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Development/Plasticity/Repair

Post-Traumatic Hyperexcitability Is Not Caused by Impaired Buffering of Extracellular Potassium

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Impaired extracellular potassium buffering has been proposed as one of the major mechanisms underlying the increased risk for temporal lobe epilepsy after brain injury (D'Ambrosio et al., 1999). The present study systematically tested this hypothesis by measuring the resting $[K^+]_o$ and recovery of the stimulation-evoked $[K^+]_o$ increases in the dentate gyrus after experimental head trauma, using a combination of whole-cell recordings and ion-selective microelectrode recordings in rat hippocampal slices. Despite the presence of hyperexcitability, the resting $[K^+]_o$ was not increased after injury. The faster rate of increase and larger amplitude of the orthodromically evoked $[K^+]_o$ elevation after head trauma occurred in association with a greater population spike with shorter response latency. Contrary to the assumption in previous studies that the evoked activity in control and injured neuronal circuits is the same during antidromic activation, stimulation of granule cell axons in glutamate receptor antagonists evoked a greater $[K^+]_o$ increase and a larger population spike. Although perforant path stimulation resulted in a larger $[K^+]_o$ elevation after injury, the rate of clearance of the $[K^+]_o$ transients evoked either by neuronal activity or by external application of potassium was not compromised. The $[K^+]_o$ increase evoked by activation of the presynaptic afferents in isolation was not increased. In addition, the postsynaptic neuronal depolarization and firing evoked by exogenous potassium application was decreased after trauma.

These results show that the regulation of $[K^+]_o$ is not impaired after injury and indicate that the larger $[K^+]_o$ increase evoked by neuronal activity is a consequence, rather than the primary mechanism underlying post-traumatic hyperexcitability.

Key words: **potassium; buffering; head trauma; FPI; dentate gyrus; seizures**

Introduction

Head injury is an important risk factor in the etiology of temporal lobe epilepsy (Jennett, 1975; Annegers and Coan, 2000). Although concussive brain trauma causes a distinct pattern of cellular injury in the dentate gyrus (Margerison and Corsellis, 1966; Bruton, 1988), the exact mechanisms that underlie the postinjury onset of seizures are unknown.

An intriguing hypothesis suggests that elevated extracellular potassium ($[K^+]_o$) is the key to post-traumatic hyperexcitability and seizures (D'Ambrosio et al., 1999). The impaired potassium buffering theory proposes that $[K^+]_o$ increases, as a consequence of impaired glial clearance, cause neuronal hyperexcitability (Pollen and Trachtenberg, 1970; D'Ambrosio et al., 1999). Consistent with the contribution of $[K^+]_o$ to neuronal activity (Huxley and Stampfli, 1951), conditions that disrupt $[K^+]_o$ regulation by artificially elevating $[K^+]_o$ (Traynelis and Dingledine, 1988; Helekar and Noebels, 1992; McBain et al., 1993) or by manipulations that depress glial K^+-uptake (Largo et al., 1996; Janigro et al., 1997; Xiong and Stringer, 1999) can cause neuronal hyperexcitability. Recent data demonstrating postinjury reduction in glial inward rectifier potassium currents (K_{IR}) that are thought to regulate $[K^+]_o$, and the abnormal $[K^+]_o$ increases evoked by antidromic stimulation in glutamate receptor antagonists *in vitro*, a paradigm assumed to normalize neuronal activity between injured and control tissue, were presented as evidence that supported the glial impairment theory (D'Ambrosio et al., 1999). However, studies have shown that the immediate post-traumatic increase in resting $[K^+]_o$ *in vivo* recovers to control levels within a few hours (Takahashi et al., 1981; Katayama et al., 1990). Similarly, regulation of both the resting $[K^+]_o$ and the activity-dependent $[K^+]_o$ increases is not compromised in electrically induced seizure foci (Xiong and Stringer, 1999). In addition to the controversy concerning the glial impairment theory (Walz and Wuttke, 1999), results demonstrating that $[K^+]_o$ elevations often follow, rather than precede, seizure-like activity in animal models of epilepsy (Pedley et al., 1976; Somjen, 1984) call into question the role for altered $[K^+]_o$ regulation as a major mechanism for seizure generation.

The dentate gyrus regulates the neuronal signaling between the entorhinal cortex and the hippocampus (Buzsaki et al., 1983) and has been proposed to gate seizure propagation in the limbic system (Heinemann et al., 1992; Lothman et al., 1992). It is also the site of characteristic post-traumatic alterations, including hilar cell loss (Lowenstein et al., 1992; Coulter et al., 1996; Toth et al., 1997), mossy fiber sprouting (Golarai et al., 2001; Santhakumar et al., 2001), and reactive gliosis (Smith et al., 1997). Further-

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more, studies investigating neuronal excitability using the rodent fluid percussion head injury (FPI) model of concussive head trauma have demonstrated neuronal hyperexcitability (Lowenstein et al., 1992; Coulter et al., 1996; Toth et al., 1997) and modifications in the dentate interneuronal and excitatory networks (Ross and Soltesz, 2000; Santhakumar et al., 2000; Ratzliff et al., 2002). Despite the data that imply that the changes in the dentate gyrus might be pivotal to seizure generation (Coulter et al., 1996; Masukawa et al., 1999; Santhakumar et al., 2001), little is known about the efficacy of $[K^+]_o$ regulation in the dentate after head injury.

This study was performed to test the hypothesis (D'Ambrosio et al., 1999) that an impaired regulation of the resting $[K^+]_o$ and the stimulation-evoked $[K^+]_o$ transients underlies post-traumatic hyperexcitability.

Materials and Methods

Fluid percussion injury. Lateral fluid percussion head trauma on young adult (4 weeks of age) male Wistar rats was carried out as described previously (Dixon et al., 1987; Lowenstein et al., 1992; Toth et al., 1997) (all procedures described were approved by the Institutional Animal Care and Use Committee, University of California, Irvine, CA). Briefly, the rats were anesthetized, placed in a sterotaxic frame, and the scalp was sagittally incised. A 2 mm hole was trephined in the skull at −3 mm (i.e., caudal) from the bregma, 3.5 mm lateral from the sagittal suture. A Luer-Loc syringe hub with a 2.6 mm inside diameter was placed over the exposed dura and bonded with cyanoacrylate adhesive. A day later, the rats were anesthetized with halothane in a 2 liter chamber and subsequently removed from the anesthetizing chamber and immediately connected to the injury device. The animal was fully anesthetized at the time of injury, although halothane anesthesia was not actively administered at that time. All animals were immediately ventilated with room air. The fluid percussion device (Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA) was used to inject a small volume of saline into the closed cranial cavity on the intact dura and produce a brief (20 msec) displacement and deformation of the brain tissue. The magnitude of the injury was controlled by varying the height from which the pendulum in the injury device was released (in these experiments, it was 12–14°, which produced a 2.0–2.2 atm pressure wave). This resulted in a moderate level of injury that has been shown to cause a highly reproducible pattern of >50% hilar cell loss (Toth et al., 1997; Santhakumar et al., 2000).

Slice preparation. At various time points (2 d, 1 week, and 1 month) after the injury, or sham injury (Toth et al., 1997), the rats were anesthetized with halothane and decapitated. Horizontal brain slices (400 μm) were cut using a vibratome tissue sectioner (VT1000S; Leica, Nussloch, Germany), as described previously (Ross and Soltesz, 2001). The slices were sagittally bisected, and the slices ipsilateral to the side of injury were submerged in 32°C oxygenated (95% O_2-5% CO_2) artificial CSF (ACSF) composed of (in mM): 126 NaCl, 2.5 KCl, 2 $MgCl_2$, 26 $NaHCO_3$, 2 $CaCl_2$, 1.25 NaH_2PO_4, and 10 glucose, for 1–6 hr.

In vitro *electrophysiology.* The slices were transferred to the interface recording chamber and perfused with oxygenated ACSF at 36°C. In some experiments, the perfusion was switched to ACSF containing one or more of the following drugs: 20 μM bicuculline methiodide (BMI), 20 μM D-AP-5, 10 μM CNQX, 100 μM picrotoxin, 10 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX), 20 μM (2S)(+)5,5-dimethyl-2-morpholineacetic acid (SCH 50911), 500 μM (RS)-a-methyl-4-carboxyphenylglycine (RS-MCPG), and 1 μM TTX. All salts were obtained from Fluka (Buchs, Switzerland). AP-5, CNQX, NBQX, picrotoxin, RS-MCPG, SCH 50911, and TTX were purchased from Tocris (Bristol, UK); QX-314 was obtained from Calbiochem (La Jolla, CA); and BMI from Sigma--Research Biochemicals International (Natick, MA).

Single-barrel ion-selective microelectrodes (ISME) were silanized, backfilled with 3 mM KCl, and the tip was filled with valinomycin-based membrane solution selective for potassium ions (Cocktail B; Fluka; Voipio et al., 1994). Signals from the ISMEs were recorded using a custom-built electrometer amplifier that has a bias current of <50 fA, an input impedance three orders of magnitude higher than the electrode resistance, and a feedback circuit for electrode capacitance compensation. The ISMEs (1–10 GΩ) were calibrated using solutions containing 2.5, 5, and 10 mM potassium. Only electrodes with a slope in the range of 54–60 mV/10-fold change in $[K^+]$ were used. The ISME capacitance was compensated using the feedback circuit of the amplifier. A double electrode holder (Narishige, Tokyo, Japan) was used to position the ISME and a reference electrode at a constant tip separation of 5 μm. Field potential recordings were obtained using the reference electrode coupled to the ISME. The field responses and $[K^+]_o$ measurements were obtained from the crest of the dentate granule cell layer at a depth of 150 μm from the surface of the tissue. Level-matched slices from head-injured and age-matched sham-operated control animals were recorded alternately on the same day. Orthodromic population spikes in granule cells were evoked by constant–current stimuli (1–8 mA, 50 μsec) at 0.1 Hz using a bipolar tungsten-stimulating electrode placed in the perforant path, on the entorhinal side of the fissure, at the junction of the dorsal blade, and at the crest. Control experiments conducted in the absence of the slice showed that stimulation in the bath did not induce an artifactual ion–electrode signal ($n = 6$). For antidromic activation of granule cells, the stimulating electrode was located in the hilus, halfway between the tip of the upper blade and the crest of the granule cell layer, and midway between the granule cell layer and CA3 (at ~60–70 μm from the recording electrode), and the slices were perfused with ionotropic glutamate and GABA receptor antagonists (20 μM AP-5, 10 μM CNQX, and 20 μM BMI; or 20 μM AP-5, 10 μM NBQX, and 100 μM picrotoxin). Similarly, CA3 cells were stimulated antidromically by an electrode placed in the Schaffer collaterals in 20 μM AP-5, 10 μM CNQX, and 20 μM BMI. Either single-shock stimuli (1–6 mA, 50 μsec) at 0.1 Hz or 50 msec trains of 5–10 stimuli (at 100–200 Hz) at 6 mA stimulation intensity were used to evoke antidromic firing. Tetanic stimulation consisted of a 5 sec, 100 Hz train of stimuli applied to the perforant path with a pulse width of 0.1 msec at 8 mA stimulus intensity. Exogenous potassium was applied using a glass pipette with a 10 μm diameter tip opening, containing ACSF with either 10 or 120 mM potassium (replacing potassium for equimolar sodium to maintain osmotic balance). The potassium application pipette was placed in the bath (with the tip above the surface of the slice) on the hilar side of the granule cell layer 20 μm (for experiments shown in Fig. 3) or 50 μm (for experiments shown in Fig. 7) from the recording electrode. In separate experiments (similar to those in Fig. 7), pressure application of ACSF containing 120 mM potassium for 10 msec was found to cause over a 6 mM increase in potassium at a depth of 250 μm in the granule cell layer ($n = 3$ slices). A pressure ejection pump (Picospritzer) was used to apply the potassium solution at 4–8 psi. Representative traces of $[K^+]_o$ transients are plotted as voltage changes from the ISME recordings, and the corresponding $[K^+]_o$ (as a micromolar increase from resting $[K^+]_o$; calculated from the mV change from rest) is indicated on a Nernstian scale. For simplicity, the summary data in bar graph form show the relative increase in $[K^+]_o$ (in mM) above the resting $[K^+]_o$ on a linear scale. Blind whole-cell recordings were obtained as described previously (Toth et al., 1997; Santhakumar et al., 2000; Chen et al., 2001), using patch pipettes filled with an internal solution that consisted of (in mM) 140 K-gluconate, 2 $MgCl_2$, and 10 HEPES. The correction for the junction potential was not performed. The sodium and nonspecific potassium channel blocker QX-314 (3 mM) was included in the internal solution in some experiments summarized in Figure 7.

Analysis. Recordings were filtered at 3 kHz and digitized at 20 kHz using the Strathclyde Electrophysiology software (courtesy of Dr. J. Dempster, University of Strathclyde, Glasgow, UK) and Synapse software (courtesy of Dr. Y. De Koninck, McGill University, Montreal, Canada). Half-time of recovery of $[K^+]_o$ transients, a measure of the rate of potassium clearance, was defined as the time taken for $[K^+]_o$ to decline to half-maximum amplitude (Xiong and Stringer, 1999). To assess whether the decay of the $[K^+]_o$ transients was better fit with single or double exponentials, the sum of squared errors (SSE) improvement function (Hollrigel et al., 1996; Chen et al., 2001) was used. Briefly, an F-test was used to assess the improvement in the ratio $(SSE_1\text{-}SSE_2)/SSE_2$

where SSE_1 and SSE_2 are the sum of squared errors of fits with one and two exponentials. When SSE improvement was not significant, a single exponential described the decay. Statistical analyses were performed with SigmaPlot or SPSS for Windows (SPSS, Inc., Chicago, IL). The significance of differences in field and whole-cell responses and $[K^+]_o$ changes in control and injured animals was evaluated using the Student's *t* test. The Mann–Whitney *U* test was used to assess the significance of differences in $[K^+]_o$ increase in response to tetanic stimulation. The statistical significance of changes in potassium-evoked firing was tested using the nonparametric z-test. The level of significance was set at $p < 0.05$. Data are presented as mean ± SEM.

Results

Absence of increase in the resting $[K^+]_o$ after head trauma

It has been reported previously that the resting $[K^+]_o$ in hippocampal slices is significantly elevated after FPI (D'Ambrosio et al., 1999). The study by D'Ambrosio et al. (1999) was conducted in CA3 2 d after head injury. The present study examined the resting $[K^+]_o$ in the dentate gyrus at various time points after head trauma. Additionally, the resting $[K^+]_o$ was also measured in the CA3 pyramidal cell layer 2 d after injury to facilitate comparison with previous results (D'Ambrosio et al., 1999).

The resting $[K^+]_o$ was measured in the absence of external stimulation in slices from injured and control animals in which we established the presence of post-traumatic neuronal hyperexcitability. The perforant path-evoked population spike amplitude (Fig. 1*A,B*) in the animals used in the present study was larger 1 week after FPI (Fig. 1*A,B*), in agreement with the results of earlier studies (Lowenstein et al., 1992; Toth et al., 1997; Santhakumar et al., 2000). However, the resting $[K^+]_o$ in the granule cell layer of slices from head-injured animals measured in control ACSF was not increased at the same time point (Fig. 1*C*) [CON: 100 ± 3.3%, *n* = 16; FPI: 97.94 ± 3.13%, *n* = 18; for easier comparison, the data are presented as percentage of control, i.e., normalized to control mean; the absolute $[K^+]_o$ was 2.70 ± 0.09 mM (CON) and 2.64 ± 0.08 mM (FPI)]. Similar to the results at 1 week, the resting $[K^+]_o$ in the dentate gyrus was not different from controls either 2 d or 1 month after head trauma (Fig. 1*D*) (2 d: CON, 100 ± 0.68%, *n* = 12; FPI, 100.13 ± 1.57%, *n* = 12; 1 month: CON, 100 ± 3.23%, *n* = 5; FPI, 99.81 ± 0.81%, *n* = 5), indicating that an elevation of the resting $[K^+]_o$ cannot underlie the long-term decrease in seizure threshold (Santhakumar et al., 2001). Consistent with our findings in the dentate gyrus and in contrast to the results of D'Ambrosio et al. (1999), there was no change in the resting $[K^+]_o$ in the CA3 pyramidal cell layer either 2 d (CON: 100 ± 2.08%, *n* = 8; FPI: 96.94 ± 0.71%, *n* = 8) or 1 month after FPI (CON: 100 ± 1.98%, *n* = 5; FPI: 99.16 ± 1.25%, *n* = 5).

Previous results have shown hyperexcitability of the dentate excitatory circuit (Santhakumar et al., 2000) even when the post-traumatic differences in fast inhibition (Toth et al., 1997) are abolished in the presence of $GABA_A$ receptor antagonists. Therefore, we examined the possible role for resting $[K^+]_o$ increase in the neuronal hyperexcitability observed in $GABA_A$ receptor antagonists (20 μM BMI) 1 week after FPI. Once again, there was no postinjury increase in the resting $[K^+]_o$, even in BMI and the NMDA receptor antagonist AP-5 (Fig. 1*E*) [CON: 100 ± 4.41%, *n* = 10; FPI: 101.02 ± 4.00%, *n* = 12; note that 20 μM AP-5 was included only to conform to our previous study (2000)]. Additionally, there was no increase in the resting $[K^+]_o$ after head injury when the $GABA_A$ channel blocker picrotoxin (100 μM) was substituted for BMI (Fig. 1*E*) (inset; CON: 100 ± 1.13%, *n* = 7; FPI: 98.46 ± 1.12%, *n* = 9) to control for any potential nonspecific effects of potassium channel block by BMI (Khawaled et al., 1999) on $[K^+]_o$.

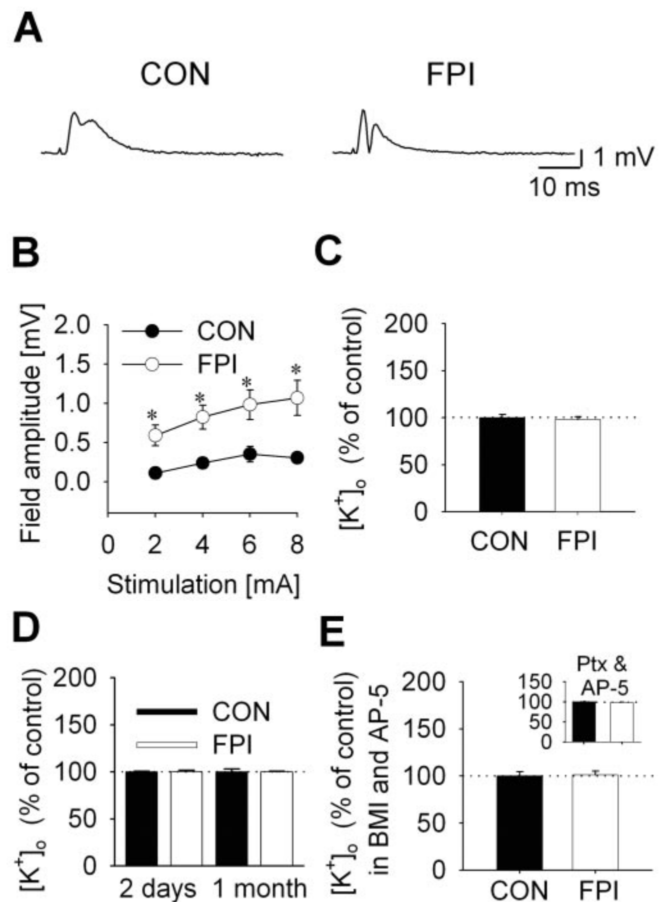

Figure 1. Resting $[K^+]_o$ is not increased in the post-traumatic dentate gyrus. *A*, Field recordings of perforant path-evoked granule cell responses are shown, 1 week after moderate head injury in slices from a fluid percussion-injured animal (FPI) and an age-matched sham-control animal (CON) in ACSF (at 6 mA stimulation intensity). *B*, Summary data obtained from experiments similar to those in *A*. Note that the amplitude of the population spike in slices from head-injured animals is larger than controls, indicating the presence of post-traumatic hyperexcitability. *C*, The resting $[K^+]_o$ in the granule cell layer from the same slices as in *B* was not increased 1 week after head trauma. *D*, There was also no increase in the resting $[K^+]_o$ 2 d and 1 month after injury. *E*, Resting $[K^+]_o$ in the granule cell layer in 20 μM BMI and 20 μM AP-5 was not different between head-injured and control animals 1 week after FPI. [Inset, Similar results were obtained in 100 μM picrotoxin (Ptx) and 20 μM AP-5.]

Taken together, these data demonstrate that the resting $[K^+]_o$ is not increased at any time point after injury.

Clearance of stimulation-evoked increases in $[K^+]_o$ is not impaired after head injury

The next series of experiments were performed to examine the post-traumatic changes in the buffering of $[K^+]_o$ transients evoked by neuronal activity, in control medium. The rate of clearance of the tetanic stimulation-evoked $[K^+]_o$ increases was measured in the granule cell layer 1 week after FPI. As shown by representative $[K^+]_o$ traces in Figure 2*A*, the amplitude of the orthodromically evoked $[K^+]_o$ elevation (above the resting $[K^+]_o$) was larger after head trauma (Fig. 2*B*) (CON: 0.87 ± 0.14 mM, *n* = 29; FPI: 1.35 ± 0.22 mM, *n* = 30), suggesting an increase in $[K^+]_o$ elevation as a result of neuronal hyperexcitability or a deficiency in the clearance of $[K^+]_o$. Indeed, the granule cell population spike was increased after head trauma, as measured after single-shock stimulation of the perforant path (Fig. 2*B*, inset) (at 8 mA stimulation intensity; CON, 0.51 ± 0.04 mV; FPI, 1.06 ± 0.20 mV), suggesting that the enhanced orthodromically

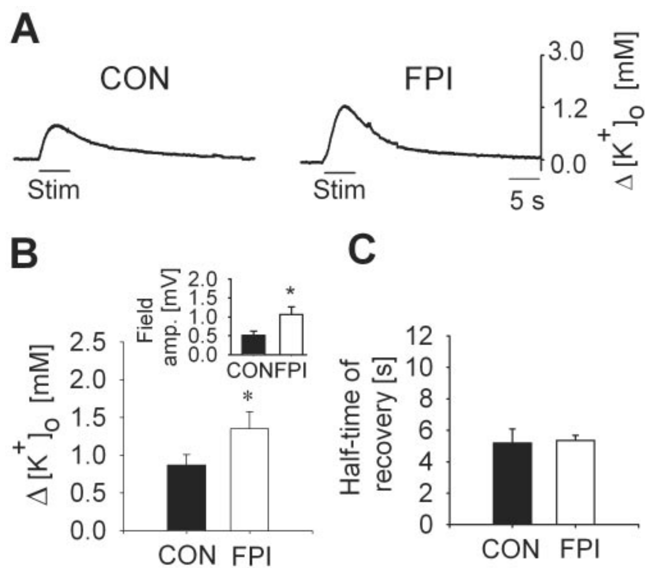

Figure 2. Absence of postinjury decrease in the rate of clearance of tetanic stimulation-evoked $[K^+]_o$ increase. *A*, Representative recordings of $[K^+]_o$ in the granule cell layer evoked by tetanic stimulation of the perforant path at 8 mA stimulation intensity (for 5 sec at 100 Hz) reveal a larger $[K^+]_o$ elevation 1 week after trauma (FPI) compared with controls (CON) (the *y*-axis shows $[K^+]_o$ elevation above resting $[K^+]_o$ on a Nernstian scale). *B*, Summary of data demonstrate an increase in the amplitude of the evoked $[K^+]_o$ transient (above the resting $[K^+]_o$) after head injury. The asterisk indicates significance (Mann–Whitney *U* test). Inset, Summary plot showing that the single-shock, perforant path-evoked population spike amplitude (at 8 mA simulation intensity), from the same slices as in *B*, was larger after head trauma. *C*, The half-time of recovery of the tetanus-evoked $[K^+]_o$ increase was not prolonged in slices from head-injured animals.

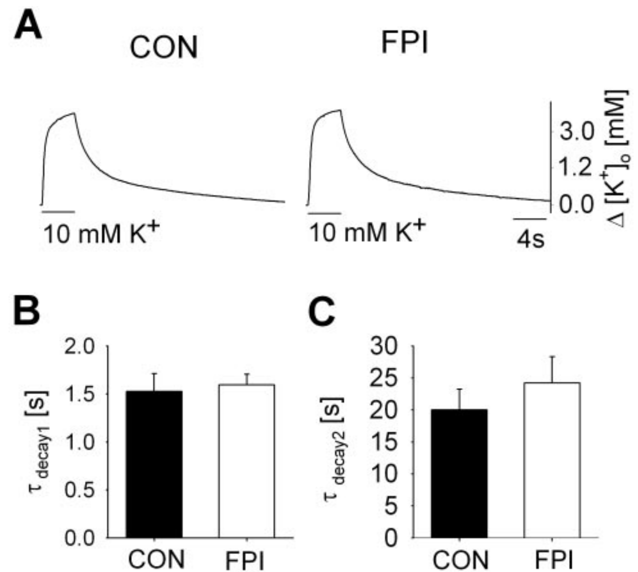

Figure 3. The rate of clearance of externally applied potassium is not impaired after head injury. *A*, Example of traces showing $[K^+]_o$ elevation and clearance in the granule cell layer during pressure application of ACSF containing 10 mM potassium in 1 μM TTX 1 week after injury (FPI) or sham operation (CON) (the *y*-axis showing $[K^+]_o$ elevation above rest as a micromolar concentration is on a Nernstian scale). *B, C*, Summary data showing no difference in the either the fast (*B*) or the slow (*C*) exponential decay time constants of the externally applied potassium between slices from injured and control animals.

evoked neuronal activity might contribute to the larger $[K^+]_o$ increase (Fig. 2*A,B*). In contrast, the half-time of recovery of the evoked $[K^+]_o$ transients (time taken for the $[K^+]_o$ elevation to decay to half the peak amplitude) was not increased (Fig. 2*C*) (CON, 5.19 ± 0.89 sec; FPI, 5.34 ± 0.33 sec), showing that buffering of the stimulation-evoked $[K^+]_o$ transients was not compromised. These findings indicate that increased neuronal firing, and not an impaired buffering of $[K^+]_o$, is likely to underlie the greater $[K^+]_o$ increase after tetanic stimulation of afferents after head trauma.

In the experiments described above, the postinjury increase in the orthodromic population spike amplitude (Fig. 2*B*, inset) is an obvious confounding factor in assessing the rate of clearance of the stimulation-evoked $[K^+]_o$ transients (Dietzel and Heinemann, 1986). Therefore, we examined the rate of decay of $[K^+]_o$ transients evoked by pressure application (see Materials and Methods) of ACSF containing 10 mM potassium (for 4 sec) in the presence of the sodium channel blocker TTX (1 μM) to block action potential firing. As shown by the representative $[K^+]_o$ recordings (Fig. 3*A*), potassium application evoked $[K^+]_o$ increases of similar amplitude head-injured and control tissue (CON: 4.24 ± 0.49 mM, $n = 11$; FPI: 3.48 ± 0.43 mM, $n = 11$). The rate of decay of the $[K^+]_o$ transient after external application of potassium was described by the sum of two exponentials, because the SSE improvement (see Materials and Methods; CON, 65.51 ± 28.22%, FPI, 63.30 ± 10.73%) was significant. Importantly, the rate of decay of the externally applied potassium was not decreased after head trauma (Fig. 3*B*) ($\tau_{decay\ (fast)}$; CON, 1.53 ± 0.18 sec; FPI, 1.59 ± 0.11 sec) (Fig. 3*C*) ($\tau_{decay\ (slow)}$; CON, 20.22 ± 3.24 sec; FPI, 24.20 ± 4.12 sec). Taken together, these data demonstrate that the buffering of $[K^+]_o$ increases is not impaired after head injury.

Larger and faster postinjury increase in orthodromically evoked $[K^+]_o$ transients

In an effort to determine whether the postinjury increase in the orthodromically evoked $[K^+]_o$ transients was a consequence of the enhanced neuronal activity, we examined the early increase in $[K^+]_o$ (within 50 msec of stimulation). Because the resolution of the early time course of $[K^+]_o$ elevation is difficult when tetanic stimulation is used, the rise and latency of the $[K^+]_o$ responses in the dentate granule cell layer were examined in response to single-shock perforant path stimulation. The experiments described in Appendix (available at www.jneurosci.org) and illustrated in Figure 8 established the temporal properties of the ISME and made it possible to measure the latency of the $[K^+]_o$ transients evoked by single-shock stimulation.

The amplitude of the single-shock, perforant path-evoked $[K^+]_o$ responses and population spikes were measured in the presence of the $GABA_A$ receptor antagonist BMI (and the NMDA receptor antagonist AP-5), because the orthodromically evoked $[K^+]_o$ transient in control ACSF could not be resolved above the electrical noise. As shown in Figure 4*A* (top traces) and as described previously (Santhakumar et al., 2000), the amplitude of the perforant path-evoked granule cell population spike was enhanced 1 week after head injury. In agreement with the larger neuronal firing, the amplitude of the orthodromically evoked $[K^+]_o$ transient was also increased after head trauma (Fig. 4*A*, middle and bottom traces) (Fig. 4*B*, $[K^+]_o$ 50 msec after a 2 mA stimulation) (CON: 0.06 ± 0.01 mM, $n = 9$; FPI: 0.15 ± 0.03 mM, $n = 10$). Despite the enhanced amplitude, there was no increase in the time constant for exponential decay of the $[K^+]_o$ transient (Fig. 4*C*) (CON: 180.28 ± 22.81 msec, $n = 4$; FPI: 178.62 ± 35.38 msec, $n = 7$). Therefore, similar to the results of the tetanic stimulation experiments described above, these data also suggest that the greater stimulus-evoked $[K^+]_o$ increase after head injury cannot be a result of decrease in the rate of clearance of $[K^+]_o$.

After the measurement of the amplitude and decay of the

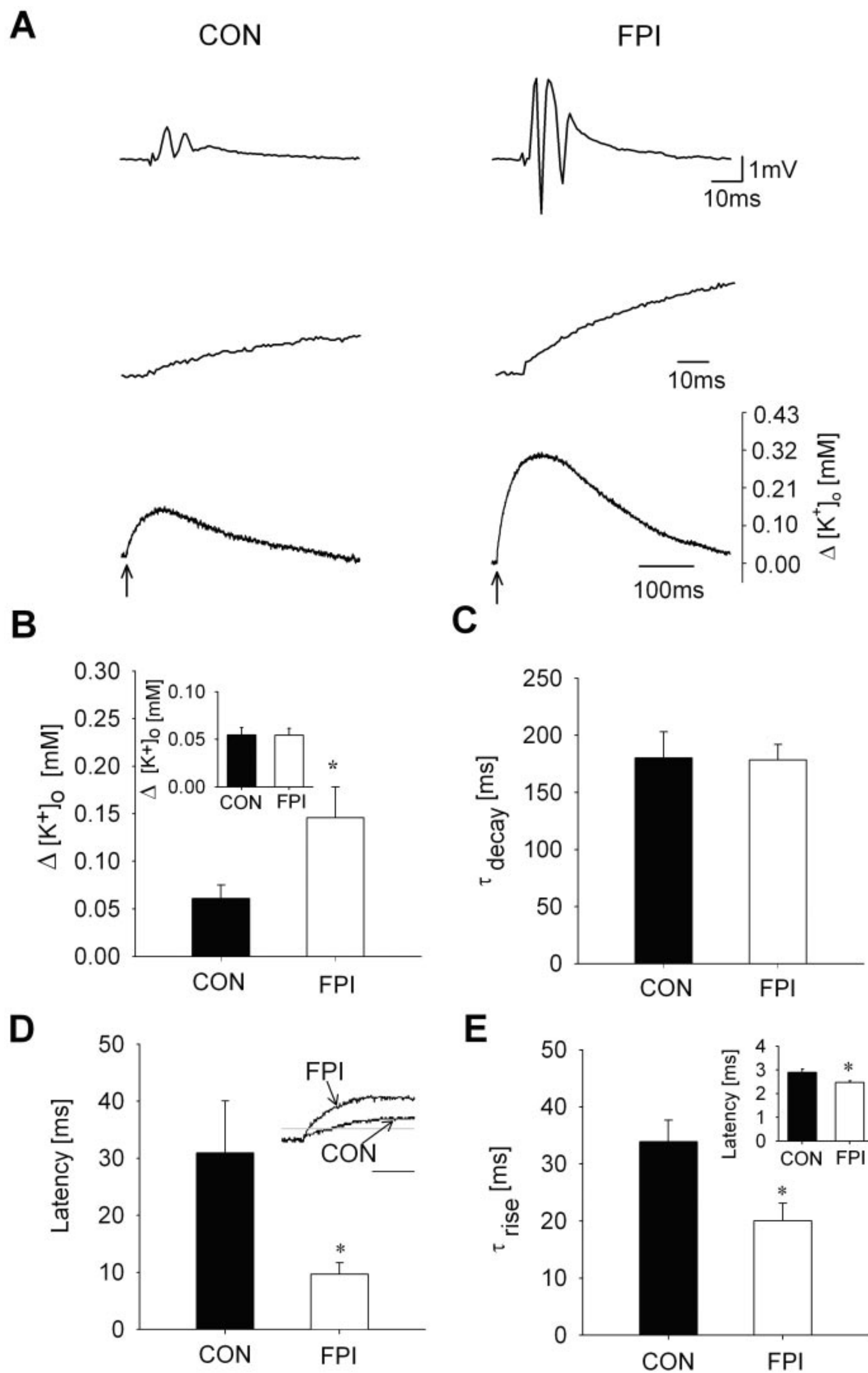

Figure 4. Faster rise and larger amplitude of evoked $[K^+]_o$ increase 1 week after head trauma. *A*, Example traces of granule cell population spikes (top) evoked by perforant path stimulation (stimulation intensity, 2 mA) show the enhanced excitability in BMI (20 μM) and AP-5 (20 μM) after injury (FPI) compared with controls (CON). Representative $[K^+]_o$ recordings at the same time scale as the field response (middle) and at a longer time scale (bottom) show a post-traumatic increase in the amplitude of the $[K^+]_o$ transient (the scale for micromolar $[K^+]_o$ increase is the same for the middle and bottom panels). *B*, Summary histogram demonstrate a greater evoked $[K^+]_o$ increase after head injury, in response to low-frequency perforant path stimulation (at 2 mA). Inset, Summary data show that the presynaptic component of the $[K^+]_o$ increase in the granule cell layer, evoked by a train of 10 stimuli (at 6 mA stimulation intensity) in slices recorded in the presence of ionotropic and metabotropic glutamate and GABA receptor antagonists, was not increased after head trauma. *C*, The exponential decay time constant of single-shock perforant path-evoked $[K^+]_o$ transient was not prolonged in slices from head-injured animals. *D*, Summary bar graphs show the post-traumatic decrease in the latency to a 0.08 mM (0.75 mV) $[K^+]_o$ elevation in response to low-frequency stimulation. Inset, Overlay of representative potassium electrode recordings from injured and control animals shows the faster rate of rise and shorter latency to detect a 0.08 mM increase in the evoked $[K^+]_o$ elevation after head trauma. The horizontal line indicates a 0.08 mM increase in $[K^+]_o$ (0.75 mV depolarization). Calibration bar, 40 msec. *E*, Histogram demonstrates the postinjury decrease in the rise time constant of the $[K^+]_o$ transient. Inset, Summary data show the faster latency to onset of the population spike after head trauma.

single-shock stimulation-evoked $[K^+]_o$ elevation, the latency and rise times were examined. The early time course of the perforant path-evoked $[K^+]_o$ transient revealed that the latency to an arbitrary threshold (0.08 mM) increase in $[K^+]_o$ (Fig. 4*D*) (CON, 30.94 ± 9.12 msec; FPI, 9.67 ± 2.03 msec; see inset for representative traces), time to peak (CON, 78.37 ± 5.79 msec; FPI, 60.22 ± 4.28 msec, $n = 7$), as well as the time constant for exponential rise of $[K^+]_o$ (Fig. 4*E*) (CON, 33.91 ± 3.78 msec; FPI, 20.06 ± 3.07 msec), were significantly faster after head trauma. Next, we examined whether an increase in the $[K^+]_o$ transient evoked by activation of the presynaptic fibers, or a decreased latency to onset of the postsynaptic activity after stimulation of the perforant path fibers, contributed to the faster rise and shorter latency of the orthodromically evoked $[K^+]_o$ increase. The component of the stimulus-evoked $[K^+]_o$ elevation that was caused by activation of the afferent fibers was determined by perfusing the slices with a mixture of ionotropic and metabotropic glutamate and GABA antagonists (20 μM BMI, 10 μM CNQX, 20 μM AP-5, 20 μM SCH, and 500 μM RS-MCPG) to block postsynaptic responses. Because the $[K^+]_o$ elevation in response to a single-shock stimulation of the afferent fibers could not be discriminated over the electrical noise, a train of 10 stimuli (at 6 mA, 200 Hz) was used in these experiments. In agreement with previous studies demonstrating $[K^+]_o$ increase by the activation of presynaptic fibers (Fisher et al., 1976; Aitken and Somjen, 1986; Jones and Heinemann, 1987; Poolos et al., 1987), stimulation of the perforant path resulted in $[K^+]_o$ elevations even in the absence of postsynaptic activity. There was no post-traumatic increase in the amplitude of the perforant path-evoked $[K^+]_o$ transient in the presence of blockers of synaptic transmission (Fig. 4*B*, inset) (peak $[K^+]_o$; CON: 0.05 ± 0.01 mM, $n = 13$; FPI: 0.05 ± 0.01 mM, $n = 13$), indicating that an increase in presynaptic $[K^+]_o$ elevation does not underlie the faster rate of rise of $[K^+]_o$ after head injury (Fig. 4*D*,*E*). As an additional control, we verified that there was no tetanus-evoked $[K^+]_o$ elevation in the granule cell layer in the presence of TTX (data not shown), indicating that electrical stimulation could not directly evoke $[K^+]_o$ increase in the absence of neuronal activity. In contrast to the lack of difference in the presynaptic component of the stimulus-evoked $[K^+]_o$ elevation, both the latency to onset (Fig. 4*E*, inset) (CON, 2.90 ± 0.14 msec; FPI, 2.47 ± 0.08 msec)

and the time to peak (CON, 4.33 ± 0.17 msec, FPI, 3.77 ± 0.18 msec) of the single-shock perforant path-evoked postsynaptic population spike (in BMI and AP-5) were shorter after head trauma. In agreement with the field recording data, the perforant path-evoked action potential firing, determined in separate experiments by whole-cell recordings from granule cells (also in BMI and AP-5), occurred at a significantly shorter latency after FPI (CON: 5.13 ± 0.25 msec, *n* = 4; FPI: 3.83 ± 0.23 msec, *n* = 6; at 4 mA stimulation intensity). However, the latency to onset of the perforant path-evoked, whole-cell-recorded EPSC in the granule cells was not shorter after injury (CON, 1.67 ± 0.05 msec; FPI, 1.89 ± 0.12 msec). Although the mechanisms underlying the decrease in the latency to onset of firing after trauma are not understood, the main point of these results is that the faster rise of $[K^+]_o$ in response to single-shock stimulation of the perforant path after head injury occurs in conjunction with an earlier onset for neuronal activity, as can be seen in Figure 4*A*.

These results show that the decay of the $[K^+]_o$ transient is not altered by trauma, indicating no alteration in the $[K^+]_o$ buffering system. Furthermore, these data also demonstrate that the earlier and faster rise of $[K^+]_o$ does not, by itself, provide evidence for decreased potassium buffering, because it occurs in conjunction with an earlier onset of neuronal firing after the activation of the perforant path.

Post-traumatic increase in antidromically evoked $[K^+]_o$ transients: reexamining protocols that attempt to normalize activity between injured and control neuronal circuits

As demonstrated by the results presented above, it is not possible to unequivocally establish the cause of the larger $[K^+]_o$ increase in the traumatized tissue using tetanic or single-shock stimulation of the perforant path, because of the temporal limitations posed by the ISME and the post-traumatic changes in the latency to onset and amount of neuronal firing. However, there is a paradigm that has been suggested previously to equalize neuronal activity in slices from control and head-injured animals (D'Ambrosio et al., 1999). The experimental protocol is based on antidromic stimulation of the axons in the presence of ionotropic glutamate receptor antagonists, an approach that is proposed to precisely control principal cell firing. However, this paradigm rests on two previously untested assumptions: first, that there is a 1:1 correspondence between the antidromic stimulation and the firing of action potentials by principal cells; and second, that the antidromic stimulation with a relatively large stimulating electrode causes a similar antidromic population spike in both control and post-traumatic hippocampi.

We implemented this paradigm to examine whether differences in evoked $[K^+]_o$ increase were eliminated when the granule cell axons were antidromically stimulated in the hilus. Surprisingly, the amplitude of the $[K^+]_o$ increase evoked by either trains of stimuli (Fig. 5*A,B*) or by a single stimulus (Fig. 5*B*, top inset) was greater after injury. The larger post-traumatic increases in the antidromically evoked $[K^+]_o$ transient could have occurred as a consequence of various factors including an increase in neuronal excitability (Santhakumar et al., 2000), a decrease in the extracellular space that would boost the ephaptic field effects (Jefferys, 1995), and/or impaired regulation of $[K^+]_o$. Control experiments showed that there was no difference in antidromically evoked $[K^+]_o$ increase when the perfusate was switched from a medium containing AP-5, CNQX, and BMI to a solution in which NBQX and picrotoxin were substituted for CNQX and BMI in either the control or injured slices (Fig. 5*B*, bottom inset) (percentage change after perfusate switch; CON: 97.69 ± 19.01%, *n* = 3; FPI: 104.89 ± 17.84%, *n* = 3). These data verified that neither the block of certain potassium channels by BMI (Misgeld et al., 1992; Khawaled et al., 1999) nor an increase in GABAergic currents by CNQX (McBain et al., 1992; Brickley et al., 2001; Maccaferri and Dingledine, 2002) affected the amplitude of the measured $[K^+]_o$ transients in slices from control and head-injured animals.

Next, the rate of clearance of the antidromically evoked $[K^+]_o$ increase was measured, to ascertain whether a post-traumatic decrease in buffering of $[K^+]_o$ contributed to the larger $[K^+]_o$ elevation after head trauma in the experiments described above. The rate of decay of the antidromically evoked $[K^+]_o$ transients was better fit by the sum of two exponentials (SSE improvement; CON, 12.02 ± 3.65%; FPI, 15.86 ± 6.30%; see Materials and Methods). Indeed, similar to the results in response to orthodromic perforant path stimulation, neither the decay time constants (Fig. 5*C*) (τ_{decay1}; CON, 1.48 ± 0.29 sec; FPI, 1.40 ± 0.28 sec; τ_{decay2}; CON, 7.76 ± 2.89 sec; FPI, 7.69 ± 1.54 sec; *n* = 12 CON and *n* = 11 FPI) nor the half-time of recovery (Fig. 5*C*, inset) (CON: 1.70 ± 0.12 sec, *n* = 15; FPI: 1.38 ± 0.10 sec, *n* = 11) of the antidromically evoked $[K^+]_o$ elevation in response to a train of 10 stimuli were increased in the slices from head-injured animals. If anything, there was a slight trend toward a faster (rather than slower) half-time of recovery of the potassium transient after injury.

The fortuitous observation that the $[K^+]_o$ elevation in response to eight stimuli in the control tissue and five stimuli in the injured tissue was similar (Fig. 5*B*) provided us with an opportunity to examine differences in the clearance of stimulation-evoked $[K^+]_o$ transients between control and traumatized tissue without the confounding effects of differences in the amplitude of evoked $[K^+]_o$ elevation. The decay time constants of the antidromically evoked $[K^+]_o$ transients of similar amplitude was not increased after head injury (τ_{decay1}; CON, 1.46 ± 0.19 sec; FPI, 1.63 ± 0.23 sec; τ_{decay2}; CON, 8.36 ± 2.05 sec; FPI, 6.61 ± 0.99 sec). These data confirmed that the clearance of $[K^+]_o$ was not compromised after 1 week after head injury and, therefore, was not the cause of the larger post-traumatic increase in the antidromically evoked $[K^+]_o$ transient. Additional experiments performed to further examine this issue at different time points and locations showed that, similar to our data from 1 week described above, the half-time of recovery of the antidromically evoked $[K^+]_o$ increases was not increased either 2 d or 1 month after injury, either in the granule cell layer (Fig. 5*D*) (half-time of recovery; 2 d after injury: CON, 1.68 ± 0.13 sec, *n* = 9; FPI, 1.60 ± 0.11 sec, *n* = 9; 1 month after injury: CON, 1.21 ± 0.16 sec, *n* = 10; FPI, 1.51 ± 0.11 sec, *n* = 11) or in the CA3 pyramidal cell layer (half-time of recovery; 2 d after injury: CON, 1.68 ± 0.17 sec, *n* = 9; FPI, 1.25 ± 0.12 sec, *n* = 9; 1 month after injury: CON, 1.36 ± 0.31 sec, *n* = 7; FPI, 1.76 ± 0.19 sec, *n* = 8). These results demonstrate that the rate of clearance of stimulation-evoked $[K^+]_o$ increase was not impaired 2 d, 1 week, or 1 month after head injury.

The fact that the antidromically evoked $[K^+]_o$ increase in ionotropic glutamate and GABA receptor antagonists was larger in the head-injured animals (Fig. 5*A,B*), despite a lack of decrease in the clearance of $[K^+]_o$, prompted us to examine whether the neuronal firing was enhanced after trauma in the paradigm used in the above experiments. As shown by whole-cell recordings from granule cells in response to a train of 10 stimuli (Fig. 5*A*, insets), each antidromic stimulus evoked a single action potential (CON and FPI, four of four cells each), confirming that the action potential firing evoked by antidromic stimulation of granule cell

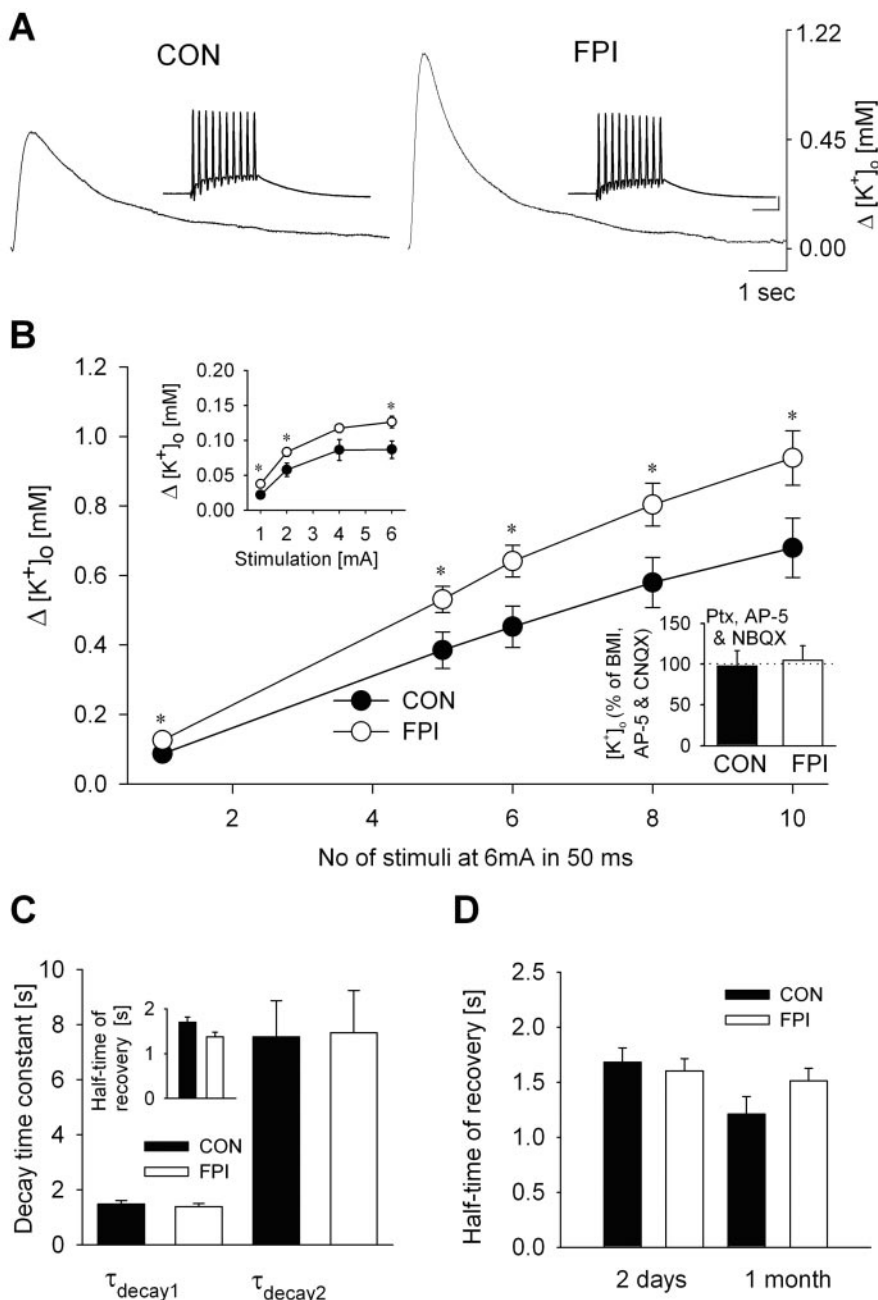

Figure 5. Larger antidromically evoked $[K^+]_o$ increase after head injury. *A*, Representative recordings of $[K^+]_o$ transients in the granule cell layer evoked by antidromic stimulation at 6 mA (10 stimuli in 50 msec) show the larger $[K^+]_o$ elevation in the fluid percussion head-injured animal (FPI) compared with the control (CON), in 20 μM BMI, 20 μM AP-5, and 10 μM CNQX ($\Delta[K^+]_o$ scale is exponential). Insets, Whole-cell recordings from granule cells in response to 10 stimuli (in 50 msec) show that each hilar stimulus evoked a single action potential in slices from both control and injured animals. Calibration: 20 mV, 10 msec. Recordings were obtained 1 week after head injury. *B*, Summary data similar to those in *A* are shown from slices from control animals (●) and FPI animals (○), in response to the increasing number of stimuli. Top inset, $[K^+]_o$ increase (as a micromolar potassium concentration indicated on the *y*-axis) in slices from control animals (●) and FPI animals (○) animals, evoked by single-shock stimulation in the hilus (see Materials and Methods) at an increasing intensity (indicated on the *x*-axis in mA). Note that the response to a 6 mA stimulation shown in the inset is the same as the response to a single stimulus in *B*. Bottom inset, Histograms show the percentage change in the $[K^+]_o$ elevation, evoked by a train of 10 stimuli (in 50 msec) to the hilus, when the perfusing medium was switched from 20 μM BMI, 20 μM AP-5, and 10 μM CNQX to 100 μM picrotoxin, 20 μM AP-5, and 10 μM NBQX. *C*, Fast and slow exponential decay time constants of the antidromically evoked $[K^+]_o$ transient were not different between head-injured and control animals 1 week after injury. Inset, The same data as in *C*, showing that the half-time of recovery of the evoked $[K^+]_o$ increase was not prolonged after FPI. *D*, Half-time of recovery of the antidromic stimulation-evoked $[K^+]_o$ transient 2 d and 1 month after FPI was not different from age-matched sham-controls.

axons was not different between injured and control animals. Although the individual granule cells fired the same number of action potentials, the antidromically evoked population spikes recorded in the granule cell layer simultaneously with the antidromically evoked $[K^+]_o$ transients (in Fig. 5) were larger after head injury (Fig. 6*A*,*B*). In addition to the larger amplitude, there was a post-traumatic decrease in the half-width (width at half-maximal amplitude) of the antidromic population spike (Fig. 6*C*) (CON: 0.73 ± 0.05 msec, $n = 15$; FPI: 0.58 ± 0.03 msec, $n = 11$), suggesting a greater synchrony in the granule cell firing after head trauma. In fact, the population spike in CA3 evoked by single-shock stimulation of the Schaffer collaterals (at 6 mA stimulation intensity) was also significantly larger 2 d after injury (data not shown; CON, 1.76 ± 0.30 mV; FPI, 2.99 ± 0.47 mV). Overall, similar to the orthodromically evoked $[K^+]_o$ increase, the larger antidromically evoked $[K^+]_o$ transient after FPI also occurred in conjunction with increased neuronal population spike. Although it is possible that a postinjury decrease in the extracellular space could contribute to both the enhanced population response and the larger $[K^+]_o$ transient (Jefferys, 1995; Nicholson et al., 2000), it is evident that the activity of populations of neurons in control and pathological conditions is not the same in the above experimental paradigm, possibly as a result of plastic changes in the neuronal networks (e.g., sprouting of the axons of principal cells after trauma) (McKinney et al., 1997; Santhakumar et al., 2001).

Post-traumatic decrease in granule cell response to $[K^+]_o$

Finally, we investigated the possibility that $[K^+]_o$ transients could still play a mechanistic role in the postinjury granule cell hyperexcitability even in the absence of impaired buffering of $[K^+]_o$, if the same $[K^+]_o$ increase evoked a larger depolarization and more action potential firing in the neurons after head injury. Brief application (10 msec) of ACSF containing 120 mM potassium through a glass pipette located at a constant distance (50 μm) from the recording electrode in the bath above the surface of the slice in the direction of flow of the perfusate, with a constant orientation of the slice, was used to evoke granule cell depolarization and firing. Whole-cell recordings were obtained from granule cells in ionotropic glutamate and GABA receptor antagonists to prevent polysynaptic activity. Surprisingly, potas-

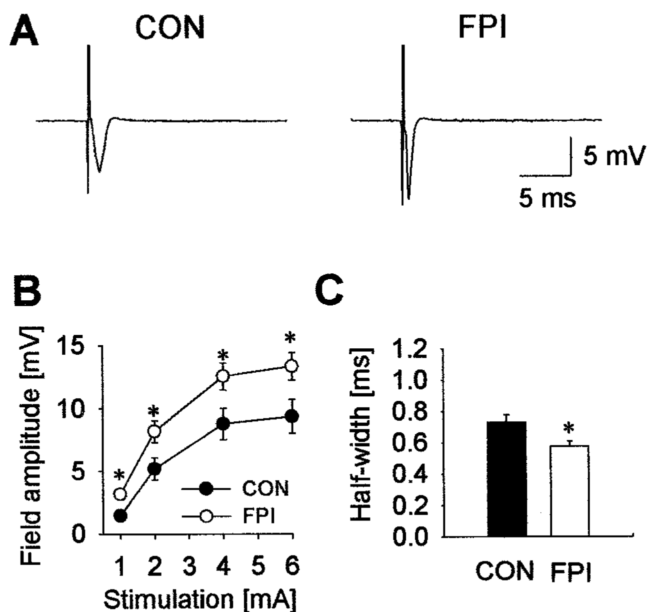

Figure 6. Hyperexcitable granule cell field responses to antidromic stimulation. *A*, Examples of population spikes evoked by antidromic stimulation of the granule cells (at 6 mA stimulation intensity) in the same slices as in Figure 5*A* from an injured animal (FPI) and sham-control animal (CON) are shown. *B*, Summary data of the antidromic population spike amplitudes during the $[K^+]_o$ recordings shown in the top inset in Figure 5*B* demonstrate an increase in the population spike amplitude 1 week after FPI. *C*, Summary plot shows that the half-width of the antidromically evoked population spike was decreased after head trauma.

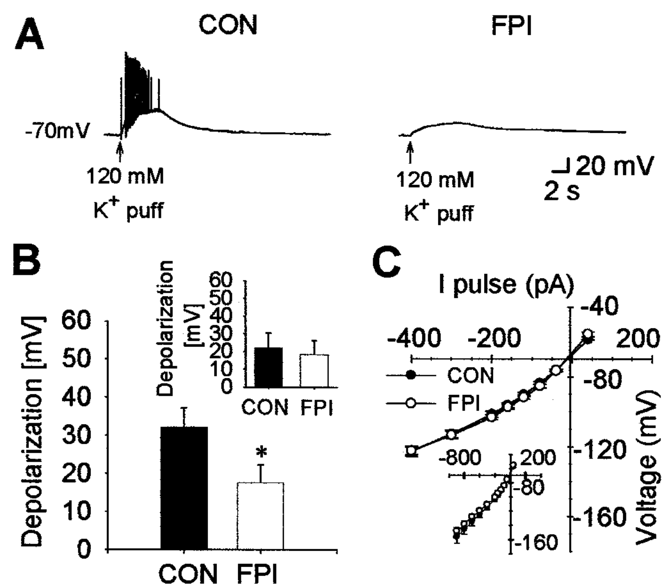

Figure 7. Post-traumatic decrease in granule cell firing in response to exogenous application of potassium. *A*, Representative voltage traces from granule cells in control animals (CON) and head-injured animals (FPI) show the post-traumatic decrease in depolarization and action potential firing evoked by exogenous potassium application. The traces were obtained by whole-cell patch-clamp recordings at −70 mV in 20 μM BMI, 20 μM AP-5, and 10 μM CNQX, 1 week after FPI. *B*, Summary data show the smaller potassium-evoked depolarization of granule cells from head-injured animals compared with controls from experiments similar to those in *A*. Inset in *B* shows that the difference in the potassium-induced depolarization was absent when QX-314 was present in the internal solution of the recording whole-cell pipette. *C*, Granule cell *I/V* plots from control animals (●; $n = 5$) and head-injured animals (○; $n = 5$) show that that there was no discernable difference in the voltage response evoked by +50 pA to −400 pA current steps (from a holding potential of −70 mV) after head trauma. Inset, Granule cell *I/V* plots from a subset of the cells, from control animals (●; $n = 3$) and injured animals (○; $n = 3$) in *C* in which larger current steps could also be tested, show that that there was no difference in the voltage response evoked by +50 pA to −700 pA current steps after head trauma.

sium application evoked a smaller depolarization (Fig. 7*A,B*) (CON: 32.25 ± 4.99 mV, $n = 12$; FPI: 17.58 ± 4.66 mV, $n = 11$) in head-injured animals. Furthermore, potassium application also evoked action potential firing in fewer granule cells after head trauma (CON: 70%, $n = 10$; FPI: 21%, $n = 14$), indicating a post-traumatic decrease in sensitivity to $[K^+]_o$ elevation. These data show that not only is there no evidence for impaired buffering of $[K^+]_o$, but the response of granule cells to $[K^+]_o$ transients is actually decreased after head trauma.

Although our main focus was to test the impaired $[K^+]_o$ buffering hypothesis of post-traumatic hyperexcitability (D'Ambrosio et al., 1999), an additional interesting point concerns the mechanisms that may cause the postinjury decrease in granule cell sensitivity to $[K^+]_o$ increases described above. What made the injured granule cells less responsive to $[K^+]_o$ increases? Previous studies have shown that the resting membrane potential, input resistance, and threshold for action potential generation are not different between granule cells from injured and control animals (Santhakumar et al., 2000). In agreement with these previous results, steady-state current–voltage (*I/V*) plots from granule cells in head-injured animals ($n = 5$ cells) and control animals ($n = 5$ cells) showed no difference in either the resting membrane potential (data not shown) or the slope of the *I/V* plots for negative current steps between +50 pA and −400 pA (Fig. 7*C*). Additionally, a subset ($n = 3$ cells each) of the granule cells that showed stable voltage responses to current injections between +50 pA and −700 pA also did not reveal a significant difference in the slope of the *I/V* plots from injured and control animals (Fig. 7*C*, inset) (note that if anything, there was a slight trend at very hyperpolarized potentials for the granule cells after injury to show less change in voltage to a given large current step, i.e., in the opposite direction of what would be expected if the potassium permeability were decreased due to fewer or less active potassium channels). However, an alternative approach for the identification of the mechanism of post-traumatic decrease in the potassium-evoked depolarization is to block the potassium channels with intracellular QX-314, a nonspecific potassium and sodium channel blocker. Intracellular application of QX-314 has been shown to decrease the neuronal responses to externally applied potassium (Smirnov et al., 1999). When QX-314 was included in the internal solution in our experiments, the differences in potassium-evoked membrane depolarization between granule cells from injured and control animals were abolished (Fig. 7*B*, inset) (CON: 21.67 ± 8.5 mV, $n = 7$; FPI: 18.31 ± 7.7 mV, $n = 6$). Furthermore, the depolarization evoked by potassium application in the control tissue was reduced to a level similar to that of the granule cells from injured animals. The fact that intracellular QX-314 inside the recorded neuron could occlude the post-traumatic decrease in the potassium-evoked depolarization indicates that the underlying mechanisms involve differences in the expression of potassium channels, even if the steady-state *I/V* plots could not reveal such differences. In addition, the lack of a complete block of the potassium-evoked depolarization by intracellular QX-314 in these experiments could be due to various factors, e.g., there may be potassium channels that are not fully blocked by intracellular QX-314.

Discussion

This study systematically tested the hypothesis that impaired $[K^+]_o$ buffering underlies post-traumatic neuronal hyperexcitability (D'Ambrosio et al., 1999). First, we reexamined the resting $[K^+]_o$ in both the dentate gyrus and CA3 at various time points

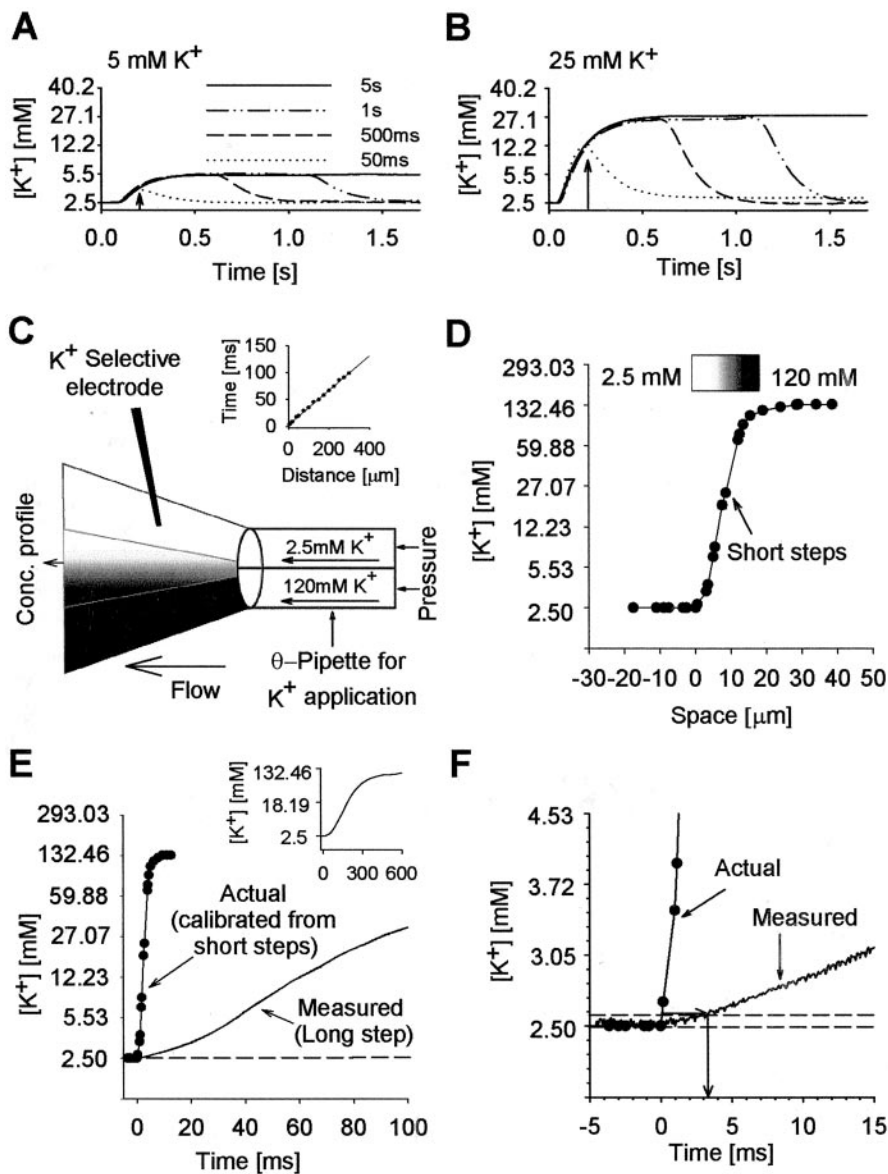

Figure 8. Detection of rapid changes in potassium concentration by ISMEs. Representative traces obtained by applying ACSF containing 5 mM (*A*) or 25 mM (*B*) potassium for increasing durations (50 msec, 500 msec, 1 sec, and 5 sec) show that brief changes in $[K^+]_o$ lasting <500 msec can be detected by ISMEs. *C*, A schematic of the experimental setup for fast application switch from normal (2.5 mM, white) to high (120 mM, black) potassium concentration is shown. Inset, Regression fit shows the linear time distance profile for the stepping motor operating at 3 μm/msec [see Appendix (available at www.jneurosci.org) for details]. *D*, Spatial profile of the potassium concentration is shown in the narrow (20 μm) interface region between the 2.5 mM and 120 mM potassium flow. Zero on the *x*-axis indicates the start of the interface region. *E*, Overlay of the actual and the measured $[K^+]$ during the long (i.e., uninterrupted) step from normal to high $[K^+]$ across the liquid interface is shown. The trace with circles is the actual K^+ concentration outside the electrode tip calculated from the spatial concentration profile of the flow (in *D*) and the speed of the fast application switch (3 μm/msec). The line trace is what the electrode measured during the long step across the liquid interface normal to high $[K^+]$. The inset shows that the electrode correctly measured the change in concentration during prolonged application of 120 mM potassium. *F*, The first 15 msec of the plots in *E* shows that a 0.08 mM (equivalent to 0.75 mV) change in $[K^+]_o$ can be detected in <4 msec. The top dashed line indicates the threshold level of 0.08 mM increase in $[K^+]_o$, and the bottom dashed line indicates the steady-state $[K^+]_o$.

after injury but found no evidence for an elevated resting $[K^+]_o$. Next, we reevaluated the experimental paradigm that was proposed to normalize neuronal activity between the control and injured circuits to study deficits in $[K^+]_o$ regulation and found post-traumatic alterations in the neuronal population activity evoked by antidromic stimulation in ionotropic receptor antagonists. Moreover, examination of the rate of clearance of $[K^+]_o$ increases evoked either by orthodromic stimulation or after exogenous potassium application did not reveal any postinjury decrease in $[K^+]_o$ regulation. In fact, the decrease in potassium-evoked depolarization in neurons from head-injured animals suggests a post-traumatic decrease in sensitivity to $[K^+]_o$ elevation.

$[K^+]_o$ buffering after head injury

Concussive brain injury causes an immediate increase in the resting $[K^+]_o$ *in vivo* that returns to control levels within a few hours (Takahashi et al., 1981; Katayama et al., 1990). In agreement with the rapid recovery of resting $[K^+]_o$, the resting membrane potential of granule cells *in vitro* is similar to controls within hours after *in vivo* trauma (Ross and Soltesz, 2000; Santhakumar et al., 2000). Our data showed no increase in the resting $[K^+]_o$ after FPI, in slices in which post-traumatic hyperexcitability in the dentate gyrus was directly verified, despite a recent study indicating that elevated resting $[K^+]_o$ in hippocampal slices was responsible for the postinjury neuronal hyperexcitability (D'Ambrosio et al., 1999). Furthermore, there was no increase in the resting $[K^+]_o$ in either the dentate gyrus or CA3, either 2 d or 1 month after trauma. The possible sources of the discrepancy between the data from D'Ambrosio et al. (1999) and the present study could be that the former study used ACSF containing 4.3 mM potassium and measured baseline $[K^+]_o$ during low-frequency stimulation (0.05 Hz) at room temperature, whereas we measured the resting $[K^+]_o$ in the absence of evoked neuronal activity at 36°C in a perfuste containing 2.5 mM potassium, comparable with the $[K^+]_o$ measured *in vivo* (Prince et al., 1973; Somjen, 1979; Somjen and Giacchino, 1985; Xiong and Stringer, 1999) and used in previous studies (Rose and Ransom, 1996; Xiong and Stringer, 2000; Brickley et al., 2001). In addition, it should be pointed out that a persistent increase in the resting $[K^+]_o$ in slices is unlikely because the bath solution would be expected to act as an infinite potassium sink and remove any post-traumatic, steady-state increase in $[K^+]_o$.

We also examined the rate of clearance of the $[K^+]_o$ elevation evoked by neuronal activity during the decay phase of the $[K^+]_o$ transient (Xiong and Stringer, 1999). The half-time of recovery of the $[K^+]_o$ transient provided a measure of the clearance of $[K^+]_o$ that was normalized for the absolute level of the $[K^+]_o$ increase. The rate of clearance of the $[K^+]_o$ increase evoked by either orthodromic or antidromic stimulation was not compromised after trauma. Similarly, there was no postinjury impairment in the clearance of $[K^+]_o$ transients after either high-frequency or single-shock orthodromic stimulation, or after exogenous potassium application. Overall, our data show that there is no decrease

in the recovery of $[K^+]_o$ after head injury at any of the time points examined.

Interestingly, whereas the decay of the single-shock stimulation-evoked $[K^+]_o$ transient was adequately fit by a single exponential, the larger $[K^+]_o$ increases, in response to both potassium application and to high-frequency, antidromic stimulation, were better fit by the sum of two exponentials, consistent with the contribution of different mechanisms of $[K^+]_o$ regulation with increasing $[K^+]_o$ (Hertz, 1978; Rose and Ransom, 1996). Several processes, including diffusion through extracellular space, spatial buffering through glial K_{IR}, and active (e.g., via the Na^+/K^+ ATPase) and passive potassium uptake, are known to regulate $[K^+]_o$ (Orkand et al., 1966; Heinemann and Lux, 1975; Ballanyi et al., 1984; Newman et al., 1984; Nicholson, 1995; Walz, 2000). However, the proportion of the $[K^+]_o$ cleared by each of these processes is not fully known in the normal brain (Walz, 2000; Steinhauser and Seifert, 2002), and their contributions might vary in pathophysiological states (Onozuka et al., 1987; MacFarlane and Sontheimer, 1997; Bordey and Sontheimer, 1998; Walz and Wuttke, 1999; Schroder et al., 1999; Xiong and Stringer, 2000; Hinterkeuser et al., 2000; Amzica et al., 2002). Nevertheless, although the role of individual regulatory mechanisms in maintaining $[K^+]_o$ homeostasis might change after brain injury, our results show that the buffering of stimulation-evoked $[K^+]_o$ elevation is not decreased, indicating that impaired clearance of $[K^+]_o$ does not underlie post-traumatic dentate hyperexcitability.

Changes in granule cell excitability during antidromic stimulation and exogenous K^+ application after head trauma

The present study examined the validity of antidromic activation of neurons in the presence of glutamate receptor antagonists (D'Ambrosio et al., 1999), a paradigm that was proposed to be useful to evaluate the $[K^+]_o$ regulation without the confounding effects of postinjury increases in neuronal excitability. Our data showed that granule cells responded with a single action potential to each antidromic stimulus under these conditions. However, the amplitude of the antidromic population spike was larger after trauma, suggesting that more neurons were firing with greater synchrony after head injury. It is likely that the presence of post-traumatic mossy fiber sprouting (Santhakumar et al., 2001) contributes to the activation of a greater population of granule cells in response to antidromic stimulation, resulting in a larger antidromic population spike. In addition, it is possible that a decrease in the extracellular volume fraction and tissue conductivity (Jefferys, 1995) contribute to the increase in the amplitude of the population spike and $[K^+]_o$ elevation evoked either by orthodromic or antidromic stimulation of granule cells after head trauma. However, the presence of increased stimulus-evoked neuronal activity and hilar cell loss after head injury confounds the meaningful comparison of the alterations in extracellular volume fraction during neuronal activity, between control and injured animals. Although the factors underlying the enhanced antidromically evoked population response after head trauma are not known, our results show that the postinjury alteration in the neuronal population activity and not a decrease in the clearance of $[K^+]_o$ underlies the enhanced $[K^+]_o$ elevation in an antidromic stimulation-based paradigm.

A mechanism by which stimulation-evoked $[K^+]_o$ elevation could trigger post-traumatic hyperexcitability, even in the absence of decreased potassium buffering, is whether $[K^+]_o$ increases caused greater firing in the granule cells from head-injured animals. Contrary to the above idea, our results show that granule cells responded to potassium application with smaller depolarization and less action potential firing after FPI. Although the steady-state *I/V* curves from granule cells in injured and control animals were similar, the potassium-evoked depolarization of granule cells from control animals was decreased to postinjury levels when potassium channels were blocked by intracellular QX-314 inside the recorded neuron. These data are consistent with a post-traumatic decrease in the potassium permeability, not revealed by the *I/V* plots, contributing to the smaller amplitude of the potassium-evoked depolarization in granule cell after head trauma. Although it is possible that the enhanced $[K^+]_o$ transients during neuronal activity may contribute to neuronal hyperexcitability by increasing the presynaptic axon terminal excitability (Noebels and Prince, 1978; Stasheff et al., 1993), these results show that granule cells from head-injured animals are hypo-excitable in response to $[K^+]_o$ increases, indicating that stimulation-evoked $[K^+]_o$ transients cannot directly underlie granule cell hyperexcitability.

Post-traumatic increase in stimulation-evoked $[K^+]_o$ transient: the cause or result of neuronal hyperexcitability?

In several experiments, we examined whether increased neuronal firing could account for the larger amplitude of the stimulation-evoked $[K^+]_o$ transients after injury. The response time of the ISME was evaluated to determine whether the electrodes could be used to study the time course of the early phase of $[K^+]_o$ elevation evoked by single-shock stimulation [see Fig. 8; also see Appendix (available at www.jneurosci.org)]. The electrodes could detect $[K^+]_o$ increases within a few milliseconds, consistent with previous studies (Prince et al., 1973; Lux and Neher, 1973; Moody et al., 1974; Amzica et al., 2002), and revealed a faster rise and a shorter latency to orthodromically evoked $[K^+]_o$ transients after injury. Although there was no post-traumatic increase in the $[K^+]_o$ transient evoked by afferent stimulation in postsynaptic receptors antagonists, the orthodromically evoked granule cell population spike occurred earlier after trauma, suggesting that the shorter latency to neuronal response could contribute to the faster postinjury $[K^+]_o$ increase. Consistent with the shorter latency to the evoked population spike, whole-cell recordings from granule cells demonstrated an earlier onset of firing in response to perforant path stimulation after head trauma. In addition to the faster onset, the greater number of postsynaptic action potentials fired in response to perforant path stimulation (Santhakumar et al., 2000) might contribute to the larger amplitude, shorter latency, and faster rise of the orthodromically evoked $[K^+]_o$ elevation. In addition, the post-traumatic hyperexcitability of certain nonprincipal cells in the dentate gyrus, e.g., the mossy cells (Santhakumar et al., 2000) or GABAergic cells (Ross and Soltesz, 2000; Santhakumar et al., 2001), could also contribute to the augmentation of the evoked $[K^+]_o$ increase. Overall, in agreement with the role for neuronal activity in postinjury epileptogenesis (Graber and Prince, 1999), our results underscore the role for the increase in neuronal activity, rather than an impaired $[K^+]_o$ buffering, as the primary mechanism for the enhanced risk for seizures after head injury.

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