Membrane trauma and Na\textsuperscript{+} leak from Nav1.6 channels

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Wang JA, Lin W, Morris T, Banderali U, Juranka PF, Morris CE. Membrane trauma and Na\textsuperscript{+} leak from Nav1.6 channels. Am J Physiol Cell Physiol 297: C823–C834, 2009. First published August 5, 2009; doi:10.1152/ajpcell.00505.2008.—During brain trauma, white matter experiences shear and stretch forces that, without severing axons, nevertheless trigger their secondary degeneration. In central nervous system (CNS) trauma models, voltage-gated sodium channel (Nav) blockers are neuroprotective. This, plus the rapid tetrodotoxin-sensitive Ca\textsuperscript{2+} overload of stretch-traumatized axons, points to “leaky” Nav channels as a pivotal early lesion in brain trauma. Direct effects of mechanical trauma on neuronal Nav channels have not, however, been tested. Here, we monitor immediate responses of recombinant neuronal Nav channels to stretch, using patch-clamp and Na\textsuperscript{+}-dye approaches. Trauma constituted either bleb-inducing aspiration of cell-attached oocyte patches or abrupt uniaxial stretch of cells on an extensible substrate. Nav1.6 channel transient current displayed irreversible hyperpolarizing shifts of steady-state inactivation [availability(V)] and of activation [g(V)] and, thus, of window current. Left shift increased progressively with trauma intensity. For moderately intense patch trauma, a ~20-mV hyperpolarizing shift was registered. Nav1.6 voltage sensors evidently see lower energy barriers posttrauma, probably because of the different bilayer mechanics of blebed versus intact membrane. Na\textsuperscript{+} dye-loaded human embryonic kidney (HEK) cells stably transfected with αNav1.6 were subjected to traumatic brain injury-like stretch. Cytoplasmic Na\textsuperscript{+} levels abruptly increased and the trauma-induced influx had a significant tetrodotoxin-sensitive component. Nav1.6 channel responses to cell and membrane trauma are therefore consistent with the hypothesis that mechanically induced Nav channel leak is a primary lesion in traumatic brain injury. Nav1.6 is the CNS node of Ranvier Nav isoform. When, during head trauma, nodes experienced bleb-inducing membrane damage of varying intensities, nodal Nav1.6 channels should immediately “leak” over a broadly left-smearred window current range.

sodium dye; stretch; shear; bleb

AFTER TRAUMATIC BRAIN and spinal cord injury (TBI/SCI), Ca\textsuperscript{2+}-protease driven breakdown of the cytoplasm in otherwise intact axons results in an untreatable state called diffuse axonal injury (4, 22, 38). When neurons grown on extensible membranes are subjected to a brief intense (TBI-like) stretch that does not rupture the axonal membranes, a rapid (<2 min) and irreversible increase in axonal calcium occurs. Strikingly, this calcium rise is tetrodotoxin (TTX) sensitive, which points to leaky voltage-gated sodium (Nav) channels (22, 62) as the primary lesion. Since the TTX-sensitive calcium rise is nearly maximal at 2 min, well before any proteolytic damage to Nav channels is detected, the primary Nav channel lesion is probably of a mechanical nature. How mechanical insult would directly render neuronal Nav channels leaky is, however, not understood. In situ, the diffuse axonal injury state develops gradually as overwhelmed sodium/calcium exchangers lead to overactivated Ca\textsuperscript{2+} proteases that attack cytoplasmic proteins, notably, spectrin. At that later stage, cleavage of the cytoplasmic Nav channel “inactivation gate” might exacerbate the leak, but our interest here is in the earliest cause of leaky Nav channels. We therefore examined the immediate responses of the recombinant form of the central nervous system (CNS) white matter Nav channel Nav1.6 (48) to cellular and membrane trauma. Ensuring fast salutary action potential propagation, these channels are present at high density at nodes of Ranvier. They likely produce window current that contributes to the small TTX-sensitive component of the resting conductance of myelinated axons (50).

At stretch-traumatized CNS nodes of Ranvier, the major membrane histopathology is the rapid appearance of “nodal blebs, axolemma limited protrusions of the nodal axoplasm into the perinodal space, in which the nodal dense undercoating has been lost” plus “disruption of the axonal cytoskeleton in nodes where blebs occur” and (as seen by freeze fracture and ATPase cytochemistry) “structural reorganization of the nodal axolemma” (31). Although trauma (3, 15) that impacted nodal Nav channels would affect many species of nodal membrane protein (12), leaky Nav channels clustered at high density in small caliber processes and operating by positive feedback would be inordinately treacherous. Not surprisingly, therefore, in animal models of TBI and SCI, various Nav channel inhibitors are neuroprotective (e.g., 2, 42).

Voltage-gated channels, including the “cardiac” Nav isoform (Nav1.5), exhibit reversible (“elastic”) kinetic responses to membrane stretch (35) consistent with intimate molecular interactions of voltage sensors and the structured lipid bilayer (35, 51). Were stretch to irreversibly alter the nanostructure of the voltage sensors’ bilayer environment, then irreversible gating changes would be expected, as recently reported for some Kv channels by Schmidt and Mackinnon (43). Importantly, the same conditions that reversibly change Nav1.5 gating cause irreversible gating changes in the skeletal muscle sodium channel isoform Nav1.4. In Nav1.4 (expressed without auxiliary β-subunits), membrane stretch causes both activation [i.e., g(V)] and steady-state inactivation [i.e., availability(V)] to irreversibly shift “leftward” toward hyperpolarized potentials (44, 52). Because Nav1.6 is similar to Nav1.4 and distant from Nav1.5 (64), we thought its susceptibility to stretch would be Nav1.4-like and have a bearing on the primary trauma-induced node of Ranvier Na\textsuperscript{+} leak.

We found that traumatic stretch caused irreversible Nav1.6 channel current left shift (without/with auxiliary β-subunits) that predicts a TTX-sensitive mechanically induced window current-based Na\textsuperscript{+} leak in CNS axons. We also asked whether human embryonic kidney (HEK) cells expressing Nav1.6 channels exhibited TTX-sensitive sodium leak in response to a trauma-like stretch episode. We discuss how, in traumatized axons, stretch/shear induced blebbing of nodal membrane
could be the primary mechanical disruption underlying the primary Nav1.6 channel leak.

METHODS

Oocyte patch-clamp electrophysiology. To monitor Nav1.6 current with trauma, cell-attached Xenopus oocyte patches were used because: 1) for characterizing stretch effects, within-preparation comparisons are needed and making whole cell recordings from a mammalian cell before and after stretch on silastic membranes is not possible, 2) macroscopic sodium current (Im) is needed to monitor stretch-induced changes in Im, and is attainable in oocyte patches but not in Nav1.6-HEK patches, 3) endogenous oocyte mechanosensitive channels can be blocked, as necessary, by La3+, whereas in mammalian cell lines mechanosensitive currents are diverse and poorly characterized (e.g., see Refs. 20 and 58), 4) oocytes lack endogenous β1-subunits, so αNav1.6 without or with auxiliary subunits can be tested. Except for the Nav1.6 currents noted in Fig. 2, A and B, and Fig. 3B, Nav1.6 currents are from oocytes coexpressing α- and β-subunits. Both the auxiliary subunit β1 (52), used previously, and CNS splice variant β1B (40), were used.

In oocytes, αNav1.6 (48) and αNav 1.4 channel kinetics (52), unlike those of αNav1.5 channels (27), are anomalously slow and right-shifted compared with what is seen in mammalian cell lines (33) and native cells but are rendered normal (left shifted, faster) by coexpression of β1-subunits.

Gigaohm seal formation, even when done gently, inevitably affects plasma membranes, but we minimized inadvertent “patch history” effects (36) by using less than −7 mmHg suction (usually −4 mmHg) to achieve seals. The term “prestretch” used in conjunction with patch recordings is used with the understanding that it ignores the membrane stretch that occurs during seal formation.

Xenopus laevis oocytes expressing mouse αNav1.6 channels and β1- or β1B- auxiliary subunits, as indicated (or no B as in Fig. 2, A and B) were prepared for patch clamp as described previously (35). The plasmid for mouse αNav1.6 (pNaScn8a) was kindly provided by Dr. Al Goldin (48). pNaScn8a was linearized with NotI and used to produce capped cRNA by in vitro transcription using T7 RNA Polymerase Ambion Message Machine (Austin, TX). Plasmids for the auxiliary subunits human β1 (hβ1-pSP64T) and human β1B (hβ1B-pAGA3) were kindly provided by Dr. A. L. George (28) and Dr. Ning Qin (40), respectively. hβ1-pSP64T was linearized with EcoRI and hβ1B-pAGA3 was linearized with HindIII. The cDNA templates were used to produce capped cRNA by in vitro transcription using SP6 (hβ1) or T7 (hβ1B) RNA Polymerase Ambion Message Machine. The α (30–100 pg per oocyte) and β1 and/or β1B-subunit RNAs were injected in a 1:8 molar ratio (when all three were coexpressed the ratio was 1:8:8) with injection volume of cRNA (in 10 mM Tris buffer, pH 8) varying between 15 and 50 nl.

Macroscopic currents were recorded from cell-attached patches using Sylgard-coated, fire-polished pipettes. P/N linear subtraction with hyperpolarizing steps was used (usually N = 8). High-K bath solution contained (in mM) 89 KCl, 0.4 CaCl2, 5 HEPES, and 0.8 MgCl2 (pH 7.5 with KOH). The recording pipette solution was the same except that KCl was substituted with 89 mM NaCl (pH adjusted with NaOH). When testing for reversible stretch effects (see Fig. 4), 1 mM LaCl3 was added to the pipette to block the oocytes’ endogenous stretch-activated cation channels. Patch membrane was stretched using pipette suction (negative pipette pressure, given in mmHg) of which its intensity was monitored by a DPM-1B transducer (Bio-Tek, Winsoski, VT).

Cell culture. HEK-293 cells stably expressing the α-subunit of human Nav1.6 (Nav1.6-HEK cells) were a generous gift from Dr. S. Burbridge (8). Channel expression was confirmed by electrophysiology (e.g., our whole cell recordings in Ref. 60). The cells, obtained at passage 25, were amplified and stored (passage 26) then used till passage 30, were cultured 3–5 days (37°C, 5% CO2) in DMEM (GIBCO, Invitrogen, Burlington, ON) with l-glutamine, supplemented with 1% essential amino acids, 1% penicillin-streptomycin, and 10% nonactivated fetal bovine serum plus G418 (GIBCO) 80 μg/ml to select for Nav1.6-expressing cells. At 70% confluency, cells were washed once with phosphate-buffered saline then dispersed in 2 ml 0.05% trypsin-EDTA buffer (Sigma-Aldrich Canada, Oakville, ON) for 2 min, harvested by a 5-min 500 rpm spin, and then resuspended in the DMEM-G418 at 106 cells/ml. A Silastic chamber was positioned across two glass-slide stacks in a dempended 100-mm culture dish (to elevate the chamber floor), 0.5 ml of cell suspension was added, and then the covered dish was incubated 24 h or until cells were 50–70% confluent. Cells were then dye loaded as described below.

Fabrication of Silastic cell chamber. Stretchable Silastic growth chambers (see Fig. 5A) were fabricated precisely as described previously by Pfister and colleagues (39) by gluing together thick and thin silicone membranes; a rectangle cut from the thick membrane formed the walls of a thin-bottomed stretchable culture chamber. Coverslips glued to this assembly provided a rigid surface for clamping on either side of the stretch zone. For consistency, cells in the midline of the chamber were imaged. The Silastic growth chambers were fabricated using the following: 127 μm (0.005") nonreinforced vulcanized silicon membrane (cut from gloss/gloss 12" × 12") pieces for the thin chamber bottom and 1.52 mm (0.06") nonreinforced vulcanized silicon sheeting (matt/matt 12" × 12") for the thick upper part (both from Specialty Manufacturing, Saginaw, MI). These were cut (by placing the transparent sheets on graph paper with appropriate-sized grids) into 50 mm × 22 mm pieces for the thick pieces and slightly larger (∼55 mm × 25 mm) for the thin pieces, using a metal straight edge and fine scalpel. Cell chambers (13 mm × 6 mm) were cut into the thick pieces and, then, working in a chemical hood, thick-thin were glued (g1, inset) with R-2615 Silicone Elastomer (NuSil Technology, Carpinteria, CA) mixed 1:1. To keep the thin floor flat, glue was applied to the thick piece, which was then positioned on the slightly larger thin piece. Forty-eight hours later, the excess was trimmed. In the hood, two 22 × 22 mm glass coverslips were then glued (g2) to the bottom along the cut-out chamber edges using RTV silicone sealant R2–1140 RTV (NuSil Technology) and dried 4 h. For this step, we inverted the chamber, spread glue on one coverslip, then lowered it starting from the cell chamber edge (visible through the bottom), and then repeated for the other side. To prepare chambers for cell culture, 0.5 ml of coating buffer [10 μg/ml poly-D-lysine (Sigma-Aldrich) in 0.1 M boric acid buffer, pH 9.6] was added to each for 4 h in a 100-mm cultured dish. Coating buffer was removed and then the chamber was washed 3× in deionized water and then 70% ethanol. For 2 h or more, the growth chambers were dried and sterilized under ultraviolet light in a culture hood.

Fluorescent Na+ dye loading, drugs, ionophores. Membrane-soluble CoroNa Green AM (C36676, Invitrogen; in vitro K, 80 mM, excitation 488 nm, emission at 516 nm) was used. CoroNa Green (50 μg) in 38 μl DMSO was divided into 5–μl aliquots and stored at −20°C. Just before dye loading, 5 μl Pluronic F-127 (Invitrogen) were added (1:1) and this was diluted in ECS (extracellular or bath solution). From preliminary tests (with 1, 2, 5, and 10 μM CoroNa Green in ECS and 10, 20, 30, 45, and 60 min) 10 μM and 30 min was chosen for loading as it yielded good signal-to-noise upon addition of a Na+ ionophore cocktail. After 30 min dye loading in a darkened damp chamber (100 mm dish) at room temperature and then a three-stage wash in ECS (see below), cells were left for another 30 min (for AM dye hydrolysis) and then 95% of the fluid was removed and replaced with fresh ECS (“posthydrolysis wash”). ECS was (in mM) 140 NaCl, 5 KCl, 1 CaCl2, 5 HEPES, and 5.5 glucose, buffered to pH 7.4 with NaOH, 290–310 mosmol/kg. Some experiments had a zero Na ECS, in which case 140 mM N-methyl-D-glucamine (NMDG) (−Cl) (Sigma) was used instead of NaCl. NMDG-ECS was substituted for ECS at the posthydrolysis wash. Likewise, drugs were included in the ECS at this point as follows. The Na+/Ca2+ exchanger
experimental ECS. TTX (100 nM or 10 μM) (Alamone Labs) and the sodium ionophore cocktail monensin (5 mg/ml) plus Gramicidin D (200 μg/ml) (both from Sigma) were always included in the ECS as indicated. The three-stage wash procedure was performed as follows: remove ~95% of the fluid by pipette, gently refill with 2 ml new solution 1× (requires ~5 min), and then 2× at 1 ml, for 5 min each.

Confocal microscopy and image data analysis. Imaging was done on a Bio-Rad MRC 1024 laser scanning confocal microscope (Hemel, Hemstead, Hertsfordshire, UK). Excitation and emission filters with peaks near 488 and 510–530 nm, respectively, were used for CoroNa Green. Scan speed was normal, zoom was held at 1.5, and 10% laser power was used. Gain and iris settings were constant throughout an experiment but adjusted between experiments to minimize photo-bleaching. Z scans (10–12 X-Y planes in 3-μm steps) were recorded for the baseline data (t = 0) and then automatically at 2, 3, 9, 15, 21, and 30 min. NIH Image software was used for data analysis. The fluorescence intensity of a field of cells was monitored, not that of

subtraction. All data points were normalized against the (background-subtracted) baseline data point for t = 0.

RESULTS

Nav1.6 channel gating is susceptible to membrane trauma. Currents through Nav1.6 channels in gently sealed (i.e., minimally traumatized) cell-attached oocyte patches were monitored while patches were subjected to traumatizing membrane stretch using pipette aspiration (suction). If excessive or prolonged suction was needed to achieve a gigahm seal, patches were considered pretraumatized and were discarded (see METHODS). The sustained stretch caused macroscopic Nav1.6 current to change irreversibly, as illustrated in Fig. 1Ai, which shows superimposed currents at ~10 mV before stretch (1st, black) then with stretch (up to 20th at 1 Hz, grey). Differences in pipette and patch geometries mean that, for a given suction intensity, stretch intensity (membrane tension) would differ among patches. Monitoring in this way therefore confirmed that suction had traumatized a given patch. Oocytes’ endogenous stretch-activated channels reverse close to ~10 mV so the during-stretch monitoring records were not contaminated by stretch channel activity. To illustrate that on the time scale of our experiments, changes were irreversible, several superimposed traces recorded with stretch a minute after the end of the previous stretch episode are shown (they are indistinguishable

Fig. 1. Ai: voltage-gated Na (Nav1.6 channel currents from an oocyte patch (αNav1.6 + β1B-subunits expressed) at ~10 mV as the transient current features changed irreversibly while the patch was stretched by ~20 mmHg suction. Current at 1 Hz was monitored before stretch (1st) and then during stretch (applied for 20 s in this case). Left inset: early part of this set of currents is expanded, showing that current amplitude increased as current accelerated; right inset: when stretch was reapplied at the same intensity 1 min later, no further changes in the current characteristics were evident (the 3 overlapping traces, shown at smaller scale, are indistinguishable from the previous 20th trace); the effect had “saturated.” Aii: currents from this patch during steps to ~40 mV, before (black) and after (grey) the 20 s of stretch. B: from the same patch, activation [from which average activation g(V) is obtained] (i) and steady-state inactivation (obtained from availability at 0 mV) (ii) seen via the early part of current families before and after the traumatizing stretch monitored in A. i, Vhold = −110 mV, 18-ms depolarizing steps at 5-mV increments, from −70 mV to +15 mV. ii, from Vhold = −110, the voltage was stepped for 210 ms to voltages from −110 to −12 mV in 7-mV increments, and then for 10 ms to the test potential 0 mV. To aid comparison, arrows highlight currents as described in the text. C: exponential decay times for an α/β1 patch (same as in Fig. 3A) during a family of step depolarizations (as per Fig. 1Bi) before (black) and after (grey) stretch; dashed lines serve to indicate that stretch reversibly left shifted the τ1/2_act (V) values by ~18 mV.
from traces 18–20). During the monitored stretch episode, current onset and decay got progressively faster (see expanded inset) before stabilizing (shortly before ~20 s of sustained stretch in this case, so that traces 18–20 do not change). Currents from this patch at ~40 mV (Fig. 1Aii) before (black) and after (grey) the monitored stretch application reveal that, dramatically and irreversibly, the stretch episode had changed the behavior of these neuronal-type Nav channels.

In some patches, the full time course of Nav1.6 current decay required double than single exponentials (e.g., the Fig. 1A, A and B patch). During the stretch-monitoring process in Fig. 1A, the relative weight of the faster component progressively increased (from ~70% to ~95%), and its decay time dropped (from 0.48 to 0.27 ms) during steps 2–20 (the fast decay time 1 min later: 0.28 ms). From a patch with a single exponential decay time course, Fig. 1C plots tau_{inact} (V) fits before and after a comparable stretch episode. In that patch, tau_{inact} during the stretch-monitoring steps at 10 mV (not shown) was initially 0.65 ms then fell to 0.40 ms by step 20 (a minute later: 0.38 ms).

Six patches expressing α only had an average tau_{inact} (10 mV) of 2.0 ms at the start of stretch (trace 2) and 0.6 ms by the last monitored step during stretch; within-patch comparisons of these tau_{inact} (10 mV) values showed that stretch caused an irreversible 3.1 ± 0.6-fold (SE; n = 6; paired two-tailed t-test, P < 0.001) increase in the speed of inactivation.

Irreversible stretch-induced changes in conductance(V) and steady-state availability(V). Figure 1B shows sets of Nav1.6 current recorded before (top) and after (bottom) a monitored application of traumatic stretch (as in Fig. 1Ai). These records are typical of responses obtained in each of >12 experiments. Patches were made from oocytes coexpressing αNav1.6 + β1, αNav1.6 + β1B or αNav1.6 + both β variants or αNav1.6 only. Data from an αNav1.6 + β1B oocyte are shown here, but responses were the same with β1, with the mix, or with no β1 subunits. At left are current-voltage (I-V) families and at right, to reveal the channel’s voltage-dependent steady-state availability, currents at a 0-mV test pulse immediately following a family of 210 ms voltage prepulses. For orientation, traces at −35 mV and at +15 mV in the I-V sets are indicated. In the availability(V) sets, traces following prepulses to −75 mV and to −110 mV are indicated.

Comparison of I-V traces before and after stretch showed that, as here, rates of current onset and decay at a given voltage were both faster poststretch [with the proviso that rising phases became limited by the system bandwidth (e.g., here at +15 mV poststretch)]. Irreversible time-course acceleration was accompanied by a hyperpolarizing shift in voltage dependence, as illustrated at −35 mV. This is further demonstrated by the trace overlays of Fig. 3C (and for two other patches in Fig. 3, Aii and B). Likewise, steady-state inactivation [i.e., availability (V)] changed irreversibly with stretch. Regardless of amplitude, the poststretch test currents all had accelerated onsets and decays. Critically, test current after prepulses that initially yielded almost full availability (e.g., see −75 mV) yielded greatly reduced availability poststretch, consistent with a hyperpolarizing shift of availability(V). The ratio [availability (−75 mV): availability (−110 mV)] was 0.48 ± 0.06 (n = 12 patches) prestretch. Stretch-induced left shift caused the ratio to fall to 0.24 ± 0.04 (n = 12). Based on within-patch comparisons, pre/poststretch, this amounted to an irreversible 2.5 ± 0.4-fold decrease in Nav1.6 current availability at −75 mV (relative to −110 mV) (n = 12, paired two-tailed t-test: P < 0.001). Poststretch availability was not necessarily saturated for prepulses to −110 mV (moreover moderate stretch intensities were used) so a 2.5-fold decrease likely constitutes a low estimate of the stretch-induced change in availability of Nav1.6 channels in a cell with a resting potential of −75 mV.

Hyperpolarizing shift of transient current operational range and hence of window current. Irreversible changes with stretch were always obtained for Nav1.6 channels coexpressed with auxiliary β-subunits, but it is unknown how or if αNav1.6 interacts molecularly with auxiliary molecules so αNav1.6 alone was used to quantify the effect. Figure 2A shows αNav1.6 families before (black) and then after (grey) traumatic stretch. As with β-subunits present, large depolarizing steps (e.g., to −35 mV, inset) that elicited little or no current prestretch elicited large transient currents poststretch. Gating of αNav1.6 channels at a given voltage became faster for both onset and decay (e.g., −25 mV, peak amplitudes normalized). Evidently, patch stretch caused kinetically significant and irreversible changes to the Nav1.6 pore subunit and/or to its immediate environment.

Figure 2B plots average availability(V) and g(V) curves for αNav1.6 channels pre- and poststretch for pipette suction of moderate intensity (approximately −20 mmHg) applied as in Fig. 1. Stretch caused ~20-mV hyperpolarizing shifts of both curves. Note that this hyperpolarizing shift would constitute a low estimate of the maximal possible stretch-induced left-shift because 1) mild stretch during gigaohm seal formation might have caused some unmonitored premeasurement shift and 2) to reduce the likelihood of patch rupture before a poststretch data set could be acquired, suction of only moderate intensity was applied. As will be seen in Fig. 4A, −20 mmHg would not necessarily maximally traumatize a patch.

Since stretch irreversibly shifted both availability(V) and g(V), any window current associated with the overlapping feet of these functions (see the Boltzmann fits to each) would also be shifted approximately ~20 mV. Arrows indicate voltage apexes of the pre- and poststretch window current ranges for the αNav1.6 data. Nav channel window current should be detectable using ramp clamp but due to small steady-state currents, our attempts with ramp clamp generally met with poor success. Nevertheless, one patch with exceptionally large transient and steady-state current (Fig. 2Cii) did yield substantial ramp currents (50–100 pA before-after stretch), and the poststretch negative resistance region was shifted as expected from transient current responses (Fig. 2Ciii).

Increased poststretch speed and the hyperpolarizing shift of gating. The increased poststretch speed of Nav1.6 currents was largely accounted for by the hyperpolarizing shift in the voltage dependence of activation and availability. Figure 3 demonstrates this for three patches (from α/β1, α-only, α/β1B-expressing oocytes, respectively) for which stretch-induced left shifts for activation and availability respectively were 20 mV; >16 mV (A); 10 mV, 11 mV (B); 35 mV, >25 mV (C), with A given in most detail. As plotted in Fig. 1C, the tau_{inact}(V) relation for this patch was left shifted by almost 20 mV after stretch. Pre/poststretch availability families for the patch are in Fig. 3Aii, ii. Below that (Fig. 3Aiii) pre/poststretch currents at −15 mV are compared. During steps to voltages where the current rising phase is limited by activation (24), double rescaling (35), as seen in Fig. 3Aiv, tests whether an experi-
mental procedure (patch trauma in our case) affects activation and fast inactivation equally. Perfect overlap would imply that the traumatized (poststretch) condition caused activation and fast inactivation to accelerate to the same extent. In the patches of Fig. 3, A–C, timebase extensions of 2.2-, 1.9- and 3.5-fold were required for current onset overlap [double rescaling of the Fig. 3, B and C, patch currents (not shown) gave poorer late-current overlap than for the 3A patch]. Figure 3A shows that over a wide voltage range, the stretch-induced left shift and 20 mV of hyperpolarization produced almost identical changes in the current traces. For the Fig. 3, B and C, patches, unlike the 3A patch, fitting current decay required two exponentials at some voltages. Not surprisingly, offset matching (using 10 and 35 mV, respectively) was more approximate in the Fig. 3, B and C patches, than in the 3A (α/β) patch.

**Cumulative trauma, progressive left shift of transient current.** We infer that window current shift accompanied hyperpolarizing-shift of the transient I\textsubscript{Na} operating range [that is, of availability (V) plus g(V)] poststretch. For pretrauma patches in oocytes expressing α+β subunits, the foot of the g(V) was near −60 mV, which is more depolarized than typical axonal resting potentials and (by definition) below the normal Nav1.6 window current range. Next we demonstrate that the extent of stretch-induced shift depended on trauma intensity. A double step in the foot voltage range (to −65 mV and −50 mV with recovery time between the steps) was used to monitor traumatic changes to g(V). I\textsubscript{Na} was measured during the double step, suction was applied (at fixed intensity) for 30 s and then released, the double-step measurement was repeated, and then another 30 s suction episode, etc. Suction-induced trauma to

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**Fig. 2.** A: αNav1.6 channel current families from a patch before (black) and after (grey) sustained stretch, obtained as in Fig. 1. A and B. Inserts: “before” and “after” comparison traces at two voltages as explained in the text. B: g(V) and steady-state inactivation [availability(V)] curves (n = 6). g(V) (open symbols) is peak Na current (I\textsubscript{Na}) at the indicated test potentials divided by the Na driving force (E\textsubscript{Na} = approximately −60 mV) and availability (solid symbols) is assessed as for Fig. 1B. Black signifies before (prestretch) and grey signifies after (poststretch). Arrowheads indicate the voltage apex of window current regions before and after traumatic stretch. These plots are akin to plots for αNav1.4 (44, 52) and for αNav1.2 when cell-attached vs. excised (traumatized?) patch currents are compared (17). Ci shows, for a patch (α + β1 subunits) with large currents, I\textsubscript{Na} at −20 mV, prestretch (black) then after −20 mmHg had ceased causing further kinetic change (grey). The time-expanded inset shows that poststretch, activation and inactivation were both faster. Cii emphasizes the late I\textsubscript{Na} amplitude. Ciii, the patch was depolarized to +100 mV and then ramped to −100 mV at 2 mV/ms (no P/N subtraction). Prestretch replicates (not shown) indicated that ramp current was stationary. Pre- and poststretch ramp currents are plotted, plus prestretch normalized to the maximal inward poststretch current.
membrane patches is cumulative (21, 52). Presumably the fraction of bilayer separated from the adherent membrane skeleton (i.e., blebbed) increases progressively during suction episodes. Current was monitored as trauma was intensified by bouts of stronger suction on 16 patches, as illustrated in Fig. 4, A and B, which chronicles an especially sturdy patch and a more fragile one. In Fig. 4, A, as labeled, a series of peak amplitude-normalized pre- and poststretch (black/grey) currents compared for steps to voltages 20 mV apart. The pre-15 mV post-35 mV pair series looks nearly like Aiv (but the Aiv pair are rescaled pre- and postcurrents at −15 mV). B: (α-only patch) amplitude-normalized pre- and poststretch currents compared 10 mV apart; tau_inact(0 mV) pre- and poststretch was 0.4 ms/0.3 ms poststretch; pre- and post-avail(0) midpoint: −76/−87 mV. C: α/β1B patch; same as Fig. 1, A and B; amplitude-normalized pre- and poststretch currents compared 35 mV apart; tau_inact(0 mV) pre- and poststretch was 1.4 ms/0.5 ms (faster component of double exponential); pre- and post-avail(0) midpoint: −70/−96 mV [post-stretch avail(0) did not saturate].

Fig. 3. Stretch-accelerated Nav1.6 currents and stretch-induced left shift of gating. A: α/β1 patch, same as Fig. 1C; Aii: availability families (as per Fig. 1Bii) with late (persistent) currents expanded (bottom left) in each. Pre- and poststretch τ_inact (0 mV) values are given, as are the pre- and poststretch availability(V) midpoints. The left-shift probably exceeded 16 mV because poststretch avail(0) did not appear saturated. Aiii: pre(black)- and post(grey)-stretch currents at −15 mV; pre- and post-tau_inact (−15 mV) values were 2.15 ms/0.81 ms. A faster-rising current at −10 mV (not shown) confirmed that the poststretch rising phase at −15 mV was not filtered. Aiv: double rescaling of Aiii traces: peaks normalized and then time base of the faster (poststretch) current extended (2.2-fold in this case) until current rising phases overlap perfectly. Av, as labeled, a series of peak amplitude-normalized pre- and poststretch currents compared for steps to voltages 20 mV apart. The pre-15 mV post-35 mV pair series looks nearly like Aiv (but the Aiv pair are rescaled pre- and postcurrents at −15 mV). B: (α-only patch) amplitude-normalized pre- and poststretch currents compared 10 mV apart; tau_inact(0 mV) pre- and poststretch was 0.4 ms/0.3 ms poststretch; pre- and post-avail(0) midpoint: −76/−87 mV. C: α/β1B patch; same as Fig. 1, A and B; amplitude-normalized pre- and poststretch currents compared 35 mV apart; tau_inact(0 mV) pre- and poststretch was 1.4 ms/0.5 ms (faster component of double exponential); pre- and post-avail(0) midpoint: −70/−96 mV [post-stretch avail(0) did not saturate].

for a patch that ruptured when suction greater than −15 mmHg was attempted, the progressive irreversible kinetic changes are abundantly clear.

In patches tested this way, tau_inact(−50 mV) was obtained from single exponential fits to the currents at −50 mV prestretch and just before patch rupture. Prestretch decay times ranged from 3.2 to 1.1 ms and poststretch decay times from 1.2 to 0.3 ms. The ratio pre-tau_inact/post tau_inact was 3.1 ± 0.5 (SE; n = 16; paired two-tailed t-test, P < 0.001). In all these patches, stretch also progressively enlarged the currents [reflecting left shifted g(V) relations] and speeded the kinetics of currents at −65 mV.

Reversible (elastic) responses to stretch: Nav1.6 channel gating is modulated by lipid stress. Stretching any object, including a patch of plasma membrane, can change it reversibly (=elastic effect) and/or irreversibly (=plastic effect). This would correspond to reversible versus irreversible changes in
the physical structure of a plasma membrane. The intact plasma membrane is structurally inhomogeneous, so some parts may experience elastic, whereas others experience plastic change. Once all plastic change has “saturated,” the membrane should respond to any further stretch episodes as an elastic object. Note that for a bilayer, reversible and irreversible structural changes equate to reversible and irreversible changes in the bilayer’s lateral pressure profile. In oocyte patches, the cardiac Nav channel isofrom (αNav1.5) does not exhibit irreversible stretch-induced gating changes like those seen for Nav1.6 [and αNav1.4 (44, 52)] channels. Nav1.5 gating is, however, reversibly modulated by stretch (35); evidently, during stretch, the Nav1.5 channel gating machinery experiences the reversible changes of the bilayer lateral pressure profile. We wondered whether, once the plasma membrane was maximally traumatized (i.e., plastic changes saturated), Nav1.6 channels, too, would reveal Nav1.5-like elastic responses to stretch. The reversible stretch-induced changes in channel gating are called stretch-modulated gating.

Stretch modulation tests were done on oocytes expressing αNav1.6 plus β1 and β1B. Patch current was monitored until  \( I_{Na} \) had demonstrably stopped changing with stretch. Since stretch-modulation of voltage-gated channel kinetics is typically small compared with the irreversible ones just shown for Nav1.6, signal averaging was used. The procedure was as follows: a “before/during/after protocol” (black/red/grey in Fig. 5) was applied. Forty runs at 1 Hz were averaged for a set of depolarizing pulses; for “during” stretch, −15 mmHg was applied continuously. This would normally correspond to a moderate (as opposed to near-lytic) increase in bilayer tension. For reference, −50 mmHg typically ruptured our patches during 40-s applications. Under these conditions, Nav1.6 channels exhibited fully reversible stretch modulation, with stretch accelerating both activation and inactivation as seen in Fig. 5. In Nav1.5, stretch accelerates activation and fast inactivation to the same extent (35). To test whether Nav1.6 current onset and decay accelerated to the same extent, the simple model-independent kinetic test used for Nav1.5 (35), double rescaling of before/during/after traces, was used, with the result illustrated. The complete overlap of stretch and no-stretch traces [e.g., for the steps to −50 mV (Fig. 5, bottom)] upon double rescaling indicated that the tightly coupled activation and fast inactivation processes remained coupled during stretch. In this case acceleration was 1.2-fold. In 4 of 4 patches (different oocytes) tested in this manner for reversible responses, current onset and decay time courses accelerated reversibly to the same extent with stretch. The simplest explanation for stretch modulation like this is that voltage sensor motions depend on bilayer mechanics and that in a stretched bilayer, the barrier to the rate-limiting voltage-dependent transition is reversibly lowered (35). We conclude that Nav1.6 channel gating, like that of other voltage-gated channels, is subject to bilayer mechanics. Given the accelerating effect of trauma, the immediate lipid microenvironment of a Nav1.6 channel might be more orderly (less fluid) in intact than in traumatized membrane.

**Fig. 4. Patch-stretch intensity.** A:  \( I_{Na} \), from a patch (αNav1.6 + β1B) stepped to −65 then −50 mV (note time interval).  \( I_{Na} \) was monitored immediately after the indicated stretch episodes (prestretch  \( I_{Na} \) is shown black, poststretch currents are grey except for post −45 mmHg, which, for contrast, is black). **Boxed inset:** amplitude-normalized (for −50 mV)  \( I_{Na} \), prestretch and post −45 mmHg; B:  \( I_{Na} \) from a patch (αNav1.6 + β1) after −10 mmHg and −15 mmHg (patch then ruptured).

**Fig. 5. Effects of elastic (reversible) membrane stretch on Nav1.6 channel current.** Before we tested for reversible stretch effects, suction was applied until monitored kinetics stopped changing. To block endogenous stretch channel activity, 1 mM La\(^{3+}\) was included in the pipette (see METHODS). 40 runs (1 Hz) were averaged before, during, and after (black/red/grey) application of −15 mmHg. The pulse pair (−50 mV, back to  \( V_{test} = −110 \) mV, −45 mV) provided within-patch confirmation of adequate operational bandwidth at the less depolarized step. Incomplete recovery from inactivation at −45 mV yielded smaller peak  \( I_{Na} \) than at −50 mV and mitigated possible excess-amplitude artifacts. Expanding the early part of the traces (right) shows that stretch-accelerated  \( I_{Na} \) at −50 mV was fully resolved (demonstrably faster currents were obtained at −45 mV). At bottom (double rescaled traces at −50 mV) full overlap of stretch and no-stretch traces was achieved by normalizing peak  \( I_{Na} \), and then expanding the during-stretch time base.
Fig. 6: A: uniaxial stretcher was identical to that of Pfister and colleagues (39) except for minor modifications to reduce apparatus weight (and a removable bar for chamber mounting). With dye-loaded cells ready for imaging in the Silastic growth chamber (exploded view of chamber gives dimensions in mm; g1 and g2 are glues listed in METHODS), the chamber was positioned into a scaffold (i.e., the two holders plus a removable rigid cross-bar whose position during mounting is indicated by dashed grey line) and mounted into the stretcher with the cell chamber above the microscope objective. Metal screws secured the fixed-side holder (black asterisk) and nylon screws (replaced for each use) secured the moving-side holder (grey asterisk), which connected to the computer-controlled actuator. The cross-bar was removed and the chamber was ready for the stretch (double-headed arrows indicate sliding components in the stretcher assembly). Low intensity epifluorescent illumination was used to locate a field of cells for confocal imaging. Baseline confocal data were taken (t = 0) and the experiment was underway. Immediately after a single 50-ms stretch (or not, for no-stretch controls), the timer was started. Strain rates during stretch were 100% in 50 ms or 50% in 50 ms, as indicated. To minimize evaporation over the next 30 min, a large coverslip was placed atop the chamber and damp toweling was placed over the stretcher just after t = 0. Bi,ii: confocal images of Nav1.6-HEK cells (no tetra-toxin) at the lower (i) and higher (ii) range of confluency used in these experiments. Images at t = 0, 2, and 30 min are shown. Between t = 0 and 2 min, a 50%/50 ms strain was applied.

us (60). The rapid uniaxial stretch episodes are intended to mimic the speed and intensity of single mechanical trauma episodes experienced during head injury in, for example, vehicle accidents. The stretcher apparatus used is diagrammed in Fig. 6A; for details about its fabrication and use, see METHODS and Fig. 6. In brief, the top left shows an exploded view of the Silastic growth chamber, which is then shown secured by four screws into the computer-controlled stretcher apparatus that mounts on an inverted confocal microscope. Figure 6Bi,ii shows fluorescent images of fields of dye-loaded cells [at the bottom (i) and top (ii) end of densities used] just before (0) then 2 and 30 min after the traumatic stretch event. Figure 7 plots Na⁺-dye intensity in Nav1.6-HEK cells (expressed as % increase) for various conditions. Dye intensity was monitored intermittently up to 30 min. For traumatized cells, a Na⁺ ionophore cocktail (gramicidin, monensin) added immediately after 30 min elicited no further intensity increase (not shown). Added to no-trauma controls at 30 min, it caused a doubling of fluorescence intensity over several minutes (Fig. 7Aiii; % increase 2, 3, and 9 min later). In cells subjected to a trauma (strain) of 100%/50 ms, Na⁺-dye intensity increased abruptly. By 2 min, intensity was already maximal (Fig. 7Aii) (subsequent values were not significantly higher). With 100 nM TTX present, approximately two-thirds of this trauma-induced increase was blocked. The nature of the TTX-insensitive component is unknown, but, since ~5% of Nav1.6 whole cell current persists in the presence of 100 nM TTX (8), part might be Nav channel related. Trauma-modified cation channels and/or rapidly resealing bilayer tears might account for some of the TTX-insensitive stretch-induced Na⁺ entry [e.g., (5)]. Similarly traumatized glial cells show a comparable unidentified fraction of trauma-induced Na⁺ entry (18).

We used 100%/50 ms trauma for consistency with studies of Ca²⁺ dye-loaded axons (62). However, for randomly aligned HEK cells (as opposed to axons along the stretch axis), we wondered whether a milder (50% strain) trauma (“50%/50 ms”) would improve signal-to-noise ratio (19). As seen in Fig. 7Aii, it did, reducing the nonspecific (i.e., TTX insensitive) fraction of trauma-induced Na⁺ increase. That fraction at 2 min was 11% for the 50%/50 ms regime versus 18% with 100%/50 ms. Fluorescence intensity was still maximal by 2 min (with/without TTX). Larger errors for 50%/50 ms simply reflect the smaller sample sizes for “50%” vs. “100%.” For both strain regimes (100%/50 ms and 50%/50 ms), expressing the 2-min fluorescence intensity data as the ratio (TTX-sensitive trauma signal)/(TTX-sensitive no-trauma signal), i.e.,

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gives a ~10-fold signal-to-noise ratio (i.e., 29/3 and 30/3). Thus with a good signal-to-noise ratio, a TTX-sensitive rise in intracellular Na⁺ concentration occurred before 2 min post-trauma. Though we plot our full data sets, cellular loss of CoroNa Green occurs over tens of minutes (32). The CoroNa Green Kᵅ for Na⁺ is ~80 mM. If the dye was saturated at 2 min due to (say) a 100 mM increase in intracellular Na⁺ in a 2-pl HEK cell, the net gain would be 2 × 10⁻¹³ moles Na⁺ (~2 × 10⁻⁸ Coulombs). A trauma-augmented 200-pA Na⁺ current flowing for 100 s (i.e., <2 min) would account for the CoroNa Green signal. In future, dynamic range might be
improved by using (say) a “25%/50 ms” trauma (19) and a ~70 mM extracellular Na+ concentration.

If plasma membrane Nav1.6 channels mediated the TTX-sensitive trauma-induced increase in Na-dye intensity, then a reversed [Na+] gradient (Na+ replaced by NMDG+ just before t = 0) should cause a trauma-induced decrease in Na+ dye intensity. Figure 7B shows that reversing the gradient caused a posttrauma Na+-dye intensity decrease that was significantly diminished by TTX.

**DISCUSSION**

Nav1.6 channel responses to experimental trauma. Although Nav channel antagonists are neuroprotective in TBI/SCI models (2, 4, 53), neuronal Nav channels have not previously been tested to determine whether traumatic cell and membrane stretch directly renders them “leaky.” Since Nav1.6 channels are the adult brain Nav channel isoform at axon initial segments and at nodes of Ranvier, we examined the immediate responses of recombinant Nav1.6 channels to traumatic perturbations.

The small TTX-sensitive background Na+ conductance of healthy myelinated axons (50) could be due to window current and/or to persistent current; likewise, for the larger TTX-sensitive Na+ “leak” in traumatized axons. The window conductance is due to transient sodium current (flowing through channels exhibiting fast inactivation along with voltage-dependent activation and deactivation), whereas the existence of a persistent current implies that the channels can also enter a gating mode(s) that exhibits voltage-dependent activation and deactivation with a low (on the time scale of experimental observations) probability of inactivation [also termed a nonabsorbing inactivation state(s)].

Our patch-clamp recordings did not point to the stretch-induced I-V changes expected if, poststretch, persistent current was larger. That outcome is sketched in Fig. 8Ai, which shows the effect of increased channel number and/or uniformly increased open probability (Popen) (as indicated, an increased area of channel-bearing membrane would also yield that outcome). Instead, our data bespoke left-shifted I-V relations (Fig. 8Aii), which give the largest fractional INa increases near the g(V) foot. Our findings suggest how transient current (including window current) left shift could underlie trauma-induced leak in free-running (i.e., nonclamped) axons. Trauma intensity would vary across any affected axon, so window current left shift would “smear out” (Fig. 8Aiii), exacerbating the Na+-leak problem. Confirming that intact cell trauma can directly alter the behavior of their Nav1.6 channels, we found that abruptly traumatized Nav1.6-expressing HEK cells exhibited an immediate TTX-sensitive Na+ leak. Electrophysiological comparisons of intact and traumatized HEK cells will be required to determine if Nav1.6 channel responses in the two model systems correspond. Preliminary findings, using osmotrauma to cause blebbing (INa is monitored by perforated patch before and after a brief extreme swell) reveal left-shifted Nav1.6 operation (W Lin, PF Juranka, and CE Morris, unpublished observation).

The same type of patch stretch that irreversibly altered Nav1.6 channel gating [as with αNav1.4 (44, 52) and certain Kv channels (43)] acts reversibly on other channels. For instance, it reversibly modulates Nav1.5 gating and reversibly activates stretch-activated channels. What is more, Nav1.5-like reversible stretch modulation was apparent for Nav1.6 channels once the irreversible changes had been achieved. This is not unique. The oocyte’s endogenous stretch-activated channels show both irreversible and reversible stretch responses (21, 47, 65) as do native neuronal background K channels (47, 50) and importantly, the reversible (elastic) behaviors prevail once membrane skeleton/bilayer interactions are disrupted. Our working hypothesis is that comparable disruptions matter for Nav1.6 channel behavior (see Fig. 8B).

For Nav1.6 channels [unlike αNav1.4 (52)], coexpression with auxiliary subunits did not protect against stretch trauma; stretch left-shifted Nav1.6 channel gating irreversibly whether or not channels were coexpressed with β1-subunits. Although stretch irreversibly diminished the weight of the slow kinetic component when double exponentials were needed to fit current decay, irreversible shift and acceleration was just as robust for patches in which a single exponential described the decay both pre- and poststretch (e.g., the α/β1 patch of Fig. 1C and Fig. 3A). Thus possible trauma-perturbed α/β1-subunit interactions are not relevant to our outcomes. Ankyrin-Na1.6

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Nav1.5-like stretch modulation. In Nav1.5, stretch reversibly shifts cholesterol right shifts, fatty acids left shift, respectively and both modulate Nav channel kinetics (25, 63); cholesterol are lipids of disordered and ordered microdomains (Fig. 8). As examples, unsaturated fatty acids and Bi inhabit lipid-disordered microdomains in the intact membrane. Perhaps their lack of response implies that they already liquid-ordered lipid microdomain (13, 26) to a blebbed membrane. Some irreversible changes in the LPP. But some voltage-gated bilayers with 0% and 50% cholesterol. Bleb formation entails C832 Nav1.6 CHANNELS AND MEMBRANE TRAUMA

interactions are another possibility since coexpressing αNav1.6 with ankyrin-G in HEK cells reduces persistent current (46). However, this is irrelevant to the trauma-augmented Na+ influx in our HEK experiments because HEK cells lack endogenous ankyrin-G and no Nav1.6/ankyrin binding is detected in HEK cells (46). Thus left-shifted window current is the most plausible source of Na+ leak. For axons in situ, neither our patch recordings nor the HEK cell experiments rule out trauma-induced disruptions of Nav1.6 interactions with modulatory proteins [ankyrin-G, β-subunits, G-proteins (29) among others].

Membrane trauma, blebs, and bilayer’s lateral pressure profile. For the bilayer adjacent to any Nav channel, lateral pressure profile (LPP) details vary with the local lipid makeup of each leaflet. In “bulk” nonraft membrane, rafts, caveolae, and other microdomains (1, 34, 61) LPPs would differ markedly, as illustrated in Fig. 8C, which shows LPPs for simulated bilayers with 0% and 50% cholesterol. Bleb formation entails some irreversible changes in the LPP. But some voltage-gated channel show no irreversible gating changes on going from a liquid-ordered lipid microdomain (13, 26) to a blebbed membrane. Perhaps their lack of response implies that they already inhabited lipid-disordered microdomains in the intact membrane (Fig. 8Bii). As examples, unsaturated fatty acids and cholesterol are lipids of disordered and ordered microdomains respectively and both modulate Nav channel kinetics (25, 63); cholesterol right shifts, fatty acids left shift.

In fully traumatized membrane, Nav1.6 channels showed Nav1.5-like stretch modulation. In Nav1.5, stretch reversibly accelerates the rate-limiting transition in the activation pathway; the tightly coupled fast inactivation process accelerates equally. Acceleration during stretch signifies that the stretched bilayer offers a reduced energy barrier for voltage-sensor movement (24, 41, 51, 61) and this would apply for both transient and persistent current Nav channels. Stretch acceleration is, however, not a foregone conclusion for voltage-gated channels. What is important is a given channel’s rate-limiting transition. For voltage-gated N (and L) type Ca and Kv3-Shaw channels current onset does not accelerate with stretch even though peak current increases reversibly (see Ref. 23). In Kv-Shaker-ILT, where voltage-dependent activation is limited by a concerted motion of four subunits, stretch reversibly slows current onset (23). For Nav1.6, liquid-disordered bilayers (trauma-induced blebs) might present an irreversibly changed LPP that lowers energy barriers for rate-limiting transitions in both activation and availability processes. What barriers might be lowered to shift availability(V) is hard to say since availability depends in part on voltage-independent transitions that are poorly understood (56). g(V) left shift, however, is consistent with sooner/faster “outward” motions of Nav1.6 voltage sensors where the bilayer is thinner, more fluid, and more disordered. A physiological implication is that nodal membrane might be “designed” cholesterol-rich (7) to provide an operational LPP for Na1.6 channels that minimizes window current at resting potential voltages.

Implications for Nav channels in stretched/sheared axons. In myelinated axons, Nav1.6 channels localize to cholesterol-rich nodes of Ranvier (7, 9, 16). Stretch-trauma (or shear) would
likely cause blebs to begin forming immediately at some nodes (31). Pipette aspiration experiments indicate that bleb formation commences at a few hundred nanometers, i.e., at submicroscopic gap sizes (45). Blebbing is also driven by the background hydrostatic pressure of the cytoplasm (12), a pressure that in a healthy myelinated axon, as in any cell, is counterbalanced by a cortical tension (45, 54, 57). The dynamic cortex maintains a high density of weak interactions between membrane cytoskeleton and bilayer molecules (10, 45); spectrin and filamin, for example, directly bind bilayer lipids. Where stretch causes gaps in these cortex/bilayer adhesions, blebs form and bleb enlargement would follow if metabolic compromise threatened the cell’s ability to sustain the cortical countershock.

“Left-smaer” of Nav1.6 window current would be expected at nodes with mixed blebbed/intact membrane and TTX-sensitive Na+ leak would result (Fig. 8Aiii). Ironically, hyperpolarizing counterinfluences that were too weak would only serve to increase the driving force on Na+ influx, thus further compromising Na+/Ca2+ exchange. To be protective, augmented K-conductances (e.g., trauma-stimulated background-K channels) would need to hyperpolarize the neuron beyond the smeared Nav1.6 window current range. Interestingly, riluzole, an antagonist of leaky Nav channels (6) currently in neurotrauma clinical trials (4) may represent a neuroprotective “one-molecule-cocktail” insofar as it also strongly activates background-K channels (14, 30).

The knock-out punch. The link between repeated head injury and chronic TBI in professional boxing is known (11), but the instantaneous nerve block to midbrain (55) axon tracts carrying consciousness-sustaining action potential traffic. Our findings suggest a possible common cause for the immediate and long-term effects of knock-out punches. Shear-induced left cortical countertension.


doubly severe and/or repeated blows (59) more intractable diffuse axonal injury would ensue.

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REFERENCES


