Distinct Effect of Impact Rise Times on Immediate and Early Neuropathology After Brain Injury in Juvenile Rats

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Traumatic brain injury (TBI) can occur from physical trauma from a wide spectrum of insults ranging from explosions to falls. The biomechanics of the trauma can vary in key features, including the rate and magnitude of the insult. Although the effect of peak injury pressure on neurological outcome has been examined in the fluid percussion injury (FPI) model, it is unknown whether differences in rate of rise of the injury waveform modify cellular and physiological changes after TBI. Using a programmable FPI device, we examined juvenile rats subjected to a constant peak pressure at two rates of injury: a standard FPI rate of rise and a faster rate of rise to the same peak pressure. Immediate postinjury assessment identified fewer seizures and relatively brief loss of consciousness after fast-rise injuries than after standard-rise injuries at similar peak pressures. Compared with rats injured at standard rise, fewer silver-stained injured neuronal profiles and degenerating hilar neurons were observed 4–6 hr after fast-rise FPI. However, 1 week postinjury, both fast- and standard-rise FPI resulted in hilar cell loss and enhanced perforant path-evoked granule cell field excitability compared with sham controls. Notably, the extent of neuronal loss and increase in dentate excitability were not different between rats injured at fast and standard rates of rise to peak pressure. Our data indicate that reduced cellular damage and improved immediate neurological outcome after fast rising primary concussive injuries mask the severity of the subsequent cellular and neurophysiological pathology and may be unreliable as a predictor of prognosis.

Key words: traumatic brain injury; neuronal cell death; electrophysiology; dentate gyrus; neuroexcititation

Traumatic brain injury (TBI) is a rapidly growing silent epidemic (Congeni, 2009; Vaishnavi et al., 2009), leading to neurological dysfunction, including impaired learning and memory, psychological disorders such as depression (Stroke, 2002; Iverson et al., 2006), and increased risk for epilepsy and Alzheimer’s and Parkinson’s diseases (Engel and Pedley, 1998; Garga and Lowenstein, 2006; Johnson et al., 2010; Christensen, 2012; Lee et al., 2012; Chauhan, 2014). Brain injury is characterized by a cascade of cellular and circuit changes (Lowenstein et al., 1992; Santhakumar et al., 2001; Winter et al., 2004; Cohen et al., 2007) leading to lasting neurological sequelae (Hicks et al., 1993; Herman, 2002; Creed et al., 2011). The biomechanics of the impact initiating TBI can vary greatly, such as a fall, automobile accident, sports concussion, or explosive blast. These variations in trauma are likely to determine the severity and progression of injury as well as neurological outcome.

The mechanical perturbation resulting in TBI can differ in several physical attributes, including the maximum force applied, the rate at which the force is delivered, and the duration of the injurious force. For instance, we know that TBI can be caused from relatively slow events like falls, during which injury can occur over hundreds of milliseconds (Zhang et al., 2006b; Wright and Laing, 2012). In contrast, high-rate shock waves from explosions attain peak pressure on the order of microseconds to a few milliseconds depending on the distance from the source (Graham et al., 2000; Chavko et al., 2007; Zhang et al., 2009a; Shridharani et al., 2012). Because brain tissue is viscoelastic (Prange and Margulies, 2002; Vappou et al., 2007), tissue response has been shown to be sensitive to both the rate and the magnitude of mechanical trauma (Morrison et al., 2003; Rashid et al., 2012). For viscoelastic tissues, the more rapid the contract grant sponsor: NIJCBIR, contract grant numbers: CBIR111PT003; 09.003-BIR1; Contract grant sponsor: NIH/NINDS, contract grant number: NS069861; Contract grant sponsor: F.M, Kirby Foundation (to V.S.).

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traumatic deformation, the higher the damaging force developed in the brain (Shuck and Advani, 1972; Arborgast et al., 1997; Magou et al., 2011). Accordingly, we hypothesized that traumatic forces generated by faster-rate injuries would lead to a neuronal injury response different from current head injury models.

To date, studies on concussive brain injury using the fluid percussion injury (FPI) model have primarily focused on how the peak magnitude of the pressure waveform influences neuropathology (Dixon et al., 1987; Povlishock et al., 1994; Huh et al., 2011). In contrast, little is known about how injury rate influences the early and long-term histological and physiological changes after TBI. Evidence from FPI models suggests that early post-traumatic anatomical and physiological changes contribute to progressive neurological dysfunction (Santhakumar et al., 2001; D’Ambrosio et al., 2004; Kharatishvili et al., 2006). However, the initial prognosis may not always indicate longer-term outcome under different injury paradigms. Although the majority of practicing physicians believe that an accurate early neurological assessment is important for medical management of TBI patients, fewer are confident that these measures reliably predict prognosis (Collaborators et al., 2008; Steyerberg et al., 2008). Curiously, whether differences in early and later cellular neuropathology following injuries at dissimilar rates contribute to inconsistencies between early neurological assessment and subsequent prognosis has not been considered. A recently developed voice-coil-driven FPI (VC-FPI) device (Abdul-Wahab et al., 2011), which allows for independent control of the magnitude and rate of the concussive waveform, enabled us to examine experimentally the effect of injury rate on the brain. Here we address the critical issue of how differences in the time of rise to peak pressure impact affect measures of immediate neurological assessment and early cellular injury in the juvenile rat following concussive brain trauma at the same peak impact pressures. We further examine whether the early cellular and neurological measures are reliable indicators of subsequent cellular and physiological pathology after primary concussive brain injury at different rise rates.

MATERIALS AND METHODS

FPI

All procedures were performed under protocols approved by the Institutional Animal Care and Use Committee of the Rutgers New Jersey Medical School, Newark, New Jersey. Lateral FPI was performed using a uniquely programmable FPI device (Abdul-Wahab et al., 2011) that permits independent control of key variables defining the waveform (Fig. 1A). This prototype device utilizes a voice-coil actuator to generate a precise temporal forcing function under closed-loop control with a proportional-integral-derivative (PID) motion controller and linear encoder with a 1-μm resolution. The voice coil is coupled to a syringe filled with water that delivers the fluid percussion injury. In this study, the device was programmed to alter the rise times to peak pressure rise, keeping the pressure peak consistent. B: Representative fast- and standard-rise pressure waveforms obtained during animal injuries using the VC-FPI device are overlaid on a trace from the pendulum-style FPI device (Virginia Commonwealth University) for comparison.

Fig. 1. Schematic of the VC-FPI device and representative FPI waveforms. A: Schematic of the programmable VC-FPI device that independently controls key variables defining the waveform and can generate high-rate injuries. The system utilizes a voice-coil actuator to generate a precise temporal forcing function under closed loop controlled with a proportional-integral-derivative motion controller and linear encoder with a 1-μm resolution. The voice coil is coupled to a syringe filled with water that delivers the fluid percussion injury. In this study, the device was programmed to alter the rise times to peak pressure rise, keeping the pressure peak consistent. B: Representative fast- and standard-rise pressure waveforms obtained during animal injuries using the VC-FPI device are overlaid on a trace from the pendulum-style FPI device (Virginia Commonwealth University) for comparison.
Histology and Immunohistochemistry

Gallyas silver stain (Gallyas et al., 1990; van den Pol and Gallyas, 1990; Toth et al., 1997) and Fluoro-Jade C staining were performed on 40-μm sections from animals perfused with 4% paraformaldehyde 4–6 hr postinjury. The range of time to perfusion after injury was kept constant among groups. Only sections ipsilateral to the side of injury were examined. Sections processed for Gallyas stain were dehydrated in 50%, 75%, and 100% 1-propanol for 5 min each; incubated at 56°C in 0.8% sulfuric acid in 1-propanol for 16 hr; and treated with 1% acetic acid for 5 min. Sections were then rehydrated and treated with 3% acetic acid and developed in a silicotungstate solution (Gallyas et al., 1990; van den Pol and Gallyas, 1990). After development, sections were treated with 1% acetic acid for 30 min, dehydrated, and mounted on slides. For Fluoro-Jade C staining, sections mounted on gelatinized slides were air dried, hydrated, and incubated in 0.06% potassium permanganate before being stained with 0.001% Fluoro-Jade C in 0.1% acetic acid in the dark for 30 min. Gallyas silver stain and Fluoro-Jade C staining were performed in separate groups of rats, which were implanted and injured by different investigators. For NeuN staining, rats were perfused 1 week after sham or head injury. Sections (50 μm) were immunostained with anti-NeuN antibody (MAB377, 1:1,000, mouse monoclonal; Millipore, Bedford, MA) and reacted with an appropriate secondary antibody to reveal staining (Yu et al., 2013). Controls in which primary antibody was omitted were routinely included.

Quantification was performed with randomized systematic sampling protocols, selecting every tenth slice along the septotemporal axis of the hippocampus on the injured side (Yu et al., 2013). Cell counts were performed with the Optical Fractionator probe of Stereo Investigator V.10.02 (MBF Bioscience) and an Olympus BX51 microscope and a ×100 oil objective. In each section, the hilus was outlined by a contour traced under a ×10 objective. Sampling parameters were set at ×100, counting frame 50 × 50 μm, dissector height 25 μm, and top guard zone 4 μm. Approximately 30 sites per contour were sampled using randomized systematic sampling protocols. In each section, the number of labeled cells was estimated based on planimetric volume calculations in Stereo Investigator (West et al., 1991; West, 1993; Yu et al., 2013). Data are plotted as number of stained neurons per section.

In Vitro Electrophysiology

One week (7–10 days) after FPI or sham injury (Santhakumar et al., 2001; Gupta et al., 2012), rats were decapitated under isoflurane anesthesia. Horizontal brain slices (400 μm) were prepared in ice-cold sucrose-artificial cerebrospinal fluid (sucrose-aCSF) containing the following (in mM): 126 NaCl, 2.5 KCl, 2 CaCl2, 2 MgCl2, 1.25 NaH2PO4, 26 NaHCO3, and 10 D-glucose. All solutions were saturated with 95% O2 and 5% CO2 and maintained at a pH of 7.4 for 1–6 hr. Field recordings were performed in an interface recording chamber (BSC2; Automate Scientific) perfused with aCSF at 34°C. Recordings in the granule cell layer of the dentate gyrus were obtained by using patch pipettes (4 MΩ, KG-33 borosilicate glass capillary with 1.5 mm outer diameter from Harvard Apparatus, pulled using a Sutter P-1000 horizontal micropipette puller) filled with recording aCSF as in earlier studies (Toth et al., 1997; Gupta et al., 2012). Responses were evoked by constant-current stimuli (0.5–6 mA, 50 μsec) delivered at 0.1 Hz through a custom-made, parallel bipolar 90-μm tungsten stimulating electrode placed in the perforant path at the junction of the dorsal blade and the crest as previously described (Santhakumar et al., 2000, 2001; Gupta et al., 2012). Recordings were obtained with an AxoPatch200B amplifier and digitized at 10 kHz with a DigiData 1440A (Molecular Devices, Sunnyvale, CA). Population spike amplitude was measured as the amplitude of the first negative deflection overriding the field excitatory postsynaptic potential (EPSP) waveform as described previously (Jester et al., 1995), (x + y)/2 – z in the fast- and standard-FPI traces in Figure 5A.

Statistical Analysis

Statistical analysis of behavioral and histological data was performed via Mann-Whitney U test or one-way ANOVA on ranks (Kruskal-Wallis test) followed by pairwise multiple comparison by Dunn’s method or Student-Newman-Keuls method as appropriate. Categorical data were analyzed via χ2 test. Two-way repeated-measures ANOVA was used to compare field recording data between experimental groups. Statistical analysis was performed in SigmaPlot 12.3. Significance was set to P < 0.05. Data are shown as mean ± SEM and median and interquartile range (IQR) where appropriate.

RESULTS

Differential Immediate Behavioral Response to Fast- and Standard-Rise FPI

Previous studies using pendulum FPI devices have shown distinctive immediate behavioral changes such as seizures and transient apnea following moderate
concussive brain injury (Dixon et al., 1987; Toth et al., 1997). Here we examine the immediate neurobehavioral responses of juvenile rats to two different rise times of FPI delivery. Using a moderate injury strength of 1.8–2.1 atm peak pressure, rats were injured with a fast-rise (3–5 msec rise to peak) and standard-rise (10–15 msec rise to peak) FPI using our voice-coil-driven FPI (VC-FPI) device. A random sampling of the FPI waveforms used in the current study revealed a statistically significant difference in the time of rise to peak pressure between fast- and standard-rise FPI waveforms (Table I). Physiologically, sham controls showed no apnea (data not shown), and rats injured using the fast waveform demonstrated shorter duration of apnea than those injured using the standard waveform (Fig. 2A; apnea in seconds, fast FPI: 14.8 ± 1.8, median = 10.0, IQR = 8.25–19.25, n = 30; standard FPI: 20.27 ± 2.77, median = 15.0, IQR = 12.0–19.75, n = 30; P < 0.05 by Mann-Whitney U test, based on pooled data from animals euthanized at the 4–6-hr and 1-week time points).

None of the rats exposed to fast-rise FPI demonstrated postinjury seizures (0 of 16 rats subject to fast-rise FPI), whereas a majority of the rats exposed to standard rate FPI developed stage 3 or higher seizures (13 of 18 rats subject to standard-rise FPI). The difference in the percentage of rats that developed seizures following fast- and standard-rise injuries was statistically significant (Table I).

Injury with a standard rate of rise to peak pressure resulted in 22.2% mortality in the rats (four of 18 rats; two rats died from postinjury apnea and two rats died from continuous stage 4 posttraumatic seizures following recovery from apnea). However, none of the rats exposed to fast-rise FPI at identical peak pressures died following injury, indicating reduced mortality following fast-rise injury (Table I). A group of animals that survived was perfused 4–6 hr after FPI for histology. Similar results were obtained with animals used for histological and physiological studies 1 week after FPI (Table I).

Rats injured using either waveform showed increase in latency of response to toe pinch and recovery of righting reflex compared with controls. However, rats subject to fast-rise FPI exhibited a significantly faster recovery of response to toe pinch than rats that underwent standard-rise FPI (Fig. 2B; latency to recovery of response to toe pinch in sec, sham: 4.6 ± 0.4, median = 4.0, IQR = 4.0–5.5, n = 5; fast FPI: 17.0 ± 1.1, median = 16.0, IQR = 15.0–19.5, n = 5; standard FPI: 70.4 ± 12.7, median = 73.0, IQR = 41.5–98.0, n = 5, P < 0.05, H = 12.61, df = 2 by one-way ANOVA on ranks followed by P < 0.05 for all pairwise multiple comparisons using Student-Newman-Keuls method). Similarly, rats that sustained fast-rise FPI exhibited earlier return of righting reflex compared with rats injured at standard-rise FPI (Fig. 2C; time to return of righting reflex in sec, sham: 26.0 ± 1.7, median = 25.0, IQR = 22.5–30.0, n = 5; fast FPI: 115.80 ± 33.5, median = 89.0, IQR = 63.0–182.0, n = 5; standard FPI: 332.4 ± 41.9, median = 287.0, IQR = 262.0–425.5, n = 5; P < 0.05, H = 12.61, df = 2 by one-way ANOVA on ranks followed by P < 0.05 for all pairwise multiple comparisons using Student-Newman-Keuls method). Together the immediate neurobehavioral data from juvenile rats suggest that, even when peak injury pressures are similar, the fast-rise injuries result in reduced early neurological deficits compared with standard-rise injuries.

### Rate of FPI Modifies the Extent of Early Hilar Neuronal Injury

Next, we examined differences in cellular responses to injury at fast and standard rise times. Previous studies have shown that moderate injuries at standard rise, using the pendulum-style FPI device, lead to instantaneous mechanical injury (Toth et al., 1997) and early degeneration (Gupta et al., 2012) of neurons in the hilus of the dentate gyrus. Similar to earlier studies (Toth et al., 1997), sections from sham-control rats treated with the Gallyas silver stain (Gallyas et al., 1990; van den Pol and Gallyas, 1990; Toth et al., 1997) revealed negligible hilar staining (sham: nine sections from three rats), in contrast to the dense staining of hilar neuronal somata and dendrites following standard-rise injury (standard FPI: n = 12 sections from three rats). Importantly, the somatic silver staining following fast-rise FPI was relatively sparse (fast FPI: n = 12 sections from three rats), suggesting that the immediate mechanical damage to dentate neurons is reduced compared with standard-rise FPI at similar peak pressures (Fig. 3A–C).

To confirm our findings, a second investigator subjected an additional group of rats to injuries at fast and standard rates for examination of early postinjury neuronal degeneration. Fluoro-Jade C staining performed 4–6 hr after FPI revealed few labeled neurons in sham controls.
Fluoro-Jade-immunoreactive dentate hilar neurons in rats subject to fast-rise FPI compared with rats injured at standard rise (Fig. 3G; estimated number of degenerating hilar neurons per section, sham: 4.8 ± 2.4, median = 0, IQR = 0.0–6.8, n = 14 sections from three rats; fast FPI: 38.0 ± 4.6, median = 38, IQR = 66.0–123.0, n = 19 sections from three rats; standard FPI: 165.2 ± 27.1, median = 118.0, IQR = 84.0–200.5, n = 20 sections from three rats; P < 0.001, H = 40.995, df = 2 by Kruskal–Wallis one-way ANOVA on ranks). Pairwise multiple comparisons by Dunn’s method showed a statistically significant difference in degenerating neurons among all three groups. Presence of a few Fluoro-Jade-immunoreactive neurons in the dentate of sham-injured rats is consistent with earlier reports in juvenile rats (Sun et al., 2013). Even when we compared the average estimated number of degenerating hilar neurons per section in each rat, fast-rise FPI resulted in significantly fewer Fluoro-Jade-immunoreactive hilar neurons than standard-rise injury (estimated number of degenerating hilar neurons per section per rat, sham: 3.8 ± 3.4, n = 3 rats; fast FPI: 38.5 ± 11.0, n = 3 rats; standard FPI: 150.9 ± 47.7, n = 3 rats; P < 0.05, F2,6 = 7.35 by Kruskal–Wallis one-way ANOVA on ranks, P < 0.05 for sham vs. standard and fast vs. standard, and P > 0.05 for sham vs. fast by pairwise multiple comparison using Student-Newman-Keuls method).

Collectively, the immediate postinjury behavioral assessment and early cellular damage demonstrate that, even when peak pressures are the same, a small change in time to rise to peak pressure influences the immediate cellular pathology and neurological assessment measures. Moreover, our data suggest that juvenile rats exposed to fast-rise FPI may have a milder injury and thus show improved neurological prognosis compared with those receiving standard-rise FPI at identical peak pressure.

Absence of Rise-Time-Specific Difference in Dentate Cell Loss and Excitability 1 Week After FPI

Next, we examined whether injury rise-time-specific differences in hilar neuronal loss were also observed 1 week after FPI. This is an important time point at which evolved cellular and physiological changes occur in the dentate gyrus after FPI using pendulum-style devices (Lowenstein et al., 1992; Toth et al., 1997; Santakumar et al., 2000, 2001; Gupta et al., 2012). In sections obtained from rats perfused 1 week after injury, NeuN staining for neuronal nuclei revealed fewer NeuN-immunoreactive neurons in the dentate hilus following both fast- and standard-rise FPI than in sham controls (Fig. 4A). Stereological cell counts revealed a significant decrease in hilar neurons after both fast- and standard-rise FPI (Fig. 4A–C; NeuN-positive hilar neurons per section, sham: 525.8 ± 38.0, median = 488.5, IQR = 368.8–549.0, n = 20 sections from three rats; fast FPI: 366.2 ± 23.9, median = 335.5, IQR = 288.3–457.5, n = 16 sections from three rats; standard FPI:

Fig. 2. Injury rate impacts immediate postinjury behavior. A: Summary plot of the average duration of apnea immediately following fast- and standard-rise FPI (n = 30 rats in each group). *P = 0.04 by Mann-Whitney U test. B,C: Summary histograms show the duration of recovery of response to toe pinch (B) and time to recovery of righting reflex (C) following injuries (n = 5 rats in each group). *P < 0.01 compared with sham by one-way ANOVA on ranks followed by pairwise multiple comparisons using Student-Newman-Keuls method. #P < 0.01 compared with fast-rise FPI by one-way ANOVA on ranks followed by pairwise multiple comparisons using Student-Newman-Keuls method. Summary data are presented as mean ± SEM.
Fig. 3. Differential early dentate neuronal injury and degeneration after fast- and standard-rise FPI within 4 hr after injury. A–C: Representative sections from the dentate gyrus of rats fixed 4 hr after sham, fast-rise, and standard-rise FPI and stained with the Gallyas silver stain to reveal neurons with mechanical injury. Note the lack of staining in controls (A), the sparse staining after fast-rise FPI (B), and the presence of numerous darkly stained cells in the dentate hilus and also a subset of the darkly stained cells in the granule cell layer (GCL) after standard-rise FPI (C). D–F: Fluoro-Jade C stained hippocampal sections from rats perfused 4–6 hr after FPI show few labeled hilar neurons in the sham control (D), several labeled neurons following fast-rise FPI (E), and numerous degenerating neurons in the dentate hilus after standard-rise FPI (F). G: Summary histogram of stereological counts of Fluoro-Jade C-labeled hilar neurons per section in animals subject to the injury paradigms (n = 14 sections from three rats in sham, 19 sections from three rats after fast-FPI, and 20 sections from three rats after standard FPI). *P < 0.05 compared with sham; #P < 0.05 compared with fast-rise FPI by one-way ANOVA for ranks followed by post hoc pairwise comparison by Dunn’s method. Summary data are presented as mean ± SEM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
322.1 ± 27.4, median = 287.0, IQR = 216.0–411.0, n = 23 sections from three rats; P < 0.05, H = 17.7, df = 2 by Kruskal-Wallis one-way ANOVA on ranks). Pairwise multiple comparison by Dunn’s method showed that differences in the number of surviving NeuN-positive neurons between sham and standard-rise FPI and between sham and fast-rise FPI were statistically significant. However, the number of NeuN-labeled neurons was not different between fast- and standard-rise FPI 1 week after injury. Similar results were obtained when statistical analysis was conducted on the estimated number of hilar neurons per section in each rat (estimated number of NeuN-positive hilar neurons per section per rat, sham: 520.7 ± 13.9, n = 3 rats; fast FPI: 371.7 ± 48.5, n = 3 rats; standard FPI: 319.1 ± 18.4, n = 3 rats; F2,6 = 11.37, P < 0.05 by one-way ANOVA, P < 0.05 for sham vs. standard and sham vs. fast, and P > 0.05 for standard vs. fast by pairwise multiple comparison using Holm-Sidak method). Given the disparity between hilar neuronal degeneration following fast- and standard-rise FPI at 4 hr, this could suggest recovery of some mechanically injured neurons following standard-rise FPI (Toth et al., 1997). However, because Fluoro-Jade labels irrevocably degenerating neurons, our data likely indicate a greater progression of hilar cell loss within the week following fast-rise FPI. Fluoro-Jade staining conducted on sections from rats 1 week after fast- or standard-rate FPI showed no labeling of neuronal profiles (number of degenerating hilar neurons per section per rat, sham: 0, nine sections from three rats; fast FPI: 0, nine sections from three rats; standard FPI: 0, nine sections from three rats) indicating that there are no actively degenerating neurons 1 week after fast- and standard-rate FPI.

Earlier studies, by us and by other groups, have consistently demonstrated an increase in dentate network excitability and altered inhibition 1 week after FPI when using the pendulum-style device (Lowenstein et al., 1992; Toth et al., 1997; Santhakumar et al., 2000, 2001; Gupta et al., 2012). The hilar neuronal loss and increase in dentate excitability have been suggested to predict development of long-term neurological complications such as posttraumatic epilepsy (Lowenstein et al., 1992; Toth et al., 1997). Therefore, we examined the granule cell population response to afferent activation to assess the effect of injury rise time on neurophysiological outcomes 1 week after FPI. As illustrated in Figure 5A, although the granule cell population response evoked by perforant path stimulation at 4 mA showed no population spikes in seven of 12 slices from sham-control rats, afferent activation reliably elicited dentate population spikes in all slices 1 week after fast- and standard-rise FPI. Summary data demonstrate that the afferent-evoked granule cell population spike amplitude in injured rats was significantly enhanced compared with that in sham controls. Two-

![Fig. 4. Dentate hilar neuronal loss 1 week after fast- and standard-rise FPI. A–C: Representative NeuN-stained hippocampal sections from rats perfused 1 week after injury show significantly more neurons in the hilus of sham controls (A) compared with rats exposed to either fast-rise (B) or standard-rise (C) FPI. D: Summary data compare stereological counts of NeuN-labeled neurons per sections from sham controls and rats subject to fast- and standard-rise FPI (n = 20 sections from three rats in sham, 16 sections from three rats after fast FPI, and 23 sections from three rats after standard FPI). GCL, granule cell layer. *P < 0.05; N.S. indicates P > 0.05 by one-way ANOVA for ranks followed by post hoc pairwise comparison by Dunn’s method. Summary data are presented as mean ± SEM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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Fig. 5. Absence of rate-specific difference in dentate excitability 1 week after FPI. A: Examples of dentate granule cell field responses evoked by a 4-mA stimulus to the perforant path. Recordings were performed in control aCSF. Population spike amplitude was calculated as $(x + y)/2 - z$ (Jester et al., 1995). B: Summary plot of afferent evoked population spike amplitude in aCSF. C: Dentate population responses evoked by perforant path stimulation at 4 mA with the GABA_A receptor antagonist gabazine (10 μM, SR95531). D: Summary plot of perforant path-evoked granule cell population spike amplitude with gabazine (10 μM). In B,D, *$P < 0.05$ in fast FPI, **$P < 0.05$ in standard FPI compared with sham for effect of injury by two-way repeated-measures ANOVA. In A,C, triangles indicate truncated stimulus artifact. E: Summary histogram of the number of population spikes in response to 6-mA stimulation in the presence of gabazine. *$P < 0.05$ by one-way ANOVA on ranks followed by post hoc pairwise multiple comparison using Dunn’s method. Summary data are presented as mean ± SEM.
way repeated-measures ANOVA identified significant effects of treatment (injury) at $F_{2,179} = 7.42$ ($P < 0.01$), stimulus intensity at $F_{4,179} = 54.03$ ($P < 0.001$), and interaction between treatment and intensity at $F_{8,179} = 6.43$ ($P < 0.001$). Pairwise multiple comparisons by Tukey’s test within each stimulus intensity level revealed that both fast and standard FPI were significantly different from sham at 2, 4, and 6 mA stimulation (population spike ampli
tude in millivolts evoked by 4 mA stimulation: sham: $0.31 \pm 0.13$; fast FPI: $1.42 \pm 0.34$; standard FPI: $1.2 \pm 0.18$, 6 mA stimulation: sham: $0.35 \pm 0.14$; fast FPI: $1.56 \pm 0.27$; standard FPI: $1.53 \pm 0.13$, n = 12 slice each from five sham, six fast-FPI, and six standard-FPI rats). Interestingly, slices from rats injured with fast-rise or standard-rise FPI produced similar postinjury population spike amplitudes (Fig. 5B; $P > 0.5$ by two-way repeated-measures ANOVA followed by pairwise multiple comparison within each stimulus intensity level by Tukey’s test).

Previous work has identified that brain injury with the pendulum-style FPI device enhances excitability of the dentate excitatory network, measured in the presence of a GABAA receptor (GABAAR) antagonist (Santhakumar et al., 2000). To determine whether injuries at fast and standard rise differentially impacted the excitability of the dentate excitatory network, we examined the perforant path-evoked granule cell field responses in slices that were perfused with gabazine (10 μM, SR95531), a GABAAR antagonist. Once again, the amplitude of the first population spike in response to afferent activation was greater in slices from head-injured rats compared with sham controls (Fig. 5C). Two-way repeated-measures ANOVA identified significant effects of treatment (injury) at $F_{2,224} = 4.53$ ($P < 0.05$), stimulus intensity at $F_{4,224} = 49.52$ ($P < 0.001$), and interaction between treatment and intensity at $F_{8,224} = 3.3$ ($P < 0.005$). Pairwise multiple comparisons within each stimulus intensity level by Tukey’s test revealed significant differences between sham and both fast and standard FPI at 4 and 6 mA stimulation (Fig. 5D); population spike amplitude in mV evoked by 4 mA stimulation: sham: $2.35 \pm 0.23$, n = 15 slices from six rats; fast FPI: $2.85 \pm 0.35$, n = 15 slices from six rats; standard FPI: $3.44 \pm 0.34$, n = 15 slices from six rats; 6 mA stimulation: sham: $2.54 \pm 0.24$ n = 15 slices; fast FPI: $3.16 \pm 0.36$ n = 15 slices; standard FPI: $3.71 \pm 0.41$ n = 15 slices). However, the population spike amplitudes within each intensity was not different between fast and standard FPI (Fig. 5D; $P > 0.5$ by two-way repeated-measures ANOVA followed by pairwise multiple comparisons within each stimulus intensity level by Tukey’s test). Additionally, the slices from rats subject to FPI showed an increase in the number of afferent-evoked population spikes (Fig. 5E). The number of population spikes in response to perforant path stimulation at 6 mA was significantly higher in both fast- and standard-FPI groups compared with sham but similar in slices from rats exposed to fast- and standard-rise FPI (Fig. 5E; number of population spikes in response to 6 mA stimulation: sham: $1.07 \pm 9.30$, n = 15 slices from six rats; fast FPI: $2.67 \pm 0.32$, n = 15 slices from six rats, standard FPI: $3.07 \pm 0.34$, n = 15 slices from six rats; significance determined by one-way ANOVA on ranks followed by post hoc pairwise multiple comparisons using Dunn’s method).

Together our studies on fast- and standard-rise FPI at similar peak impact pressures in juvenile rats indicate that, although the improved neurobehavioral outcome and reduced cellular injury measures within hours following fast-rise injury suggest that fast-rate trauma leads to a milder TBI than slower-rate injury, the extent of cell loss and physiological dysfunction after fast-rise injury are comparable to that of moderate TBI within a week after injury.

**DISCUSSION**

The use of a novel programmable VC-FPI device allowed us to examine a fundamental question concerning whether differences in the rate of delivery of a fluid percussion wave alter neurological outcome following brain injury. We demonstrate, for the first time, that increasing the rate of injury, by decreasing the rate of rise to the same peak pressure, alters the immediate behavioral assessment as well as cellular injury and neuronal degeneration within 4 hr after injury. This initially suggests that fast-rate injuries lead to reduced neurological damage compared with slower-rate injuries. Studies performed 1 week after FPI, however, demonstrate a similar level of injury as shown by the extent of dentate hilar cell loss and increase in network excitability following fast- and slower-rise injuries.

Our findings in juvenile rats suggest that a better immediate neurological outcome following fast injury may mask the severity of neuropathology at later time points. Strategies for evaluation of injury severity rely primarily on early clinical measures (Saatman et al., 2008), so our data suggest the need to consider the effect of injury rate in shaping the cellular and physiological response at later time points while managing patients sustaining fast- and slower-rate concussive injuries.

Brain injury can result from a wide spectrum of insults ranging from falls that occur at relatively slow impact velocities (Wright and Laing, 2012) to sports injuries and traffic accidents, which represent higher-rate impacts (Zhang et al., 2006a, 2009b). Extremely rapid shock waves generated by blasts represent the farthest end of the spectrum of high-rate impact (Zhang et al., 2009a; Shridharani et al., 2012). Indeed, fast-rate TBI following exposure to explosive blasts is the signature injury of the recent wars (Elder and Cristian, 2009) and a growing issue facing veterans. The primary impact waves of blast TBI have a considerably faster rise to peak pressure than non-blast concussive TBI (Chavko et al., 2007; Zhang et al., 2009a; Cernak and Noble-Haeusslein, 2010; Shridharani et al., 2012; Wright and Laing, 2012). Most contemporary studies use devices such as shock tubes to generate fast-rise injuries (Cernak et al., 2001; Risling et al., 2011; Rubovitch et al., 2011; Skotak et al., 2013). The more...
commonly employed FPI devices (Dixon et al., 1987; Garga and Lowenstein, 2006) are used to deliver fixed concussive waveforms to model closed-head injury from trauma such as automobile accidents or falls. The inability to control rate and magnitude in the pendulum-style FPI device independently and the use of different devices to model injuries at drastically different rates render it difficult directly to compare rise time-specific changes in neuropathology without differences in peak pressure and device-dependent confounding factors. The prototype VC-FPI device (Abdul-Wahab et al., 2011) enabled us to overcome these technical obstacles and demonstrate that, even at constant peak pressures, the rate of fluid percussion directly affects the immediate neurological response. Our study presents the first demonstration that even small changes in injury rate can modify the ability of early neurological measures to predict the subsequent neuropathology.

Rate sensitivity to injury is likely because brain tissue behaves like a viscoelastic material (Prange and Margulies, 2002; Vappou et al., 2007; Rashid et al., 2012). Indeed, some biomechanical investigations are beginning to suggest that the brain’s response to mechanical loading is sensitive to both the rate and the magnitude of the trauma (Morrison et al., 2003; Lusardi et al., 2004; Magou et al., 2011; Rashid et al., 2012). Viscoelastic tissues are soft and compliant under slow mechanical loading, where small forces can lead to large deformations in the brain. Under higher rates of mechanical loading, however, the time dependence of the viscoelastic brain acts more like a stiff solid, developing larger internal forces under the same deformation constraints (Shuck and Advani, 1972; Arbogast et al., 1997; Magou et al., 2011). Accordingly, we should assume that, with variations in the rate of mechanical trauma, the deformation and damaging forces within the brain tissue will differ. These variations in the biomechanical loading conditions of the brain tissue likely contribute to the variety of neuronal damage and neurological outcome seen in head-injured patients. Similar to how increases in the magnitude of trauma, such as peak pressure in FPI, lead to increased injury, alterations in loading rate should also lead to alterations in the injury sequelae. Our results suggest that increases in the rate of injury appear to influence a milder injury acutely but progress into neuronal injury and dysfunction similar to the lower-rate injury at the same injury magnitude.

A potential caveat is that, although the peak pressures are similar, the fast-injury waveform in the current study had a shorter duration than the standard wave. Thus the impulse, a measure of energy transfer determined as the area under the pressure curve, in fast-rise FPI is lower than in standard-rise FPI. This difference is realistic, in that high-rate injuries such as blast waves usually have a brief duration compared with the standard concussive waveform modeled by the pendulum-style FPI device (Elder and Cristian, 2009; Zhang et al., 2009a). Previous studies with the pendulum-style FPI device have demonstrated that dentate neuronal loss measured 1 week after injury increases progressively with increase in injury severity, classified based on injury peak pressure from mild to moderate to severe (Lowenstein et al., 1992). Additionally, it has been shown that the cell loss after brain injury using the pendulum-style FPI device occurs early after injury and does not increase further beyond 1 week postinjury (Toth et al., 1997). Thus, if the fast-rise, low-impulse injury leads to just a milder form of TBI, we would expect that the dentate hilar cell loss 1 week after FPI would be lower in rats exposed to fast-rise FPI than those subject to standard-rise FPI. However, it is notable that fast-rise FPI produced cellular and physiological pathology at 1 week comparable to that of standard-rise, higher-impact injuries, indicating that fast-rise FPI is not merely a milder version of standard-rise FPI. Moreover, although the immediate neurobehavioral compromise after fast-rise FPI is significantly milder than that after standard-rise FPI (Fig. 1B,C), animals examined 1 week after FPI showed similar dentate hilar cell loss and alterations in network excitability regardless of the injury rate (Figs. 4, 5). Measures of postinjury neurological responses such as the Glasgow Coma Scale are widely used to assess severity of neurological injury and predict prognosis following TBI (Stembach, 2000; Saatman et al., 2008; Elder and Cristian, 2009). Our data indicate that early postinjury criteria, which seem to predict prognosis reliably after increasing magnitudes of impact at similar rates (Dixon et al., 1987; Povlishock et al., 1994; Huh et al., 2011), may have to consider the effect of impact rate for fast-rate injuries such as blast TBI. Moreover, our findings suggest that early clinical assessment may not provide an accurate evaluation of the subsequent pathology and indicate that followup assessments of patients conducted several days after the trauma could aid in evaluating long-term prognosis of brain-injured patients.

Our histological studies 4 hr after injury demonstrate that the immediate cellular injury and degeneration are considerably milder after fast-rise FPI than after standard-rise FPI. What are the mechanisms that could account for the subsequent absence of rate-specific difference in hilar cell loss and network excitability 1 week after injury? Structural characteristics of cytoskeletal elements in somatodendritic compartments may underlie the difference in mechanical damage induced by fast- and slower-rise FPI. It is possible that axons, which have distinctive cytoskeletal components (Stiess and Bradke, 2011), are more vulnerable to injury during fast-rise TBI. Consistent with this possibility, diffuse axonal injury takes a longer time to manifest (Leonard et al., 1997; Carbonell and Grady, 1999) compared with considerable early somatodendritic injury evident in silver-stained sections from rats exposed to standard-rise injury. Additionally, high-rate TBI is typically associated with extensive white matter injury (Koliasos et al., 2011; Jorge et al., 2012). An equally possible alternative is that fast-rise injuries lead to delayed excitotoxic cell death (Manev et al., 1989; Palmer et al., 1993; Yi and Hazell, 2006), leading to increased network excitability. However, the lack of Fluoro-Jade C-stained neurons 1 week after both fast and standard FPI indicates that, as with standard injury (Toth et al., 1997), the cell...
loss after fast FPI may be complete by 1 week. Concussive injury leads to distinctive apoptotic responses in juvenile and aged rats (Sun et al., 2013), so it is possible that injury rate modified the apoptotic responses in the juvenile rats in the current study. Although the exact mechanisms remain to be elucidated, it is evident that the progression of neuropathology following fast-rise FPI in juvenile rats is different than that after slower-rise TBI. The observed divergence between early postinjury assessment and subsequent cellular and physiological outcome following fast- and standard-rise FPI suggest that early behavioral measures used to predict prognosis after slower-rate TBI may underestimate the severity of long-term neurological outcome after faster-rate injuries such as blast-related TBI.

CONCLUSIONS
We have demonstrated that the rate of rise to peak pressure modifies the immediate neurobehavioral and early cellular response to concussive brain injury in juvenile rats. We show that, despite a better immediate neurological outcome following fast injury, the long term cellular and physiological pathology after injuries with fast- and slow-rising waveforms are not different. Our findings suggest dissimilarities in the progression of cellular pathology after fast- and slow-rising injuries.

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