Opposing Modifications in Intrinsic Currents and Synaptic Inputs in Post-Traumatic Mossy Cells: Evidence for Single-Cell Homeostasis in a Hyperexcitable Network

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Head trauma is a major cause of epilepsy in the adult population and represents the etiology of ≈20% of epilepsy cases in the general population (Hauser et al. 1991). Although the link between head trauma and temporal lobe epilepsy is well established, the mechanisms of epileptogenesis following head injury have not been fully elucidated (Garga and Lowenstein 2006). Fluid percussion injury (FPI), a rodent model of head trauma, has been instrumental in revealing various synaptic and cellular processes that mechanistically link the traumatic event to the development of persistent limbic hyperexcitability. Importantly, the dentate gyrus in FPI exhibits many pathological hallmarks of human head trauma and temporal lobe epilepsy, including mossy fiber sprouting, hilar cell loss, and increased in vitro and in vivo hyperexcitability (Lowenstein et al. 1992; Santhakumar et al. 2000, 2001; Toth et al. 1997).

Mossy cells, excitatory neurons located in the dentate hilus, are known to be some of the most vulnerable neurons in the entire mammalian brain, and significant mossy cell loss is observed in human epileptic tissue as well as after experimental head trauma (Blumcke et al. 2000; Lowenstein et al. 1992; Toth et al. 1997). However, several lines of evidence suggest that some mossy cells survive trauma (Blumcke et al. 2000; Ratzliff et al. 2002; Toth et al. 1997) and that these surviving mossy cells may spread or amplify dentate network hyperexcitability. First, mossy cells that survive traumatic head injury have been shown to respond to perforant path stimulation with an increased number of action potentials (Santhakumar et al. 2000). Second, mossy cells and granule cells form a recurrent excitatory loop as the vast majority of mossy cell terminals synapse onto granule cell dendrites, and granule cells project to mossy cells (Wenzel et al. 1997). Not only can mossy cells spread excitability to intralamellar granule cells, but they are in a unique position to spread excitability throughout the hippocampus due to their long-distance associative and commissural projections (Blasco-Ibanez and Freund 1997; Buckmaster et al. 1996; Frotscher et al. 1991; Ratzliff et al. 2002; Ribak et al. 1985; Soltesz et al. 1993). Recent results revealed that the net physiological effect of mossy cells is to excite intralamellar and extralamellar granule cells (but see Sloviter et al. 2003) because the acute deletion of mossy cells from horizontal and longitudinal hippocampal slices leads to decreased granule cell activity (Ratzliff et al. 2004). Finally, consistent with the experimental data indicating that mossy cells contribute to granule cell hyperexcitability in an epileptic brain, a recent anatomically and physiologically realistic computational modeling study showed that the spread of granule cell hyperexcitability was decreased if mossy cells were “killed” in the model (Santhakumar et al. 2005). These data support the idea that mossy cells can spread the hyperexcitability of granule cells through the dentate network.

In this study, we found that mossy cells did not show significant changes in their I-F and I-V curves after trauma despite the presence of a hyperexcitable dentate network. Yet on closer inspection, extensive, opposing alterations were found in various membrane currents that together resulted in the unchanged I-F and I-V relationships observed in posttraumatic mossy cells. Miniature and spontaneous excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) also displayed wide-ranging, but nonrandom, systematic and opposing

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changes. These results point to homeostatic regulatory mechanisms influencing the properties of single cells in the hyperexcitable dentate gyrus.

**Methods**

**Mossy cell labeling and fluid percussion injury**

All procedures were performed under protocols approved by the University of California Irvine Institutional Animal Care and Use Committee. Mossy cell labeling (Ratzliff et al. 2004) and preparation for lateral fluid percussion injury (FPI) head trauma (Dixon et al. 1987; Lowenstein et al. 1992; Santhakumar et al. 2001; Toth et al. 1997) were performed on juvenile (postnatal days 13–15) Wistar rats (Charles River, Wilmington, MA) in one combined surgery. Briefly, the rats were placed in a stereotaxic frame under ketamine-xylazine anesthesia. For the mossy cell labeling (Ratzliff et al. 2004), a 2 × 2-mm hole was trephined into the right parietal bone, and Hamilton syringes were used to inject 1.5 µL of 7.5% (wt/vol) fast 3,3′-dilinoleoylcarbocyanine perchlorate in DMSO (DiO; Invitrogen, Carlsbad, CA) at two sites in the right dentate gyrus. The hole was sealed with bone wax and a plastic cap to prevent movement of the brain outside the skull during the injury. The animals were then prepared for FPI: on the left side, a second 2-mm hole was trephined to the skull at 3 mm caudal to bregma, 3.5 mm lateral from the sagittal suture. Two steel screws were placed 1 mm rostral to bregma and 1 mm caudal to lambda. A Luer-Loc syringe hub with a 2.6 mm ID was placed over the exposed dura and bonded to the skull with cyanoacrylate adhesive. Dental acrylic was poured around the injury tube and skull screws and allowed to harden. Neopore® was applied to the wound, and the animal was returned to its home cage. One day later, animals were anesthetized with halothane and decapitated. For the mossy cell labeling (Ratzliff et al. 2004; Toth et al. 1997), the rats were anesthetized with halothane and decapitated. Drugs used in each experiment are listed in the appropriate figure legends and were purchased from Tocris (Ellisville, MO). The following pipette solutions (pH 7.2–7.25, 265–275 mOsm) were used (concentration in mM). For most experiments, unless otherwise specified: 140 K-glucuron, 2 MgCl2, and 10 HEPES; for Na currents: 140 CsCl, 10 EGTA, 10 HEPES, 2 MgCl2, and 2 ATP; for K-currents: 120 K-glucuronate, 20 KCl, 10 HEPES, 2 MgCl2, and 20 EGTA; for evoked EPSCs in granule cells: 140 Cs-glucuronate, 2 MgCl2, and 10 HEPES; for mIPSCs and sIPSCs: 140 CsCl, 2 MgCl2, and 10 HEPES; for depolarization-induced suppression of inhibition (DSI): 140 CsCl, 2 MgCl2, 10 HEPES, 2 EGTA, 3 QX-314, 0.2 EGTA, 1 ATP, and 0.2 GTP. Whole cell recordings were performed using IR-DIC visualization techniques (Stuart et al. 1993) with a Zeiss Axioskop FS microscope, using a ×40 or ×60 water-immersion lens. For all experiments that examined firing rate, input resistance, and action potential waveforms, the cells were maintained at ~60 mV with small current injections. The test pulse consisted of 500-ms current injections, except for Fig. 2B, where single action potentials were elicited with 20-ms current injections. Nucleated patches were pulled from whole cell patches, as described previously (Martina and Jonas 1997). Recordings were made using an AxoPatch 200B amplifier, filtered at 4 kHz using a Bessel filter, and digitized at either 10 or 100 kHz with a Digidata 1320A analog–digital interface (Molecular Devices, Sunnyvale, CA). To induce DSI, cells were voltage clamped at −60 mV and depolarized to 0 mV for 100 or 500 ms (Chen et al. 2003; Pilter and Alger 1992). For evoked EPSC experiments in granule cells, synaptic events were evoked with an ACSF-filled patch pipette, using an A360 stimulus isolator (WPI, Sarasota, FL). The stimulation strength was 0 Hz delivered with a monopolar stimulation electrode placed in the inner molecular layer (30–50 µm from the granule cell layer at a lateral distance of ≥100 µm from the recording site). Stimulus intensities were adjusted to 30–50% of the maximal response, typically between 100 µA and 1 mA. Series resistance was monitored periodically (initial series resistances ranged from 8 to 12 MΩ), and recordings were rejected if it changed by >15%.

**Measurement of action potential threshold**

Three methods were used to detect the AP threshold. The first method, “change in dV/dt” is to take the dV/dt of the voltage trace, and take the SD of the dV/dt for 50 ms before the AP. The threshold is reached once the dV/dt reaches: mean(dV/dt) + 2*SD(dV/dt) (Athern and Bevan 2005). The second method, “dV/dt threshold” is to set a cutoff point of the dV/dt, such as 30 mV/ms, as the AP threshold (Fig. 2A, dotted line) (Cooper et al. 2003; Metz et al. 2005). The final method, “maximum of second derivative” is to take the maximum of the second derivative of the voltage trace with respect to time (Fig. 2A, gray line) (Mainen et al. 1995).

**Data analysis**

Synaptic currents were analyzed for frequency, amplitude, rise time, and τ decay using MiniAnalysis 6.0 (Synaptosoft, Decatur, GA). DSI was analyzed with a custom-written software program that measures the synaptic charge transfer after the depolarization as a percentage of baseline charge transfer for 3 s before the depolarization. Analysis of action potential waveforms and threshold was performed with scripts written in Matlab 7.0 (The MathWorks,
Natick, MA). Statistics were performed using either Student’s t-test or the Kolmogorov-Smirnov test, with significance set at $P < 0.05$. $N$ refers to number of cells (for physiology) or number of animals (cell counts). Sodium channel permeability was calculated from the Goldman-Hodgkin-Katz current equation $I_{Na} = I_{Na}^{\infty}(RT)/(2eEF^5)(1 - \exp(-FV/RT))/[[Na] - [Na]_0[\exp(-FV/RT)]$, (Martina and Jonas 1997). The sodium channel activation curve was fitted with a Boltzmann function raised to the third power: $f = 1/[1 + \exp(-(V - V_{\theta}))/k]^3$, and the steady-state inactivation curve was fitted with a simple Boltzmann function $f = 1/[1 + \exp(-(V - V_{\theta}))/k]$ (Martina and Jonas 1997).

Computational modeling

The model network was implemented using the NEURON 5.6 simulation environment (Hines and Carnevale 1997). The large-scale, anatomically and biophysically realistic model network used in this study was identical to the one described previously (Dyhrfjeld-Johnsen et al. 2006). Briefly, the multi-compartmental single-cell models were taken from the 500-cell network described in Santhakumar et al. (2005) and were incorporated into a network including 50,000 granule, 1,500 mossy, 500 basket, and 600 HIPP cells, i.e., in biologically realistic proportions as described in Dyhrfjeld-Johnsen et al. (2006). The distribution and synaptic conductances were based on those in Santhakumar et al. (2005) and were scaled to reflect the larger number of cells (Dyhrfjeld-Johnsen et al. 2006). The parameters used in the model were based on available physiological and anatomical data from our lab and from the literature (for details, see Dyhrfjeld-Johnsen et al. 2006; Santhakumar et al. 2005). Perforant path stimulation was simulated by a single synaptic input to 5,000 granule cells, 10 mossy cells, and 50 basket cells (situated in the middle lamella of the model network) at $t = 5$ ms after the start of the simulation. For the “FPI only” network, strictly identical to the “50% sclerosis” functional model network in Dyhrfjeld-Johnsen et al. (2006), mossy fiber sprouting and hilar cell loss were added to the model. Mossy fiber sprouting was modeled by adding synaptic connections from granule cells to the proximal dendrites of granule cells. Because FPI produces moderate mossy fiber sprouting, the degree of mossy fiber sprouting in the model was set at 50% of the maximal sprouting observed in the pilocarpine model of epilepsy (Buckmaster et al. 2002), which exhibits a very high-density of sprouting. Hilar cell loss was modeled by randomly removing 50% of hilar interneurons (HIPP cells) and 50% of mossy cells, which is the approximate degree of cell loss observed after FPI in our hands (Toth et al. 1997). Additional details of the model can be found in Dyhrfjeld-Johnsen et al. (2006).

Results

Basic input-output curves of mossy cells are unchanged after injury

To examine whether mossy cells exhibit any major changes in their intrinsic properties, the mean firing rate was measured.
in response to 500-ms depolarizing pulses, when cells were held at $-60$ mV (representative traces in Fig. 1A). As shown in Fig. 1B, there was a trend toward increased firing rates in FPI cells, but this increase was not significant despite a large number of cells in both groups (control: $n = 20$; FPI: $n = 20$). Several additional parameters of the firing patterns were measured to explore whether more subtle alterations occurred. There were no differences between FPI and control mossy cells in the mean interspike interval, the first interspike interval, the adaptation of the interspike interval, or the latency to the first spike (Fig. 1, C–E). Next, the current-voltage relationship of mossy cells was measured in response to 500-ms hyperpolarizing pulses when cells were held at $-60$ mV. The responses of FPI cells to these pulses were not different from the responses of control cells (measurements taken at the end of the pulse; control: $n = 20$; FPI: $n = 20$; Fig. 1F). Accordingly, the apparent input resistance around and below $-60$ mV was not changed between control and FPI cells at any current amplitude tested. However, the resting membrane potential ($V_m$) of mossy cells was significantly depolarized after FPI ($V_m$ in control: $-66.6 \pm 1.4$ mV, $n = 20$; in FPI: $-63.2 \pm 0.8$ mV, $n = 20$; Fig. 1F, inset). Therefore after FPI, mossy cells have maintained their target firing rate and input resistance. This result suggests two possible interpretations. It is possible that the intrinsic properties of mossy cells simply have not changed. Alternatively, several intrinsic properties may have changed concurrently, in a coordinated manner, resulting in the unaltered $I$-$V$ and $I$-$F$ relationships.

**Alterations in action potential threshold and waveform**

To explore whether subtle changes occur to mossy cells’ intrinsic properties after FPI that cannot be detected in the current-voltage or current-firing relationships, several parameters of the mossy cell action potential (AP) in control and FPI mossy cells were measured. AP threshold can be regulated in an activity-dependent manner (Henze and Buzsaki 2001), and an altered AP threshold has been predicted in a genetic form of epilepsy (Spampanato et al. 2004). As there is no single accepted method of determining the AP threshold (Sekerli et al. 2004), three different methods were used to calculate this value (see METHODS for detailed explanation and Fig. 2A for illustration). The AP threshold of mossy cells was significantly depolarized after FPI, when single APs were evoked with 20-ms current pulses (Fig. 2B; change in 1st derivative: control = $-34.7 \pm 0.3$ mV, FPI = $-32.8 \pm 0.4$ mV; 1st derivative threshold: control = $-34.8 \pm 0.3$ mV, FPI = $-32.8 \pm 0.3$ mV; maximum of 2nd derivative: control = $-32.1 \pm 0.3$ mV, FPI = $-30.6 \pm 0.7$ mV; control, $n = 12$; FPI, $n = 19$). Note that both the change in first derivative and the first derivative threshold measures gave nearly identical values. The AP threshold was also depolarized after FPI when a train of APs was evoked by a 500-ms, 200-pA current injection, for AP numbers 2–10 (Fig. 2C; control: $n = 7$, FPI: $n = 16$).

In theory, if the AP threshold was depolarized without additional concurrent changes, mossy cells should fire fewer
APs after FPI in response to the same amount of current injection, and yet the firing rates remain similar to control values (Fig. 1B). An increase in input resistance could offset the depolarized AP threshold and allow the FPI cells to fire at the same rate as the control cells. Therefore the positive current injection experiments were repeated in the presence of TTX to block APs. The FPI cells responded to positive current injection in control: 125.8 \pm 7.1 \text{ pF}; \text{in FPI: } 154.6 \pm 4.8 \text{ M\Omega} \) because the membrane potential reached in response to a 240-pA current injection increased by 5 mV, whereas the AP threshold only was depolarized by 1.5–2.0 mV (voltage resulting from 240-pA injection: control: \(-34.5 \pm 1.4 \text{ mV}; \text{FPI: } -29.5 \pm 1.0 \text{ mV}\)).

Because differences in a cell’s AP waveform can impact neurotransmitter release from its boutons, the AP waveform was closely examined in mossy cells after FPI (Fig. 3A). There was no difference in AP height between control and FPI mossy cells (Fig. 3B). In the same cells, the AP width was analyzed at one-half of the maximum height. Although there was a trend for APs from FPI cells to be wider, the difference was not significant (Fig. 3C). However, in FPI mossy cells, there was a significant increase in the AP width at one-third of maximum height (Kamal et al. 2006) (Fig. 3D). This difference suggests that the widening of the AP after FPI only occurs at the later phases of the AP.

Therefore when examining the detailed intrinsic properties of mossy cells after FPI, the following alterations were seen: a depolarized AP threshold, an increased input resistance around AP threshold, and an increased AP width. Next, the intrinsic conductances of mossy cells were examined to find the mechanisms underlying these alterations.

**Shift in activation curve of sodium current**

To uncover the origin of the depolarized AP threshold observed after FPI (Fig. 2, B and C), the fast sodium current (\(I_{\text{Na}}\)), which contributes to the action potential threshold, was measured. To obtain accurate voltage-clamp measurements of sodium currents, nucleated outside-out patches (Martina and Jonas 1997) were obtained from mossy cells. To measure the activation curve, patches were held at \(-90 \text{ mV}, \) with a 50-ms prepulse to \(-120 \text{ mV}, \) and 20-ms test pulses to command potentials between \(-80\) and \(+40 \text{ mV}\) (representative traces shown in Fig. 4A) were applied. The capacitance of the patches was not different between control and FPI cells (capacitance in control: \(2.4 \pm 0.3 \text{ pF}, n = 8, \text{in FPI: } 2.3 \pm 0.6 \text{ pF}, n = 4\)). The maximum current amplitude was also similar between control and FPI (maximum amplitude in control: \(0.32 \pm 0.04 \text{ mV}, \text{in FPI: } 0.39 \pm 0.08 \text{ mV}\); \(I_{\text{Na}}\) shown in Fig. 4B). After FPI, the voltage of half-maximal activation of \(I_{\text{Na}}\) was significantly depolarized (\(V_{1/2}\) in control: \(-31.1 \pm 1.17 \text{ mV}; \text{FPI: } -25.3 \pm 1.28, P < 0.05; \text{Fig. 4C}\)). Therefore after FPI, the activation curve of \(I_{\text{Na}}\) is shifted by 5.8 mV in the positive direction, which is consistent with the observed depolarization of the AP threshold.

**Downregulation of a delayed-rectifier potassium current**

A downregulated potassium current could lead to the wider AP observed in mossy cells after FPI because potassium currents contribute to the repolarizing phase of the AP. Three potassium currents were isolated in control and FPI mossy cells: an A-type current (\(I_A\)) blocked by 3 mM of 4-aminopyr-
idine (4-AP), a delayed-rectifier blocked by 25 mM of TEA ($I_{\text{TEA}}$), and a residual current insensitive to both 4-AP and TEA ($I_{\text{RES}}$; representative traces shown in Fig. 5A). The amplitude of $I_{\text{TEA}}$ was decreased in FPI cells at test potentials from −30 to 0 mV (at 0 mV: $1.876 \pm 155$ pA in control, $n = 11$; $1.329 \pm 171$ pA in FPI, $n = 10$; Fig. 5B). However, the activation curve of this current was not shifted (Fig. 5B, inset). The decrease in amplitude was specific to $I_{\text{TEA}}$, as the amplitudes of $I_A$ and of $I_{\text{RES}}$ were similar between control and FPI cells (amplitude of $I_A$ at 0 mV—in control: $2.264.1 \pm 158$ pA, $n = 14$; in FPI: $2.392 \pm 184$ pA, $n = 12$; amplitude of $I_{\text{RES}}$ at 0 mV—in control: $2.152 \pm 258$ pA, $n = 9$; in FPI: $2.305 \pm 168$ pA, $n = 10$; Fig. 5, C and D).

To determine whether the downregulation of $I_{\text{TEA}}$ could lead to the observed broadening of APs, this current was pharmacologically decreased in control mossy cells by adding a low concentration (2.5 mM) of TEA to the bath and measuring AP width before and after the addition of the drug. The results show a significant widening of the AP with the addition of TEA (representative traces in Fig. 6A, summary plot in B; $n = 3$). This effect is similar to the broadening seen after FPI (compare Fig. 6B with Fig. 3D). The addition of 2.5 mM TEA to control mossy cells did not affect their AP height (Fig. 6C). Because this current is slowly inactivating, the decreased $I_{\text{TEA}}$ could contribute to the increased input resistance shown in Fig. 2D at voltages above −30 mV. Thus a delayed-rectifier potassium current is downregulated in mossy cells after FPI, and this may be a cause of the observed AP broadening.

Functional effects of decreased potassium current expression

Does a wider AP have an effect downstream of the mossy cell? Previous studies have shown that a wider somatic AP can lead to increased calcium entry in the bouton and a corresponding increase in transmitter release (Bollmann and Sakmann 2005; Borst and Sakmann 1999; Geiger and Jonas 2000). To examine whether a wider AP in mossy cells would lead to increased glutamate release onto postsynaptic granule cells, whole cell recordings were made from dentate granule cells in slices from control animals, and EPSCs were evoked by low-intensity stimulation in the inner molecular layer. As mossy cell axons are the only glutamatergic fibers in this area in control animals, these evoked EPSCs originate from mossy cell axons. TEA (2.5 mM) was then added to the bath, the concentration that leads to an AP width in control mossy cells similar to the AP width in FPI cells (Figs. 3D and 6B). Note that granule cells were recorded with a cesium-containing intracellular solution to block potassium channels to rule out postsynaptic effects of TEA. The granule cell EPSC amplitude increased significantly within 5 min of TEA addition (Fig. 6D: increase in amplitude: $51.5 \pm 13\%$, $n = 5$). Therefore the decreased expression of a TEA-sensitive delayed rectifier (in the absence of other compensatory alterations at the synapse; see discussion) is expected to lead to a significantly increased mossy cell to granule cell EPSC.

Computational modeling of modifications to intrinsic properties of mossy cells

So far we have uncovered several alterations in mossy cell physiology. Could these alterations, if they occurred separately, significantly affect the excitability of the dentate network? Experimentally, it is difficult to separate the effects of isolated posttraumatic changes in mossy cells on the dentate network. Therefore computational modeling studies were performed to examine whether any of the altered parameters seen in mossy cells after FPI could modify network activity. The simulations were run on a 52,100-cell anatomically and physiologically realistic model of the dentate gyrus (Dyrhøj-Johnsen et al. 2006). The excitability of the network was tested with a single perforant path stimulation (see methods). Reflecting the behavior of the biological dentate (Ratcliff et al. 2004; Santhakumar et al. 2001, 2005), the simulated “control” net-

![FIG. 4. Depolarizing shift in the sodium current activation curve after trauma. A: traces of sodium currents recorded from nucleated patches of a control (left) and an FPI (right) mossy cell. Scale bars for control: 1 ms, 50 pA; for FPI: 1 ms, 25 pA. B: Plots of raw maxima. Labels apply to B and C. C: plots of sodium channel permeability. The $V_{1/2}$ of activation in FPI cells is significantly depolarized with respect to the $V_{1/2}$ of control cells. All experiments were recorded in the presence of 20 mM TEA, and measurements of sodium currents were the result of the subtraction of the currents measured in TTX from the currents measured without TTX. Although calcium channel blockers were not included in the bath, no calcium currents were observed in this preparation, consistent with previous observations in CA1 pyramidal cells (Martina and Jonas 1997).](http://jn.physiology.org/doi/10.1152/jn.00511.2006)
work never spread activity to the entire network (data not shown) (see Dyhrfjeld-Johnsen et al. 2006), and the firing rate of granule and mossy cells was low (average granule cell firing: 0.2 Hz; mossy cell firing: 0.3 Hz). However, because mossy cells after FPI exist in an altered anatomical environment, simulated mossy fiber sprouting and hilar cell loss were added, to create an “FPI-only” network (i.e., without intrinsic changes; see METHODS). With these anatomical changes added, the same single stimulation led to the activation of the entire network (latency to full network activation in FPI-only network: 99.3 ± 2.0 ms, n = 3 trials; Fig. 7, A and G). In addition, the average granule cell and mossy cell firing rates over the entire 500-ms simulation were significantly increased (granule cell firing: 28.5 ± 0.6 Hz; mossy cell firing: 7.5 ± 0.1 Hz; n = 3 trials, Fig. 7, A, E, and F). The remaining simulations, which tested the effect of the altered intrinsic properties of mossy cells after FPI, were performed by modifying the physiological parameters of this FPI-only network. Specifically, we tested the effects of the depolarized \( V_m \), altered mossy cell to granule cell EPSC resulting from the increased glutamate release due to the

**FIG. 5.** TEA-sensitive delayed rectifier potassium current is downregulated. A: representative traces from control (left) or FPI (right) mossy cells, of TEA-sensitive delayed rectifier potassium current (\( I_{\text{TEA}} \), 4-aminopyridine (4-AP)-sensitive A-type potassium current (\( I_A \)), and TEA-resistant, 4-AP-resistant potassium current (\( I_{\text{RES}} \)). Scale bars: 500 pA, 50 ms. B: current-voltage plots of \( I_{\text{TEA}} \), a delayed rectifier potassium current, show a downregulation of this current in FPI cells. This current was the result of a subtraction of currents recorded in 25 mM TEA and 2.5 mM 4-AP from those recorded in 2.5 mM 4-AP. Current amplitude is significantly decreased in FPI cells for test voltages from −30 to 0 mV. B, inset: activation curves of \( I_{\text{TEA}} \) are not different in control and FPI cells. C: current-voltage plots of \( I_A \), the 4-AP-sensitive potassium current. This current was the result of a subtraction of currents recorded in 2.5 mM 4-AP from those recorded without potassium channel blockers. This current is similar in control and FPI mossy cells. D: current-voltage plots of \( I_{\text{RES}} \), a TEA-resistant, 4-AP-resistant potassium current, which is also similar in control and FPI mossy cells. All potassium currents in A–D were recorded in the presence of 20 μM 2-amino-5-phosphonovaleric acid (APV), 10 μM NBQX, 100 μM CdCl₂, and 3 mM CsCl. Leak subtraction was performed with an offline P4 protocol.

**FIG. 6.** Amplitude of mossy cells to granule cell excitatory postsynaptic currents (EPSCs) increases in TEA. A: APs from a control cell recorded in artificial cerebrospinal fluid (ACSF; black) or in the presence of 2.5 mM TEA (gray). Scale bars: 1 ms, 10 mV. B: AP width at 1/3 maximum height is increased in control cells in the presence of 2.5 mM TEA. Note the similarity to Fig. 3D. C: AP height is not changed in control cells in the presence of 2.5 mM TEA (compare with Fig. 3B). D: time course of perfusion of 2.5 mM TEA to control mossy cells, indicating a 51.5% increase in the amplitude of evoked EPSCs in granule cells within 5 min. D, inset: example traces of EPSCs in granule cells evoked by stimulation in the inner molecular layer in control cells in the absence (left) and presence (right) of 2.5 mM TEA. Scale bar: 5 ms, 50 pA.
downregulation of $I_{\text{TEA}}$ and the depolarizing shift in the $I_{\text{Na}}$ activation curve.

First, we tested whether the significant shift of $V_m$ shown in Fig. 1F could lead to increased firing in response to the same perforant path stimulation. When a 3-mV shift in $V_m$ was added to the mossy cells in the network, the network's excitability increased significantly (FPI plus 3-mV shift of MC $V_m$: latency to full network activity: 39.9 ± 0.49 ms; granule cell firing: 65.1 ± 0.5 Hz; mossy cell firing: 25.9 ± 0.2 Hz; Fig. 7, B and E–G; $n = 3$ trials).

Second, we tested the effect of the downregulation of $I_{\text{TEA}}$. Because the decrease of this conductance was shown to increase the AP width and therefore also increase the amplitude of the mossy cell to granule cell EPSC, we modeled this change by increasing the synaptic weight of the mossy cell to granule cell EPSC by 50% (from 0.3 to 0.45 nS), corresponding to the experimental data shown previously (Fig. 6D). Due to the recurrent excitatory loop formed by granule cells and mossy cells, we predicted that the increased mossy cell to granule cell EPSC caused by the increased AP width of mossy cells could have significant effects on network behavior. Accordingly, with the increased EPSC size incorporated into the network, the spread of activity through the dentate network occurred significantly faster than the FPI-only network after perforant path stimulation (FPI plus increased MC–GC synaptic weight: latency to full network activity: 74.7 ± 0.09 ms; $n = 3$ trials; Fig. 7, C and G). Additionally, both granule cells and mossy cells fired significantly more (granule cell firing: 12.3 ± 0.1 Hz; Fig. 7, C, E, and F).

Finally, a 5-mV shift in the sodium current activation curve was added to the mossy cells in the FPI-only dentate model. Predictably, the mossy cells' firing rate decreased significantly (FPI plus shift in activation of $I_{\text{Na}}$: mossy cell firing: 0.003 ± 0.0001 Hz; $n = 3$ trials; Fig. 7, D and F). The sodium current activation shift also led to a significantly lower granule cell firing rate and slower activation of the full network (FPI plus shift in activation of $I_{\text{Na}}$: granule cell firing: 16.3 ± 0.03 Hz; latency to full network activity: 175.4 ± 0.9 ms; Fig. 7, D, E, and G).

In summary, experimental data showed that despite the fact that the firing patterns were not different between control and
FPI mossy cells, the \( V_m \) was depolarized, a potassium conductance was decreased, and the sodium current’s activation curve was shifted. Computational modeling indicated that each of these isolated perturbations can lead to significant alterations in dentate network activity. Note that because several additional known and unknown factors (see following text) may have changed in these posttraumatic mossy cells, it is currently not possible to test the net combined effect of all posttraumatic alterations in mossy cells on the simulated dentate network. Thus the only purpose of the modeling experiments was to show that, individually, subtle changes in the physiology of one cell type can lead to significant changes in network activity.

Increase in the effect of the \( I_h \) blocker ZD7288 after FPI

Because the current-firing plots hid several, mutually opposing \( (i.e., \) increased \( V_m \), increased input resistance around AP threshold, depolarizing shift in \( I_{Na} \), and a downregulated delayed rectifier potassium current) alterations in mossy cells after FPI, it is possible that the current-voltage relationships at and around \(-60 \) mV also concealed some interesting changes. As mentioned in the preceding text, mossy cells showed a significantly depolarized \( V_m \) after FPI (\( V_m \) in control: \(-66.6 \pm 1.4 \) mV, \( n = 20 \); in FPI: \(-63.2 \pm 0.8 \) mV, \( n = 20 \); Fig. 8A, left). One possible cause of a depolarized \( V_m \) is an increase in the hyperpolarization-activated cation current, \( I_h \) (Beaumont and Zucker 2000; Chen et al. 2001; Maccaferri and McBain 1996; Pape 1996; Santoro and Tibbs 1999; Siegelbaum 2000). In agreement with this possibility, when mossy cells were incubated in the \( I_h \) blocker ZD7288 (ZD; 10 \( \mu \)M), \( V_m \) was hyperpolarized in both control and FPI mossy cells, and the difference between the two groups was abolished (control \( V_m \) in ZD: \(-70.0 \pm 2.3 \) mV, \( n = 16 \); in FPI: \(-69.0 \pm 1.6 \) mV, \( n = 19 \); Fig. 8A). To further examine whether \( I_h \) might be upregulated, we examined the effects of ZD on voltage changes during and after a hyperpolarizing current pulse: a slow afterdepolarization due to deactivation of the current after the pulse and a slow depolarizing sag due to activation of the current during the pulse (Fig. 8B, inset). Both the afterdepolarization and the sag were increased in mossy cells after FPI (Fig. 8B, C, and D; control: \( n = 20 \); FPI: \( n = 20 \)). To demonstrate that \( I_h \) is the current underlying the afterdepolarization and the sag in mossy cells, these experiments were repeated after incubation of the slices in 10 \( \mu \)M ZD, which allows for a complete block of \( I_h \). The afterdepolarization was nearly abolished when \( I_h \) was blocked, and there were no differences between control and FPI cells in the presence of ZD (Fig. 8D1; control: \( n = 12 \); FPI: \( n = 13 \)), and the sag was completely abolished in ZD (Fig. 8D2).

If \( I_h \) is the only conductance that has been altered in this voltage range, the input resistance of mossy cells should be decreased. However, as shown in Fig. 1F, the input resistance at around and below \(-60 \) mV is similar between control and FPI mossy cells. This would appear to contradict our suggestion that \( I_h \) is upregulated. To investigate this apparent discrepancy, the input resistance of individual mossy cells was measured in control ACSF and after a switch of the perfusate to ACSF containing a high concentration (100 \( \mu \)M) of ZD. The input resistance was larger in FPI mossy cells at most current amplitudes after the switch to the ZD-containing solution, indicating that blockade of \( I_h \) reveals a difference in input resistance (Fig. 8E; control: \( n = 9 \); FPI: \( n = 10 \)). Additionally, the change in input resistance caused by the perfusion of ZD was significantly greater in FPI at most current amplitudes (Fig. 8F) (Shah et al. 2004). In light of the unchanged input resistance at hyperpolarized voltages (Fig. 1F), these data suggest that there are two opposing forces at work: an increased \( I_h \) to decrease the input resistance and the decrease of one or more unknown conductances, such as a leak potassium conductance, to increase the input resistance back to control values.

Alterations of synaptic inputs to mossy cells after FPI

Do the properties of synaptic inputs to mossy cells change after head injury, and what are the common features of these posttraumatic synaptic alterations? Modifications to synaptic inputs to mossy cells after FPI (Fig. 8F) show that the membrane potential of mossy cells is more hyperpolarized in FPI mossy cells compared to control mossy cells (Fig. 1F). The change in membrane potential of mossy cells caused by synaptic inputs to mossy cells after FPI (Fig. 8F) is greater in FPI than in control at most current amplitudes. All experiments in this figure were performed in the presence of d-AP5 (10 \( \mu \)M) and NBQX (20 \( \mu \)M) and, for \( E \) and \( F \), in TTX (1 \( \mu \)M).
properties of other cell types have been shown to occur in the dentate gyrus after FPI (Santhakumar et al. 2000, 2001; Toth et al. 1997). Specifically, the frequency of mIPSCs (AP-independent events recorded in the presence of TTX) in granule cells is decreased after FPI and the frequency of sIPSCs (recorded in the absence of TTX) is increased in granule cells after FPI. Although alternative interpretations are possible, the most parsimonious explanation for the decreased mIPSC frequency and the increased sIPSC frequency (Santhakumar et al. 2000; Toth et al. 1997) is that the death of hilar interneurons leads to fewer GABAergic boutons available for release, yet the surviving interneurons fire more frequently as has been experimentally demonstrated (Ross and Soltesz 2000). The population of inhibitory cells that innervates granule cells is thought to be similar to the population that innervates mossy cells (Acsády et al. 2000). Therefore it was expected that the changes in frequency of mIPSCs and sIPSCs would parallel those seen in granule cells. Indeed, when mIPSCs were compared from control and FPI mossy cells, the following parameters were changed significantly: the interevent interval was increased (in control: 110.4 ± 2.0 ms, n = 13; in FPI: 181.5 ± 4.2 ms, n = 10; Fig. 9C), the amplitude was increased (in control: 59.0 ± 1.0 pA; in FPI: 65.6 ± 1.3 pA; Fig. 9E), and the 10–90% rise time was shortened (in control: 0.87 ± 0.01 ms; in FPI: 0.82 ± 0.01 ms; Fig. 9G). When sIPSCs were recorded, the same three properties were changed significantly after FPI but in the opposite direction. The interevent interval of sIPSCs was decreased (in control: 128.4 ± 6.5 ms, n = 14; in FPI: 103.8 ± 2.8 ms, n = 12; Fig. 9D), the amplitude was decreased (in control: 127.5 ± 3.3 pA; in FPI: 97.6 ± 2.7 pA; Fig. 9F), and the 10–90% rise time was lengthened significantly (in control: 0.72 ± 0.01 ms; in FPI: 0.75 ± 0.02 ms; Fig. 9H). Note that the significant difference between interevent intervals of sIPSCs is due to the presence of very large events in control but not FPI mossy cells. The decay time constant was not changed for either mIPSCs (in control: 7.12 ± 0.3 ms; in FPI 7.6 ± 0.2 ms) or sIPSCs (in control 7.42 ± 0.2 ms; in FPI 7.53 ± 0.3 ms).

Excitatory inputs to mossy cells include those from mossy fibers (granule cell axons), those from other mossy cells, and those from CA3 pyramidal cells (Scharfman 1994). After FPI, and in many other models of hyperexcitability, mossy fibers

![Figure 9](http://jn.physiology.org/)

**FIG. 9.** Properties of miniature and spontaneous inhibitory postsynaptic currents (m- and sIPSCs) in mossy cells. *A* and *B*: representative miniature (*A*) and spontaneous (*B*) IPSCs from control (black) and FPI (gray) mossy cells. *Left*: individual traces; *right*: averaged IPSCs from a single cell. Scale bars for *left*: 100 ms, 25 pA; for *right*: 2 ms, 20 pA. Cumulative probability plots for interevent interval (*C*), amplitude (*D*), and 10–90% rise time (*E*) of mIPSCs recorded in the presence of D-AP5 (20 μM), NBQX (10 μM), and TTX (1 μM). Cumulative probability plots for interevent interval (*F*), amplitude (*G*), and 10–90% rise time (*H*) of spontaneous IPSCs recorded in the presence of D-AP5 and NBQX. Note that in *D*, although the cumulative probability plots are similar, there are very large interevent intervals (IEIs) in control cells that are absent in FPI cells. These events cause the 2 distributions to differ significantly. Labels in *C* apply to *C–H*. The same number of events was selected from each cell (control: n = 13; FPI: n = 10), starting 2 min after the start of the recording (250 for IEI, 100 for amplitude and rise time, 60 for decay).
sprout (Cavazos et al. 1991; Cronin et al. 1992; Okazaki et al. 1995; Santhakumar et al. 2001) and mossy cell axons are lost due to death of mossy cells (~50% after moderate FPI) (Toth et al. 1997). Therefore it is reasonable to suppose that glutamatergic synaptic events might also change in mossy cells after FPI. Recordings of excitatory synaptic events were made in the presence of picrotoxin to block fast GABA_{A}–mediated inhibitory events. The following significant changes were seen in mEPSC properties after FPI: the interevent interval of mEPSCs was increased (in control: 202.7 ± 6.0 ms, n = 10; in FPI: 416 ± 5.3 ms, n = 9; Fig. 10C), the amplitude was decreased (in control: 71.4 ± 1.6 pA; in FPI: 50.5 ± 1.6 pA; Fig. 10E), and the 10–90% rise time was shortened (in control: 0.83 ± 0.1 ms; in FPI: 0.77 ± 0.01 ms; Fig. 10G). As was the case with the inhibitory events, the direction of the changes were reversed between mEPSCs and sEPSCs and accordingly the following three significant alterations were seen in sEPSCs after FPI: the interevent interval was decreased (in control: 118.5 ± 3.6 ms, n = 12; in FPI: 70.4 ± 1.3 ms, n = 12; Fig. 10D), the amplitude was increased (in control: 77.8 ± 1.5 pA; in FPI: 116.5 ± 4.3 pA; Fig. 10F), and the 10–90% rise time was decreased (in control: 0.78 ± 0.01 ms; in FPI: 0.83 ± 0.01 ms; Fig. 10H). There were no changes in the decay time constant for either mEPSCs (in control: 4.32 ± 0.2 ms; in FPI: 3.89 ± 0.3 ms) or sEPSCs (in control: 4.34 ± 0.2 ms; in FPI: 4.26 ± 0.2 ms). In summary, synaptic events in posttraumatic mossy cells are significantly modified in an apparently highly systematic manner as evidenced, for example, by the alterations in miniature events that are invariably opposite to those seen in spontaneous events.

**Endocannabinoid signaling in mossy cells is present but unchanged after FPI**

As noted in the preceding text, opposing alterations of m- and sIPSCs occur in mossy cells after FPI. In light of these widespread but apparently highly specific changes in synaptic inputs, we next tested if the short-term plasticity of sIPSCs also underwent significant alterations after FPI or if they stayed the same in spite of the altered m- and sIPSC properties. Although the exact source of sIPSCs is unknown, many of these events originate from perisomatically projecting interneurons, such as basket cells expressing the cannabinoid type-1 (CB1) receptors, which are known to project to mossy cells (Acsady et al.

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**FIG. 10. Properties of m- and sEPSCs in mossy cells.** A and B: representative miniature (A) and spontaneous (B) EPSCs from control (black) and FPI (gray) mossy cells. Left: individual traces; right: averaged EPSCs from a single cell. Scale bars for left: 100 ms, 25 pA; for right: 3 ms, 20 pA. Cumulative probability plots for interevent interval (C), amplitude (D), and 10–90% rise time (E) of mEPSCs recorded in the presence of picrotoxin (100 μM) and TTX (1 μM). Cumulative probability plots for interevent interval (F), amplitude (G), and 10–90% rise time (H) of spontaneous EPSCs recorded in the presence of picrotoxin. Labels in C apply to C–H. The same number of events was selected from each cell (control: n = 10; FPI: n = 9), starting at 2 min after the start of the recording (250 for interevent interval, 100 for amplitude and rise time, 60 for decay).
Cannabinoids can modulate inhibitory synapses through a process termed DSI (Kretzner and Regehr 2001; Llano et al. 1991; Ohno-Shosaku et al. 2001; Pittler and Alger 1992; Wilson and Nicoll 2001). DSI takes place when the postsynaptic cell is depolarized and releases endocannabinoids that are thought to diffuse to the presynaptic membrane. These cannabinoids bind to the CB1 receptor, and transiently suppress GABA release via the inhibition of voltage-gated Ca²+ channels. Recently, the cannabinoid signaling system has been shown to exhibit long-term plasticity in different models of epilepsy (Bernard et al. 2005; Chen et al. 2003; Wallace et al. 2003), and cannabinoids have been shown to have neuroprotective and antiepileptic effects (Bernard et al. 2005; Blair et al. 2006; Marsicano et al. 2003; Shaforoodi et al. 2004). Therefore, to determine whether DSI undergoes alterations after trauma, mossy cells were examined for the presence of DSI in the control condition and for persistent plasticity of this cannabinoid-mediated signaling after FPI.

Because DSI has been demonstrated in mossy cells only recently (Hofmann et al. 2006), we first carried out experiments to characterize DSI in these neurons under our conditions. On depolarization of control mossy cells from −60 to 0 mV for 100 or 500 ms, a transient decrease in the sIPSC charge transfer was observed (Fig. 11, A, C, E, and F). Because DSI was completely blocked by the postsynaptic mossy cells and unchanged after trauma. Therefore, DSI in mossy cells was similar to DSI recorded in CA1 pyramidal cells from control animals under the same conditions (percentage baseline charge transfer for 3-100 ms depolarization with a 100-ms pulse: 72.9 ± 5.4%, n = 7; with a 500-ms pulse: 68.7 ± 6.9%, n = 5), indicating that cannabinoids have comparable effects on the GABAergic inputs arising from CB1-containing interneurons to both mossy cells and CA1 pyramidal cells.

Next, DSI in mossy cells was examined after FPI. The mean amplitude of DSI was not changed after FPI (Fig. 11, B and D–F: percentage of baseline charge transfer for 3-s postdepolarization with a 100-ms pulse: 81.2 ± 5.1%, n = 17; 500-ms pulse: 73.4 ± 7.2%, n = 16). Additionally, there was no alteration in the percentage of cells expressing DSI (percentage of DSI-expressing cells in control: 66.7% of 15 cells for 100 ms, 68.75% of 16 cells for 500 ms; in FPI: 64.7% of 17 cells for 100 ms, and 62.5% of 16 cells for 500 ms). DSI in FPI mossy cells was completely blocked in the presence of AM251 (Fig. 11, D–F). In summary, mossy cells exhibit cannabinoid-mediated inhibition of spontaneous inhibitory events, but this form of short-term plasticity is not changed after head trauma, in spite of the extensive alterations in intrinsic and synaptic properties of posttraumatic mossy cells.

**Discussion**

In this paper, we examined the plasticity of intrinsic and synaptic properties in single mossy cells after traumatic brain injury. The experiments were performed 1 wk after head injury because previous research has established the presence of robust hyperexcitability at this time point (Santhakumar et al. 2001; Toth et al. 1997) and also because this time point is after the immediate posttraumatic period characterized by hilar cell loss and transient changes in single-cell properties (Ross and Soltész 2000; Toth et al. 1997). Our current data show that mossy cells display a large number of significant alterations in their properties after traumatic brain injury. The individual alterations take place in an apparently coordinated manner that suggests that certain regulatory mechanisms are likely to underlie the observed intrinsic and synaptic modifications. Concerning the intrinsic properties, we found extensive modifications in various ion channels that occur without changes in either I-F or I-V curves. In the depolarizing direction, where there is no change in the I-F relationship (Fig. 1B), a depolarized AP threshold, due to the depolarizing shift in $I_{Na}$, is apparently opposed by a depolarized Vm and an increased input resistance. In the hyperpolarizing direction, where there is no change in the I-V relationship (Fig. 1F), an increase in $I_h$ is apparently opposed by the downregulation of an unidentified conductance. Regarding the synaptic events, the presence of increased sEPSC frequency and amplitude indicate that the mossy cells experienced sustained increases in incoming excitation in the posttraumatic dentate gyrus. Furthermore, the identified posttraumatic alterations in mEPSC and mIPSC amplitudes are exactly as could be predicted from culture experiments on homeostatic responses after the artificial elevation of network activity (Turrigiano and Nelson 2004; Turrigiano et al. 1998). Similarly, the decreased frequency of miniature IPSCs and EPSCs is apparently opposed by an increased frequency of spontaneous I/EPSCs. In the subsequent sections, we first discuss how modifications in intrinsic and synaptic properties in mossy cells described in this paper...
may play a role in dentate network hyperexcitability, and we integrate these new findings in the context of current theories of mossy cell functions in seizure-propagation. Second, we discuss how the current data on intrinsic and synaptic changes in posttraumatic mossy cells can be considered as evidence for homeostatic regulatory mechanisms shaping the plasticity of single-cell properties in hyperexcitable networks.

Role of surviving mossy cells in the hyperexcitable dentate gyrus

Mossy cells are one of the most vulnerable cell types in the mammalian brain. Indeed, mossy cells are lost under a variety of pathological conditions, including epilepsy, trauma and ischemia (Buckmaster and Jongen-Relo 1999; Ratzliff et al. 2002; Sutula et al. 2003). The precise nature of the contributions of mossy cells to hyperexcitability in the dentate gyrus is not fully understood. One theory (the dormant basket cell hypothesis) (Sloviter et al. 2003) focused on the effects of mossy cell loss, arguing that the loss of mossy cells deprives GABAergic interneurons of their excitatory input, resulting in a hyperexcitable dentate gyrus through the hypoexcitation of basket cells and hypoinhibition of granule cells. An alternative theory (the irritable mossy cell hypothesis) (Ratzliff et al. 2002; Santhakumar et al. 2000) proposed that mossy cell loss itself leads to hyperexcitability in granule cells, and that it is the surviving mossy cells that play key roles in seizure propagation. The basic tenet of the irritable mossy cell hypothesis, i.e., that mossy cells contribute an overall pro-excitatory influence onto granule cells, is supported by single-cell deletion data from both experiments and computational network models showing that the removal of mossy cells invariably leads to decreased granule cell excitability (Ratzliff et al. 2004; Santhakumar et al. 2005). According to the irritable mossy cell hypothesis, mossy cells either passively distribute granule cell hyperexcitability arising from other pro-excitatory modifications such as mossy fiber sprouting or they are intrinsically hyperexcitable, in which case they could actively amplify incoming excitatory signals. Therefore it is important from this respect that our current data demonstrate that mossy cell intrinsic cellular excitability does not exhibit significant alterations after trauma as determined by the unchanged I-F and I-V curves. Although these results may be at first regarded as evidence that mossy cells do not actively amplify incoming excitation, several caveats should be noted. First, it is possible that EPSPs generated on dendrites may be differentially amplified after trauma because our current data indicate significant alterations in Ih in posttraumatic mossy cells, and seizure-induced changes in Ih have been shown to exert major effects on the processing of both inhibitory and excitatory synaptic inputs (Chen et al. 2001; Shah et al. 2004). Second, posttraumatic mossy cells may amplify incoming signals by translating each action potential to larger EPSCs in their postsynaptic target cells due to the wider action potentials resulting from the downregulated delayed-rectifier potassium current. Although our results show that a decreased delayed rectifier current enhances the mossy cell to granule cell EPSCs, it is possible that additional compensatory mechanisms may have taken place at the mossy cell to granule cell synapse. The mossy cell to granule cell EPSC amplitude, and the effect of TEA on these EPSCs were not measured from FPI animals because stimulation of the inner molecular layer after trauma evokes responses from both the sprouted mossy fibers and mossy cell axons. Therefore we cannot rule out the possibility that counteracting mechanisms have taken place to decrease the amplitude of the mossy cell to granule cell synapse to adjust for the increased AP width in mossy cells. For example, calcium channels could be downregulated in mossy cell boutons to decrease transmitter release presynaptically or AMPA receptors could be removed from the granule cell membrane to decrease the EPSC amplitude postsynaptically. Thus the absence of alterations in I-F and I-V curves cannot be considered as final evidence against the idea that mossy cells actively contribute to dentate hyperexcitability. In any case, however, it should be emphasized that it has already been established using computational modeling techniques that surviving mossy cells play critical roles in long-range synchronization of the dentate gyrus even without changes in their intrinsic properties (Dyhrfjeld-Johnsen et al. 2006; Santhakumar et al. 2005). Indeed in the hyperexcitable FPI-only baseline model used in this paper, the intrinsic and synaptic properties of mossy cells were exactly as in control networks that do not demonstrate hyperexcitability, and the removal of these mossy cells clearly decreases the perforant path-evoked hyperexcitatory responses in granule cells (Dyhrfjeld-Johnsen et al. 2006; Santhakumar et al. 2005), indicating their overall pro-excitative network effects in dentate hyperexcitability.

Does homeostasis occur in mossy cells after trauma?

Recent studies have shown that neurons demonstrate the ability to powerfully regulate themselves to maintain a target level of activity or excitability through homeostatic regulation of synaptic and intrinsic cellular parameters (Desai et al. 1999; MacLean et al. 2005; Niven et al. 2003; Pulver et al. 2005; Turrigiano and Nelson 2004). In this context, homeostatic plasticity refers to changes in synaptic or intrinsic properties that allow a neuron to maintain its firing within a target range. Under healthy normal conditions, both synaptic scaling and regulation of intrinsic conductances have been demonstrated to be involved in homeostatic plasticity (Lissin et al. 1998; O’Brien et al. 1998; Turrigiano et al. 1998; Watt et al. 2000; Wierenga et al. 2005). The key observations from such studies on homeostatic responses that are especially relevant to our situation are that after induction of increased network activity, intrinsic excitability and mEPSC amplitude decrease (Turrigiano et al. 1998), whereas mIPSC amplitude should theoretically increase (Kilman et al. 2002; Turrigiano and Nelson 2004). The overall interpretation of these results is that cells respond to increased incoming excitation by decreasing their intrinsic excitability and the postsynaptic responses to glutamate, and increase their postsynaptic responses to GABA, in an apparent attempt to restore their original firing rates.

The existence of hyperexcitable states, such as epilepsy, clearly shows that networks under pathological conditions can persistently stay outside of the normal, “target” range of activity. This presents an interesting paradox: why do some changes in activity levels lead to neuronal homeostasis, and yet others lead to pathology? There are least three distinct possible solutions to this paradox. First, it is possible that in hyperexcitable states, homeostatic mechanisms have not been enlisted. Because the triggers for homeostatic plasticity have not been
fully characterized, it is possible that the pathological changes occurring in the hyperexcitable brain do not elicit the homeostatic response. For example, although significant advances are beginning to emerge (Desai et al. 2002; Echegoyen et al. 2005), little is known about the presence of homeostatic regulation in adult mammalian systems in vivo. A second possible solution to the paradox is that homeostatic plasticity has been engaged in hyperexcitable states, but the initial insult was so severe that the result is an imperfect homeostasis. For example, acute deafferentation of the crustacean somatogastric ganglion leads to the loss of oscillatory behavior, but remarkably, the neurons in this ganglion “self-tune” their conductances to re-establish oscillations within a few hours. However, this tuning is not ideal, as both the period of the oscillations and the phase relationships between neurons have changed (Luther et al. 2003; Mizrahi et al. 2001). A third possible solution to the paradox of homeostasis in single cells within hyperexcitable networks is that homeostasis occurs, but hyperexcitability persists for other reasons. Indeed, as mentioned in the preceding text, computational modeling techniques using anatomically and biophysically realistic, large-scale models of the dentate gyrus clearly demonstrated that robust hyperexcitability can arise in the dentate gyrus even without changes in intrinsic properties of any of the cell types, purely due to modifications in network architecture arising from mossy fiber sprouting and hilar cell loss (Dyhrfjeld-Johnsen et al. 2006; Santhakumar et al. 2005).

Our current data are most consistent with the third of these alternative solutions, i.e., that the homeostatic regulatory mechanisms resulted in unchanged intrinsic excitability in mossy cells that reside within the hyperexcitable, posttraumatic dentate gyrus. The best evidence in favor of homeostasis in mossy cells is that the $I-F$ and $I-V$ curves are unchanged after trauma, but numerous changes in distinct membrane currents suggest that significant reconfiguration of these currents took place after trauma. Several observations are reminiscent of homeostatic plasticity: the increased voltage response to positive current pulses is counterbalanced by a depolarized AP threshold, and the possible increase in $I_h$ is counterbalanced by the downregulation of an unidentified conductance (as evidenced by the increased input resistance in the presence of ZD). Therefore although it is not possible to provide incontrovertible evidence that these intrinsic alterations are indeed produced as a result of homeostatic plasticity, the seemingly finely tuned, mutually opposing alterations in distinct ion channels are certainly consistent with homeostatic processes set in motion by the insult. The observations from our simulation experiments that the relatively small alterations in the conductances of a single cell type can have robust effects on the excitability of the network as a whole further emphasize the importance of coordination among the individual plasticity events affecting distinct ion channel subtypes. It is interesting to note in this regard that recent computational and experimental studies have shown that there are many solutions to the problem of how a cell can preserve its proper firing pattern with different combinations of intrinsic conductances (Marder and Goaillard 2006; Marder and Prinz 2003). For example, the expression levels of $I_A$ and $I_h$ are co-regulated in both lobster and crab somatogastric ganglion cells to ensure the proper rhythmic firing of these neurons (MacLean et al. 2003, 2005; Schulz et al. 2006). In addition, in cultured neocortical cells, the blockade of activity with TTX leads to homeostatic responses through alterations of $I_{Na}$ and $I_{TEA}$ (Desai et al. 1999), two currents that were also shown to be altered in our system.

Are the data concerning the alterations in miniature and spontaneous I/EPSCs consistent with homeostasis? Based on results from experiments using externally imposed changes in network activity in cultures (Kilman et al. 2002; Turrigiano and Nelson 2004; Turrigiano et al. 1998), homeostatic responses in single cells to network hyperexcitability are expected to include decreases in mEPSC amplitudes and increases in mIPSC amplitudes. Consistent with hyperexcitable responses from both granule and mossy cells to perforant path stimulation (Santhakumar et al. 2000, 2001; Toth et al. 1997), the presence of increased sEPSC frequency and amplitude indicates that posttraumatic mossy cells experience sustained increases in the levels of excitatory inputs from other neurons in the posttraumatic dentate gyrus network. Accordingly, the posttraumatic mossy cells showed decreased mEPSC amplitude and increased mIPSC amplitude as would be expected from homeostatic responses in single cells to network hyperexcitability. Regarding the additional alterations in synaptic inputs that our data revealed in posttraumatic mossy cells, the following interpretations may be considered. Both the mEPSC and the mIPSC frequencies were decreased (interevent intervals increased), which is consistent with the loss of release sites after the death of presynaptic neurons after the trauma (although alternative explanations, e.g., changes in release probabilities, cannot be excluded). In spite of the decreased mEPSC and mIPSC frequencies, the sEPSC and sIPSC frequencies were both increased. The most parsimonious explanation for the opposing changes in miniature and spontaneous event frequencies is that the surviving cells that are presynaptic to mossy cells increased their firing rates. The exact mechanism underlying this apparent compensatory alteration is not known although the additional excitatory drive arising from the sprouting of mossy fibers onto granule cells and directly or indirectly onto GABAergic neurons is likely to be a major factor.

Summary and outlook

Mossy cells give rise to the associational and commissural pathway of the dentate gyrus, innervating ~30,000 granule cells along the septo-temporal axis on the ipsilateral side (Buckmaster et al. 1996; Wenzel et al. 1997). Based on this extensive connectivity and their high vulnerability, these cells are considered to be a key element in the dentate circuit under both healthy (Lisman 1999) and pathological conditions (Ratzliff et al. 2002, 2004). The current study revealed important information concerning a number of mossy cell properties from control animals, including resting membrane potential, input resistance, action potential accommodation properties, $Na^+$ current parameters, amplitude and kinetic measures of miniature excitatory and inhibitory events, and the properties of DSI. These parameters from control mossy cells will be incorporated into the large-scale anatomically and biophysically realistic network models that are being developed and constantly updated (Dyhrfjeld-Johnsen et al. 2006; Santhakumar et al. 2005). In addition, our data identified a large number of posttraumatic alterations in mossy cell properties after head injury, yielding important information concerning the roles of...
mosaic cells in hyperexcitable states in the dentate gyrus. Finally, when taken together, our results indicate that mosaic cells after trauma undergo a series of changes in a manner consistent with homeostatic mechanisms as evidenced by the functionally opposing changes in membrane currents and synaptic events. Although we do not fully understand the nature of the underlying regulatory processes, it is likely that such homeostatic and compensatory processes play considerable roles in shaping the intrinsic and synaptic plasticity phenomena in hyperexcitable states.

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