and the consistency and intensity of TMS-evoked phosphenes were scored by the subjects at each TMS intensity level. OD was tested before and immediately after patching. After 48 h an fMRI session was performed. Details of methods and results are described in another context. The control experiment involved an identical time line of measurements but without any manipulation of the normal visual input.](image)
MD, SI50% increases, indicating a reduction in cortical excitability. After removal of the patch SI50% returns to preinterventional baseline.

To enable evaluation of the score as a continuous function of TMS intensity a generalized logistic function (Richards 1959) was fitted to the TMS response data.

\[
x(p) = \frac{S_{\text{max}}}{1 + Qe^{-R(p-M)}} + B,
\]

where \( x \) is the score as a function of the TMS power \( p \), \( S_{\text{max}} \) is the maximum score obtainable (30), and \( Q, R, M \), and \( B \) are parameters to be determined by standard nonlinear least squares fitting. In order to ensure that the reported score was a nondecreasing function of the applied TMS intensity, an isotonic regression was applied to the data prior to fitting the model. The resulting function allowed us to determine the threshold for inducing phosphenes increases during 48 h of MD (measurements between the 2 horizontal bars) along with a reduction in phosphene size and brightness. Restoration of normal vision reverses these changes within 3 h. (B) The right panel gives the corresponding stimulus–response curves of the same participant. The sum of phosphene scores ranging from 0 to 30 (y-axis) is plotted, as a function of TMS intensity, ranging from 30% to 80% of maximal stimulation output (x-axis). The green line indicates the TMS intensity required to reach a phosphene score of 15, which corresponds to 50% of the maximally possible phosphene score. This TMS intensity is termed SI50%. During MD, SI50% increases, indicating a reduction in cortical excitability. After removal of the patch SI50% returns to preinterventional baseline.

**Results**

All participants tolerated the MD and TMS procedures without major side effects. However, all participants complained about double vision immediately after removal of the patch. The Porta test revealed that 3 participants had changed to left eye dominance after MD. All participants had right eye dominance without double vision within a few hours after removal of the patch in 2 of the 3 subjects, while the exception of one participant where the dominance shift persisted for approximately 36 h. In all participants, it was possible to elicit phosphenes with paired-pulse TMS of the occipital cortex. The location of origin of the phosphenes was always in the periphery of the visual field contralateral to the stimulation site. At high intensity of TMS, participants consistently reported an expansion of the induced phosphene, including the central part of the visual field and sometimes even a small part of the ipsilateral visual field. The brightness also gradually increased with TMS intensity (Fig. 2 A). Interspersed stimulation over a control site never produced phosphenes.

**Change in Phosphene Induction with Monocular Deprivation**

Measurements of the stimulus–response curve revealed that MD altered the perception of TMS-induced phosphenes. Relative to preinterventional baseline, a 48-h period of visual deprivation of the right eye induced a rightward shift of the stimulus–response curve, indicating a reduction in visual cortex excitability (Fig. 3). The rightward shift in the stimulus–response curve after MD was present in the right and left occipital cortex.

The change in visual cortex excitability induced by MD was formally assessed using the SI50% as dependent variable. The group data of SI50% are illustrated in Figure 4. Monocular visual deprivation of the right eye produced a consistent increase in SI50% during the 48-h period of MD, which gradually returned to preinterventional baseline levels during the 3-h period after the end of MD. Although the deprivation-induced increase in SI50% was stronger when phosphenes were elicited over the...
left visual cortex contralateral to the deprived eye (Fig. 4A), a consistent increase in SI50% with MD of the right eye was also evident when phosphenes were elicited with TMS over the ipsilateral right visual cortex (Fig. 4B). There was also a slight nonsignificant increase in SI50% in the control experiment without MD, but the increase in SI50% was consistently greater with MD (Fig. 4C,D). Accordingly, repeated measures ANOVA showed a main effect of TIME ($F_{2.25,33.69}=12.211, P<0.001$) and an interaction between INTERVENTION and TIME ($F_{2.18,32.74}=6.527, P=0.001$) due to a stronger increase in SI50% with MD as opposed to the control condition. The ANOVA provided no evidence for an asymmetrical effect of MD on SI50% in the right and left visual cortex. There was no significant main effect of HEMISPHERE and no interaction between HEMISPHERE and the other experimental factors (INTERVENTION or TIME). Separate repeated measures analyses of variance for the intervention and control group revealed a significant effect of TIME in the intervention group ($F_{1.98,29.75}=11.80, P<0.001$) and no evidence for the effect of TIME in the control group ($F_{1.10,32.65}=1.19, P=0.318$), indicating that differences in phosphenic induction over time were driven by the intervention.

**Discussion**

To our best knowledge, our results provide first-time evidence for a rapid bilateral decrease in cortical excitability of the adult human visual cortex during a 48-h period of MD of the right dominant eye.

The 2 Phases of Ocular Dominance Plasticity

The response pattern is in good agreement with the first phase of the OD plasticity in adult animals. This early phase of OD plasticity is dominated by LTD and increased gamma-aminobutyric acid (GABA)ergic inhibition (He et al. 2006; Sato and Stryker 2008) and weakens the neuronal responses evoked through inputs from the deprived eye during the first 3–5 days of deprivation (Heynen et al. 2003; Smith et al. 2009). We hypothesize that MD resulted in a patchy decrease in excitability in those parts of the right and left visual cortex that are normally processing the input from the deprived eye due to deprivation-induced LTD. This in turn reduced the efficacy of double-pulse TMS to induce phosphenes during MD.

As outlined in the introduction, animal work has shown that the early phase of OD plasticity is followed by a second phase characterized by a dominant role of LTP and an increase in glutamate levels. The second phase strengthens the synaptic responses evoked through inputs from the nondeprived eye (Kirkwood et al. 1996). Therefore, one might expect that the decrease in visual cortex excitability during the first 2 days of MD might be attenuated or even be reversed, if the period of MD is prolonged. In the present experiment, the decrease in visual cortex excitability was stable across the 48 h of MD. Three participants showed a reversible shift in OD which might indicate that these subjects were entering the second phase of the bidirectional plasticity with increased glutamergic activity. However, these 3 individuals still showed suppressed cortical excitability just as the other participants. Future experiments need to monitor visual cortex excitability with TMS over longer periods to specifically test whether MD induces a biphasic plasticity pattern in human visual cortex that in analogy to the animal studies, shows an initial decrease and a subsequent increase in visual cortex excitability.

**Monocular Versus Binocular Deprivation**

The finding that MD produces an acute decrease in visual cortex excitability is in sharp contrast to previous TMS studies.
on the consequences of binocular deprivation (BD). These studies consistently reported an increase in visual cortical excitability during the first 3 h of BD (Boroojerdi, Bushara, et al. 2000; Pitskel et al. 2007). Pitskel et al. (2007) studied the cortical excitability during a longer period of BD. They found that the initial increase of cortical excitability was followed by a slow progressive decrease which became significant after 5 days of blindfolding (Boroojerdi, Bushara, et al. 2000; Pitskel et al. 2007). We speculate that differential effects on the intracortical balance between GABA-mediated inhibition favoring LTD and glutamergic activity promoting LTP might explain the differential effects of MD and BD on visual cortex excitability. In contrast to MD, BD may acutely reduce GABAergic inhibition (Rosier et al. 1995) and thereby gate the induction of LTP (Massie et al. 2003). The different effects of MD and BD must be ascribed to the partially preserved visual input in MD, which reaches both visual cortices from the nondeprived eye. The striking difference in the response pattern of the visual cortex to MD and BD indicate that there is no stereotypic response of the visual cortex to visual deprivation. The available evidence rather suggests that the plastic changes that can be induced in the visual cortex by visual deprivation critically depend on the pattern of visual deprivation. Here, studying these differential responses of the visual cortex with TMS can provide valuable insights into the adaptive properties of the human visual cortex in vivo.

**Possible Effects of Sleep**

When comparing monocular versus BD it has to be born in mind that the 48-h period of MD also included 2 nights in which the visual input was totally disrupted during normal sleep. This raises the question whether the normal sleep-related modulation of visual input might have interacted with the MD effects. Of note, the decrease in visual cortex excitability developed rapidly within a few hours after onset of MD and was already present before the first night of sleep. Therefore, we argue that the MD effect on visual cortex excitability was not sleep dependent. Moreover, sleep does not appear to counteract the suppressive effect of MD on visual cortex excitability because phosphene measurements performed 24 and 28 h after MD onset showed a stable reduction in visual cortex excitability. Apart from causing a disruption of visual input, a growing body of research has provided evidence that sleep-specific cortical states (e.g., slow-wave activity) are associated with synaptic downscaling (Tononi and Cirelli 2003; Liu et al. 2010). Therefore, sleep might play an active role, shaping MD-induced plasticity in visual cortex. Our study was not designed to tackle this interesting question. Future experiments which manipulate the amount of sleep during a period of MD and assess a circadian profile of MD-induced changes in phosphene threshold might help to answer how much MD-induced plasticity is shaped by sleep.
Symmetrical Changes in Visual Cortex Excitability

Although the observed changes tended to be more consistent in the left hemisphere contralateral to MD, there was no statistical difference between the 2 hemispheres regarding the perception of TMS-induced phosphenes. The slightly more pronounced effect in the hemisphere contralateral to MD can be explained by the fact that the density of ganglion cells in the nasal part of retina is 1.5-3 times greater than in the temporal part, and the ratio of crossed to uncrossed fibers in chiasm is 3:2 (Connolly and Van 1984; Toosy et al. 2001). Likewise, earlier visual evoked potential studies of MD have also shown less excitability of the contralateral visual cortex compared with the ipsilateral side (Tyler and Kaitz 1977; Mitzdorf and Singer 1980; Sclar et al. 1986). However, these moderate asymmetries within the afferent visual pathway did not result in a hemispherical asymmetry of MD-related plasticity. On the contrary, MD induced a consistent reduction in visual cortex excitability in both hemispheres.

Cortical Plasticity Induced by Unilateral Sensorimotor Deprivation

In recent years, TMS has been widely used to study cortical plasticity of the motor system by measuring the motor evoked potentials (MEPs) (Siebner and Rothwell 2003; Leocani and Comi 2006). Repeated recordings of the MEP have revealed that unilateral immobilization of the upper limb also results in a consistent decrease of corticospinal excitability (Zanette et al. 2004; Granert et al. 2011). In contrast to the effects of MD, unilateral limb immobilization causes an asymmetric suppression of motor cortex excitability which is limited to the contralateral hemisphere. If any, the motor cortex ipsilateral to immobilization SM1 displays an opposite response pattern showing an increase in excitability (Zanette et al. 2004). This difference in the asymmetry of deprivation-induced plasticity between the visual and sensorimotor system can be readily explained by the strict lateralization of the somatosensory input and motor output (Liepert et al. 1995), while the visual input from one eye project into the visual cortex bilaterally. We conclude that TMS can be used to trace the consequences of unilateral sensory deprivation in different sensory modalities and that the manifestation depends on the neuroanatomical architecture of the deprived sensory system.

Methodological Considerations

Participants also showed a slight but nonsignificant decrease in excitability in the control condition without MD during serial phosphen measurements. It is possible that the repeated application of double-pulse TMS at a very low frequency might on its own have a moderate effect on visual cortex excitability. Repetitive TMS (rTMS) at frequencies of 1 Hz or more is a well-established tool to modulate cortical excitability (Pascual-Leone et al. 1994; Siebner and Rothwell 2003). Low-frequency rTMS of the occipital cortex has been shown to increase the phosphenes in healthy subjects (Boroojerdi, Prager, et al. 2000; Brighina et al. 2002). Although the repetition rate of double-pulse TMS was far below 1 Hz, a conditioning effect of double-pulse TMS cannot be excluded. Another possible explanation for the nonspecific increase in $S_{50\%}$ in the control condition is an increased familiarity to the procedure lowering the subject's attention to report phosphenes over time. Whatever the explanation, the change in phosphen perception in the MD condition was significantly larger than in the control condition without MD, confirming that MD had a specific effect on visual cortex excitability over and above the nonspecific changes found in the control condition. Another factor which might have influenced MD-induced changes of visual cortex excitability in female participants is the menstrual phase (Smith et al. 1999; Inghilleri et al. 2004; Chehab et al. 2007). We did not assess the menstrual phase in our female participants. We did not assess the menstrual phase in our female participants. However, under the assumption that the menstrual phase was random across the female participants this should not produce any systematic effects on the observed changes in visual cortex excitability.

Clinical Implications

Our demonstration of a rapid and robust decrease in cortical excitability in unilateral visual impairment may have important clinical implications. Unilateral visual deprivation is a common clinical condition and more knowledge is needed about the functional consequences of MD in terms of central visual processing. The deprivation-induced change in visual cortex excitability may adversely affect sensory or cognitive function. On the other hand, TMS studies of MD-induced visual cortex plasticity might also be useful to study the pathophysiology of neurological disorders that are characterized by abnormal cortical excitability, such as migraine (Antal et al. 2008; Palermo et al. 2009), epilepsy (Brodbeck et al. 2010), and photosensitivity (Siniatchkin, Groppa, et al. 2007; Siniatchkin, Moeller, et al. 2007). It is conceivable that the plastic changes in response to MD might deviate from the normal response pattern found in healthy subjects, showing a less prominent or absent suppression of cortical excitability.

Funding

Rigshospitalets Research Foundation; Danish Eye Health Society; P.A. Messerschmidt and Hustru Foundation; Director Jacob Madsen and Hustru Olga Madsen Foundation. H.R.S. was supported by a Grant of Excellence “ContAct” from Lundbeckfonden (R59 A5399).

Notes

The Elsass Foundation is acknowledged for the donation of the Magstim Rapid Transcranial Magnetic Stimulator. Conflict of Interest: None declared.

References


