

Transcorneal alternating current stimulation induces EEG “aftereffects” only in rats with an intact visual system but not after severe optic nerve damage

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Sergeeva EG, Fedorov AB, Henrich-Noack P, Sabel BA. Transcorneal alternating current stimulation induces EEG “aftereffects” only in rats with an intact visual system but not after severe optic nerve damage. *J Neurophysiol* 108: 2494–2500, 2012. First published August 8, 2012; doi:10.1152/jn.00341.2012.—Noninvasive alternating current stimulation can induce vision restoration in patients with chronic optic nerve damage and results in electroencephalogram (EEG) aftereffects. To better understand the mechanisms of action, we studied such EEG “aftereffects” of transcorneal alternating current stimulation (tACS) at the chronic posttraumatic state in rats. EEG baseline was recorded from visual cortex under ketamine/xyzazine narcosis of healthy rats and rats with chronic severe optic nerve crush. One week later, both groups were again anesthetized and stimulated transcorneally twice for 12 min each time. tACS-induced changes were compared with baseline EEG. Over the course of 65 min narcosis baseline EEG revealed a shift from a dominant delta power to theta. This shift was significantly delayed in lesioned animals compared with healthy controls. tACS applied during the late narcosis stage in normal rats led to significantly increased theta power with a parallel shift of the dominating peak to higher frequency which outlasted the stimulation period by 15 min (aftereffects). EEG in lesioned rats was not significantly changed. In rodents, tACS can induce neuroplasticity as shown by EEG aftereffects that outlast the stimulation period. But this requires a minimal level of brain activation because aftereffects are not seen when tACS is applied during deep anesthesia and not when applied to animals after severe optic nerve damage. We conclude that tACS is only effective to induce cortical plasticity when the retina can be excited.

optic nerve damage; noninvasive current stimulation; EEG; anesthesia; vision restoration

NONINVASIVE CURRENT STIMULATION is emerging as a new tool to manipulate central nervous system plasticity and repair (Antal et al. 2011; Chrysikou and Hamilton 2011; Coslett and Hamilton 2011; Nair et al. 2011; Nitsche and Paulus 2011; Oliveri 2011; Olma et al. 2011; Rogers et al. 2011; Sabel et al. 2011a; Song et al. 2011; Zaghi et al. 2011). Transcranial direct current stimulation, for example, enhances excitability in motor cortex (Chaieb et al. 2011; Nitsche and Paulus 2000) and improves motor performance in stroke patients (Reis and Fritsch 2011). In the visual system, alternating currents enhance excitability, which is seen by lowered phosphene thresholds in normal subjects (Antal et al. 2003; Chaieb et al. 2008; Kanai et al. 2008), and after visual system damage, noninvasive alternating current stimulation has therapeutic effects. Fujikado et al.

(2006) found improvement of visual acuity after transcorneal electrical stimulation in patients with non-arteritic ischemic optic neuropathy or traumatic optic neuropathy, and Oono et al. (2011) reported improved visual function in eyes with branch retinal artery occlusion mainly in chronic cases. Most recently, in our own laboratory we have induced recovery of vision by applying noninvasive repetitive transorbital alternating current stimulation (tACS) to patients with optic nerve damage. This was documented by a single case study by Gall et al. (2010), a retrograde data analysis of 466 patients (Fedorov et al. 2011), and in a prospective randomized controlled clinical trial (Gall et al. 2011; Sabel et al. 2011a). Here, optic neuropathy patients with up to 6-year-old lesions were treated with 10 days of tACS, which led to increased detection ability in perimetric testing. It was proposed that visual field improvements were mediated by increased neuronal synchronization of residual visual system structures and higher cortical areas (Fedorov et al. 2010; Sabel et al. 2011a). This is in line with the proposal by others that neuroplasticity mechanisms, like long-term potentiation and depression, may underlie excitability shifts in residual structures (Paulus 2004), and this may lead to restoration of neuronal networks (Fregni and Pascual-Leone 2007; Hummel and Cohen 2006).

The study of cellular mechanisms of noninvasive brain current stimulation is, however, not possible in human subjects, but, rather, animal studies are needed. Despite the high clinical relevance, there are only very few such preclinical studies, and these experiments were focused on neuroprotective actions only in the acute and not the chronic lesion stage (Morimoto et al. 2005, 2007, 2010; Ni et al. 2009). Also, these studies did not address the issue of synaptic plasticity in visual cortex or downstream pathways. Their main findings were enhanced survival of axotomized retinal ganglion cells under transcorneal electrical stimulation conditions (Morimoto et al. 2005, 2007, 2010; Ni et al. 2009), but behavioral and electrophysiological effects have not been studied. Thus, it is unknown if enhanced cell survival provides a realistic basis for functional benefit, particularly considering that (visual) functions can improve despite the number of ganglion cells declining (Sabel et al. 1997; Eysel et al. 1999).

We therefore studied tACS in rats with optic nerve crush (ONC) damage, which is a model of visual system recovery (Sabel 1999; Sabel and Aschoff 1993; Sabel et al. 1997; Sautter and Sabel 1993; Sautter et al. 1991). Considering that the tACS effects in clinical studies were elicited at the chronic stage (Fedorov et al. 2010, 2011; Gall et al. 2011; Sabel et al. 2011a), we have now studied mechanisms of action in rats with

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chronic ONC. Particularly in view of the observation in humans that noninvasive current stimulation can induce cortical excitability changes and enhance synchronization as indicated by EEG "aftereffects," in our animal study we were interested in possible cortical electrophysiology changes after tACS.

Because brain current stimulation effects are state dependent, tACS effects may be reduced under anesthesia (Gersner et al. 2011; Rennaker et al. 2007). We correlated the electrophysiological effects obtained by EEG with the stage of narcosis. In addition, we recorded EEG in fully sighted (normal) and chronically lesioned rats after ONC that were treated with tACS under anesthesia to determine if electrophysiological activity of the visual system is required for tACS to unfold its effects.

MATERIALS AND METHODS

Adult Lister hooded rats were kept on a 12-h light-12-h dark cycle at an ambient temperature of 24–26°C and humidity of 50–60%. Food and water access was ad libitum. The lesioned animals were morphologically and functionally blind as revealed by *in vivo* confocal neuroimaging of retina and behavioral testing (P. Henrich-Noack, S. Lazik, E. Sergeeva, S. Wagner, N. Voigt, S. Prilloff, A. Fedorov, B.A. Sabel, unpublished observations). All procedures conformed to the requirements of the German National Act on the use of Experimental Animals.

Surgery

Bilateral ONC was performed in ten 8-wk-old rats as described (Sautter et al. 1991; Sautter and Sabel 1993). Under anesthesia with ketamine (75 mg/kg ip) and xylazine (10 mg/kg ip), a lateral canthotomy was made and the optic nerve exposed by blunt dissection. To induce an ONC, a calibrated forceps (Martin Instruments, Tuttlingen, Germany) was used for 30 s at a distance of 2–3 mm from the eye with the jaws of the forceps 0.1 mm apart (the "lesioned" group).

Six months later, these rats and 10 unlesioned animals of comparable age were anesthetized (ketamine/xylazine), and two stainless steel screws with a shaft diameter of 1.17 mm (Fine Science Tools, Heidelberg, Germany) were stereotaxically inserted into the skull without piercing the dura. They were implanted bilaterally over the visual cortex 7 mm posterior to bregma and 2 mm lateral from midline and served as measuring electrodes. Around the screws a plastic ring was placed to prevent the skin from covering the assembly, which was fixed with dental cement. Two stainless steel surgical clamps were used as reference, and neutral electrodes and were attached to the ears and the skin over the nose bone, respectively.

tACS Protocol

Under anesthesia with ketamine/xylazine, a ring-shaped gold electrode for transcorneal electrical stimulation was placed on the eye (Roland Consult, Brandenburg, Germany) with Vidisic optical gel (M. Pharma, Berlin, Germany) beneath for protection and better conductance. The reference electrode was fixed on the ear. The electrical stimuli were generated by an A-M Systems 2100 stimulator. The stimuli consisted of biphasic square-wave pulses (pulse duration: 1 ms/phase, intensity: 100 μ A) with changing frequency. The stimulation protocol was based on clinical experience where a similarly modulated frequency schedule was used in patients with optic nerve damage.

We used a total of 12 min of tACS of 30-s trains with 10-s breaks in between each train. The frequency of each such train was altered in the following order: 10, 12, 9, 11, 8, 10, 9, 12, followed by a 2-min break and another series in the same order.

EEG Recording

Baseline EEG recording. To investigate the dynamics of bioelectrical brain activity under narcosis in naïve and lesioned animals, rats were anesthetized with ketamine/xylazine intraperitoneally. Active electrodes were connected with implanted screws, reference electrodes were placed on the ears, and neutral electrode was placed on the nose bone. Ten minutes after injection of the anesthetic, EEG recordings were started under normal room light conditions and continued up to awakening (usually ~65 min) using electroencephalograph Encephalan-131-03, modification 9, by Medicom MTD (Russia).

To address the question of whether the lack of functional visual input alone influences the dynamic pattern of bioelectrical activity under anesthesia, we carried out the same EEG recording in five unlesioned rats with eyes closed. Eyes were covered with non-transparent spectacles fixed onto the head.

tACS and EEG recording. One week later, tACS was applied to the same animals at 18 and 36 min after narcosis induction. Again, EEG recordings commenced from 10 min after ketamine/xylazine injection.

To align the data properly, the schedule of handling and data acquisition was identical in both groups and at both the day of baseline assessment and the day of stimulation.

Statistical Analysis

EEG files were recorded with sampling rate 256/s and processed in Vision Analyser software by Brain Products. Automatic artefacts detection, low-pass filter 30 Hz, and high-pass filter 0.25 Hz were used. Then, fast Fourier transform was performed for every 4-s segment with frequency pitch 0.25 Hz. The data were statistically analyzed by PASW Statistics 18.0 (SPSS). To describe the dynamics of bioelectrical activity under narcosis in normal and lesioned animals, averaged EEG power spectral densities were calculated for every 5-min segment starting 10 min after narcosis administration up to the time of awakening and compared with Mann-Whitney U-test. For comparison of EEG with tACS and baseline EEG recording in normal and lesioned rats, the EEG power bands of the following segments of recording were taken: before tACS (PRE), after first stimulation (POST1), after second stimulation (POST2), and at three further data collection points (FU1, FU2, FU3; follow-ups) until awakening (see Fig. 3). Power spectral densities were compared with multiple comparisons with the Kruskal-Wallis test and post hoc Mann-Whitney U-test.

RESULTS

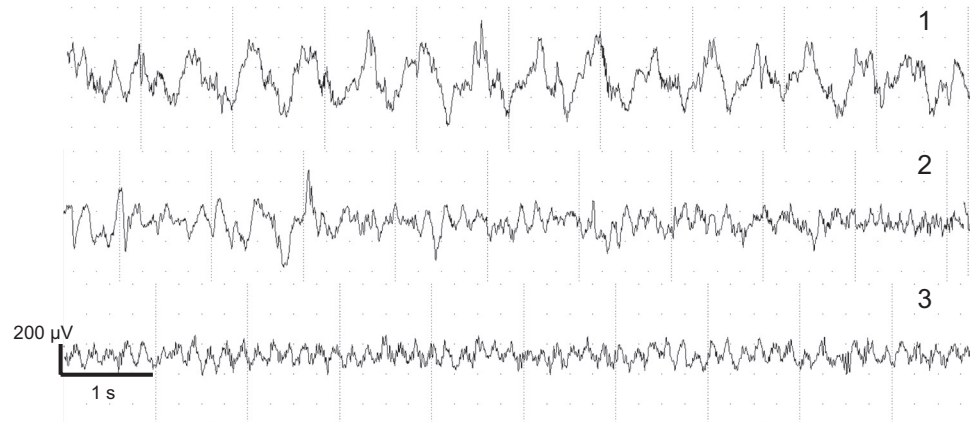
EEG Dynamics Under Ketamine/Xylazine Anesthesia in Normal and Lesioned Rats

Intraperitoneal ketamine/xylazine anesthesia caused extinction of tail and hind paw pinch withdrawal reflex within 4–5 min. During the following 60–65 min, the predominantly slow EEG oscillations changed gradually into faster waves (Fig. 1). In typical EEG recordings, three narcosis stages were seen: 1) slow wave sleep, with delta band prevailing, 2) transition period, and 3) theta stage (Fig. 1).

Fast Fourier transform allowed retracing the dynamic of these changes and separating them according to the distinct ranges of standard bands. Figure 2 demonstrates the changes of the dominant power bands over the narcosis period within delta (1.25–2 Hz) and theta (5.25–6.25) bands.

Both in unlesioned and lesioned animals, the EEG pattern during anesthesia was characterized by a distinct decrease of the delta band (Fig. 2A) that was paralleled by an increase of theta (Fig. 2B). This allowed us to define the transition period,

Fig. 1. Typical electroencephalograms (EEGs) recorded from right occipital cortex in rat under ketamine/xylazine anesthesia: slow wave stage (1), transition period (2), theta stage (3).



i.e., the time of theta power exceeding delta, which was observed typically between 35 and 60 min after anesthesia induction. There was a significant difference between normal controls and post-ONC rats regarding this transition period: in ONC rats it was shifted to a later time point compared with the normal control rats.

In normal rats with eyes closed, we found that the temporal dynamics of changes in power bands during narcosis was close to what was seen in normal control rats with open eyes. In the specified transition period, the power of theta was therefore also significantly lower in lesioned compared with unlesioned rats, irrespective if eyes were open or closed. Thus, the EEG transition point shift was a result of the lesion and not caused by the absence of functional visual input.

EEG Response After tACS in Normal and Lesioned Rats

To understand how and if tACS affects brain activity, we compared different EEG segments before and after tACS with exactly the same segments and in the same animals that were collected during baseline (unstimulated) EEG recording performed 1 wk earlier. This comparison of segments was necessary to be able to compare the dynamics of EEG power spectral density in the different narcosis stages. Six segments were included in our analysis: 1) PRE: a 3-min baseline recorded 10 min

after injection, 2) POST1: a 2-min EEG segment recorded immediately upon terminating the first 12 min of tACS, 3) POST2: a 2-min EEG segment recorded immediately upon terminating the second 12 min of tACS, and 4) FU1–3: three follow-up recordings (FU1, FU2, FU3) for 5 min each at 50–65 min after anesthesia induction (Fig. 3).

In general, the replacement of slow wave sleep (delta activity) by theta activity across the narcosis stages as seen during baseline recording was also revealed in the experiment with tACS treatment. Also, the delayed transition point in lesioned rats compared with control rats, which was detected during the baseline EEG recordings, was found under stimulation condition (Fig. 3, POST2).

After the first tACS was completed, i.e., at 18–30 min after injection of ketamine/xylazine, no differences were noted in EEG power bands between the PRE and POST1 session within the groups, and this also did not differ between the two groups (control vs. lesioned) at these two data collection points. The delta peak prevailed in both groups and at both time points (Fig. 3, POST1). The second tACS was applied 36–48 min into narcosis, which coincided with the transition period in the EEG. At POST2, the control rats and lesioned rats had different patterns in the EEG power spectra: the theta band power was higher in normal animals compared with lesioned animals, which still showed significant delta band power (Fig. 3, POST2).

During the follow-up sessions, a significant increase in theta band power was seen in normal tACS-treated animals compared with baseline EEG values (Fig. 3, FU1, FU2, FU3).

Figure 4 displays the power of four separate frequencies within the theta band (4, 5, 6, 7 Hz) for the period 48–65 min into narcosis. This is the poststimulation period after the second tACS application in the stimulation experiment and the corresponding period in baseline EEG experiment. A Kruskal-Wallis test revealed highly reliable differences between all analyzed groups (Baseline EEG Normal, Baseline EEG Lesioned, tACS Normal, tACS Lesioned): for 4 Hz – $\chi^2 = 50.9$; Asymp. sign. = 5.1×10^{-11} ; for 5 Hz – $\chi^2 = 8.8$; Asymp. sign. = 0.032; for 6 Hz – $\chi^2 = 36.9$; Asymp. sign. = 4.9×10^{-8} ; for 7 Hz – $\chi^2 = 32.1$; Asymp. sign. = 4.9×10^{-7} . The theta band power in the baseline EEG recording was almost the same in normal and lesioned animals (only 5 Hz power significantly prevailed in unlesioned; Fig. 4, Baseline), and this pattern of power spectra was not significantly different in the stimulation experiment after the second tACS was applied in

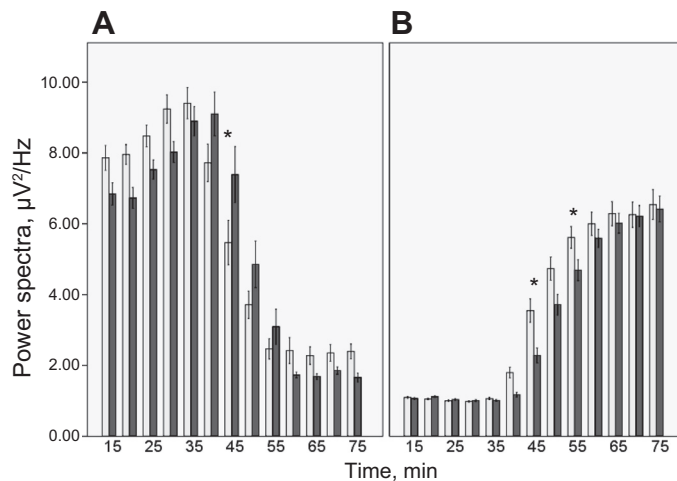


Fig. 2. Temporal dynamics of EEG power spectral density in delta (A) and theta (B) bands under ketamine/xylazine anesthesia from intraperitoneal application to awakening in normal (pale gray bars) and lesioned rats (dark gray bars). Significances with Mann-Whitney U-test, $*P < 0.05$. Error bars: means \pm SE.

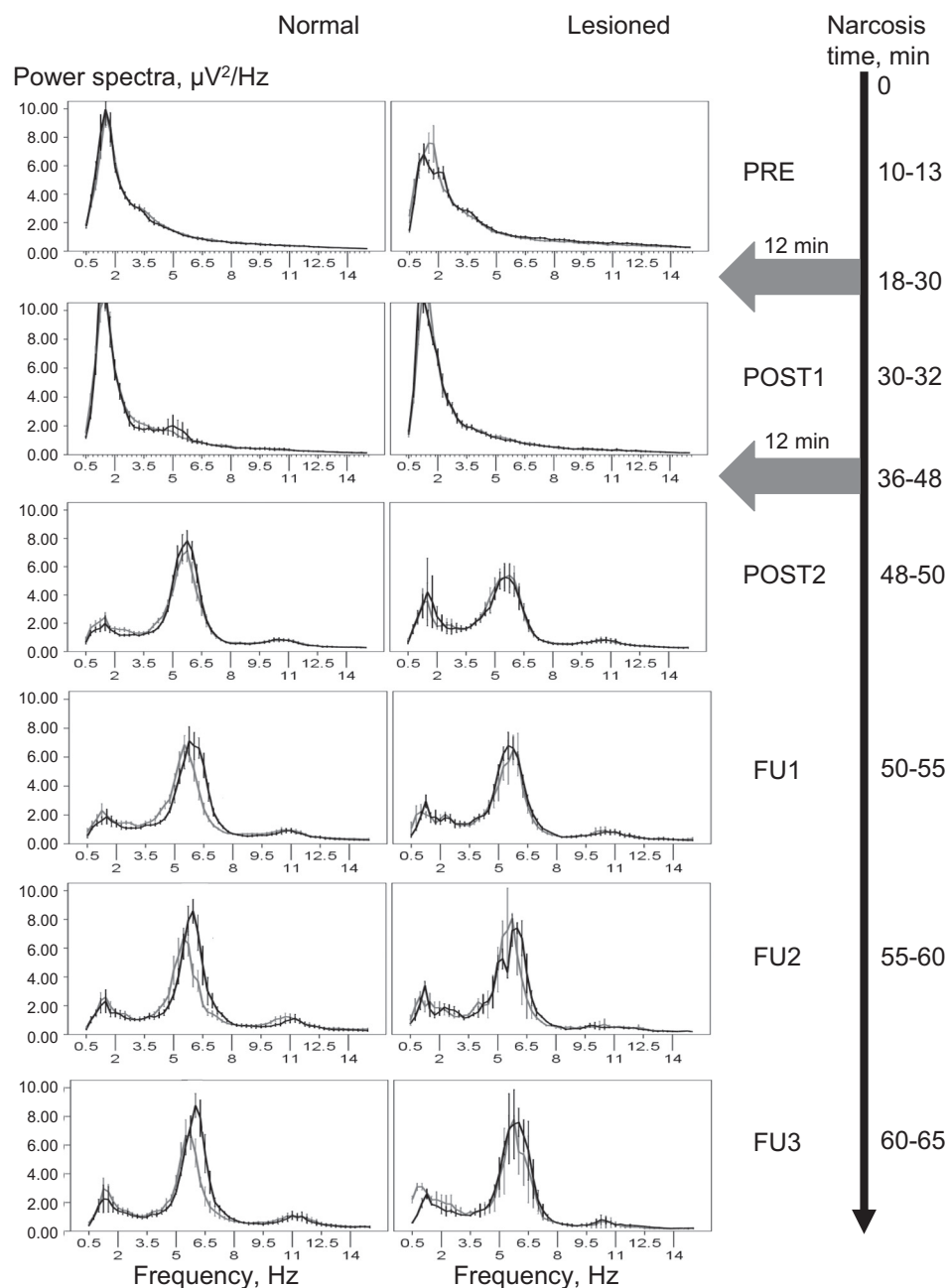


Fig. 3. Power spectra EEG recorded in normal and lesioned rats at different time points: PRE and POST1, before and after the first tACS application, respectively; POST2 and FU1–3, after the second tACS (black lines). Gray lines show the spectra for the same time points in baseline EEG (unstimulated).

lesioned rats (Fig. 4, tACS). In contrast, unlesioned animals revealed a significant increase in the power spectra of 5, 6, and 7 Hz when the post-tACS recordings were compared with baseline (Fig. 4, Normal). This illustrates the mentioned EEG shift in theta band after tACS in normal rats, which was not seen in animals with ONC.

DISCUSSION

With the present experiment performed in anesthetized animals, we demonstrated that tACS can alter EEG power bands at a time after current stimulation is already completed (aftereffect). This is in line with work by Fedorov et al. (2011), Sabel et al. (2011a), and Zaehle et al. (2010), who observed aftereffects following tACS in humans. Fedorov et al. (2011) showed that in patients, the EEG spectral profile showed

increased alpha power after tACS. It was proposed that alternating current stimulation shifted the bioelectrical brain activity, which had been altered by the traumatic damage, towards the range of more "normal" visual function, and this might provide a possible mechanism for vision restoration. In the Zaehle et al. (2010) study, ACS using alpha band (8–12 Hz) stimulation led to a temporary elevation of alpha power that lasted for 3 min beyond the stimulation period (aftereffect) in healthy humans. Therefore, with our preclinical model we were able to mimic the neurophysiological signs of plasticity following tACS treatment as observed in humans.

In our rat experiment, we observed not only increased power in the prevailing EEG band but also a moderate shift of the dominant frequency peak toward faster theta. This might be attributed to the fact that we applied stimulation frequencies in

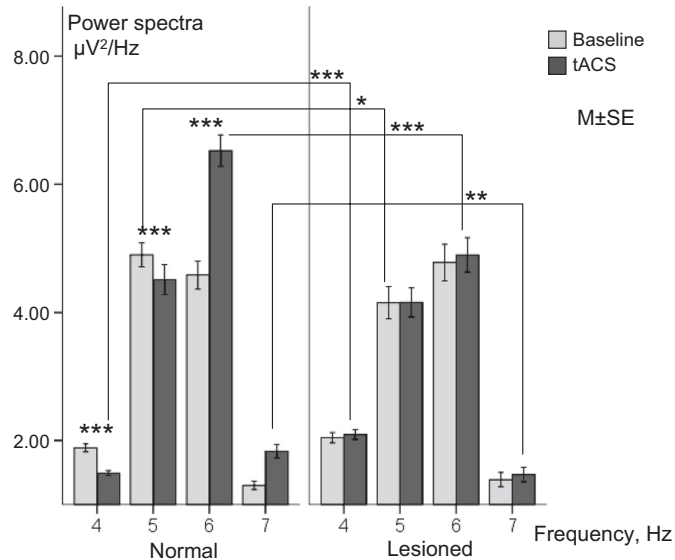


Fig. 4. Baseline EEG vs. poststimulation EEG and normal vs. lesioned group; analysis of power spectral density in 4 sub-bands within theta (4, 5, 6, 7 Hz). Baseline, unstimulated EEG (gray bars); tACS, recording after the second tACS application (black bars) in normal and lesioned animals. Significances with post hoc Mann-Whitney U-test: * $P = 0.011$, ** $P = 0.001$, *** $P < 0.001$. Error bars: means \pm SE.

the alpha range, which is associated with visual activity in humans, whereas in rats theta frequencies are prevailing.

In this study, we made the following new observations: 1) the tACS-induced EEG shift is only observed during the late narcosis stage, but not during earlier, slow wave sleep; and 2) the EEG shift is observed only in normal rats, not in rats with chronic and severe ONC.

The greatest difference of our rat studies to human studies is that tACS was applied during anesthesia. While this is also what others have done (Morimoto et al. 2005, 2007, 2010; Ni 2009), our study shows that anesthesia imposes limitations to study brain current stimulation effects preclinically. We have therefore paid particular attention to the different narcosis stages, i.e., observing the whole time span from administration up to awakening as a dynamic progression of the brain's functional state. We witnessed slow wave oscillations of the delta band prevailing up to ~ 35 min after ketamine/xylazine intraperitoneal injection, which was then followed by theta waves dominance until the point of awakening. These narcosis stages were analyzed separately in rats with and without tACS application.

During the early narcosis delta EEG stage, we did not find any differences in EEG bands in animals treated and not treated with tACS (POST1), whereas during later theta stage, EEG changes were induced by tACS. Since no EEG change was seen after the first tACS application, we hypothesize that an additive effect of two subsequent stimulations is needed to alter brain activity, i.e., the first stimulation alone was not enough. However, there is also an alternative explanation as to why that narcosis state may have a significant influence: Gersner et al. (2011) observed that repetitive transcranial magnetic stimulation increased trophic factor levels in awake but not anesthetized animals. Our results are in line with this. We propose that the unsuccessful stimulation during the delta stage is, in all probability, due to the well-known reduced functional state of

brain under narcosis, which contributes to lowering the responsiveness of the central nervous system to electrical stimulation. This supports the argument that the functional state of the brain is a key factor for the success of tACS treatment. If we assume that LTP-like strengthening of synaptic transmission (Paulus 2004) in residual tissue is the physiological substrate of tACS-induced restoration of vision (Sabel et al. 2011b), then this could explain the presence of stimulation effects in unanesthetized patients as well as the absence in deeply anesthetized rodents. In line with this argument, synaptic plasticity in local cortical network is dependent on the level of neuronal activity (Crochet et al. 2005).

While on one hand we found that anesthesia seems to be a limiting factor, on the other hand we benefited from this unexpected finding. It is known that unanesthetized animals have different behavioral states relating to different levels of vigilance varying from deep sleep, light sleep, motionless wakefulness, search behavior, feeding behavior, orientation response, to arousal. Treatment with electrical stimulation is believed to induce excitability changes in the brain (Antal et al. 2004, 2006, 2011; Chaieb et al. 2008, 2011; Nitsche et al. 2003; Nitsche and Paulus 2011) and to give rise to plasticity in neural networks (Chaieb et al. 2008, 2011; Paulus 2004, 2011), which are, in turn, influenced by the level of neuronal activity, the state of networks (Crochet et al. 2005; Silvanto et al. 2008), the level of vigilance (Steriade et al. 1993; Steriade and Timofeev 2003), and, finally, behavioral states (Steriade 1999). Effects of brain stimulation are even dependent on the functional state of the stimulated regions (Silvanto 2008). Apparently, the relationship of stimulation effects and activity state follow an inverted U-shape curve function: not only low background neuronal activity, but also exceedingly high arousal levels both can reduce the plasticity potential. The consequence of this is that behavioral states that are at the extremes on an activity/non-activity scale (such as stress or pain on one side of the spectrum and deep sleep on the other side) are expected to reduce tACS-induced outcomes. From this point of view, a deep narcosis state is not suitable for testing effects of noninvasive brain stimulation effects, but rather, the light narcosis provides a useful condition for tACS to be effective.

We therefore proposed that tACS applied during the later transition period of narcosis, when the theta band dominates, provides a better condition to study ACS effect.

Interestingly, the EEG power band shifted after electrical stimulation when applied during the transition period in normal control rats but not in rats with ONC. This raises the issue of, why brain bioelectrical activity can be altered by tACS only in rats with intact optic nerves, whereas it has no impact after severe optic nerve damage? Actually, at first glance our findings in lesioned rats contrast the findings in humans where tACS-induced plasticity was observed in patients with optic nerve damage (Sabel 2011a). However, the severity of injury in patients was only partial (mild), and we not only treated the affected eye with tACS but also the fellow (often normal) eye as well. In our rats, the injury was very severe with no or almost no residual vision left. The number of surviving cells dropped to 9% at 4 wk (P. Henrich-Noack, S. Lazik, E. Sergeeva, S. Wagner, N. Voigt, S. Prilloff, A. Fedorov, B.A. Sabel, unpublished observations), which is probably below a critical activity threshold for tACS effects to take place. Such

massive cell death is very rare in partially blind patients. Also, in rats, both eyes were injured, i.e., there was not treatment of the normal (fellow) eye as in the clinical studies. Therefore, the very low level of residual structures after ONC in rats is probably an important difference to our patient work (note that completely blind patients are typically excluded from our clinical studies). Thus, the amount of retinofugal activation after bilateral severe optic nerve lesion was probably too low to produce sufficient physiological drive in higher (cortical) visual areas. This downside of our experimental protocol, however, also had an upside: the lack of a modulatory effect of tACS in lesioned rats indicates that tACS effects are mediated by neuronal processing in the retina and optic nerve and not by a nonspecific, nonneuronal current flow into the brain, which directly affects plasticity in higher regions.

Our results do not imply that tACS is an inappropriate treatment for cases of severe visual system damage. In fact, even after complete optic nerve cut, some positive impact of current stimulation on RGCs survival was shown in animal experiments by Morimoto et al. (2005). Similar neuroprotective effects of tACS were achieved with our protocol of severe ONC (P. Henrich-Noack, S. Lazik, E. Sergeeva, S. Wagner, N. Voigt, S. Prilloff, A. Fedorov, B.A. Sabel, unpublished observations). But this improved cell survival was seen after severe injury when current stimulation was applied immediately after damage, i.e., at the acute stage of injury. Here, ACS promotes neuroprotection of RGCs rather than inducing synaptic plasticity (Morimoto et al. 2005). But the experimental protocol used in the current study was chosen to mimic the chronic stage of damage in a "stabilized" visually deafferentation state, well beyond the early phase of spontaneous recovery of vision, which occurs usually within the first 3 wk (Sabel et al. 1997; Sabel 1999).

However, in addition to the lesion severity issue, another hypothesis is conceivable as to why there may be a lack of tACS effects in posttraumatic animals. It is possibly that the timing of tACS may have been wrong because neuroplasticity is state dependent (Crochet et al. 2005; Silvanto et al. 2008; Steriade et al. 1993; Steriade 1999; Steriade and Timofeev 2003). In our study, stimulation mainly influenced the theta band in normal rats during the later stage of narcosis. In contrast, in lesioned animals, where the tACS is applied at the same time with respect to induction of narcosis, it is, however, applied at a different narcosis stage because the transition from delta band to theta band dominance is delayed in rats post-ONC, and our tACS application was given during a relatively deep narcosis, as indicated by delta band dominance. Thus, in the lesioned animals, we may have missed the effective time window that then prevented neuroplasticity as seen in control rats.

When interpreting the effects of tACS, one should keep in mind that rats are not primarily "visual" animals and rather rely primarily on the sense of touch by their vibrissae and smell, especially when they suffer acute vision deficits. Following ONC, visual function is lost, and it is conceivable that the brain switches from vision to other senses (Prilloff et al. 2010). Residual visual structures "not in use" also lead to structural rearrangement due to dramatic reduction of visual afferents. For instance, in experiments with enucleated rabbits, the depressed mode of cortical activity due to interruption of visual input and long-standing visual deprivation was described

(Zislina and Novikova 1971). Also, a depressed EEG alpha rhythm was found in blind humans (Kaplan et al. 1970). In the current work, we demonstrate that the transition time in anesthesia is longer in lesioned rats than in control animals. This suggests that the impaired brain "wakes up" later, perhaps due to reduced (visually induced?) excitability. Therefore, the lack of tACS effects in lesioned rats may be based on a depressed brain state due to long-term visual deprivation. This may leave neuronal structures not receptive to tACS, and brain activity can thus not be altered as easily.

In conclusion, we used a new animal model and showed that tACS can influence bioelectrical activity of the brain that outlasts the stimulation period (aftereffects), which is in line with human studies. But this tACS effect probably depends on some minimal physiological activation state of the brain, because when applied during slow waves sleep stages of narcosis, tACS does not alter EEG activity. Furthermore, when the retinofugal fibers are severely injured, which reduces activation input to the brain and leaves the animals blind, then tACS is also not effective. These results emphasize that the functional state of the brain is a crucial matter in tACS treatment protocols and that retinal activation seems to be required to produce a cortical plasticity response. Although this issue needs further study, when viewed in the context of prior studies, it becomes clear that a (yet not defined) minimum activation level (vigilance) is required for tACS to unfold its full therapeutic potential in the field of vision restoration following visual system damage.

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DISCLOSURES

B. A. Sabel is consultant and shareholder of EBS Technologies GmbH (Kleinmachnow, Germany), manufacturer of an AC stimulation device. A. B. Fedorov is an employee of EBS Technologies GmbH. The other authors have no competing interests. The study was funded by the Otto-von-Guericke University.

AUTHOR CONTRIBUTIONS

E.G.S., A.B.F., P.H.-N., and B.A.S. conception and design of research; E.G.S. performed experiments; E.G.S. analyzed data; E.G.S., A.B.F., P.H.-N., and B.A.S. interpreted results of experiments; E.G.S. prepared figures; E.G.S. drafted manuscript; E.G.S., A.B.F., P.H.-N., and B.A.S. edited and revised manuscript; E.G.S., A.B.F., P.H.-N., and B.A.S. approved final version of manuscript.

REFERENCES

- Antal A, Kincses TZ, Nitsche MA, Bartfai O, Paulus W. Excitability changes induced in the human primary visual cortex by transcranial direct current stimulation: direct electrophysiological evidence. *Invest Ophthalmol Vis Sci* 45: 702–707, 2004.
- Antal A, Kincses TZ, Nitsche MA, Paulus W. Manipulation of phosphene thresholds by transcranial direct current stimulation in man. *Exp Brain Res* 150: 375–378, 2003.

- Antal A, Nitsche MA, Paulus W.** Transcranial direct current stimulation and the visual cortex. *Brain Res Bull* 68: 459–463, 2006.
- Antal A, Paulus W, Nitsche MA.** Electrical stimulation and visual network plasticity. *Restor Neurol Neurosci* 29: 365–374, 2011.
- Chaieb L, Antal A, Paulus W.** Gender-specific modulation of short-term neuroplasticity in the visual cortex induced by transcranial direct current stimulation. *Vis Neurosci* 25: 77–81, 2008.
- Chaieb L, Antal A, Paulus W.** Transcranial alternating current stimulation in the low kHz range increases motor cortex excitability. *Restor Neurol Neurosci* 29: 167–175, 2011.
- Chaieb L, Paulus W, Antal A.** Evaluating aftereffects of short-duration transcranial random noise stimulation on cortical excitability. *Neural Plast* 2011: 105927, 2011.
- Chryssikou EG, Hamilton RH.** Noninvasive brain stimulation in the treatment of aphasia: exploring interhemispheric relationships and their implications for neurorehabilitation. *Restor Neurol Neurosci* 29: 375–394, 2011.
- Coslett HB, Hamilton RH.** Non-invasive brain current stimulation in neurorehabilitation. *Restor Neurol Neurosci* 29: 361–363, 2011.
- Crochet S, Chauvette S, Boucetta S, Timofeev I.** Modulation of synaptic transmission in neocortex by network activities. *Eur J Neurosci* 21: 1030–1044, 2005.
- Eysel UT, Schweigart G, Mittmann T, Eyding D, Qu Y, Vandesande F, Orban G, Arckens L.** Reorganization in the visual cortex after retinal and cortical damage. *Restor Neurol Neurosci* 15: 153–164, 1999.
- Fedorov A, Chibisova Y, Szymaszek A, Alexandrov M, Gall C, Sabel BA.** Non-invasive alternating current stimulation induces recovery from stroke. *Restor Neurol Neurosci* 28: 825–833, 2010.
- Fedorov A, Jobke S, Bersnev V, Chibisova A, Chibisova Y, Gall C, Sabel BA.** Restoration of vision after optic nerve lesions with noninvasive transorbital alternating current stimulation: a clinical observational study. *Brain Stimul* 4: 189–201, 2011.
- Fregni F, Pascual-Leone A.** Technology insight: noninvasive brain stimulation in neurology—perspectives on the therapeutic potential of rTMS and tDCS. *Nat Clin Pract Neurol* 3: 383–393, 2007.
- Fujikado T, Morimoto T, Matsushita K, Shimojo H, Okawa Y, Tano Y.** Effect of transcorneal electrical stimulation in patients with nonarteritic ischemic optic neuropathy or traumatic optic neuropathy. *Jpn J Ophthalmol* 50: 266–273, 2006.
- Gall C, Fedorov AB, Ernst L, Borrmann A, Sabel BA.** Repetitive transorbital alternating current stimulation in optic neuropathy. *NeuroRehab* 27: 335–341, 2010.
- Gall C, Sgorzaly S, Schmidt S, Brandt S, Fedorov A, Sabel BA.** Noninvasive transorbital alternating current stimulation improves subjective visual functioning and vision-related quality of life in optic neuropathy. *Brain Stimul* 4: 175–188, 2011.
- Gersner R, Kravetz E, Feil J, Pell G, Zangen A.** Long-term effects of repetitive transcranial magnetic stimulation on markers for neuroplasticity: differential outcomes in anesthetized and awake animals. *J Neurosci* 31: 7521–7526, 2011.
- Hummel FC, Cohen LG.** Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? *Lancet Neurol* 5: 708–712, 2006.
- Kanai R, Chaieb L, Antal A, Walsh V, Paulus W.** Frequency-dependent electrical stimulation of the visual cortex. *Curr Biol* 18: 1839–1843, 2008.
- Kaplan AI, Novikova LA, Pevzner MS.** Electrical activity of the brain in basic clinical forms of childhood blindness. *Ophthalmol Zh* 25: 381–382, 1970.
- Morimoto T, Fujikado T, Choi JS, Kanda H, Miyoshi T, Fukuda Y, Tano Y.** Transcorneal electrical stimulation promotes the survival of photoreceptors and preserves retinal function in royal college of surgeons rats. *Invest Ophthalmol Vis Sci* 48: 4725–4732, 2007.
- Morimoto T, Miyoshi T, Matsuda S, Tano Y, Fujikado T, Fukuda Y.** Transcorneal electrical stimulation rescues axotomized retinal ganglion cells by activating endogenous retinal IGF-1 system. *Invest Ophthalmol Vis Sci* 46: 2147–2155, 2005.
- Morimoto T, Miyoshi T, Sawai H, Fujikado T.** Optimal parameters of transcorneal electrical stimulation (TES) to be neuroprotective of axotomized RGCs in adult rats. *Exp Eye Res* 90: 285–291, 2010.
- Nair DG, Renga V, Lindenberg R, Zhu L, Schlaug G.** Optimizing recovery potential through simultaneous occupational therapy and non-invasive brain-stimulation using tDCS. *Restor Neurol Neurosci* 29: 411–420, 2011.
- Ni YQ, Gan DK, Xu HD, Xu GZ, Da CD.** Neuroprotective effect of transcorneal electrical stimulation on light-induced photoreceptor degeneration. *Exp Neurol* 219: 439–452, 2009.
- Nitsche MA, Liebetanz D, Antal A, Lang N, Tergau F, Paulus W.** Modulation of cortical excitability by weak direct current stimulation—technical, safety and functional aspects. *Suppl Clin Neurophysiol* 56: 255–276, 2003.
- Nitsche MA, Paulus W.** Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol* 527: 633–639, 2000.
- Nitsche MA, Paulus W.** Transcranial direct current stimulation - update 2011. *Restor Neurol Neurosci* 29: 463–492, 2011.
- Oliveri M.** Brain stimulation procedures for treatment of contralesional spatial neglect. *Restor Neurol Neurosci* 29: 421–425, 2011.
- Olma MC, Kraft A, Roehmel J, Irlbacher K, Brandt SA.** Excitability changes in the visual cortex quantified with signal detection analysis. *Restor Neurol Neurosci* 29: 453–461, 2011.
- Oono S, Kurimoto T, Kashimoto R, Tagami Y, Okamoto N, Mimura O.** Transcorneal electrical stimulation improves visual function in eyes with branch retinal artery occlusion. *Clin Ophthalmol* 5: 397–402, 2011.
- Paulus W.** Outlasting excitability shifts induced by direct current stimulation of the human brain. *Suppl Clin Neurophysiol* 57: 708–714, 2004.
- Paulus W.** Transcranial electrical stimulation (tES - tDCS; tRNS, tACS) methods. *Neuropsychol Rehabil* 21: 602–617, 2011.
- Prilloff S, Henrich-Noack P, Kropf S, Sabel BA.** Experience-dependent plasticity and vision restoration in rats after optic nerve crush. *J Neurotrauma* 27: 2295–2307, 2010.
- Reis J, Fritsch B.** Modulation of motor performance and motor learning by transcranial direct current stimulation. *Curr Opin Neurol* 24: 590–596, 2011.
- Rennaker RL, Carey HL, Anderson SE, Sloan AM, Kilgard MP.** Anesthesia suppresses nonsynchronous responses to repetitive broadband stimuli. *Neuroscience* 145: 357–369, 2007.
- Rogers LM, Madhavan S, Roth HR, Stinear JW.** Transforming neurorehabilitation of walking following stroke: the promise of non-invasive brain stimulation - a review. *Restor Neurol Neurosci* 9: 507–516, 2011.
- Sabel BA.** Restoration of vision. I. Neurobiological mechanisms of restoration and plasticity after brain damage - a review. *Restor Neurol Neurosci* 15: 177–200, 1999.
- Sabel BA, Aschoff A.** Functional recovery and morphological changes after injury to the optic nerve. *Neuropsychobiology* 28: 62–65, 1993.
- Sabel BA, Fedorov AB, Naue N, Borrmann A, Herrmann C, Gall C.** Non-invasive alternating current stimulation improves vision in optic neuropathy. *Restor Neurol Neurosci* 29: 493–505, 2011a.
- Sabel BA, Henrich-Noack P, Fedorov A, Gall C.** Vision restoration after brain and retina damage: the “residual vision activation theory.” *Prog Brain Res* 192: 199–262, 2011b.
- Sabel BA, Kasten E, Kreutz MR.** Recovery of vision after partial visual system injury as a model of postlesion neuroplasticity. *Adv Neurol* 73: 251–276, 1997.
- Sautter J, Sabel BA.** Recovery of brightness discrimination in adult rats despite progressive loss of retrogradely labeled retinal ganglion cells after controlled optic nerve crush. *Eur J Neurosci* 5: 680–690, 1993.
- Sautter J, Schwartz M, Duvdevani R, Sabel BA.** GM1 ganglioside treatment reduces visual deficits after graded crush of the rat optic nerve. *Brain Res* 565: 23–33, 1991.
- Silvanto J, Muggleton N, Walsh V.** State-dependency in brain stimulation studies of perception and cognition. *Trends Cogn Sci* 12: 447–454, 2008.
- Song S, Sandrini M, Cohen LG.** Modifying somatosensory processing with non-invasive brain stimulation. *Restor Neurol Neurosci* 29: 427–437, 2011.
- Steriade M.** Coherent oscillations and short-term plasticity in corticothalamic networks. *Trends Neurosci* 22: 337–345, 1999.
- Steriade M, McCormick DA, Sejnowski TJ.** Thalamocortical oscillations in the sleeping and aroused brain. *Science* 262: 679–685, 1993.
- Steriade M, Timofeev I.** Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. *Neuron* 37: 563–576, 2003.
- Zaehle T, Rach S, Herrmann CS.** Transcranial alternating current stimulation enhances individual alpha activity in human EEG. *PLoS One* 5: e13766, 2010.
- Zaghi S, Thiele B, Pimentel D, Pimentel T, Fregni F.** Assessment and treatment of pain with non-invasive cortical stimulation. *Restor Neurol Neurosci* 29: 439–451, 2011.
- Zislina NN, Novikova LA.** Effect of visual deafferentation on background and evoked activity of cerebral cortical neurons in the rabbit. *Zh Vyssh Nerv Deiat Im I P Pavlova* 21: 1298–1306, 1971.