Neuroscience 133 (2005) 571–581

TETANUS-INDUCED RE-ACTIVATION OF EVOKED SPIKING IN THE POST-ISCHEMIC DENTATE GYRUS

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Abstract—This study aimed at investigating and influencing the basic electrophysiological functions and neuronal plasticity in the dentate gyrus in freely moving rats at several time-points after global ischemia. Although neuronal death was induced selectively in the cornu ammonis, subfield 1 (CA1)-region of the hippocampus, we found an additional loss of the population spike in the dentate gyrus after stimulation of the perforant path. Input/output-measurements revealed that as early as 1 day post-ischemia population spike generation in the granular cell layer is greatly decreased when compared with pre-ischemic values and to sham-operated animals, despite an apparently intact morphology of granular cells as evidenced by Nissl-staining. In contrast, the synaptic transmission (excitatory postsynaptic field potential) shows no significant difference when comparing values before and after ischemia and ischemic and sham-operated animals. Despite reduced output function, indicated by very small population spike amplitudes, long lasting potentiation can be induced 10 days after ischemia. Surprisingly, even "silent" populations of neurons, which appear selectively post-ischemia and do not show any evoked population spike, can be re-activated by tetanisation which is followed by a normal appearing long-term potentiation. However, this functional recovery seems to be partial and transient under current conditions: population spike-values do not reach pre-ischemic values and return to the low pre-tetanic baseline values the next day. Electrophysiological measurements ex vivo after ischemia indicate that the neuronal dysfunction in the dentate gyrus is not due to locally destroyed structures but that the activity of granular cells is merely suppressed only under *in vivo* conditions.

In summary, global ischemia leaves a neighboring morphologically intact input area, functionally impaired. However, neuronal function can be partially regenerated by electrophysiological tetanic stimulation. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: hippocampus, degeneration, functional regeneration, electrophysiology, plasticity.

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Abbreviations: ACSF, artificial cerebrospinal fluid; CA1, cornu ammonis, subfield 1; DG, dentate gyrus; fEPSP, excitatory postsynaptic field potential; iNOS, inducible nitric oxide synthase; I/O, input/output; LTP, long-term potentiation; NO, nitric oxide; PS, population spike; 2-VO, two-vessel occlusion; 4-VO, four-vessel occlusion.

0306-4522/05$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.02.044

Although the pharmacology and pathophysiology of cerebral ischemia have been investigated for decades, knowledge about the pathological mechanisms is still insufficient and only a few successful therapeutic strategies have emerged (e.g. Dirnagl et al., 1993; Hata et al., 2000; Henrich-Noack et al., 2001; Krieglstein, 2002; Tang et al., 2002). The discrepancy between experimental findings and subsequent clinical results may, in part, result from the almost exclusive reliance of many experimental studies on histological parameters to assess protection/regeneration. Clinically, however, the ultimate parameter is not the histological but the functional recovery from ischemia-induced deficits. Behavioral studies have shown that histological and functional criteria do not always match (Kawamata et al., 1996; Hunter et al., 1998; DeVries et al., 2001). In the current study we therefore evaluated both histological appearance and neuronal function and tried to influence these by applying electrical stimulation/tetanisation.

To date, electrophysiological characterizations of neuronal damage have been performed primarily *in vitro* or ex vivo (Hori and Carpenter, 1994; Jensen et al., 1991; Mittmann and Eysel, 2001; Neumann-Haefelin and Witte, 2000; Opitz et al., 1995; Sabelhaus et al., 2000; Shinno et al., 1997; Urban et al., 1989). However, to study the pathophysiology of ischemia it is essential to have *in vivo* data, since, in some cases, artificial *in vitro* conditions may have a significant influence on the parameters evaluated. This may result, for example, from traumatic and ischemic stimuli during decapitation, the temperature, constant ion milieu, and the presence/absence of neurotrophic factors (McManus et al., 2004; Richerson and Messer, 1995; Danzer et al., 2004; Whittingham et al., 1984). As far as we are aware, data from *in vivo* experiments combining ischemia and electrophysiology in animals with chronically implanted electrodes are quite rare although this technique provides the unique advantage of measurements in awake, freely moving animals and direct comparison of data from before and after ischemia. In two pioneering papers (Buzsàki et al., 1989; Suyama, 1992), data must still be considered fragmentary because of the lack of statistics and because the results of the two studies are in part contradictory. Suyama (1992) showed the enhancement of recurrent inhibition whereas Buzsàki et al. (1989) observed an impairment of paired pulse depression.

Although delayed neuronal death occurs selectively in the cornu ammonis, subfield 1 (CA1)-region of the hippocampus after global ischemia, the dentate gyrus (DG) as a part of the trisynaptic cascade is said to be involved in mediating excitotoxic damage (Diemer et al., 1993; Johansen et al., 1986) and is therefore an interesting structure to

investigate. Moreover, although neurons of the target structure CA1 die a few days after induction of ischemia, for future regenerative strategies (Nakatomi et al., 2002) it is important to know whether the DG is functionally intact.

EXPERIMENTAL PROCEDURES

Electrophysiological preparations

The implantation of the electrodes was performed as described by Manahan-Vaughan and Reymann (1996). Male Wistar rats (250–300 g at the time of delivery; Harlan-Winkelmann, Germany) were used. The animals were fed with laboratory chow (Altromin, Lage, Germany) and water *ad libitum* and maintained in a thermoregulated environment (22 °C) during a 12-h light/dark cycle. Animals were anesthetized with sodium pentobarbitone (40–50 mg/kg, Sigma) and placed in a stereotaxic frame (Stoelting, IL, USA). A scalp incision of approximately 1 cm in length was made from a point between the eyes, along the midline toward the back of the skull. The periosteum was then scraped off the skull and the surface was cleaned with 3% hydrogen peroxide (Merck, Darmstadt, Germany). Two stainless steel screws (1.5 mm diameter; Optotec, Rathenow, Germany), to which pin-socket connectors were subsequently attached, were inserted contralaterally to electrodes in the skull via a drill hole without piercing the dura. One served as the ground screw electrode and the other was used as the reference electrode. A monopolar recording electrode and a bipolar stimulating electrode were made from an isolated stainless-steel wire (0.1 mm diameter). The free ends of the electrodes were passed through a rubber pin-socket connector attached to an oscilloscope (HAMEG GmbH, Frankfurt/Main, Germany) and stimulator (Science Products, Frankfurt/Main, Germany), respectively. Drill holes were made for the recording electrode (2.8 mm posterior to bregma and 1.8 mm lateral to midline) and for stimulating electrodes (6.9 mm posterior to bregma and 4.1 mm lateral from midline). The dura was pierced through both holes and the recording and stimulating electrodes were lowered into the granular layer of the DG region and the perforant path, respectively. To assure that the electrodes were placed correctly in the hippocampus to allow recordings from the DG, their location was carefully monitored throughout the process of electrode implantation. Once verification of position of the electrodes was completed, the entire assembly was sealed and fixed to the skull with dental cement (Heraeus Kuelzer, Hanau, Germany). The animals were allowed to recover at least 8 days before electrophysiological testing started. Throughout the experiments the animals could move freely, being contained in purpose-designed boxes (40×40×40 cm), with the implanted electrodes connected by a flexible cable to a stimulation unit and an amplifier. Evoked potentials were displayed and analyzed via a PC.

Global ischemia

At least 14 days after electrode implantation we induced transient global ischemia under normothermic conditions by two-vessel occlusion combined with systemic hypotension as described by Dirnagl et al. (1993). After initial anesthesia with 3–4% halothane (Sigma), the rats were intubated and connected to a small animal ventilator (Sachs Electronic, March, Germany) that delivered 1% halothane in a nitrous oxide/oxygen mixture. Both common carotid arteries were isolated and the tail artery was cannulated (tube rinsed with 50 IU heparin; Ratiopharm, Ulm, Germany) for measurement of blood gases (Radiometer, Copenhagen, Denmark) and blood pressure (Foehr Medical Instruments, Seeheim, Germany). The lower body of the animal (excluding the thorax) was housed in a negative pressure chamber which was connected to a vacuum pump. Ischemia was induced by transiently clamping both common carotid arteries and reducing the mean arterial blood pressure to 35–40 mm Hg by controlled negative pressure simultaneously (venous pooling). After 12 min, the carotid clamps were removed and normal blood pressure was restored. The animals were removed from the respirator as soon as they regained spontaneous respiration and kept for 90 min under 30 °C environmental temperature. Sham-operated animals had both common carotid arteries exposed under anesthesia without clamping them and without inducing hypotension.

Histology

On days 10–11 post-ischemia or sham-operation the brains were removed, fixed by immersion (ethanol/paraformaldehyde/glacial acid; Merck) and embedded in paraffin. Life/death staining was performed on 10 μm hippocampal slices with Toluidine Blue/fuchsin acid (Sigma). Slightly blue-stained neurons with distinct round nuclei were evaluated as morphologically intact neurons and were counted in the CA1 region and the DG of both hemispheres in a blind manner. Data are given as number of healthy neurons within a 500 μm area in the CA1 region or 250 μm in the granular layer of the DG (mean±S.E.M.). The electrode position was confirmed in each animal on slices stained with Toluidine Blue only.

Measurement of evoked potentials

The excitatory postsynaptic field potential (fEPSP) slope function was employed as a measure of excitatory synaptic transmission and the population spike (PS) amplitude was employed as an indication of the level of excitation of the granule cell population in the DG. Input–output (I/O) curves were registered after applying three stimuli at different intensities, ranging from 100 to 900 μA every 5 min. Stimulus intensities which elicited a PS of 40% of the maximum amplitude determined by the I/O curve were chosen to evoke the baseline responses in the DG granule cell layer. Stimulation was performed at a low frequency of 0.1 Hz with single biphasic square-wave pulses of 0.1 ms duration per half wave, generated by a constant current isolation unit. For each time-point measured during the experiments, five recordings of evoked responses were averaged. The amplitude of PS was measured from the peak of the first positive deflection of the evoked potential to the peak of the following negative potential. fEPSP slope function was measured as the maximal slope through the five steepest points obtained on the first positive deflection of the potential. Sampling frequency of Cambridge Electronic Design, analog-to-digital converter was 10 kHz. Baseline data were registered by applying five stimuli every 15 min for at least 1 h. Animals were tested for I/O-curve and baseline 1 day before ischemia and on days 1, 2, 7 and 10 post-ischemia.

Tetanisation protocol

Potentiation was induced by a tetanus of 200 Hz (10 bursts of 15 stimuli, 0.2 ms stimulus duration, 10 s interburst interval) at the stimulus intensity used for baseline recordings. Since after ischemia several animals did not show any PS response during I/O-curve measurements at the highest stimulus strength, tetanisation was performed at a stimulus intensity of 500–600 μA, which is in the upper range of intensities used in animals with regular I/O-curves. Sufficiently low variability in our recordings enabled us to perform data analysis with absolute values; this was necessary since several animals did not exhibit baseline responses 10 day after ischemia (it is not possible to calculate percentage of potentiation when all baseline values are zero). Animals were tetanised 1 day before ischemia and on day 10 post-ischemia. In another set of experiments, tetanic stimulations were applied on day 2 post-ischemia.

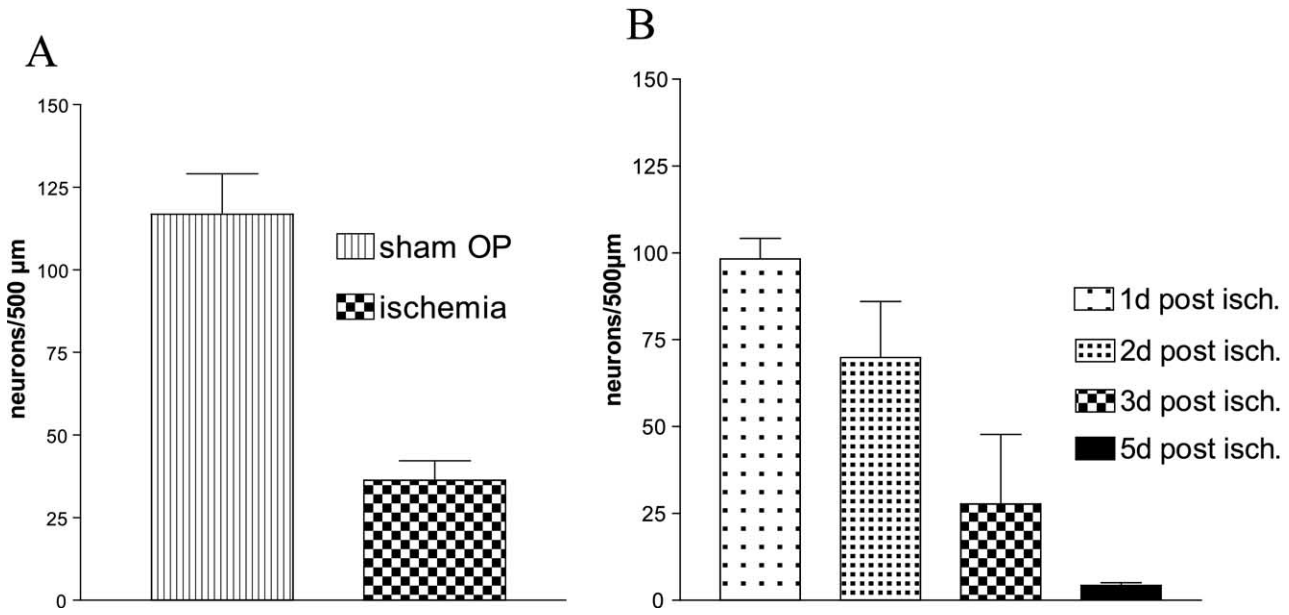

Fig. 1. A shows the extent of morphological damage in the CA1 area after 10–11 days post sham-operation (left bar) or ischemia (right bar); in ischemic animals the number of surviving neurons is significantly lower than in sham-operated animals ($n=12$–20; $P<0.001$, Mann-Whitney, U test). B illustrates the development of delayed neuronal death in CA1 as evaluated in an extra set of experiments where animals were decapitated at earlier time-points (days 1, 2, 3, 5 post-ischemia). In addition to the continuous reduction in cell number, the morphology of the neurons also showed the development of damage.

Ex vivo electrophysiology

Transient global ischemia was performed in rats as described above. Ten days after occlusion of both common carotid arteries the animals were killed by a blow to the neck. After decapitation, the brain was quickly removed and placed into ice-cold artificial cerebrospinal fluid (ACSF) with the following composition (in mM): NaCl 124, KCl 4.9, $MgSO_4$ 1.3, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.6, D-glucose 10, saturated with 95% O_2, 5% CO_2, pH 7.4). Both hippocampi were isolated and transverse hippocampal slices (400 µm thickness) were prepared using a tissue chopper with a cooled stage. The slices were transferred into a submerged type recording chamber, where they were allowed to recover for at least 1 h before the measurements were started. The chamber was constantly perfused with ACSF at a rate of 2.5 ml/min at 33±1 °C. PS were elicited in the inner blade of the DG by stimulation of the perforant path using lacquer-coated stainless steel stimulating electrodes and recorded with glass electrodes (filled with ACSF, 1–4 MΩ) in the granular cell layer. Test stimuli were adjusted to elicit a PS of about 40% of its maximum amplitude. The PS amplitude was evaluated by calculating the voltage difference between the negative peak and the positive peak preceding it. During baseline recording, three single stimuli (10 s interval) were averaged every 5 min. Once a stable baseline had been established, LTP was induced by three tetanisations at 2-min intervals (10 bursts of eight stimuli per tetanus, interburst interval 200 ms, interstimulus interval 5 ms, width of the single pulses of 0.2 ms). After tetanisation, recordings were taken every 5 min.

Statistics

ANOVA with repeated measures was used for comparison of electrophysiological data at different time-points and the Mann-Whitney U test was used to compare the ischemic group with the sham-operated group. Histological data were also analyzed with the Mann-Whitney U test. All animal procedures have been approved by the ethics committee of the German federal country of Sachsen-Anhalt and are in accordance with the European Communities Council Directive (86/609/EEC).

Assiduous efforts were made to keep the number of animals needed as low as possible and to minimize suffering of the animals.

RESULTS

Global ischemia/histology

Clamping both common carotid arteries for 12 min with combined hypotension led to a significant decrease in the number of pyramidal cells in the CA1-region of the hippocampus (animals decapitated on days 10–11 post-ischemia; numbers are given as neurons/500 µm), sham: 116.8±12.25 ($n=12$); ischemia: 36.3±5.9 ($n=20$); $P<0.001$, Mann-Whitney U test, Fig. 1A. Sham-operated animals did not exhibit signs of neurodegeneration in the CA1-region. Ischemic animals showed typical changes in behavior and motor function as described by Smith et al., 1984. They adopted a “hunchback” posture, walked with extended paws and fur was ruffled up. As evaluated by counting healthy neurons and consistent with the literature (e.g. Kirino and Sano, 1984) in another series of experiments we confirmed that neuronal death in CA1 is delayed. On day 1 post-ischemia histologically detectable damage was virtually absent while on day 3 we observed a significant reduction in cell number (Fig. 1B; $P<0.01$, ANOVA; $n=4$–5 per time-point) and obvious changes in morphology (irregular shape, dark blue or red staining). On day 5 there was mainly debris left in most of the CA1 area.

There was no obvious damage in the granular layer of the DG of sham-operated or ischemic animals and morphology did not show irregular-shaped, dark blue- or red-

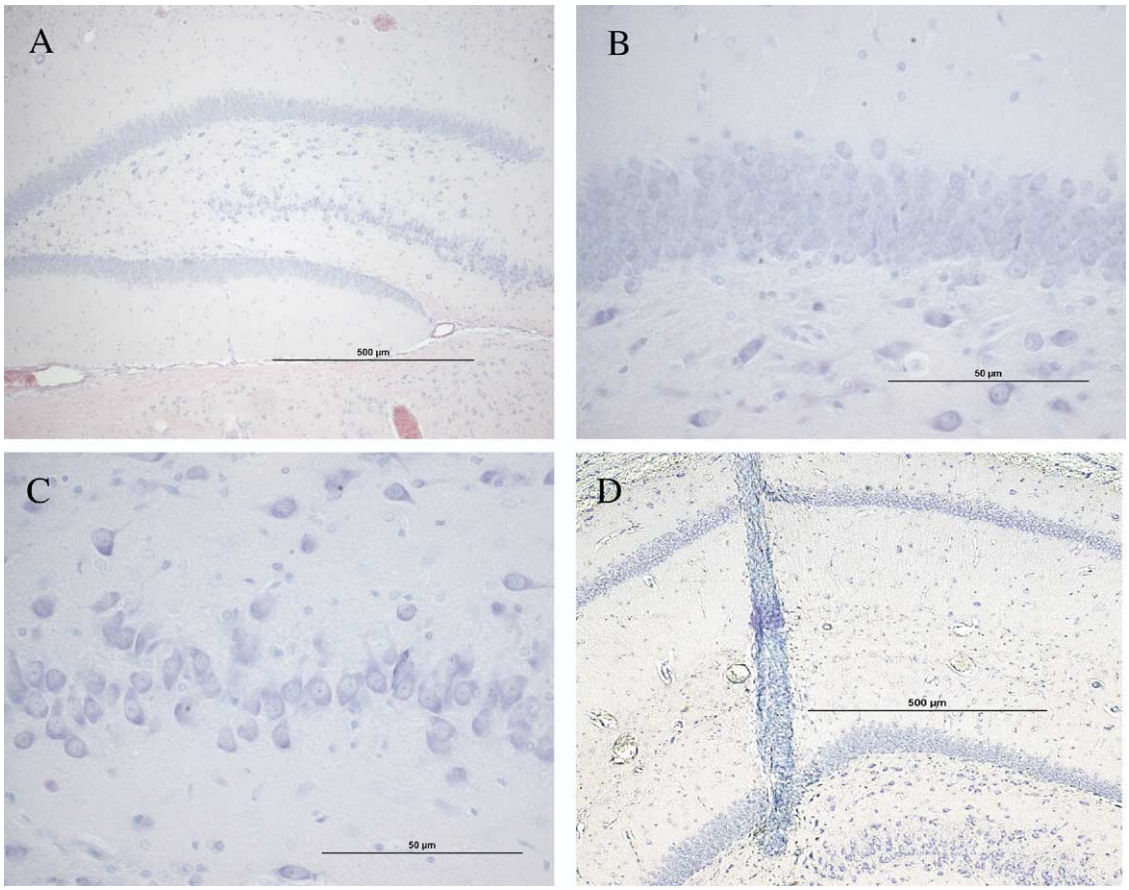

Fig. 2. Representative photomicrographs of the Nissl-stained dentate area 10 days after ischemia in 10-fold magnification (A) and 40-fold (B, C) magnification. With the applied staining technique no obvious damage can be detected in the granular layer (B) as well as in the hilar region (C). In (D) a trace of the recoding electrode can be seen.

stained cells in this area (Fig. 2A–D). Quantification of neuronal density in the DG did not reveal significant differences between sham-operated and ischemic animals (sham: 124.25±7.23; ischemia: 129.34±3.44; neurons per 250 µm, upper and lower band of granular layer, counted 4×250 µm (2× upper, 2× lower layer) in each hemisphere from two slices per animal; n=7–12).

Electrophysiology—measurement of I/O-curve and baseline

Changes in PS amplitude. A fast and pronounced decrease of the PS amplitude in the DG was observed after ischemia (Fig. 3A). On day 1, after clamping both common carotid arteries, the amplitudes of the PSs already were significantly lower than pre-ischemia. In general, values measured during I/O curve generation before ischemia were significantly different from all corresponding I/O curve values after ischemia (P<0.001; ANOVA with repeated measures). The response elicited during I/O testing with maximum stimulation intensity (900 µA) was only 32% of the response measured at 900 µA before ischemia. We did not see any recovery until day 10. In sham-operated animals there were only minor changes in the I/O characteristics regarding the PS amplitude at the five time-points measured (Fig. 3B). After operation, the I/O-values of the sham-operated animals were higher than the respective values of ischemic animals on all days (from a stimulation intensity of 300 µA onward differences are significant, Mann-Whitney U test.).

Changes in fEPSP slope. In contrast to the pronounced changes in the PS amplitude, we did not observe a significant decrease in fEPSP slope (Fig. 3C) in the ischemia group. Values of fEPSP in I/O-curves were not significantly different between ischemic and sham-operated animals at any time-point and at any stimulation intensity (Fig. 3C, D). Also unlike the PS-responses, at highest stimulation intensity there was no significant difference between values from each time-point measured (days 1, 2, 7 and 10 post-ischemia; ANOVA with repeated measures followed by Tukey's multiple comparison test). In the ischemic as well as in the sham-operated group we see significantly lower responses at the lowest stimulation strength (100 µA) when comparing values before and after the operation, a result that is apparently due to surgery. Plotting PS-amplitude against fEPSP values (E-S-curve), we could detect a massive decrease in the maximum PS values and, moreover, a distinct shift of the E-S-curve to the right (Fig. 4). This effect was transient and by day 7 post-ischemia the curve showed a partial overlay with the

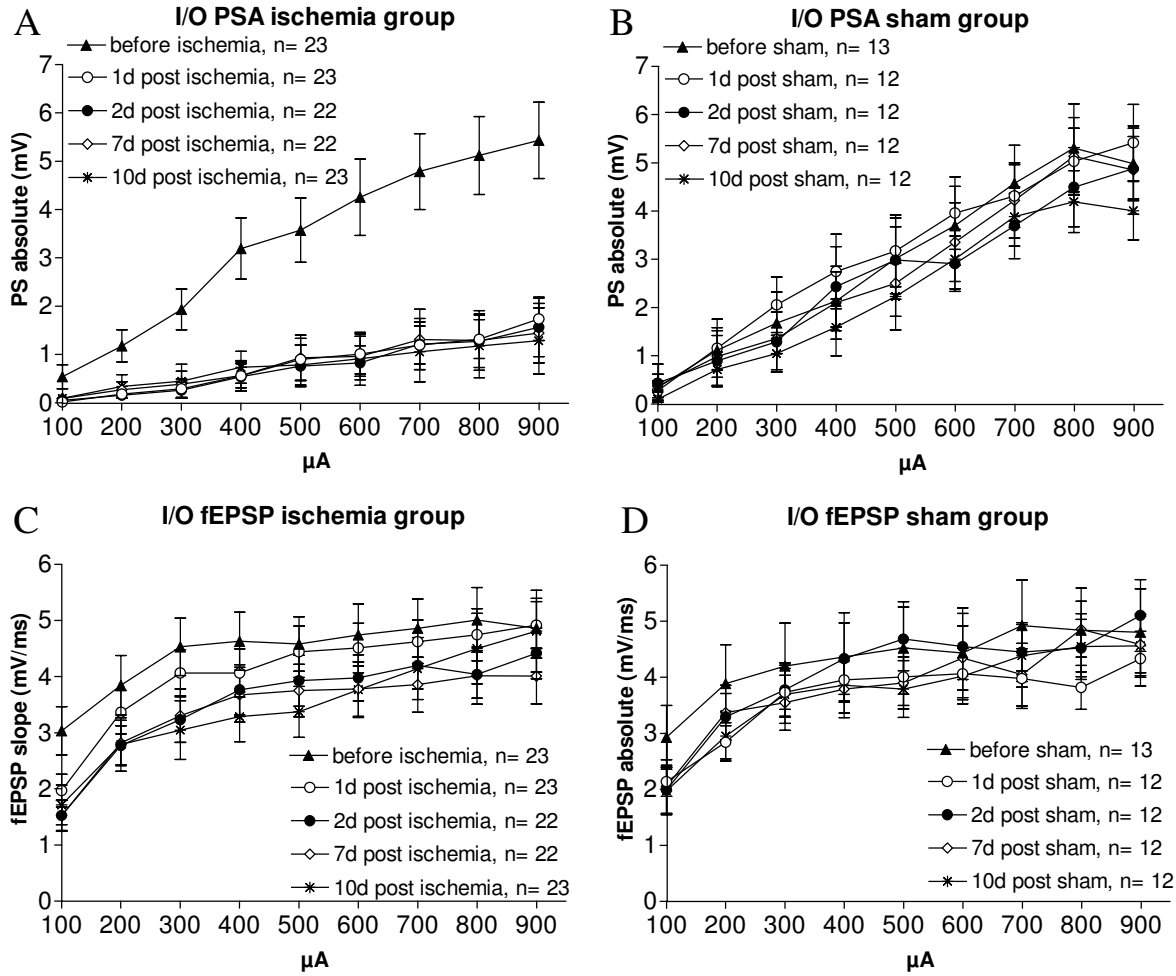

Fig. 3. A and B show the dramatic decline of the I/O-curve of the PS in DG after global ischemia (A) and only minor changes in the slope of the I/O-curve after sham-operation (B). In C and D the line-graphs show that the slopes of the fEPSP in the I/O curve also decrease after ischemia (C), but that there is, in contrast to PS-responses, a small effect detectable only at lower stimulation intensities and not at higher intensity (900 µA). Moreover, decreased responses at lower stimulation intensities are also seen in sham-operated animals (D), and therefore are not a specifically ischemia-induced phenomenon.

curve calculated from measurements made the day before ischemia.

Electrophysiology—tetanisation

In this set of experiments we applied high frequency stimulation, which induces a potentiation that persisted during the following 8 h of measurement, which is a feature of long-term potentiation (LTP). Regular measurements at 24 h post-tetanisation showed that the induced potentiation returned to baseline by the next day. We chose tetanisation-parameters to induce this type of LTP for two reasons:

(1) We applied tetanisation 1 day before ischemia in order to generate a "baseline-LTP" that can be compared with the responses after ischemia. However, because we wanted to induce potentiation several times in the same animal, we needed a schedule where potentiation does not last for many days.

(2) We wanted to avoid subjecting neurons to ischemia, which are still potentiated, because the potential influence of plastic changes on neurodegeneration is not clear.

Although baseline values were greatly reduced on day 10 post-ischemia, we could induce a potentiation of the PS showing a time course similar to the LTP in the pre-ischemic experiments (i.e. lasting for several hours). However, the absolute values of the PSs were significantly lower than in the sham-operated group and when compared with values measured before clamping both common carotid arteries. The mean baseline values 10 days after ischemia were only 20.5±1.7% of the pre-ischemic values (pre-ischemia: 1.67±0.06 mV versus 0.34±0.02 mV post-ischemia; $n=6$ baseline values), and the maximum-potentiation was only 38% of the corresponding pre-ischemic value (pre-ischemia: 3.85±0.42 mV versus 1.43±0.36 mV post-ischemia; $n=18–22$ animals). On the other hand, LTP of the PS expressed as percentage of baseline values was much more pronounced post-ischemia: the maximum value after tetanisation was 428% of the preceding baseline value (mean of six baseline

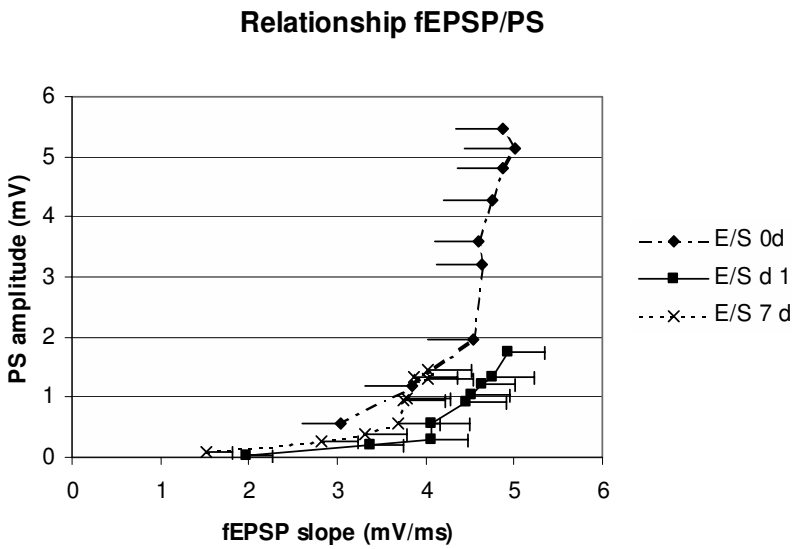

Fig. 4. The values of the PS are plotted against the slope of the fEPSPs (E-S-curve). The shift to the right on day 1 indicates a decrease in excitability.

measurements: 0.343±0.02 mV; average maximum value after potentiation: 1.47±0.4 mV). Pre-ischemia, this relative value was only 230% (mean of six baseline measurements before tetanisation: 1.67±0.06 mV; average maximum value after potentiation: 3.85±0.42 mV). This effect became even more pronounced when animals that did not show standard damage in CA1 despite being challenged by standard ischemia were eliminated from the analysis (definition for outliers: neuronal density at least 65% of value from sham-operated animals with double the number of neurons compared with the average ischemic animal; this definition applied to four animals: neurons per 500 μm: 83.3±2.1). In the remaining "standard-CA1-damage-group" (neurons per 500 μm: 17.3±1.3), we found a potentiation of the PS that was more than 2000% compared with the baseline (mean of six baseline measurements before tetanisation: 0.048±0.01 mV; average maximum value after potentiation: 1.13±0.03 mV). This extraordinarily high potentiation is mainly due to the extremely low baseline values measured in these animals (Fig. 5A, B). Interestingly, there were several rats in this group that did not show any PS response in I/O-curve at all (up to 900 μA stimulation intensity) but showed a normally shaped—although small—PS after tetanisation (Fig. 6). In eight ischemic animals, baseline measurements were performed 24 h after the post-ischemic tetanisation. In these animals we no longer found any increased responses. Sham-operated animals did not show any major differences in the extent of potentiation before or after operation (pre-operation, 231% potentiation compared with baseline; post-operation, 206% potentiation compared with baseline; Fig. 5C, D). To check whether we could induce functional regeneration by tetanisation at earlier time-points, another set of experiments was performed in which we tetanised animals 2 days post-ischemia. At this time-point the PS amplitude in baseline recordings was greatly reduced, but unlike on day 10 post-ischemia we were not able to induce potentiation by applying high frequency stimulation. As expected, we were able to induce potentiation of PSs in the sham-operated animals. The tetanisation on day 2 had no influence on the further decrease in function of granular neurons of ischemic animals as shown by I/O-curve measurements on the days after tetanic stimulation (days 3 and 8 post-ischemia: Fig. 7A, B).

Electrophysiology—ex vivo experiments

Unlike the *in vivo* experiments, there was no significant difference in the course of the I/O curves measured in slices from ischemic and sham-operated animals 10 days after the event (Fig. 8B). Preparations from both groups showed significantly elevated responses after tetanisation and the extent of potentiation was not significantly different. Just before stimulation the PS amplitudes were −0.96±0.14 mV (n=4) in slices of sham-operated animals and −0.93±0.13 mV (n=6) in slices from animals 10 days after ischemia. The PS amplitudes from both groups remained similar 60 min after tetanisation (sham: −1.71±0.27 mV; ischemia: −1.86±0.33 mV; Fig. 8A). Also paired pulse depression of the second PS after paired pulse stimulation of the medial perforant path (interpulse interval 50 ms) was similar for sham-operated and ischemic animals before and 2 h after high-frequency stimulation (PS2 vs. PS1, sham before tetanus: 0.82±0.07; sham after tetanus: 0.89±0.05; ischemic before tetanus: 0.81±0.07; ischemic after tetanus: 0.97±0.04), indicating that recurrent inhibition also is preserved in ischemic animals.

DISCUSSION

Specification of the models used

The ischemia model used in this study mimics the damage seen in humans after circulatory failure (e.g. after transient cardiac arrest) and is associated with memory impairment. It induces selective neuronal loss in the hippocampal formation, which, due to its anatomical structure, is well suited for extracellular electrophysiological recordings. In

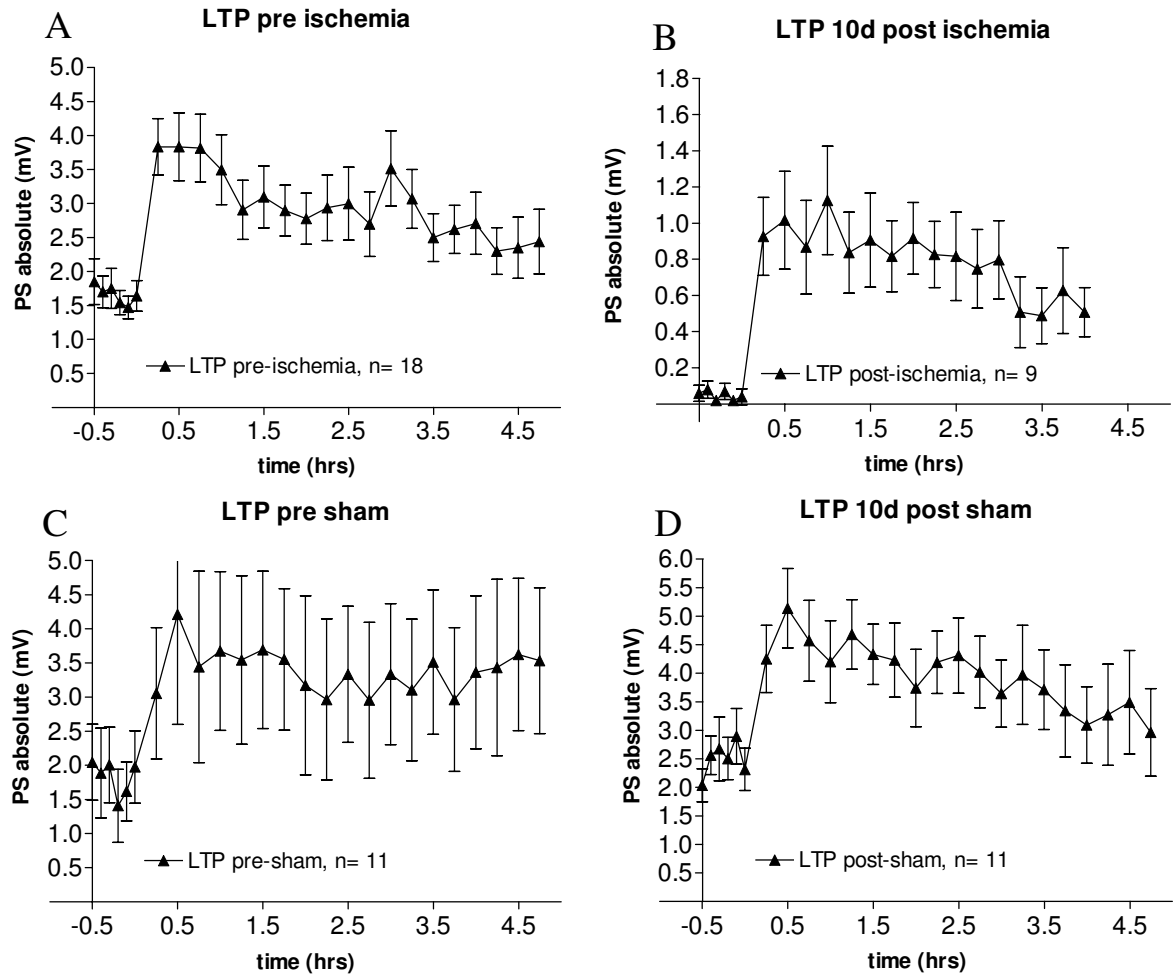

Fig. 5. A shows the PS values before and up to 4 h after tetanisation in animals which were subjected to ischemia 1 day later. Recordings from day 10 post-ischemia in animals with standard ischemic damage in CA1 are shown in Fig. 5B; a highly significant increase in, or even induction, of PS-values can be seen (i.e. zero response in the I/O-curve but a measurable PS after tetanisation). However, absolute values are significantly lower than on day 1 pre-ischemia and lower than values from sham animals on day 10 post-operation (Mann-Whitney). For comparison, C and D provide data from sham-operated animals which did not show any pronounced differences in their plastic abilities.

rats, two standard models of global ischemia are used: (1) four-vessel occlusion with electrocauterisation of vertebral arteries (4-VO) and (2) two-vessel occlusion with simultaneous hypotension (2-VO). Since it is conceivable that the high current necessary for electrocauterisation in the 4-VO model might affect the physiological state and post-ischemic pathophysiology of neurons, we chose the 2-VO model with combined hypotension for our present work to avoid any possible unintentional effects on our results.

Since narcosis was shown to have a significant influence on electrophysiological responses and LTP (Riedel et al., 1994) and may thus be a source of artifacts, we performed our measurements in unanaesthetised animals.

Changes in basic transmission

By evaluating the electrophysiological function of the DG before and at several time-points after ischemia, we find that granular cells generate a significantly reduced PS at an early time-point (1 day post ischemia) when CA1-neurons, the indirect target-neurons of the dentate granular cells, are still morphologically intact. This dysfunction continues *in vivo* for at least 11 days. Interestingly, these results differ partially from the effects described in one of the first papers in the field (Suyama, 1992). In his work electrophysiological impairments appear delayed and are less pronounced. One possible explanation of such differences in the extent and time-course of impairments might be that we use 2-VO combined with hypotension whereas Suyama (1992) performed 4-VO where the vertebral arteries are electrocauterized 1 day before clamping both common carotid arteries. The stress of this pre-ischemic operation and/or the permanently reduced blood supply might lead to a preconditioning effect and cause some difference in neurodegeneration. This is indicated by the fact that on day 7 post-ischemia the existence of pyknotic cells is described by Suyama (1992) whereas we can detect only debris in CA1 at this time-point. Subsequently, the different time-course of morphological degeneration might cause differences in electrophysiological impairments.

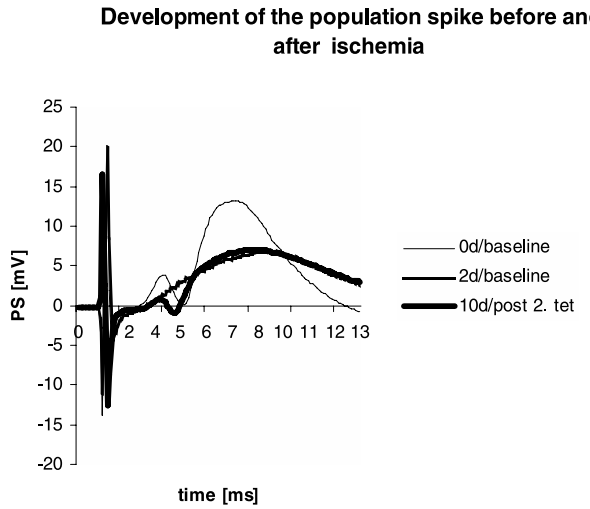

Fig. 6. An illustration of analog signals from one animal before ischemia, 2 days after ischemia and after post-ischemic tetanisation. These results demonstrate the disappearance of the PS after ischemia and its re-appearance after tetanisation.

Our experiments show that contrary to the early and massive decrease in PS amplitude the dendritic (input-) function (fEPSP) is mainly preserved after clamping both common carotid arteries. Plotting both parameters in an E-S-curve shows a shift to the right on day 1 post-ischemia, indicating a decrease in neuronal excitability at this early post ischemic time-point. Even a saturated fEPSP at 900 μA stimulation intensity was not always accompanied by a PS-induction. This might be due to a very deep cell inhibition, such as, e.g. pronounced hyperpolarisation of membranes. Also unlike the PS changes, the fEPSP does not show a pronounced decline for up to 10 days. These data indicate that the generation of PS is selectively impaired in granule cells by global ischemia whereas glutamatergic synaptic transmission is not influenced to any extent by such an insult.

From the work of Aoyagi et al. (1998) similar results are reported regarding early impairment of PS-amplitude. However, in this paper also a significant long-lasting fEPSP reduction is shown. We think that two points may account for this difference. Firstly, due to the permanent occlusion of the vertebral arteries, blood flow to the brain is permanently impaired (Ueda and Matsunaga, 1995) in ischemic as well as in sham-operated animals in the experiments of Aoyagi et al. (1998). This does not lead to obvious (additional) morphological damage, however, the function of the neurons in the DG might be already influenced: either fEPSP-responses in post-ischemic animals might be reduced or the stress of reduced blood-flow might lead to an increased signal in the sham-operated animals, which also makes fEPSP-responses look like being reduced in ischemic animals. Secondly, in the experiments described by Aoyagi et al. (1998), stimulus intensities up to 3000 μA were used, whereas we stimulated only until 900 μA to see a typical saturation curve. Effects which appeared beyond 1000 μA stimulation intensity are therefore difficult to compare with our data.

Our data do not support the notion that there is a prolonged hyperexcitability in the tri-synaptic circuit after transient global ischemia, which is driven by increased input from the DG (Jorgensen et al., 1987; Diemer et al., 1993). However, we cannot rule out that there is a very early hypersensitivity of CA1 neurons that permits these pyramidal cells to suffer from hyperexcitation despite normal or even reduced input via the tri-synaptic cascade.

There are several hypotheses for the explanation of the reduced PS in DG:

(I) Inhibitory mechanisms in the DG are increased after ischemia; the paper by Suyama (1992) gives evidence for an increase in recurrent inhibition. Additionally, death of hilar neurons is of interest regarding inhibition. It is known that selective damage can appear in the hilar region after a lesion (Mody et al., 1995; Borges et al., 2003). A reduc-

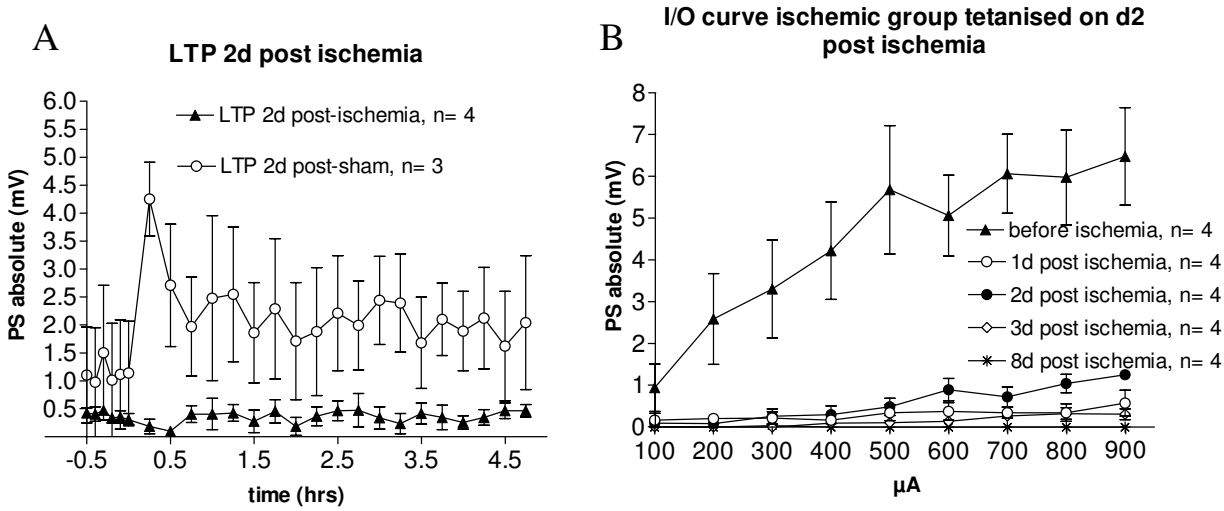

Fig. 7. (A) Rats were subjected to the tetanisation paradigm (200 Hz; 10 bursts of 15 stimuli, 0.2 ms stimulus duration, 10 s interburst interval) on day 2 post-ischemia. It was not possible to induce potentiation at this time-point and tetanisation did not alter the impairment in I/O-curve on subsequent days (B).

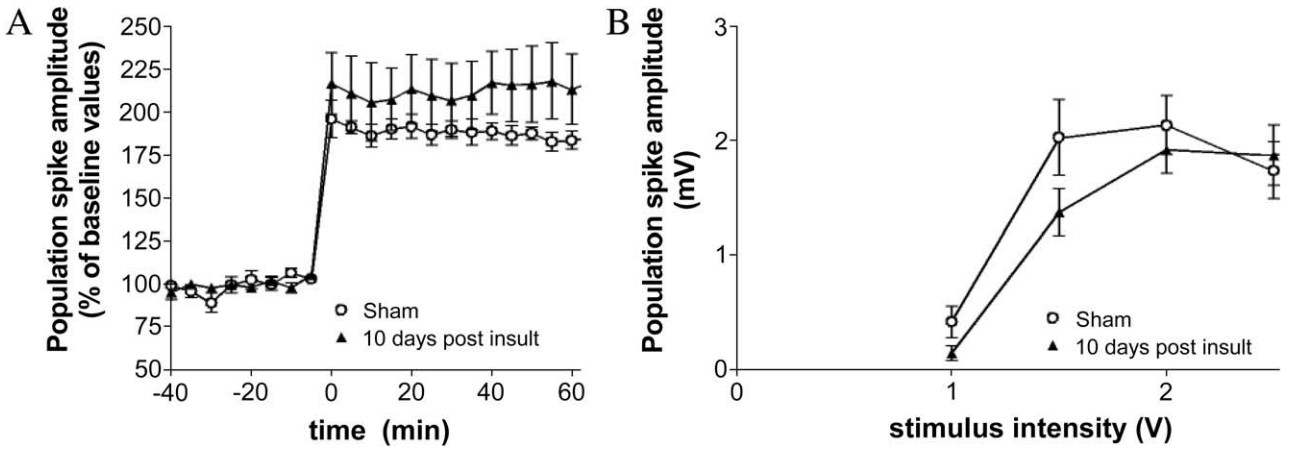

Fig. 8. Results of ex vivo preparation showing that *in vitro* potentiation in slices from ischemic animals is similar to the potentiation in slices from sham-operated animals (A). Unlike the *in vivo* situation, I/O curve from ischemic animals is similar to the I/O-curve of sham-operated animals (B).

tion in mossy cell subpopulations (Ratzliff et al., 2004) or an increase in GABA-receptors (Reeves et al., 1997) might cause a reduced output function of dentate granular neurons. Interestingly, results from trauma-research indicate that inhibition can be selectively increased in the perforant path/DG pathway (Borges et al., 2003) after injury.

(II) There is an increased inhibitory influence from structures outside the dentate area. It is conceivable that damaged/dying CA1-neurons reduce the activity of granule neurons or increase the activity of interneurons in the DG by a retrograde message. Nitric oxide (NO) would be such a putative retrograde modulator from CA1-neurons (Endoh et al., 1994a) as it is able to depress neuronal activity in the hippocampus after stress (Takeya et al., 2003). But it is also possible that an NO-induced suppression is mediated via reactive astrocytes, which were shown to express inducible nitric oxide synthase (iNOS) after ischemia (Endoh et al., 1994b). The increased inhibitory influence might also originate from extra-hippocampal structures, such as the raphe nucleus, which innervates interneurons in the hippocampus (Freund, 1992).

(III) It also is conceivable that an ischemic challenge damages granular cells in DG, albeit in a way which cannot be detected by Nissl-staining at this stage and the neurons might regenerate or die at a later time-point after a very slow degenerating process. There are reports showing that there are more subtle forms of death and dysfunction and that, e.g. damaged dendrites can also contribute to functional impairment (Corbett and Nurse, 1998; Geddes et al., 1994). However, it seems that at later time-points (after 4 weeks) the dentate granule cells may regenerate rather than degenerate since it has been shown that PS-function in the DG can recover (Aoyagi et al., 1998).

(IV) The micromilieu in the DG is changed due to neurogenesis. There is evidence from recent publications that endogenous progenitors can play a role in repair mechanisms after ischemia (Liu et al., 1998; Nakatomi et al., 2002). Activation in the neurogenic DG might, in such a case, influence electrophysiological properties toward a suppression of PS generation. NO might also be the mediator of this effect; Zhu et al. (2003) reported that enhanced dentate neurogenesis is associated with activation of iNOS. Conversely, it has been shown that the lack of action-potential does not reduce proliferation in the dentate area (Raineteau et al., 2004).

Ex vivo experiments

The results of our ex vivo experiments give some evidence against hypotheses I (recurrent inhibition enhanced, death of hilar neurons) and III (subtle damage of granular neurons). Since paired-pulse inhibition is intact in slices from ischemic animals, death of hilar neurons and consequently enhanced inhibition does not seem to be the main reason of our reduced/abolished PS-signal *in vivo* (hypothesis I). Also, if there were subtle morphological damage (e.g. to dendrites, hypothesis III), PS signals should be reduced also in the slice preparations. Since this is not the case, the functional impairment *in vivo* is more likely due to (a) retrograde messengers from other hippocampal areas, (b) inhibitory influences from extra-hippocampal structures (e.g. raphe nucleus, amygdala) (c) changes in the micromilieu (hypotheses II and IV). These mechanisms cannot exert their spike-suppressive effects in the ex vivo experiments, as according structures are removed in the *in vitro* preparation or released substances will be washed out during perfusion.

Post-lesional neuronal plasticity

In our experiments, tetanic stimulation on day 10 post-ischemia is able to potentiate the PS amplitude. Absolute values are lower in comparison to LTP-induction before ischemia but, relative to baseline, potentiation 10 days after ischemia is extremely high. This result is partially consistent with data from other laboratories (Aoyagi et al., 1998) but our recordings show an interesting new fact. Even at maximal stimulation intensity in the I/O-curve, we cannot elicit any PS in approximately one third of all animals after ischemia, but we are able to *induce* PS occurrence post-ischemia by tetanisation in these animals. Since, in part, we do not only enhance, but *induce* PS

response, in a strict sense we cannot term the phenomenon seen in our experiments as LTP, because LTP is defined as a long lasting *enhancement* in the magnitude of the cell response to further stimulation. This also leads to the suggestion that there might be substantial differences between the mechanisms behind this post-lesional potentiation and normal LTP. In general, it is assumed that LTP maintenance is mediated by increases in both the amount of transmitter released per presynaptic impulse and the degree of postsynaptic responsiveness to a fixed amount of transmitter. In our experiments, evaluation of I/O-curve indicates that increased presynaptic transmitter release induced with increasing stimulation intensity up to 900 μA does not have any influence on the extent of the PS signal: it remains zero although fEPSP-signals show nearly normal I/O-curve like in sham-operated animals. After tetanisation, however, a PS is induced although fEPSP is not significantly changed. Therefore, it is reasonable to conclude that our phenomenon of post-lesional plasticity is mainly elicited by postsynaptic changes. Further investigations are necessary to define the character of these postsynaptic changes more closely.

Since all animals investigated 24 h after post-ischemic tetanisation did not show a continuously elevated PS-response, but returned to baseline, we think that the influence of tetanisation according to our current protocol is only transient.

The reason why potentiation of PS is possible on day 10 but not on day 2 is unknown (Fig. 7A, B). Perhaps the neurons are not excitable during the first post-ischemic-days but after this “silence” it is again possible to induce potentiation. Interestingly, during this period after ischemia, neurogenesis is enhanced in the neurogenic subgranular layer of the DG as well as in the CA1 region (Liu et al., 1998; Nakatomi et al., 2002; Schmidt and Reymann, 2002). It may be, therefore, that changes in micromilieu (hypothesis IV) are even more pronounced than at day 10 post-ischemia and prevent induction of a long-lasting potentiation.

In summary, our results show that after ischemia “silent” neurons can be promoted to reach a transient functional state by tetanisation. Especially in respect to regenerative strategies, it will be interesting to pursue this line of research and evaluate additional strategies that may induce a permanent return of function in the post-ischemic brain.

Acknowledgments—We are greatly indebted to Dr. M. Sokolov and Dr. S. Frey for helpful discussions and ideas and to Dr. Roy Johnson for reading and correcting the manuscript. We thank Katrin Böhm for her complete commitment to work. This work was supported by: Land Sachsen-Anhalt, grant number: LSA 3480A/1202M; RBRF grant 040449455a; President RF grant 1989.2003.6 (Leading Scientific School). The work of Dr. A.G. Gorkin was also supported by DFG (SFB426).

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(Accepted 18 February 2005)
(Available online 10 May 2005)