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Research Report
Electrophysiology and neuronal integrity following systemic arterial hypotension in a rat model of unilateral carotid artery occlusion[☆]
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ABSTRACT

Patients with carotid artery stenosis may be particularly susceptible to hypotension-associated cerebral ischemia and subsequent neurological sequelae. Measuring somatosensory evoked potentials (SEP), electroencephalogram (EEG), direct current (DC) potential, and histology, we compared the temporal evolution of cortical functional perturbations as well as neuronal integrity in a model of unilateral carotid artery occlusion and systemic hypobaric hypotension (HH) at the lower limit of cerebral blood flow autoregulation (50 mm Hg). Serial measurements of EEG power spectra as well as SEP-amplitudes and latencies of N10.3 were performed before, during, and up to 60 min after 30 min-HH ($n=7$) or the control condition ($n=7$) in male Wistar rats. In two additional groups (with [$n=7$] or without [$n=7$] HH), cortical spreading depressions (CSD) were elicited to ascertain their contribution to brain injury. Hematoxylin–Eosin (H&E) staining was used to assess neuronal cell death at 5 days after surgery. Relative to baseline, HH attenuated ipsilateral EEG power spectrum (by maximally 62%), increased SEP-latencies (by ~6–10%) and amplitudes (by ~57–70%), and induced selective neuronal cell death in the cerebral cortex and hippocampus ($P<0.05$ vs. contralateral). Spontaneous CSD occurred in ~30% of HH-animals. Repolarization of the DC-potential during HH was significantly prolonged relative to normotensive conditions (10.3 ± 11.5 min, $P<0.001$). Our model may help to understand underlying pathophysiology and improve outcome in a clinical subset of patients with carotid artery stenosis and transient systemic hypotension.

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1. Introduction

The pathophysiology of borderzone- or watershed-distribution strokes is not well understood. Accounting for less than 10% of all strokes in the general population, incidence rates

may be considerably higher in patients undergoing carotid endarterectomy (CEA) or carotid artery stenting (CAS) (Krul et al., 1989; Lovblad et al., 2000; Paciaroni et al., 2003; Yamamoto et al., 1999). Proposed mechanisms include hemodynamic compromise and/or artery-to-artery embolism (Krul et al.,

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1989; Momjian-Mayor and Baron, 2005). Furthermore, patients with carotid artery stenosis may have significantly impaired autoregulation of cerebral blood flow (White and Markus, 1997), which may partly explain the more frequent occurrence of borderzone distribution strokes in this patient population (Krul et al., 1989; Momjian-Mayor and Baron, 2005). In addition, hemodynamic depression that is unrelated to systemic hypovolemia, bleeding, or cardiac failure is well recognized to occur during CEA and CAS (Angell-James and Lumley, 1974; Gupta et al., 2006; Melgar et al., 2005; Tarlov et al., 1973) and has been shown to be associated with subsequent focal cerebral ischemia and neurological deficits (Dangas et al., 2000; Gold et al., 1995; Gottesman et al., 2006; Gupta et al., 2006).

To improve postoperative outcome, the degree of periprocedural brain ischemia is regularly assessed by monitoring brain function through electroencephalogram (EEG) or somatosensory evoked potentials (SEP), which are non-invasive, easily implemented in the operating room, and can provide nearly continuous measurement of brain function (Florence et al., 2004; Rowed et al., 2004; Zampella et al., 1991). However, using these techniques cerebral ischemia may remain undetected in a significant number of patients (Florence et al., 2004; Horsch et al., 1990). Hence, it is crucial to understand the underlying pathophysiology to develop novel treatment strategies to reduce the impact of periprocedural hypotension on brain integrity and function. However, only few experimental studies have ascertained the effects of systemic hypotension in combination with unilateral carotid artery occlusion on brain pathophysiology (Engelhard et al., 2004; Graham et al., 1990; Ishikawa et al., 2006; Mendelow et al., 1984). Furthermore, these studies induced severe hypotension (Engelhard et al., 2004; Graham et al., 1990; Ishikawa et al., 2006), which rarely occurs during CEA or CAS (Gupta et al., 2006; Melgar et al., 2005) and/or used only very brief observation periods (Ishikawa et al., 2006; Mendelow et al., 1984).

Employing EEG, SEP, and standard Hematoxylin–Eosin (H&E) staining, this study sought to specifically elucidate the impact of borderline (i.e., at the lower threshold for CBF autoregulation ([Gold et al., 1995; Heimann et al., 1994]) systemic hypotension in conjunction with unilateral common carotid artery occlusion (CCAO) on the brain's electrophysiology and neuronal integrity. In addition, the contribution of cortical spreading depression (CSD) was assessed as these spontaneously occurred during brain ischemia and may promote ischemic injury (Hossmann, 1996).

2. Results

2.1. Physiological variables

There were no statistical differences in blood gases (PaO₂, PaCO₂, and pH), glucose, or electrolytes before and after HH or between groups (data not shown). There were no differences in MABP between normotensive groups (Control and RSD) or between hypotensive groups (HYP and HYP-RSD), respectively. In normotensive groups, the MABP remained between 85 and 100 mm Hg throughout the study ($P > 0.05$). In contrast, MABP was lowered to 50 mm Hg in groups with HH ($P < 0.001$ vs. baseline) for 30 min. Following hypotension, significant

hypertension between 100 and 105 mm Hg for ~10 min was observed relative to baseline as well as groups without HH ($P < 0.001$). CSD-induction did not alter MABP.

2.2. Median nerve SEP

Fig. 1 shows a representative SEP recorded under baseline conditions. The first three positive deflections were labeled I, II (P4.4), and III. The assumed generator structures for these waveforms are of subcortical origin (Sakatani et al., 1990; Schwerdtfeger et al., 1999). They were followed by a positive–negative–positive waveform (P8, N10.3, and P14.6) resembling the first primary cortical waves (Sakatani et al., 1990). In some recordings, additional positivities (IV, V, and VI) could be detected. We opted to limit further investigations to N10.3 because it was (i) clearly detectable in all animals, (ii) has been found to be constantly affected by cortical ischemia (Sakatani et al., 1990), and (iii) waveforms preceding P8 or following N10.3 were only invariably present. Measurements are expressed as percent of the mean baseline value.

Fig. 2 summarizes the results from N10.3-latency measurements. In Control rats, peak latencies did not differ over time or between hemispheres (Fig. 2A). In HYP animals, N10.3-latencies of the occluded hemisphere increased significantly by ~6–10% relative to baseline during HH ($P < 0.01$, Fig. 2B). Ipsilaterally elicited CSD significantly increased the peak latency by ~13–23% in normotensive rats ($P < 0.05$, Fig. 2C). Combination of HH and CSD caused the most profound increases in N10.3-latency by ~24–60% ($P < 0.05$, Fig. 2D). Contralateral latencies remained unaffected in all investigated groups ($P > 0.05$ vs. baseline) with the exception of a nonsignificant latency increase in HYP-RSD rats.

Fig. 3 summarizes the results from N10.3-amplitude measurements. The N10.3-amplitude did not differ over time as well as between hemispheres in controls (Fig. 3A). Unexpectedly, during HH a significant increase in ipsilateral peak amplitudes was observed in HYP animals (by ~57–70%, $P < 0.05$, Fig. 3B). Amplitudes of the contralateral, open side did not differ from baseline values (Fig. 3B). In contrast, CSD significantly decreased ipsilateral amplitudes in RSD-animals to ~37–38% of baseline values (significant only from the 5 to 9,

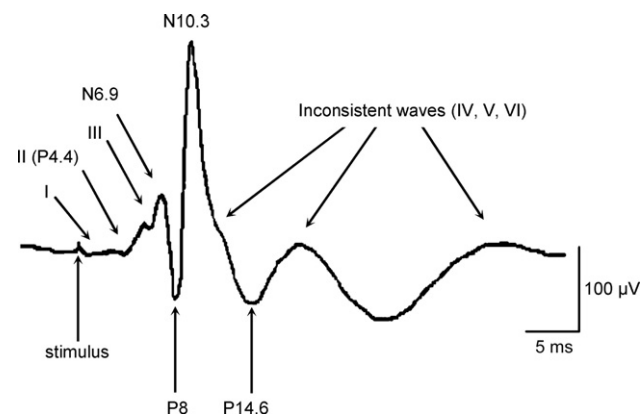


Fig. 1 – Representative median nerve SEP of one animal under baseline conditions. N10.3 was chosen for further serial investigations of its amplitude and latency.

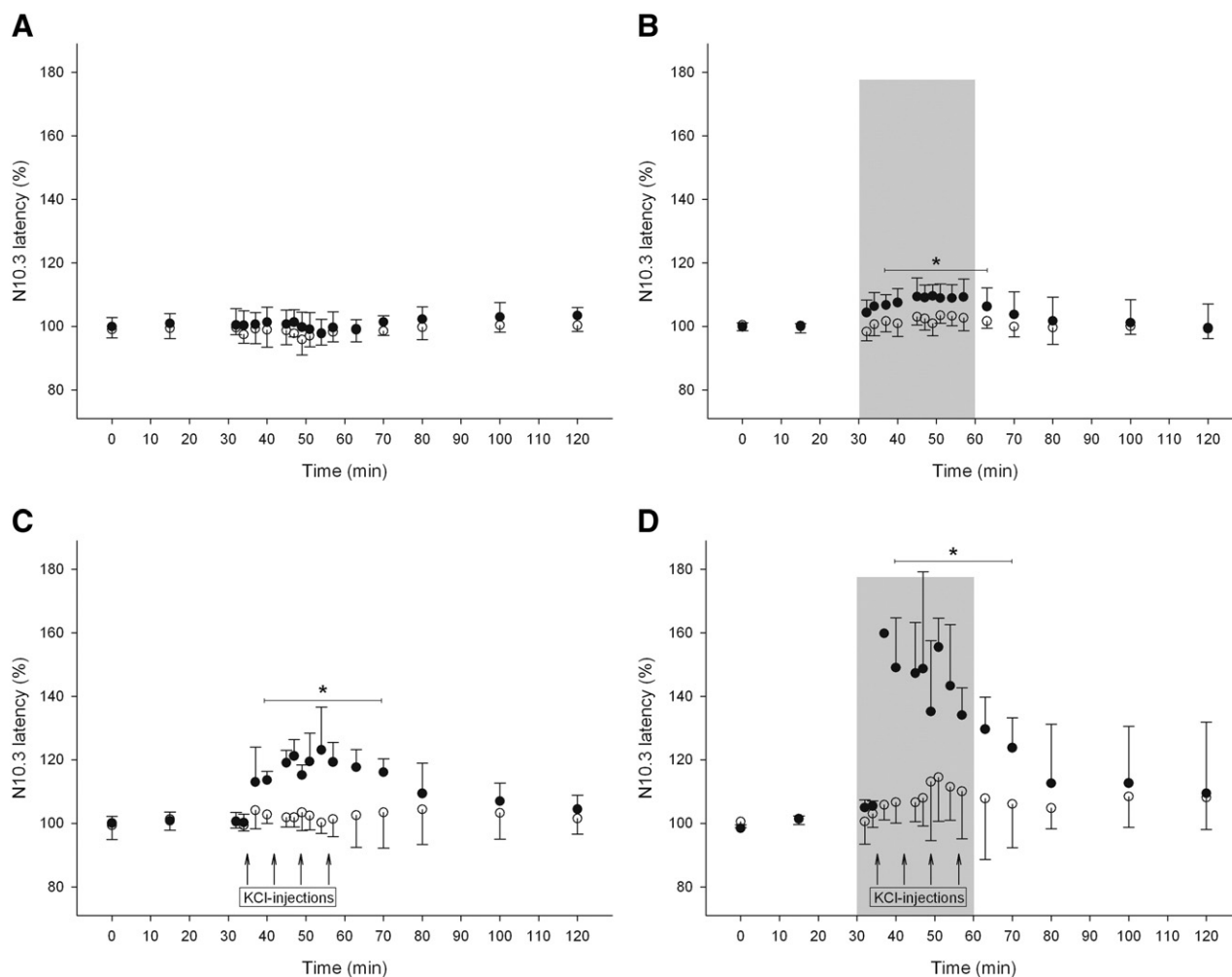


Fig. 2 – Temporal evolution of relative changes in N10.3-latencies of groups Control (A), hypobaric hypotension (HYP, B), repetitive spreading depressions (RSD, C), and systemic hypotension plus spreading depressions (HYP-RSD, D). Transient (30 min) hypotension to 50 mm Hg is indicated by the grey shaded area. Filled circles indicate ipsilateral, occluded side; open circles indicate contralateral, open side. Arrows indicate injection of potassium chloride (KCl). * $P < 0.05$ vs. baseline.

and 19 min, $P < 0.05$, Fig. 3C). Although contralateral amplitudes showed a tendency to increase, this was not statistically significant (Fig. 3C). Combination of HH and repetitive CSD reduced mean N10.3-amplitudes by ~65–98% ($P < 0.001$, Fig. 3D). However, in some animals, the SEP was completely abolished, corresponding to a reduction by 100% (data not shown). Furthermore, contralateral amplitudes significantly increased by ~70% ($P < 0.05$, Fig. 3D).

2.3. EEG

Fig. 4 summarizes the EEG results. In Control rats, no significant differences in power spectra were observed over time or between hemispheres (Fig. 4A). In HYP animals, however, ipsilateral EEG power spectra were significantly reduced (by maximal 62%) within 10 min after induction of HH and recovered within 5 min post HH ($P < 0.05$, Fig. 4B). In normotensive rats, CSD immediately diminished the power spectrum by 61% and values remained reduced by 12 min after the last CSD was elicited ($P < 0.05$, Fig. 4C). As observed with the SEP,

combination of HH and repetitive CSD caused the most profound EEG-alterations. The power spectrum was attenuated by 82% after the first CSD and values remained abnormal up to 70 min ($P < 0.05$, Fig. 4D). Contralateral EEG showed no significant changes in all investigated groups relative to baseline; however, nonsignificantly decreased power spectra were observed in HYP and HYP-RSD rats during HH (Figs. 4B and D).

2.4. DC-potential

Spontaneous spreading depressions occurred within 5 min after the start of HH in three HYP animals (they were replaced), two HYP-RSD-animals, and zero RSD-animals. Hence, 5 of 17 (29%) HH-rats had spontaneous CSD. Notably, spontaneous CSD did not have different characteristics than induced ones (data not shown). In RSD-animals, the DC-potential returned to baseline values within 2.5 ± 2.0 min (range 0.6–7.5 min) after each CSD. In contrast, in HYP-RSD-animals, the DC-potential remained abnormal for 10.3 ± 11.5 min (range 1.2–34.8 min) after each CSD ($P < 0.001$ vs. group RSD).

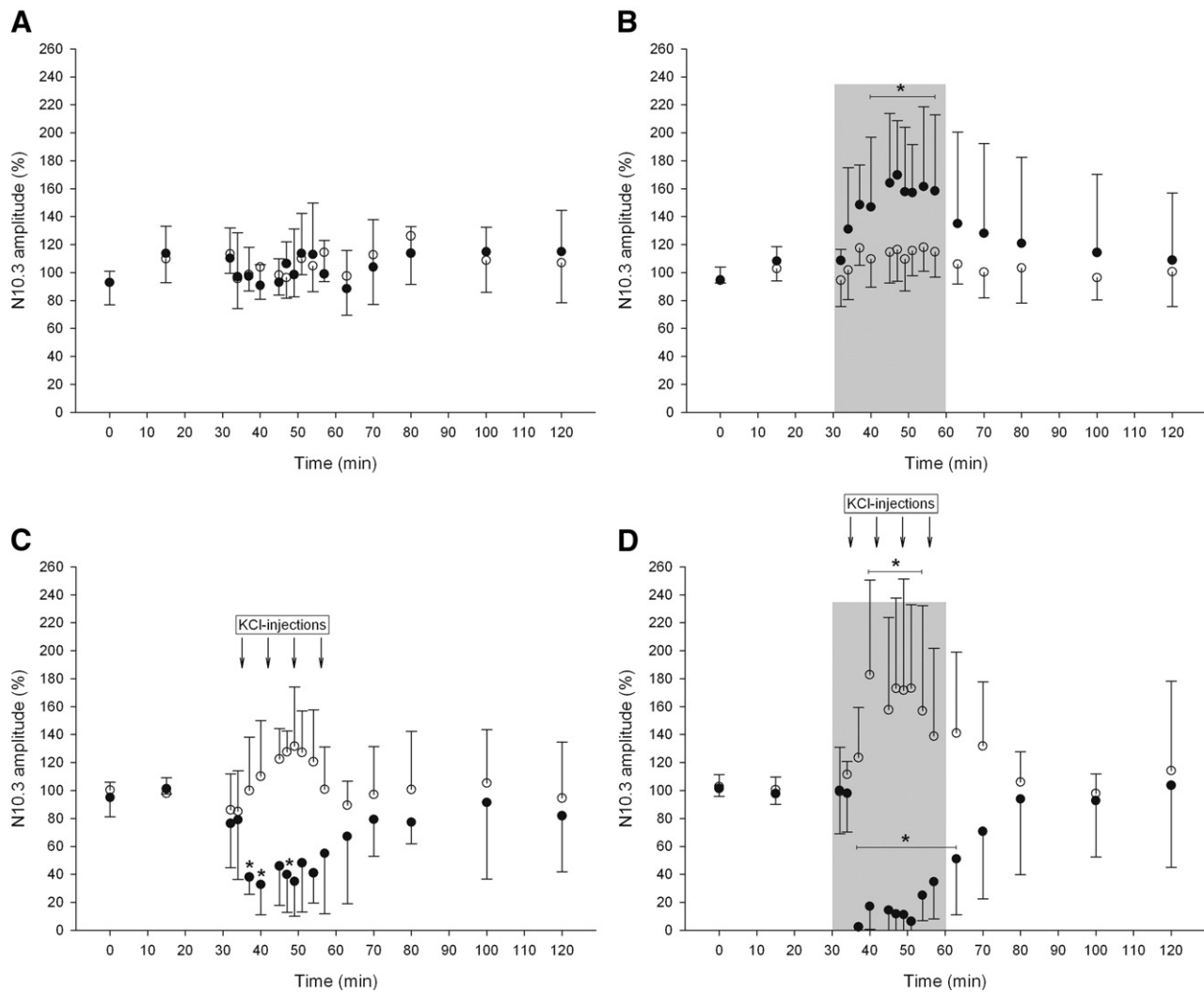


Fig. 3 – Temporal evolution of relative changes in N10.3-amplitudes of groups Control (A), hypobaric hypotension (HYP, B), repetitive spreading depressions (RSD, C), and systemic hypotension plus spreading depressions (HYP-RSD, D). Transient (30 min) hypotension to 50 mm Hg is indicated by the grey shaded area. Filled circles indicate ipsilateral, occluded side; open circles indicate contralateral, open side. Arrows indicate injection of potassium chloride (KCl). * $P < 0.05$ vs. baseline.

Fig. 5 summarizes the ipsilateral DC changes in the individual groups. Before the experiment was started, the DC-potential was set as 0.00 mV. The DC-potential measured in one animal of group Control was 0.00 ± 0.01 mV under baseline conditions and remained relatively stable throughout the experiment (Fig. 5A). In group HYP, the DC-potential almost immediately increased nonsignificantly to 0.18 ± 0.29 mV after induction of HH and did not recover completely until after 70 min (Fig. 5B). As expected, the DC-potential increased in group RSD significantly for 1–2 min (to ~ 0.2 – 0.3 mV) with each KCl-injection ($P < 0.05$, Fig. 5C). CSD elicited under HH-conditions (HYP-RSD group) significantly increased the DC-potential to ~ 0.6 mV, which did not recover until after the end of HH ($P < 0.05$, Fig. 5D).

2.5. Histology

Since the borderzone area between the territories of the main supplying brain vessels is most vulnerable to acute hypo-

tension below the threshold for autoregulation in the presence of preexisting extracranial vascular occlusion, we specifically sought to investigate cortical neuronal damage in the watershed zone between the middle cerebral artery (MCA) and anterior cerebral artery (ACA) territories (Hossmann, 2006; Momjian-Mayor and Baron, 2005; Scremin, 1995). In addition, we investigated neuronal integrity within the hippocampus because it is well known that this region is particularly sensitive to acute cerebral ischemia (Hossmann, 1998).

Table 1 summarizes the neuronal counts obtained from the defined ROIs in the cerebral cortex and hippocampus. There were no between-group differences in corresponding contralateral ROIs. Normotensive groups (Control and RSD) did not show a significant cell loss in the examined ROIs relative to contralateral, with the exception of Cortex3 in RSD rats ($P < 0.05$). In contrast, hypotensive groups (groups HYP and HYP-RSD) demonstrated significant reductions in cell numbers in all examined cortical ROIs as well as CA1 and CA3 ($P < 0.05$ vs. contralateral). Neuronal counts were reduced in

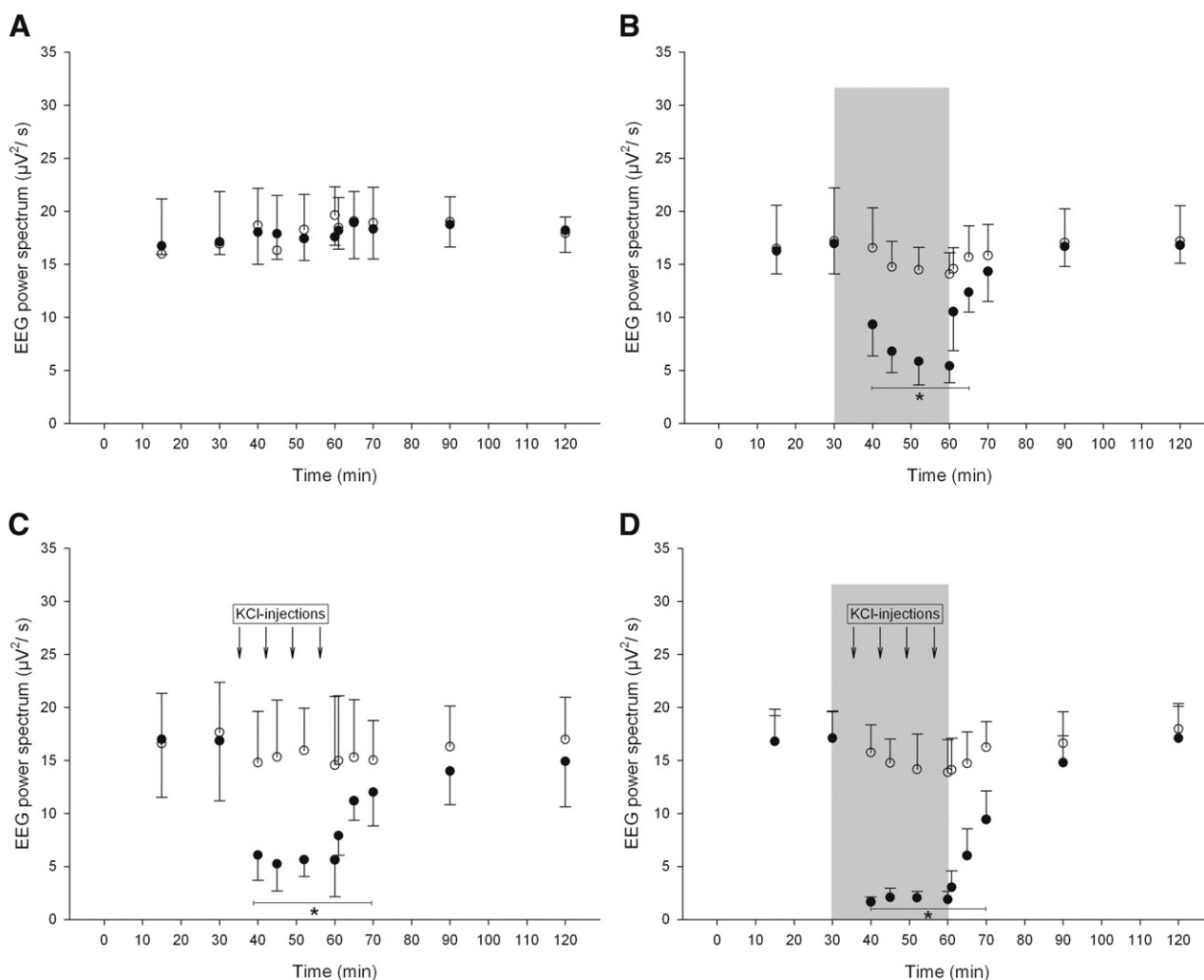


Fig. 4 – Temporal evolution of quantitative changes in the EEG power spectrum of groups Control (A), hypobaric hypotension (HYP, B), repetitive spreading depressions (RSD, C), and systemic hypotension plus spreading depressions (HYP-RSD, D). Transient (30 min) hypotension to 50 mm Hg is indicated by the grey shaded area. Filled circles indicate ipsilateral, occluded side; open circles indicate contralateral, open side. Arrows indicate injection of potassium chloride (KCl). * $P < 0.05$ vs. baseline.

the ipsilateral CA2, however, these changes were only significant in group HYP-RSD ($P < 0.05$ vs. contralateral). There were no differences in cell counts between respective normotensive and hypotensive groups, indicating that CSD did not contribute to neuronal cell death in the used model.

3. Discussion

The brain's ability to regulate its cerebral blood flow (CBF) has been shown to be significantly impaired in patients with unilateral carotid artery occlusion (White and Markus, 1997), which may partly explain the more frequent occurrence of borderzone distribution strokes in this patient population as well as their increased vulnerability to periprocedural hypotension (Dangas et al., 2000; Gold et al., 1995; Gottesman et al., 2006; Gupta et al., 2006; Krul et al., 1989; Momjian-Mayor and Baron, 2005). To assess the underlying pathophysiology, several animal models have been introduced. For example, Ishikawa et al. (2006) demonstrated ipsilateral infarction of the

MCA-territory with severe (35 mm Hg) hypotension and unilateral CCAO and, using less severe hypotension (40–50 mm Hg), Mendelow et al. (1984) revealed selective ipsilateral ischemic cell death in the neocortex and hippocampus. However, these models have the common disadvantage that they used hemorrhagic hypotension, which rarely occurs during procedures such as CEA or CAS. Furthermore, this technique may not allow for exact compensation of blood pressure fluctuations and may cause trauma to blood cells, which may have confounding effects. In addition, both experiments were terminated after only 2 (Mendelow et al., 1984) and 8 h (Ishikawa et al., 2006), respectively, which may underestimate eventual lesion extent (Li et al., 1999). Lastly, these studies did not ascertain SEP and EEG (Ishikawa et al., 2006; Mendelow et al., 1984), which are widely recognized as powerful tools for early detection and evaluation of periprocedural cerebral ischemia (Florence et al., 2004).

It would be desirable to investigate the effect of hypotension in conjunction with unilateral CCAO on these clinically commonly studied parameters. Understanding the pattern of

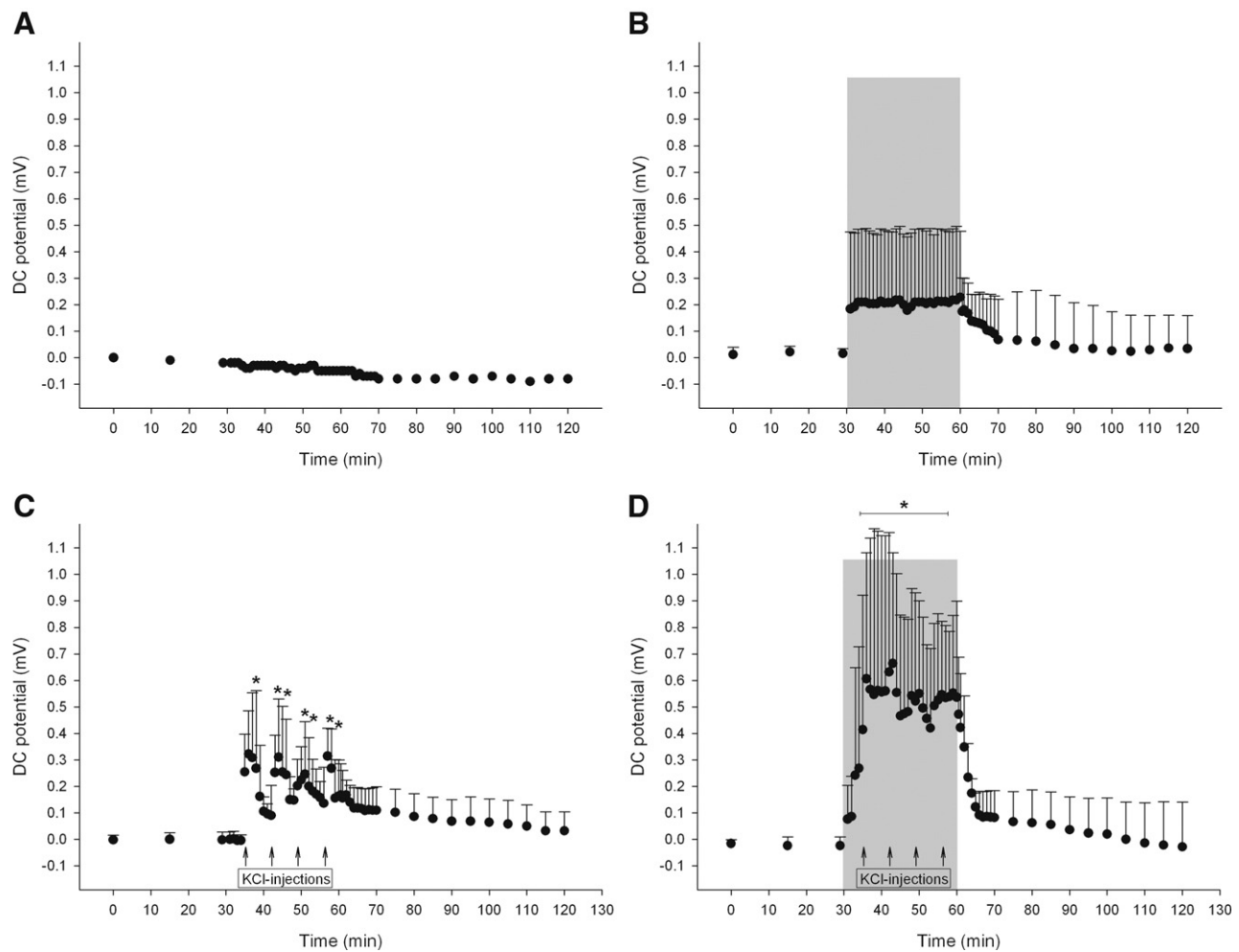


Fig. 5 – Temporal evolution of quantitative changes in the ipsilateral DC-potential of groups Control (A), hypobaric hypotension (HYP, B), repetitive spreading depressions (RSD, C), and systemic hypotension plus spreading depressions (HYP-RSD, D). Transient (30 min) hypotension to 50 mm Hg is indicated by the grey shaded area. Arrows indicate injection of potassium chloride (KCl). * $P < 0.05$ vs. baseline.

electrophysiological alterations may enhance our ability to increase patient safety and develop novel treatment strategies to mitigate the impact of periprocedural hypotension on brain integrity and function.

To overcome the previously mentioned limitations, we employed hypobaric hypotension at the lower limit of cerebral autoregulation in conjunction with unilateral CCAO as previously described (Kempinski et al., 1999). Using this highly controlled, noninvasive rat model, which allows for simulation of critical components of human periprocedural hypotension, we sought to elucidate acute electrophysiological alterations and chronic neural integrity. We specifically tracked changes in cortical SEP, EEG, and DC as well as quantified neuronal death in the cortex and hippocampus. In addition, the impact of concomitantly occurring cortical spreading depressions was studied. The major results of this study were: (i) Contralateral SEP, EEG, and neuronal survival remained unaffected by hypotension at the lower limit of autoregulation. (ii) Hypotension produced ipsilateral changes in EEG and SEP-latency similar to those frequently observed clinically with the exception of unexpectedly increased SEP-

amplitudes (Florence et al., 2004). (iii) Selective cell death was seen exclusive to ipsilateral ROIs. (iv) Though cortical spreading depressions occurred spontaneously in ~30% of hypotensive animals, they did not promote selective neuronal cell death.

Transient hypotension exclusively perturbed SEP and EEG and induced selective neuronal cell death in the hemisphere ipsilateral to CCAO indicating that our model is relevant for some human hemodynamic strokes with respect to the constellation of ischemic pathophysiological findings (Dangas et al., 2000; Gold et al., 1995; Gottesman et al., 2006; Gupta et al., 2006; Krul et al., 1989; Momjian-Mayor and Baron, 2005). Our findings corroborate the notion that CBF-dysfunction in patients with carotid artery disease can occur at CBF-levels that are not within a clearly ischemic range (Minhas et al., 2004; White and Markus, 1997) and our data may help to understand underlying pathophysiology and improve periprocedural monitoring. For example, it may be speculated that periprocedural hypotension can induce pathology not only in the cerebral cortex – which is routinely assessed by EEG or SEP – but also in more distant areas such as the hippocampus.

Table 1 – Histology

ROI	Side	Experimental group			
		Control	HYP	RSD	HYP-RSD
Cortex1	Contralateral	1563±227	1357±233	1456±336	1488±213
	Ipsilateral	1567±644	936±288*†	1272±207	991±337*†
Cortex2	Contralateral	1107±326	1029±488	1106±196	1157±241
	Ipsilateral	1043±213	703±267*	972±338	839±117*
Cortex3	Contralateral	905±210	869±229	890±179	904±314
	Ipsilateral	926±170	660±212*	774±166*	694±136*
Cortex4	Contralateral	954±219	956±417	950±197	937±151
	Ipsilateral	820±150	755±459*	950±247	673±198*
CA1	Contralateral	2589±410	2333±286	2410±234	2499±282
	Ipsilateral	2515±521	1960±371*†	2437±309	1935±328*†
CA2	Contralateral	2188±499	2095±475	2100±725	2142±460
	Ipsilateral	2114±560	1673±411	1988±609	1507±181*
CA3	Contralateral	1604±425	1602±268	1573±288	1624±295
	Ipsilateral	1663±535	1247±262*	1567±269	1330±295*

Neuronal cell counts in investigated regions of interest (ROI) 5 days after the experiment. Note that cortical ROIs were positioned within the MCA-ACA borderzone to assess neuronal damage. Contralateral numbers of non-intact neurons did not differ significantly between groups. Scattered non-intact neurons constituted a statistically insignificant number of cells in both hemispheres of Control and RSD-rats as well as the contralateral hemisphere of HYP and HYP-RSD rats and were likely artifacts resulting from removal and histological preparation/fixation of brain tissue. HYP=hypobaric hypotension, RSD=repentive spreading depressions. Values are mean±S.D. *P<0.05 vs. contralateral; †P<0.05 vs. control.

Indeed, the herein observed selective neuronal cell loss in the hippocampus, which plays a critical role in learning and memory formation, may help explain the reported high incidence of postprocedural neuropsychological deficits in the absence of focal neurological deficits in patients undergoing CEA or CAS (Brinkman et al., 1984; Cushman et al., 1984; Heyer et al., 1998, 2002; Jacobs et al., 1983; Lind et al., 1993; Vanninen et al., 1996). Furthermore, ipsilateral SEP-amplitudes increased during hypotension, which may indicate that there is no linear correlation between amplitude changes and ischemia at borderline blood pressure levels. Hence mild, yet critical cerebral hypoperfusion may go undetected if the significance of ischemia is solely based on amplitude decreases in experimental (Sakatani et al., 1990; Wagener et al., 1990) as well as clinical studies (Florence et al., 2004). A plausible explanation for these results is that inhibitory systems are more sensitive to ischemic stress than excitatory systems and cease function at relatively higher CBF values, leading to disinhibition of cortical electric activity (Wang et al., 1995).

CSD consistently diminished ipsilateral EEG and median nerve SEP (i.e., increased latency and decreased amplitude of the N10.3 peak). These results are in line with the observation that CSD render neurons transiently unresponsive, resulting in suppression of cortical electric activity (Hossmann, 1996; Mun-Bryce et al., 2001; Somjen, 2001). In accord with previous studies, ionic homeostasis and thus electrical activity were partially restored in normotensive animals following each CSD as indicated by the incomplete, yet significant, recovery of SEP-amplitudes (Somjen, 2001). Conversely, CSD under hypotensive condition induced prolonged and severe suppression of cortical activity, without significant recovery during the hypotension period. This can be explained by reduced hemodynamic capacity of the collateral system, which prevented adequate coupling of blood supply to the metabolic needs of the tissue resulting in

recalcitrant restoration of the membrane potential (Hossmann, 2006; Somjen, 2001).

Interestingly, contralateral SEP-amplitudes increased after CSD, which may have been the result of transhemispheric disinhibition caused by cerebral diaschisis (Buchkremer-Ratzmann et al., 1996). Whereas it has been shown that focal ipsilateral lesions increase contralesional excitability, which may serve as an endogenous source of stimulation to counteract contralateral loss of function (Buchkremer-Ratzmann et al., 1996), we show, to the best of our knowledge, for the first time that global ipsilateral oligemia can produce contralateral hyperexcitability, corroborating the notion that transcortical diaschisis may be a common consequence after different modes of brain ischemia (Reinecke et al., 1999). Hence, our model may help explain recovery processes in a relevant clinical scenario similar to those observed after focal cerebral ischemia in patients (Koerner and Meinck, 2004).

In contrast to the ischemic brain, in which CSD promote neuronal injury (Hossmann, 1996; Somjen, 2001), CSD can be provoked many times in the normal brain without obvious harm (Nedergaard and Hansen, 1988). Conversely, remote neuronal damage in the hippocampus was observed after CCAO with superimposed CSD (Iijima et al., 1998). Based on the findings that CBF could decrease in the striatum and thalamus ipsilateral to the CSD (Duckrow, 1991), the authors hypothesized that restricted blood flow may have promoted the observed remote injury (Iijima et al., 1998). However, since Iijima et al. (1998) did not assess blood flow in the hippocampus, which possesses a significantly different blood supply from that of the striatum and thalamus (Coyle, 1976; Scremin, 1995), it remains to be shown that their observation of hippocampal cell damage was indeed a result of CBF-alterations. In contrast to our experiment, the former study elicited CSD for 7 days (Iijima et al., 1998). However, the hippocampus is particularly susceptible to metabolic compromise and spreading depressions (Somjen, 2001), which, together with

the recent finding of hippocampal depolarization after CSD (Henning et al., 2005), suggests an alternate explanation: in contrast to brief (minutes to hours) very prolonged (days), CSD-induction could induce hippocampal damage due to the pronounced metabolic demands that accompany depolarization. Hence, our findings of normal neuronal cell counts in group RSD corroborate the notion that CSD do not readily induce neuronal cell death and further indicate that unilateral CCAO under normotensive conditions does not produce significant hemodynamic compromise in the used model.

Though we observed spontaneous CSD during hypotension similar to previous observations by Trabold et al. (2006), our results are surprising and in contrast to their data in that CSD did not negatively affect neuronal survival in our study. However, this apparent contradiction is consistent with existing data on spreading depressions. For example, Trabold et al. (2006) used a more severe model that combined systemic hypotension with a focal cortical lesion. This is insofar an important distinction because it is well established that CSD contribute to the growth of focal cerebral lesions (Hossmann, 1996; Somjen, 2001); however, they do not seem to stringently induce significant neuronal necrosis in states of compromised oxygen or glucose supply without focal injury (Gido et al., 1994a,b). Alternately, it is of note that under certain conditions (photothrombotic focal cerebral ischemia), CSD induce vasodilation in the ischemic brain and improve collateral flow, which subsequently translates to reduced neuronal damage (Buresh et al., 1999; Somjen, 2001). Since we positioned cortical ROIs in watershed areas, it is possible that some CSD-associated improvement in collateral flow occurred, which offsets the otherwise negative CSD-effects on neuronal integrity in this region. However, this hypothesis remains speculative as we did not directly ascertain CBF in these areas and future studies are needed to investigate this intriguing phenomenon. Regardless, our data clearly demonstrate that CSD, though significantly altering cortical electrophysiology, do not necessarily contribute to neuronal death in the studied scenario and developing neuroprotection strategies to foremost mitigate CSD-effects may not be warranted.

It is noteworthy that unilateral CCAO in the used animal model was acute, while in patients carotid artery stenosis usually occurs over a significantly longer period of time allowing for the development of collaterals. However, rats are particularly resistant to cerebral ischemia and chronic hypoperfusion usually requires bilateral CCAO with or without vertebral artery occlusion (Sarti et al., 2002), which is not always present in patients and would also have made it impossible to use one hemisphere as in internal control. In addition, hemodynamic depression with subsequent significant ischemia is well recognized to occur acutely during CEA and CAS (Gupta et al., 2006; Melgar et al., 2005) and hence our approach is a fair approximation of at least some pathophysiological events reported from clinical studies. Secondly, due to space limitation we could not ascertain CBF in this study as our setup with multiple electrodes did not allow for placement of a laser Doppler probe. Nonetheless, we have demonstrated that in the used model CBF is ipsilaterally reduced to values corresponding to the lower threshold for autoregulation (Dirnagl et al., 1993; Heimann et al., 1994). Importantly, to account for this limitation, we used the same setup as

reported (e.g., same rat strain, anesthetic protocol, surgical approach) allowing for valid interpretation of the herein obtained results (Heimann et al., 1994). Lastly, though our results give new insight in the pathophysiology associated with unilateral CCAO and transient systemic hypotension, no behavioral testing was performed. Measuring functional outcome is a relevant and necessary means to assess the consequences of cerebral ischemia. Hence, future studies would benefit from inclusion of neurological scoring to determine the functional significance(s) of the observations reported herein.

4. Experimental procedures

4.1. Animal preparation

Experiments were carried out according to the German animal protection legislation (Bezirksregierung Rheinhessen-Pfalz, Germany). Male Wistar rats ($n=31$, 315 ± 45 g, Charles River, Germany) were housed in individual cages and allowed free access to food and water before and after surgery. After premedication with 0.5 mg atropine, animals were anesthetized by intraperitoneal injection of chloral hydrate (36 mg/100 g). Thereafter, anesthesia was maintained by chloral hydrate (3.6 mg/100 g) given through a peritoneal PE-50 tube at 20, 80, and 120 min. Rats were orotracheally intubated and ventilated with 70% nitrogen/30% oxygen using a rodent ventilator (AP-10, Effenberger, Pfaffing/Attel, Germany) under control of end expiratory PaCO_2 (Artema MM206C, Heyer, Sweden). Body temperature was monitored continuously during anesthesia with a rectal probe and maintained at 36.0 ± 0.5 °C with a thermostatically controlled heating pad. PE-50 polyethylene tubing was inserted into the common carotid artery for functional, permanent occlusion of this vessel, monitoring of mean arterial blood pressure (MABP), and for obtaining blood samples to measure blood gases (pH, PaO_2 , PaCO_2) as well as electrolytes at 0, 25, 65, and 105 min. No blood samples were obtained between 30 to 60 min to avoid blood pressure fluctuations. Rats were mounted in a stereotaxic frame (Stoelting, Wood Dale, IL, USA) and a right frontoparietal cranial window, with the dura left intact, was made for access to the brain surface using a high-speed drill under continuous cooling of the drill tip with saline to avoid thermal injury to the cortex.

4.2. Experimental groups and protocol

After right CCAO, animals were randomly assigned to either control (CON, $n=7$) or 30 min hypotension (HYP, $n=7$). Three animals randomized into group HYP showed spontaneous cortical spreading depressions during HH and were replaced. However, this observation prompted us to investigate two additional groups in which repetitive spreading depression was elicited at predefined time points without (RSD, $n=7$) or with hypotension RSD (HYP-RSD, $n=7$). After a baseline period of 30 min, HH (groups HYP and HYP-RSD) or largely normal MABP (groups Control and RSD) was sustained from 30 to 60 min, followed by normal MABP in all groups up to 120 min.

4.3. DC-potential measurement

The DC-potential electrode was made of a glass micropipette (GB150F-10, Science Products GmbH, Hofheim, Germany) using a micropipette puller (Flaming/Brown Model P-97, Sutter Instrument, USA). A silver/silver chloride (Ag/AgCl) wire was inserted into the saline filled micropipette and the latter injected into the cortex (0.2–0.4 mm depth). The potential between this electrode and an Ag/AgCl electrode placed in the neck muscle was recorded during the experiment. For grounding the stereotaxic frame was used. The signal was fed to a DC amplifier (Gould, Inc., Cleveland, OH, USA) and recorded on a chart recorder (BBC, Goerz Metrawatt, Germany). Ten minutes of a stable potential was allowed after DC-potential electrode insertion, before the potassium chloride (KCl)-injector (see below) was inserted and the experiment started.

4.4. SEP and EEG recording

For SEP-recordings, the median nerves were stimulated at the paws via subdermal needles. Supramaximal stimulation was achieved with 3.0 mA with an impulse duration of 0.05 ms. Records opposite the side of stimulation were taken from ball shaped Ag/AgCl electrodes (diameter of 1.0 mm) placed in small depressions drilled 3.0 mm lateral to the Bregma on the intact skull. The reference electrode was placed 13.0 mm anterior and 1 mm left to the Bregma. For optimized contact between bone and electrodes, recording gel (Elefix, Nihon Kohden, Tokyo, Japan) was used. Signals were amplified and further processed after analogue-to-digital conversion. Stimulus frequency was 1/s and analogue filters were set to a bandpass of 1 to 500 Hz. Both sides were recorded sequentially but continuously, this means 20 consecutive sweeps with the stimulation of one median nerve were averaged, then stimulation switched to the other side, thereafter the cycle was restarted. Thus, for each side an SEP could be obtained every 4 min. Peak identification, measurement of peak latencies, and amplitudes were carried out manually using Neuropack II software (Nihon Kohden, Tokyo, Japan). For EEG-recordings the same montages as for the SEP-records were used. Epochs of 10 s were amplified and after analogue-to-digital conversion, they were Fourier-transformed (Dasy Lab 3.00 for Windows, Datalog GmbH, Mönchengladbach, Germany). Six epochs were averaged and power spectra ($\mu V^2/s$) were calculated.

4.5. Induction of hypobaric hypotension

The protocol for hypobaric hypotension has been described previously (Heimann et al., 1994; Kempinski et al., 1999) and allows for exact reduction of arterial blood pressure to any desired level below the physiological MABP (Heimann et al., 1994; Soehle et al., 1998). Briefly, the lower portion of the animal was placed in a sealable chamber connected to an electronically controlled vacuum pump, allowing to reduce the barometric pressure down to -30 cm H₂O. Thereby, causing a pooling of venous blood in the lower body portion of the rat the arterial pressure could be fine-tuned to 50 ± 2 mm Hg, which corresponds to the lower threshold for CBF

autoregulation in this model (Dirnagl et al., 1993; Heimann et al., 1994). In order to study the effects of MABP reduction without CCAO, the left cortex was studied. Similar to previously published clinical hypoperfusion times (Melgar et al., 2005), we investigated 30 min of systemic hypotension.

4.6. Induction of cortical spreading depressions

The KCl-injector was connected to a microinjection pump (CMA/100, Carnegie Medicine, Stockholm, Sweden), placed at equal distance to the SEP/EEG-electrodes, and inserted into the right lateral parietal cortex. Starting at 35 min (i.e., 5 min after induction of hypotension or control condition), four KCl-injections (150 mmol/L, 7.0 μ L) were given into the cortex at 7 min intervals unless the DC-potential did not return to baseline values, in which case further injections were forgone. In a preliminary experiment (data not shown) introduction of the KCl-injector as well as injection of vehicle (saline) into the cerebral cortex induced at least one spreading depression in most animals. Therefore, no additional control group for vehicle injection was investigated. Note that the DC-potential was measured in only one control rat. Lastly, if injection of KCl failed to induce a spreading depression at any prespecified timepoint, the animal was replaced.

4.7. Histological evaluation

On the fifth day after operation, animals were deeply anesthetized and perfused through the ascending aorta with saline for 10 min and then with ice cold phosphate-buffered 4% paraformaldehyde (PFA) for 10 min. Brains were removed from the cranium, postfixed overnight in the same fixative, and then embedded in paraffin to obtain coronal sections of the parietal region. Sections were stained with H&E. The histological evaluation was performed in one 15 μ m-thick section by light microscopy. A CCD camera (Sony, Tokyo, Japan) and a Maxigen Genlock interface (Merkens EDV, Bad Schwalbach, Germany) were used to project microscopic images onto the screen of an Amiga 2000 computer (Commodore, Braunschweig, Germany). The image analysis system was calibrated with a microscope ruler (Ernst Leitz, Wetzlar, Germany). For quantitative assessment of brain injury, cells were counted in 7 nonoverlapping regions of interest (ROI) in the hippocampus (CA1, CA2, and CA3) and cortex (Cortex1 to Cortex4) as previously described in detail (Soehle et al., 1998). The investigated hippocampal areas were 0.023 mm² (CA1 to CA3) and the cortical areas were 0.065 mm², respectively. Neurons were classified as necrotic when they exhibited pyknosis, karyorrhexis, karyolysis, cytoplasmic eosinophilia ("red neuron"), or loss of affinity for hematoxylin ("ghost neuron") (Li et al., 2000).

4.8. Statistical analysis

Data are expressed as means \pm S.D. Sequential changes within the groups were statistically evaluated using repeated measures analysis of variance (RM-ANOVA) with post hoc Bonferroni or Dunn's test, where appropriate. One-way ANOVA with post hoc Holm-Sidak test was used to study between group differences in neuronal counts (Sigma-Stat;

Jandel Scientific, Erkrath, Germany). $P < 0.05$ was considered significant.

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