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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Submitted 25 April 2012; accepted in final form 4 August 2012

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 threshold values 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 therapeutical 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) function 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...
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, Tuttingen, 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 surgical clamps were 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 conductivity. The reference electrode was fixed on the ear. The electrical brain activity under narcosis in naïve 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 wakening 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 Hz) 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.
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 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 \times 10^{-11}; for 5 Hz – \( \chi^2 = 8.8 \); Asymp. sign. = 0.032; for 6 Hz – \( \chi^2 = 36.9 \); Asymp. sign. = 4.9 \times 10^{-8}; for 7 Hz – \( \chi^2 = 32.1 \); Asymp. sign. = 4.9 \times 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 \( \pm SE \).
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 simulation is already completed (after-effect). 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
the unsuccessful stimulation during the delta stage is, in all
treated animals. Our results are in line with this. We propose that
Gersner et al. (2011) observed that repetitive transcranial magnetic stimula-
tion during the delta stage, but not during earlier, slow wave sleep; and
the EEG shift is only observed 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 stage, we did not find any
differences in EEG bands in animals treated and not treated
with tACS (POST1), whereas during later delta 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 anesthet-
ized 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 respon-
siveness 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 unanesthet-
tized 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.
2003; Nitsche and Paulus 2011) and to give rise to plasticity in
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 func-
tional state of the stimulated regions (Silvanto 2008). Appar-
etently, 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 find-
ings in lesioned rats contrast the findings in humans where
tACS-induced plasticity was observed in patients with optic
erve 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

![Power spectra](image)
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 the 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 (Zisлина 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 anesthetism 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.

ACKNOWLEDGMENTS

We thank Sylvia Prilloff and Uta Werner for excellent technical assistance. The valuable contribution from Dr. Gorkin regarding EEG recording in rats is much appreciated.

GRANTS

E. G. Sergeeva was supported by a DAAD postdoctoral fellowship. P. Henrich-Noack received funding from the Sibylle Assmus Foundation.

DISCLOSURES

B. A. Sabel is consultant and shareholder of EBS Technologies GmbH (Kleinhanns, 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


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