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The impact of transcranial direct current stimulation on cerebral vasospasm in a rat model of subarachnoid hemorrhage

Vesna Malinova[1], Kim Bleuel[1], Christine Stadelmann[2], Bogdan Iliev[1], Ioannis Tsogkas[3,4], Marios N Psychogios[3,4], Veit Rohde[1] and Dorothee Mielke[1]

Abstract

Transcranial direct current stimulation (tDCS) has been shown to induce changes in cortical excitability and perfusion in a rat ischemic stroke model. Since perfusion disturbances are a common phenomenon, not only in ischemic but also in hemorrhagic stroke, tDCS might have a possible beneficial effect on cerebral perfusion in hemorrhagic stroke as well. We applied tDCS in a rat model of subarachnoid hemorrhage (SAH) and evaluated its impact on vasospasm. SAH was induced using the double-hemorrhage rat model. TDCS was applied on day 3 and 4. For vasospasm assessment magnetic resonance angiography was performed on day 1, day 2 and day 5. A total of 147 rats were operated, whereat 72 rats died before day 5 and 75 rats survived the whole experiment and could be analyzed. The cathodal group consisted of 26 rats, the anodal group included 24 rats. Thirteen rats served as controls without tDCS, and twelve rats underwent a sham operation. The cathodal group revealed the lowest incidence of new vasospasm on day 5 ($p = 0.01$), and the lowest mean number of vasospastic vessels per rat ($p = 0.02$). TDCS influences the vasospasm incidence in an SAH-model in rats, where cathodal-tDCS was associated with a lower vasospasm incidence and severity.

Keywords

Cerebral vasospasm, experimental subarachnoid hemorrhage, neuromodulation, stroke, transcranial direct current stimulation

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Introduction

Neuromodulation is an emerging field in neuroscience with an increasing number of clinical indications, due to constant technical development and a growing knowledge concerning the physiological and pathophysiological mechanisms behind the observed effects. Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulation technique with an observed effect on motor function after ischemic stroke.[1–4] Furthermore, several animal studies have reported polarity-specific changes of cortical excitability and cerebral perfusion after tDCS in ischemic stroke models.[5–8] Whether these perfusion-related effects are evoked by neurovascular coupling mechanisms or if they are related to a direct effect on the vessel wall, remains unclear. However, the possibly beneficial effects of tDCS-induced perfusion changes in the context of different cerebrovascular diseases justifies the evaluation of tDCS not only in ischemic but also in hemorrhagic stroke model. Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating

[1]Department of Neurosurgery, University Medical Center Göttingen, Göttingen, Germany
[2]Department of Neuropathology, University Medical Center Göttingen, Göttingen, Germany
[3]Department of Neuroradiology, Clinic of Radiology and Nuclear Medicine, University Medicine Basel, Basel, Switzerland
[4]Department of Neuroradiology, University Medical Center Göttingen, Göttingen, Germany

Corresponding author:
Vesna Malinova, Department of Neurosurgery, University Medical Center Göttingen, Robert-Koch-Straße 40, Göttingen 37075, Germany.
Email: vesna.malinova@gmail.com

cerebrovascular disease affecting younger patients compared to other stroke types with a high mortality and morbidity rate with subsequent functional disability.[9,10] The primary brain injury caused by the initial bleeding itself as well as delayed ischemic complications, occurring in the following 14 to 21 days, are mainly determining the patients' outcome after the bleeding. However, despite ongoing research in this field, effective preventive and therapeutic options for delayed ischemic complications after aSAH are still scarce.[11–15] Since cerebral perfusion has a leading role during the pathophysiological processes after aSAH, tDCS-induced modulation of cerebral perfusion might have a positive effect on delayed ischemic complications after aSAH as well. In contrast to ischemic stroke, reports on tDCS in the setting of aSAH are currently lacking. In this study, we applied tDCS in a SAH-model in rats and assessed its impact on the incidence and severity of cerebral vasospasm, as one of the main contributors to delayed ischemia and poor outcome after aSAH. The rationale of our study was to transfer the existing concepts to the experimental setting of aSAH and to assess possible interactions with cerebrovascular disturbances after aSAH. Our hypothesis was that tDCS induces polarity-specific changes of the cerebral vessels' lumen with a subsequent impact on the incidence of cerebral vasospasm after aSAH.

Materials and methods

Ethical statement

All experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals of the NIH" and were ethically approved by the Government of Lower Saxony (AZ 13/1055). The article was written according to the ARRIVE guidelines.[16]

Experimental setting

The experiments included 147 male Sprague-Dawley rats (Charles River, Germany), whereof 135 rats were assigned to the SAH-group and 12 rats to the sham-group. Seventy-two out of 135 rats in the SAH-group died before day 5 and 63 rats with aSAH survived the whole experiment and could be analyzed. The cathodal group consisted of 26 rats, the anodal group included 24 rats, 13 rats served as controls without tDCS, and twelve rats received a sham operation. The mortality rate in the SAH-group was 53%, which was comparable to the previously reported mortality rate of 50% in the double blood injection model.[17] The rats were randomly assigned to the SAH-group or the sham-group. The animals were housed in a temperature- and humidity-controlled room on a 12-hour light/12-hour dark cycle in single cages under standard laboratory conditions, with food and water ad libitum. All rats were delivered with a body weight of 200 g and had one week of accommodation time before the experiments started. The duration of the experiment was 5 days: on day 1 and day 2 the blood was injected; on day 3 and day 4, the stimulation was performed according to the randomly assigned treatment group. On day 5, the experiment was finished by transcardial perfusion and removal of the brain.

Induction of subarachnoid hemorrhage

Anesthesia was applied by intraperitoneal injection of an anesthetic cocktail (1 ml), consisting of 0.3 ml medetomidine (Cepetor® 1 mg/ml) and 0.7 ml ketamine (Ketamin® 10% Solution) with a dosage of 0.1 ml per 100 g body weight. Since this anesthesia does not compromise the animals' respiration, an intubation of the rats was not necessary. During the procedure, the body temperature was maintained between 36.5 and 37.5 °C by a heating blanket, controlled by a rectal temperature probe (Homeothermic Monitor, Harvard Apparatus, Hugo Sachs Elektronik, Germany). SAH was induced using the modified double hemorrhage model, as first described by Güresir et al and recently utilized by our study group.[17,18] This model includes the injection of 0.25 ml autologous arterial blood into the cerebellomedullary cistern on two consecutive days. In the sham-group, the same volume of saline solution was injected instead of blood. The blood/saline solution injection was performed via a suboccipital approach through a midline incision. The tip of the injection catheter was placed within the right cerebellomedullary cistern and not in the midline position in order to avoid injury of the medulla oblongata. Post-surgery, the animals were positioned in a prone position with 15–30° head down in order to facilitate a better blood distribution within the subarachnoid space. For postoperative pain control, all rats received buprenorphine (Temgesic®, RB Pharmaceuticals Limited, Berkshire, United Kingdom) (0.03-0.05 µg/kg to 0.1 µg/kg body weight s.c.) as well as 5 ml saline solution s.c. twice per day, metamizole (1.33 mg/ml drinking water p.o.) was continuously administered via drinking water. The animals were clinically examined at least three times per day by a veterinarian and buprenorphin was additionally injected, if clinical signs of pain were detected.

tDCS-protocol

TDCS was applied on day 3 and on day 4. The duration of every single tDCS-session was 15 minutes. The tDCS intensity has been set at 400 µA. These stimulation parameters were chosen according to the findings

of previously published safety studies of our working group evaluating different stimulation protocols.[6,8]

The rats were randomly assigned to the following stimulation groups: anodal tDCS and cathodal tDCS with a number of stimulation sessions ranging from once a day to four times per day, respectively. The time interval between two stimulation sessions was one hour. During the stimulation period, the rats received no anesthesia or any other type of sedation. During the stimulation period, the tDCS electrode was connected to a tube that was fixed to the skull using glass inomer cement (Ketac™, Cem Maxicap™, ESPE Dental AG, Seefeld, Germany). The position of the electrode was standardized on the right skull; 2 mm behind the coronal suture and 4 mm lateral to the sagittal suture. The surface area of the epicranial electrode was 3.5 mm^2, as described in a previous publication of our study group.[6,8] For the application of tDCS, a constant current stimulator (CX-6650, Schneider Electronics, Gleichen, Germany) was used. The tube was filled with saline solution in order to allow a sufficient electrode contact area. The tubing was attached to the skull directly after the second injection (blood or saline solution), which was performed in all rats (SAH group and sham group as well). The setting is being illustrated in Figure 1.

Imaging protocol

Magnetic resonance imaging (MRI) was performed on day 1 before the first injection as a baseline measurement, on day 2 after the second injection and on day 5 at the end of the experiment. The MRI was done in all rats including the sham group. The MRI examinations were performed on a 3-Tesla MRI scanner (3.0 Tesla, Magnetom TIM Trio, Siemens Germany). The following sequences were included: T1-weighted, T2-weighted, T2*-weighted and time-of-flight (TOF) sequence. The MRI-data were analyzed by two blinded neuroradiologists (one with more than five years of experience) to the treatment groups focusing on the incidence of large vessel vasospasm (internal carotid artery [ICA], middle cerebral artery [MCA], anterior cerebral artery [ACA] and basilar artery [BA]) as well as on the detection of ischemic lesions. Vasospasm was defined as vessel lumen narrowing of the basal cerebral arteries, visualized by MRI TOF-sequences, of at least 50, compared to the baseline data from day 1. Our aim was to evaluate possible effects of tDCS on vasospasm in general as well as on moderate to severe vasospasm in particular. Therefore, vasospasm severity was divided into three severity grades: mild (arterial narrowing of $\leq$50%), moderate (arterial narrowing of 50–75%) and severe (arterial narrowing of >75%). In a previous publication, we have already defined the reference values for the assessment of vasospasm based on MR-angiography (MRA) in rats.[18] Examples of normal and spastic vessels are shown in Figure 2.

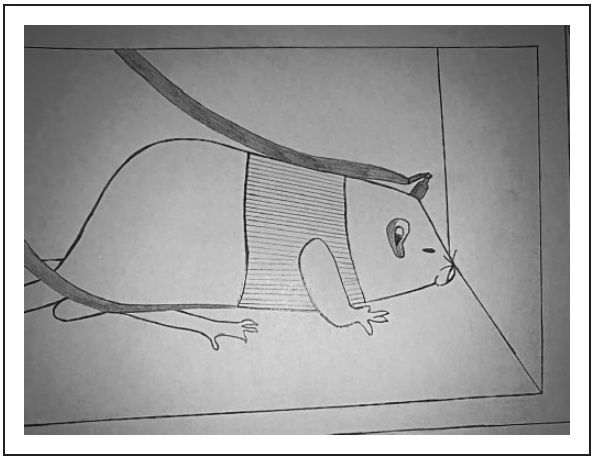

Figure 1. Setting of transcranial direct current stimulation application in an awake rat, sitting in a glass box to reduce free movement (the upper cable is the stimulation electrode, the lower cable is connected to the earthing electrode on the chest).

Primary outcome parameters

The primary outcome was the development of new vasospasm on day 5, after the application of tDCS on day 3 and day 4. Secondary endpoints were the deterioration or amelioration of preexisting vasospasm on day 5 that was already detected on day 2, the severity of vasospasm on day 5 and the number of vessels affected by vasospasm, respectively. In order to assess the effect of the applied stimulation on the incidence and severity of vasospasm, we focused separately on the incidence of new-onset vasospasm on day 5, on the deterioration or reduction of pre-existing vasospasm (i.e. changes in vasospasm severity) and on the number of vasospastic vessels within the individual treatment groups. A neurological assessment was performed on day 3, day 4 and day 5 using a neuroscore (ranging from 0 to 4) for experimental subarachnoid hemorrhage.[19] The neuroscore was calculated according to the following parameters: normal motor activity (neuroscore 0), pathologic (disturbed) postural reflex (neuroscore 1), rotation to the contralateral side (neuroscore 2), fall to the contralateral side (neuroscore 3), no spontaneous motor activity (neuroscore 4).

Statistical analysis

All analyses were performed with the statistic software R (version 3.6.1; R Core Team 2018) using the R-package lme4 (version 1.1.18.1; Bates et al. 2015) for the mixed effect linear and logistic regression, glmmTMB

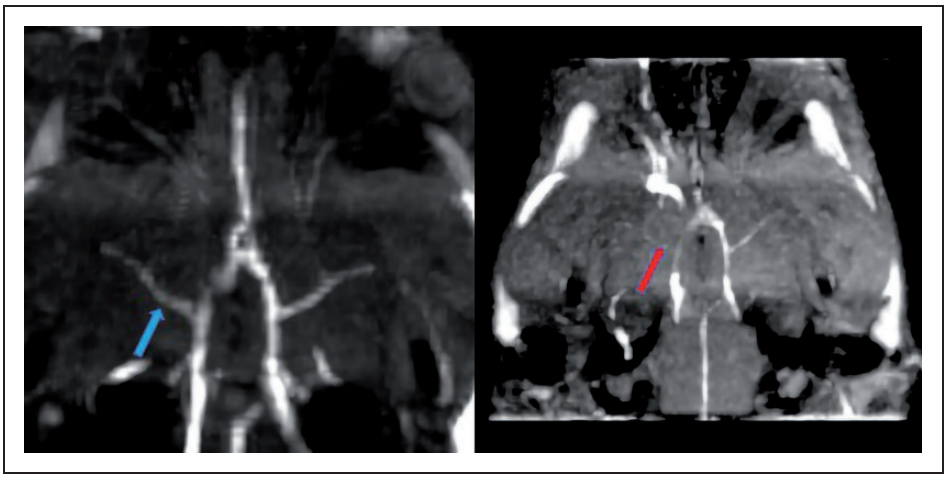

Figure 2. The images are showing the Willis circle in rat depicted by MRA with normal right middle cerebral artery (blue arrow on the left image) and with severe spastic right middle cerebral artery (red arrow on the right image), respectively.

Table 1. Overview of the treatment groups with number of rats included in each group.

	SAH-group			
Treatment group	*Control group*	*Cathodal group*	*Anodal group*	Sham group
tDCS once a day n (%)	0	5 (19)	5 (21)	12 (100)
tDCS twice a day n (%)	0	5 (19)	5 (21)	
tDCS three times a day n (%)	0	5 (19)	4 (16)	
tDCS four times a day n (%)	0	11 (43)	10 (42)	
All n (%)	13 (100)	26 (100)	24 (100)	

(version 0.2.1.0; Brooks et al. 2017) for some of the mixed effect logistic regression models, and ggeffects (version 0.12.0; Lüdecke 2018) for the marginal effect predictions. Binary outcomes were modeled using mixed effect logistic regression analysis; continuous outcomes were modeled using mixed effect linear regression models as well.[20–23] The animal was included as a random factor, accounting for correlated measurements in the same individuals across time. The data showed no normal distribution. Therefore, non-parametric tests were applied. For the evaluation of differences between the treatment groups one-way ANOVA analysis was used. Factor of interest was the treatment group or the stimulation frequency within each group and its interaction with time. The significance level was set to alpha = 5% for all statistical tests.

Results

The experiment was conducted in 147 rats (12 rats with sham operation and 135 rats with SAH induction) with a mortality rate of 51% resulting into a total of 75 rats, which survived the whole experiment and could be considered for the analysis in this study. A total of 75 rats were included in this study, whereof 63 rats were in the SAH-group and 12 rats received a sham operation. A summary of the treatment group composition is provided in Table 1.

A clinical deterioration with pathological neuroscore on day 3 was found in 34.9% of all SAH-rats, of whom 4.7% had a neuroscore of 3, 27.2% a neuroscore of 2, and 68.1% had a neuroscore of 1. The mean neuroscore on day 3 and on day 5 is presented in Table 2. We found no statistically significant difference in neuroscore between the experimental groups neither on day 3 (one-way ANOVA analysis, $p = 0.92$), nor on day 5 (one-way ANOVA analysis, $p = 0.79$).

Incidence of vasospasm before (day 2) and after tDCS (day 5)

We found no vasospasm in the sham group. The overall vasospasm incidence on day 2 of all SAH-rats was 65% (49/63 rats), whereof 13 rats (27%) had severe, 10 rats (20%) moderate, and 22 rats (53%) mild vasospasm. There was no significant difference in the vasospasm incidence on day 2 between the three groups

Table 2. Differences in vasospasm incidence and severity as well as in neuroscore between the three experimental groups on day 5 compared to day 2.

Parameters	Treatment groups			*p*-value
	Control group	Anodal tDCS group	Cathodal tDCS group	
Vasospasm on day 2				
Overall vasospasm incidence on day 2	76.9% (10/13)	75% (18/24)	80.7% (21/26)	0.88
Moderate to severe vasospasm on day 2	38.4% (5/13)	25% (6/24)	38.4% (10/26)	0.62
Number of vasospastic vessels on day 2				
- Mean	2.92	3.67	2.85	0.19
- SD	1.66	0.87	2.17	
- 95%CI	1.92–3.92	3.30–4.03	1.97–3.72	
Vasospasm on day 5				
Newly developed vasospasm on day 5	53.8% (7/13)	75% (18/24)	50% (13/26)	0.17
Newly developed moderate to severe vasospasm on day 5	38.5% (5/13)	45.8% (11/24)	26.9% (7/26)	0.003*
Number of vasospastic vessels on day 5				
- Mean	1.15	1.66	0.85	0.02*
- SD	1.28	1.30	1.19	
- 95%CI	0.38–1.93	1.11–2.22	0.37–1.33	
Vasospasm aggravation rate on day 5 of already existing vasospasm on day 2	15% (2/13)	33% (8/24)	27% (7/26)	0.50
Vasospasm reduction rate on day 5 of already existing vasospasm on day 2	54% (7/13)	42% (10/24)	58% (15/26)	0.52
Neuroscore				
Neuroscore on day 3				
- Mean	0.46	0.42	0.50	0.68
- SD	0.66	0.83	0.71	
- 95%CI	0.06–0.86	0.06–0.77	0.21–0.79	
Neuroscore on day 5				
- Mean	0.08	0.08	0.04	0.79
- SD	0.28	0.28	0.19	
- 95%CI	−0.09–0.24	−0.03–0.20	−0.04–0.12	

(one-way ANOVA analysis, $p = 0.88$). We found no statistically significant difference in the mean number of vasospastic vessels on day 2 (one-way ANOVA analysis $p = 0.19$). The vasospasm incidence and severity in the three experimental groups are summarized in Table 2. On day 5, vasospasm was present in 87% (55/63 rats) of all SAH-rats, whereof 14.5% (8/55 rats) had mild, 21.8% (12/55 rats) moderate, and 63.6% (35/55 rats) severe vasospasm. New vasospasm, that was not already present on day 2, occurred in 60.3% (38/63 rats) of all SAH-rats on day 5, whereof 42.1% (16/38 rats) had mild vasospasm, 18.4% (7/38 rats) moderate, and 39.5% (15/38 rats) severe vasospasm. Referring to the evaluated vessels (a total of 441 vessels in 63 rats), on day 2 vasospasm was detected in 164 (37.1%) and new vasospasm occurred on day 5 in 87 of the 277 vessels (31.4%) without pre-existing vasospasm on day 2. New vasospasm occurred on day 5 in 19.7% (18/91 vessels) in the control group, in 26.1% (44/168 vessels) in the anodal group and in 13.7% (25/182 vessels) in the cathodal group ($p = 0.01$, Figure 3). Moderate to severe vasospasm was newly found on day 5 in 45.8% (11/24 rats) within the anodal group, which was significantly higher compared to the incidence (26.9%, 7/26 rats) in the cathodal group ($p = 0.003$), whereas the vasospasm incidence was not significantly different between the control group (38.5%, 5/13 rats) and the cathodal group ($p = 0.10$). The mean number of vasospastic vessels on day 5 was statistically significant lower in the cathodal group compared to the anodal group ($p = 0.02$, Figure 4). A pathological neuroscore (1–4) was observed in 28.9% (11/38) of the rats with new vasospasm on day 5. There was no difference in the presence of pathological neuroscore between the treatment groups (29.4% in the anodal group, 28.5% in the cathodal group, and 28.5% in the control group).

An aggravation of already existing vasospasm occurred on day 5 in 26.9% (17/63 rats) of all SAH-rats. With regard to the vessels affected by vasospasm, a deterioration of vasospasm was found in 43.5% (17/37 spastic vessels) in the control group, in 26.5%

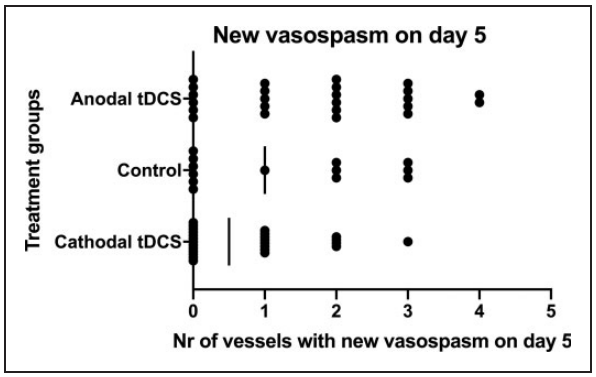

Figure 3. Incidence of new vasospasm on day 5 independent on the vasospasm severity showing significantly lower incidence in the cathodal group compared to the control group ($p = 0.01$).

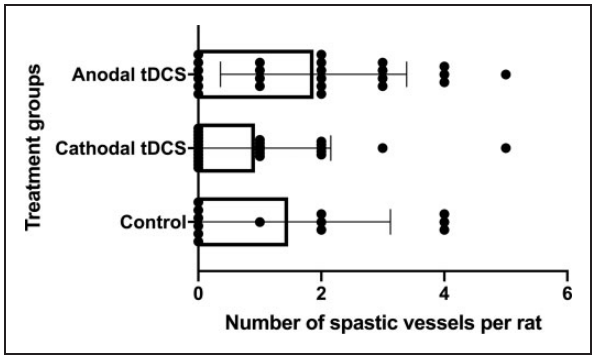

Figure 4. Number of spastic vessels per rat showing significantly lower median number of spastic vessels in the cathodal group (median 0.5, 95%CI 0 to 1) compared to the control group (median 1, 95%CI 0 to 4), and the anodal group (median 2, 95%CI 1 to 3), Kruskal-Wallis test of one-way ANOVA, $p = 0.02$.

(17/64 spastic vessels) in the anodal group, and in 20.8% (15/72 spastic vessels) in the cathodal group, $p = 0.59$. A reduction of already existing vasospasm was observed on day 5 in 50.7% (32/63 rats) of all rats. With regard to the spastic vessels, an amelioration of vasospasm was found in 28.2% (11/37 spastic vessels) in the control group, in 31.2% (20/64 spastic vessels) in the anodal group, and in 46.6% (30/72 spastic vessels) in the cathodal group, $p = 0.27$.

Vasospasm incidence in relation to frequency and side of applied stimulation

In all side-specifically evaluated vessels (ICA, MCA, and ACA), the vessel diameter was significantly narrowed on the right side compared to the left side ($p < 0.0001$). On day 2 vasospasm was found in 52% (98/189 vessels) on the right side and in 30.2% (57/189 vessels) on the left side. A deterioration of pre-existing vasospasm occurred in 31.6% (31/98 vessels) of the

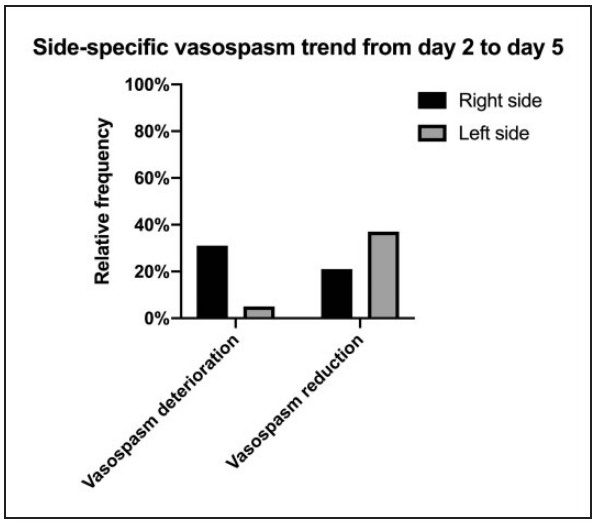

Figure 5. Side-specific vasospasm deterioration showing significantly higher deterioration rate on the right side compared to the left side (Fisher Exact test, $p = 0.001$); Side-specific vasospasm amelioration showing significantly higher amelioration rate on the left side compared to the right side (Fisher Exact test, $p < 0.0001$).

affected vessels on the right side and in 8.7% (5/57 vessels) on the left side ($p = 0.001$, Figure 5). An amelioration of pre-existing vasospasm was found in 21.4% (21/98 vessels) on the right side and in 64.9% (37/57) on the left side ($p < 0.0001$, Figure 5). Looking at subgroups receiving anodal tDCS with an increasing frequency (range 1–4 times per day), the likelihood to develop vasospasm within the ACA was 1.6 fold higher ($p = 0.04$) in the subgroup with anodal stimulation once per day, compared to the subgroups with a stimulation frequency of 2–4 times per day; the likelihood to develop vasospasm within the ICA was significantly higher (1.8 fold, $p = 0.001$) in the subgroup with anodal tDCS twice per day compared to the other subgroups. In the cathodal group, we found no significant difference in the vasospasm incidence between the subgroups receiving cathodal tDCS with an increasing frequency (range 1–4 times per day).

Discussion

The field of neuromodulation is experiencing a constantly growing number of experimental and clinical applications in neuroscience. TDCS is a neuromodulation technique, whose application has been demonstrated to be safe in animals and humans, resulting into its broad spectrum as a therapeutic tool in several neurological fields.[24–27] Several tDCS induced effects that have been reported so far might be possibly useful in the context of SAH as well. On a neurophysiological level, these effects are primarily based on the

electrical properties of neurons, where tDCS induces polarity-specific excitability changes, which translate into changes in cortical activity and subsequently into a modulation of the cerebral perfusion. This neurophysiological pathway implies the involvement of neurovascular coupling, which is a frequently assumed, but still not proven scientific explanation for the mentioned effects. Another hypothesis to explain these effects is a possible direct stimulation effect on the vessel wall. This assumption has its origin in earlier experiments in the 1970s, demonstrating a vasodilatation induced by direct current stimulation.[28] While tDCS has been extensively studied in ischemic stroke, data on a possible impact on the pathophysiological processes induced by SAH are missing. In this study, we used tDCS in the experimental setting of SAH and for the first time, we were able to demonstrate that tDCS has an impact on the incidence of vasospasm and its severity after experimental SAH. Whereupon, cathodal tDCS revealed the lowest incidence of new-onset vasospasm, but also the lowest number of vasospastic vessels, compared to both, the control and the anodal group.

Cerebral vasospasm in experimental SAH models

Cerebral vasospasm has been detected in different experimental SAH models with the lowest incidence found in the perforation model and the highest incidence accounted to the double hemorrhage model.[29,30] In order to properly assess the impact of tDCS on cerebral vasospasm, we used the double hemorrhage SAH model in rats. Previous studies were able to demonstrate that this specific model facilitates the reliable induction of severe SAH with a high incidence of vasospasm, making it specifically suitable for the evaluation of this phenomenon.[17,18,31] In the present study, we found an overall high incidence of cerebral vasospasm on day 2 (65%) as well as on day 5 (86%), which again reflects the aforementioned benefits of this experimental SAH-model. Considering vasospasm severity, expectedly, severe vasospasm was seen more often on day 5 (49%) compared to day 2 (21%).

Attempted pathophysiological explanation of beneficial cathodal tDCS-effects on vasospasm in SAH models

In our study, the cathodal tDCS was associated with beneficial effects on the occurrence as well as on the severity of vasospasm. These findings are more likely related to a direct tDCS effect on the vessel wall with a subsequent vasodilatation, than with an activation of neurovascular coupling mechanisms through a reduction of cortical activity and a consecutive vasoconstriction, followed by a decrease in cerebral perfusion. Two previously published studies have demonstrated polarity-specific changes in vasomotor reactivity (ability of dilation and constriction) of cerebral vessels, whereat anodal tDCS caused a reduction and cathodal tDCS an increase in vasomotor reactivity.[32,33] An activation of the nitric oxide synthase (NOS) has been proposed to be responsible for the aforementioned effects.[32] While the incidence of vasospasm and its severity were lower in the cathodal group, anodal tDCS was associated with a significant increase in the incidence of cerebral vasospasm. On a pathophysiological basis, these findings would imply a potentiation of an inverse neurovascular coupling that has already been demonstrated to occur after SAH, showing that neuronal activity rather constricts than dilates cerebral vessels.[34] In a recently published study, a persistent loss of neurovascular coupling could be demonstrated in a mice SAH perforation model, which should be considered as a relevant factor impacting the results' interpretation in our study on tDCS effects.[35] Furthermore, the correlation of tDCS effects with the function of neurovascular coupling merits a dedicated evaluation in a future study.

This pairs with the finding that intraparenchymal arterioles rather constrict than dilate in response to neuronal activation in an experimental SAH-model.[36] An increased voltage-dependent calcium channel activity after SAH was suspected to be the underlying cause for this unexpected finding. This is contradictory to the reported tDCS effects under physiological conditions and in the setting of ischemic stroke, where neuronal activity leads to vasodilation with a subsequent increase in cerebral perfusion.[1,7] However, the pathophysiological processes in ischemic stroke differ from those occurring after SAH, which might be an explanation for the contradictory results. The role of tDCS in the context of SAH and especially its potential interaction with the mechanisms of neurovascular coupling still remain unclear. Since our study was not primarily conducted to investigate the direct impact on neurovascular coupling, the results are not deemed to provide a final answer to the questions concerning the pathomechanisms of the observed tDCS effects. Future studies are needed to address these questions separately.

Considering the results of human studies, showing a beneficial effect of anodal tDCS on motor function during rehabilitation after ischemic stroke, we assumed a positive impact of anodal tDCS in the setting of SAH as well, due to a tDCS-induced increase in cerebral perfusion.[1–4] A logical explanation for the seemingly contradictory findings might be provided by the fact that brain tissue responds differently to tDCS during the acute stroke phase, characterized by impaired autoregulation and neurovascular communication,

compared to the post-stroke rehabilitation period. Since cortical activity demands energy, which cannot properly be delivered in the acute phase, further tissue damage might be a consequence. On the other hand, an increase in cortical activity might lead to motor function improvement during the rehabilitation period. Additionally, synergistic effects have been reported by combining tDCS with increased attention, which led to long lasting network effects during the rehabilitation phase. Positive effects of increased excitability by anodal tDCS on fatigue also supports the motor improvement during post-stroke rehabilitation.[37]

Side-specific tDCS effects

Interestingly, we observed side-specific differences concerning the vasospasm incidence in relation to the side of the applied stimulation, whereby a significantly higher vasospasm incidence was found on the stimulation side. A reduction of already existing vasospasm occurred more often on the left side in both stimulation groups (cathodal and anodal tDCS), compared to the control group without tDCS application, whereat the reduction of vasospasm was most frequently found on both sides. These findings might be related to side-specific tDCS effects in terms of transcallosal inhibition. In a previously published study, anodal tDCS increased the interhemispheric inhibition from the stimulated hemisphere to the non-stimulated hemisphere, whereas cathodal tDCS reduced the interhemispheric inhibition in both directions.[38] These findings are partially in conjunction with the results of our study. Further studies are warranted to evaluate changing stimulation effects by including bi-hemispheric and left-sided tDCS. In our study, the cathodal tDCS-effects were independent of tDCS frequency. Further studies are necessary to evaluate, whether a prolonged stimulation period beyond five days instead of only two days could potentiate the positive effects of cathodal tDCS.

Limitations of the study and future perspectives

We provide first experience with tDCS in the setting of SAH, demonstrating a polarity-specific impact on the incidence of cerebral vasospasm in an experimental SAH rat model with a beneficial effect of cathodal tDCS. We applied a previously published neuroscore for the assessment of the neurological status in rats with experimental subarachnoid hemorrhage. However, the assessment of clinical symptoms in humans with SAH cannot be directly transferred to that in rats. Furthermore, rodents usually show fast clinical recovery, which was also the case in this study. Almost all rats achieved full clinical recovery on day 5. The development of a neuroscore allowing a more comprehensive assessment of SAH-specific symptoms in rats would allow us to better evaluate the correlation of vasospasm treatment with the neurological status of the rats. This is an important clinical issue, since only symptomatic and/or hemodynamically relevant vasospasm requires treatment. In a recently published study, the development of large vessel cerebral vasospasm was evaluated by serial intravenous catheter angiography in the SAH perforation model in mice.[39] The authors found a negative correlation of large vessel vasospasm and cerebral perfusion measurement with the survival time of the animals. The correlation of vasospasm with cerebral perfusion as well as survival time is a relevant issue for clinical practice. Since the animals in our study died prior to the MRI examination, directly after the first or second blood injection, a correlation of vasospasm and survival time could not be evaluated.

A limitation of the study is the small number of rats included in some of the treatment groups. Additionally, we did not have the possibility to directly monitor tDCS-induced changes in cortical activity, e.g. by electroencephalography or conventional angiography to directly visualize tDCS-induced effects on the vessel wall. Therefore, the findings of our study do not allow to formulate definite conclusions concerning the origin of the observed effects on cerebral vasospasm, which merits further evaluation in future experiments. Last but not least, a better understanding of tDCS-interactions with neurons, astrocytes and microglia cells might shed light on tDCS-effects on neurovascular communication in the setting of SAH.

Conclusion

TDCS has an influence on the incidence and severity of cerebral vasospasm in a SAH rat-model, whereby cathodal tDCS was associated with a reduced vasospasm incidence and severity. Future experiments are necessary to study the underlaying physiological mechanism, which would open the possibility to potentiate these effects with a subsequent risk reduction of SAH-induced ischemic lesions.

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Declaration of conflicting interests

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Authors' contributions

V.M. contributed to the conceptional planning of the experiments, worked out technical details, performed the largest part of the experiments and the analysis of the data, was involved in the interpretation of the results and wrote the manuscript with input of all authors. K.B. and B.I. performed a large number of the experiments. I.T. and M. N. P. contributed to the conceptional planning of the MRI-analysis and performed the vasospasm analysis based on MR-angiography. C.S. contributed to the interpretation of the results. V.R. was involved in the planning of the study and contributed to the manuscript drafting. D.M. mainly planned the study, received the external funding, supervised the work and gave input to the manuscript and data analysis. All authors discussed the results and contributed to the final manuscript.

Ethics approval

All applicable international, national and institutional guidelines for the care and use of animals were followed. All experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals of the NIH" and were ethically approved by the Government of Lower Saxony (AZ 13/1055).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All relevant data and materials are presented in the manuscript.

Code availability

Not applicable.

References

1. Nitsche MA and Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology* 2001; 57: 1899–1901.
2. Paulus W. Transcranial direct current stimulation (tDCS). *Suppl Clin Neurophysiol* 2003; 56: 249–254.
3. Fregni F, Boggio PS, Mansur CG, et al. Transcranial direct current stimulation of the unaffected hemisphere in stroke patients. *Neuroreport* 2005; 16: 1551–1555.
4. Hummel F, Celnik P, Giraux P, et al. Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain* 2005; 128: 490–499.
5. Kim SJ, Kim BK, Ko YJ, et al. Functional and histological changes after repeated transcranial direct current stimulation in rat stroke model. *J Korean Med Sci* 2010; 25: 1499–1505.
6. Wachter D, Wrede A, Schulz-Schaeffer W, et al. Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat. *Exp Neurol* 2011; 227: 322–327.
7. Peruzzotti-Jametti L, Cambiaghi M, Bacigaluppi M, et al. Safety and efficacy of transcranial direct current stimulation in acute experimental ischemic stroke. *Stroke* 2013; 44: 3166–3174.
8. Mielke D, Wrede A, Schulz-Schaeffer W, et al. Cathodal transcranial direct current stimulation induces regional, long-lasting reductions of cortical blood flow in rats. *Neurol Res* 2013; 35: 1029–1037.
9. Rinkel GJ and Algra A. Long-term outcomes of patients with aneurysmal subarachnoid hemorrhage. *Lancet Neurol* 2011; 10: 349–356.
10. Van Donkelaar CE, Bakker NA, Birks J, et al. Prediction of outcome after aneurysmal subarachnoid hemorrhage. *Stroke* 2019; 50: 837–844.
11. Rinkel GJ. Management of patients with aneurysmal subarachnoid hemorrhage. *Curr Opin Neurol* 2016; 29: 37–41.
12. Loan JJM, Wiggins AN and Brennan PM. Medically induced hypertension, hypervolaemia and haemodilution for the treatment and prophylaxis of vasospasm following aneurysmal subarachnoid hemorrhage: systematic review. *Br J Neurosurg* 2018; 32: 157–164.
13. Jabbarli R, Pierscianek D, Rölz R, et al. Endovascular treatment of cerebral vasospasm after subarachnoid hemorrhage: more is more. *Neurology* 2019; 93: e458–e466.
14. Roelz R, Scheiwe C, Coenen VA, et al. A novel rescue therapy for cerebral vasospasm: cisternal nimodipine application via stereotactic catheter ventriculocisternostomy. *J Clin Neurosci* 2019; 63: 244–248.
15. Li K, Barras CD, Chandra RV, et al. A review of the management of cerebral vasospasm after subarachnoid hemorrhage. *World Neurosurg* 2019; 126: 513–527.
16. Kilkenny C, Browne WJ, Cuthill IC, et al. Improving bioscience research reporting the ARRIVE guidelines for reporting animal research. *PLoS Biol* 2010; 8: e1000412.
17. Güresir E, Vasiliadis N, Dias S, et al. The effect of common carotis artery occlusion on delayed brain tissue damage in the rat double subarachnoid hemorrhage model. *Acta Neurochir* 2012; 154: 11–19.
18. Malinova V, Psychogios MN, Tsogkas I, et al. MR-angiography allows defining severity grades of cerebral vasospasm in an experimental double blood injection subarachnoid hemorrhage model in rats. *PLoS One* 2017; 12: e0171121.
19. Jeon H, Ai J, Sabri M, et al. Neurological and neurobehavioral assessment of experimental subarachnoid hemorrhage. *BMC Neurosci* 2009; 10: 103.
20. Bates D, Mächler M, Bolker B, et al. Fitting linear mixed-effects models using Lme4. *J Stat Soft* 2015; 67: 1–48.

21. Brooks ME, Kristensen K, van Benthem KJ, et al. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J* 2017; 9: 378–400.
22. Lüdecke D. Ggeffects: tidy data frames of marginal effects from regression models. *Joss* 2018; 3: 772.
23. R Core Team. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing, 2018.
24. Liebetanz D, Klinker F, Hering D, et al. Anticonvulsant effects of transcranial direct current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. *Epilepsia* 2006; 47: 1216–1224.
25. Manto M and Ben Taib NO. A novel approach for treating cerebellar ataxias. *Med Hypotheses* 2008; 71: 58–60.
26. Antal A, Kriener N, Lang N, et al. Cathodal transcranial direct current stimulation of the visual cortex in the prophylactic treatment of migraine. *Cephalalgia* 2011; 31: 820–828.
27. Nitsche MA, Cohen LG, Wassermann EM, et al. Transcranial direct current stimulation: state of the art 2008. *Brain Stimul* 2008; 1: 206–223.
28. Fox JL and Yasargil MG. The experimental effect of direct electrical current on intracranial arteries and the blood-brain barrier. *J Neurosurg* 1974; 41: 582–589.
29. Sehba FA. Rat endovascular perforation model. *Transl Stroke Res* 2014; 5: 660–668.
30. Vatter H, Weidauer S, Konczalla J, et al. Time course in the development of cerebral vasospasm after experimental subarachnoid hemorrhage: clinical and neuroradiological assessment of the rat double hemorrhage model. *Neurosurgery* 2006; 58: 1190–1197.
31. Mielke D, Bleuel K, Stadelmann C, et al. The ESAS-score: a histological severity grading system of subarachnoid hemorrhage using the modified double hemorrhage model in rats. *PLoS One* 2020; 15: e0227349.
32. Giorli E, Tognazzi S, Briscese L, et al. Transcranial direct current stimulation and cerebral vasomotor reserve: a study in healthy subjects. *J Neuroimaging* 2015; 25: 571–574.
33. Vernieri F, Assenza G, Maggio P, et al. Cortical neuromodulation modifies cerebral vasomotor reactivity. *Stroke* 2010; 41: 2087–2090.
34. Balbi M, Koide M, Wellman GC, et al. Inversion of neurovascular coupling after subarachnoid hemorrhage in vivo. *J Cereb Blood Flow Metab* 2017; 37: 3625–3634.
35. Balbi M, Vega MJ, Lourbopoulos A, et al. Long-term impairment of neurovascular coupling following experimental subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 2020; 40: 1193–1202.
36. Wellman GC and Koide M. Impact of subarachnoid hemorrhage on parenchymal arteriolar function. *Acta Neurochir Suppl* 2013; 115: 173–177.
37. Yamaguchi T, Moriya K, Tanabe S, et al. Transcranial direct-current stimulation combined with attention increases cortical excitability and improves motor learning in healthy volunteers. *J Neuroeng Rehabil* 2020; 17: 23.
38. Tazoe T, Endoh T, Kitamura T, et al. Polarity specific effects of transcranial direct current stimulation on interhemispheric inhibition. *PLoS One* 2014; 9: e114244.
39. Weyer V, Maros ME, Kronfeld A, et al. Longitudinal imaging and evaluation of SAH-associated cerebral large artery vasospasm in mice using micro-CT and angiography. *J Cereb Blood Flow Metab* 2020; 40: 2265–2277.