Slowly inactivating component of Na⁺ current in peri-somatic region of hippocampal CA1 pyramidal neurons

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The peak conductance of $I_{NaS}$ was proportional to the peak amplitude of $I_{NaT}$ but was much smaller in amplitude. Hexanol, riluzole, and ranolazine, known Na⁺ channel blockers, were tested to compare their effects on both $I_{NaS}$ and $I_{NaT}$. The peak conductance of $I_{NaS}$ was preferentially blocked by hexanol and riluzole, but the shift of half-inactivation voltage ($V_{1/2}$) was only observed in the presence of riluzole. Current-clamp measurements with hexanol suggested that $I_{NaS}$ was involved in generation of an action potential and in upregulation of neuronal excitability.

It is difficult to study the different components of Na⁺ current in isolation for multiple reasons. First, there is no specific pharmacological blocker to definitively isolate the separate components; all are sensitive to tetrodotoxin (TTX). Second, it is impossible to obtain an adequate space clamp (all membrane controlled at a set potential) of a pyramidal neuron with a complex topology (Spruston et al. 1993; Williams and Mitchell 2008). We chose to utilize the nucleated-patch technique (Martina and Jonas 1997) to achieve good space clamp and make quantitative measurements of the Na⁺ current. Both step voltage commands and voltage ramps with varying velocities were used to separate the different components on the basis of their gating kinetics. We utilized ramp voltage commands to unveil the slower components of the Na⁺ current that might play a role near threshold. The pharmacological agents hexanol, riluzole, and ranolazine were utilized to further separate the different components. In experiments designed to measure the roles of the different components of the Na⁺ current, we used current-clamp recording to measure action potential generation, frequency of firing, and response to simulated synaptic inputs.
MATERIALS AND METHODS

Slice preparation and recording conditions. Transverse hippocampal slices were prepared from 4- to 8-wk-old male Sprague-Dawley rats according to a protocol approved by the University of Texas at Austin Institutional Animal Care and Use Committee (IACUC) using methods previously described (Magee and Johnston 1995). All recordings were performed in the recording chamber, which was continuously perfused with artificial cerebrospinal fluid (ACSF). Boro-silicate glass of 2.0-mm OD and 1.16-mm ID (Sutter Instrument, Novato, CA) was used to pull 4- to 6-MΩ patch electrodes with a Flaming/Brown P-97 micropipette puller (Sutter Instrument). All recordings were carried out near physiological temperature (31–34°C), and electrodes were wrapped in Parafilm to within ~200 μm from the tip to reduce electrode capacitance. For some experiments, 0.2% Neurobiotin was included in the recording pipette and the slices were fixed after recording with 3% glutaraldehyde. The fixed slices were processed with an avidin-HRP system activated by diaminobenzidine (DAB, Vector Labs) and then visualized with an AxioImager Z1 upright microscope (Carl Zeiss, Thornwood, NY).

Whole cell voltage-clamp recordings. Somatic whole cell voltage clamp recordings were performed in CA1 pyramidal neurons. Bath saline contained (in mM) 125 NaCl, 3 KCl, 25 NaHCO3, 2 NiCl2, 1 MgCl2, 10 dextrose, 1.3 ascorbic acid, and 3 Na2 pyruvate, aerated with 95% O2-5% CO2. Pipette solution included (in mM) 10 CsCl, 120 Cs-glucuronate, 10 HEPES, 4 NaCl, 0.1 3,4-diaminopyridine, 4 Mg-ATP, 0.3 Na-GTP, and 7 K2-phosphocreatine, with pH 7.3 adjusted with TEA-OH. A slow ramp voltage protocol of 10 mVs velocity was applied to inactivate all the transient Na+ currents, leaving persistent inward Na+ current. INa was isolated either by subtracting traces recorded in the presence of TTX (TTX subtraction) or by subtracting a fitted line to a point more negative than ~60 mV from the whole trace (linear leak subtraction). For some cases, the apical dendrite of the neuron was cut by a motorized blade under the microscope (Kang and Schuman 1996). The ramp current signals were amplified with an Axopatch 200B (Axon Instruments; Molecular Devices, Sunnyvale, CA), sampled at 10 or 50 kHz, and filtered off-line at 0.5 or 1 kHz. The measured liquid junction potential of ~7.5 mV was not corrected. Input resistance (Ri) was monitored by a 10-mV hyperpolarized pulse in 30 ms from a holding potential (Vhold) of ~65 mV. Cell capacitance was calculated by integrating a transient capacitive current evoked by the same pulse. Series resistance was estimated by dividing the time constant of the single-exponential fit to the transient capacitive current by the calculated cell capacitance. Series resistance (23.0 ± 2.5 MΩ; n = 30) was compensated by the built-in circuit of the amplifier (up to 85% prediction and 70% correction).

Nucleated-patch voltage-clamp recordings. Nucleated-patch recordings were made by slowly withdrawing the electrode while applying negative pressure after forming a whole cell recording (Martina and Jonas 1997). Bath saline and pipette solutions were the same as those in whole cell voltage-clamp experiments. Nucleated patches had Rg around 3 GΩ (2.92 ± 0.13 GΩ; n = 69), and patch capacitance was calculated from the patch size in the image captured from a Newvicon tube camera (Dage-MTI, Michigan City, IN), assuming 1 μF/cm2. Series resistance (8.1 ± 0.5 MΩ; n = 12) was compensated up to 85% prediction and 60% correction, and maximum voltage error was <3 mV.

Currents in response to ramp voltage commands were corrected for leakage current by subtracting traces recorded in the presence of TTX (TTX subtraction) or by subtracting a line fit to points more negative than ~60 mV from the whole trace (linear leak subtraction). Currents in response to step commands were corrected for leakage and capacitive currents by subtracting the scaled leak traces generated by 1/10 or 1/5 amplitude of voltage commands from the raw traces (P–N protocol, N = 5 or 10). For the activation conductance-voltage (G–V) curves, a series of depolarizing step voltage commands from ~70 to +10 mV in steps of 10 mV for 30 ms with Vhold = −80 mV were applied with a 2- to 3-s interval (activation protocol). The conductance (gNa) was calculated by Ohm’s law, gNa = INa/(Vhold − ENa) where Vhold is the command voltage and ENa is the Na+ reversal potential (−93.87 mV calculated from [Na+]lump and [Na+]outs). The normalized conductance was plotted against voltage command. For the steady-state inactivation curve, 500-ms prepulses to voltages varying from −90 mV to −10 mV in steps of 10 mV were followed by a constant 30-ms test pulse to 0 mV with Vhold = −90 mV (inactivation protocol). Peak amplitude of current during a test voltage command was normalized and plotted versus prepulse potential. The voltage command protocol for the onset of INa inactivation was consisted of Vhold of ~80 mV, a prepulse (ranged from −60 to −10 mV by 10 mV) to test membrane potential (Vtest) with various time intervals (Δt; 1–1,000 ms), and a following test voltage step to 0 mV in 30 ms. The peak amplitudes of INa traces elicited by Vtest with increasing durations were normalized by that of INa with Δt = 0. The fraction of the Na+ channels available was then plotted against Δt. The kinetics of recovery of INa inactivation was measured by the standard double-pulse protocol consisting of Vhold of ~80 mV, a conditioning pulse to −0 mV in 500 ms, a pulse to Vtest (ranged from −80 to −60 mV by 10 mV) with various time intervals (Δt; 1–1,000 ms), and a following test pulse to 0 mV in 30 ms. The peak amplitudes of INa traces elicited by the test pulse after holding at Vtest with increasing durations (Δt) were normalized by that of INa by the conditioning pulse. The fraction of the Na+ channels recovered from the inactivation was then plotted against Δt. The INa traces for the kinetics of deactivation were elicited by voltage protocols: −80 mV hyperpolarizing in 50 ms followed by 300-μs depolarization at 0 mV and repolarization at various potentials from −40 mV to −100 mV by 10 mV in 30 ms. The ramp current signals were processed in the same manner as whole cell voltage-clamp experiments. The step current signals amplified by the Axopatch 200B were filtered at 5–20 kHz and sampled at 50 or 200 kHz.

Whole cell current-clamp recordings. The ACSF contained (in mM) 125 NaCl, 3 KCl, 1.25 NaH2PO4, 25 NaHCO3, 2 CaCl2, 1 MgCl2, 10 dextrose, 1.3 ascorbic acid, and 3 Na2 pyruvate, aerated with 95% O2-5% CO2. The pipette solution contained (in mM) 20 KCl, 120 K-glucuronate, 10 HEPES, 4 NaCl, 4 Mg-ATP, 0.3 Na-GTP, and 7 K2-phosphocreatine, with pH adjusted to 7.3 with KOH. Voltage signals were amplified and filtered at 5 kHz with a BVC-700A amplifier (Dagan, Minneapolis, MN) and sampled at 40 kHz. Access resistance and resting membrane potential were monitored throughout the experiment online. Access resistance was compensated with the amplifier’s bridge circuit and was ≤30 MΩ. Resting membrane potential was in the range of −68 to −60 mV.

Drug application. TTX (0.5 μM) and hexanol (1.4 mM) were bath applied for current-clamp experiments. For nucleated-patch recordings, 2 μM TTX, 2.8 mM hexanol, 10 μM riluzole, or 30 μM ranolazine was included in a puffer pipette that also contained (in mM) 149 NaCl, 3 KCl, 1 MgCl2, 10 HEPES, 2 NiCl2, 10 dextrose, 1.3 ascorbic acid, and 3 Na2 pyruvate, with pH adjusted to 7.3 with NaOH. A pair of puffer pipettes of 3- to 4-μm diameter was pulled, and one electrode was loaded with Fast Green dye in order to confirm the diffusion pattern of puffing. The other electrode was loaded with the drug being used for that experiment, and the patch was located in the drug diffusion region identified by the dye. Drugs were applied by a pressurized injection system (Picospritzer II, General Valve, Fairfield, NJ) or passively delivered via a check valve. There was often a small effect on measured currents during puffer application of saline for control experiments (presumably due to mechanical effects). Therefore, all measurements were compared to the saline controls. When it was necessary for a neuron to be isolated from the network, all AMPA, NMDA, GABA<sub>A</sub>, and GABA<sub>B</sub> receptor-mediated synaptic transmissions were blocked by CNQX (10 μM) or DNQX (20 μM), DL-AP-5 (50 μM), bicuculline (10 μM in DMSO) and picrotixin (10 μM in ethanol), and (2S)-3-[(1S)-1-(3,4-dichlorophenyl)ethyl]-
amino-2-hydroxypropyl (phenylmethyl)phosphonic acid (CGP55845; 2 μM in DMSO), respectively. A riluzole stock at 1 mM was made by dissolving it into DMSO and diluted with puffing solution to the final concentration of 10 μM right before the experiment. The total amount of DMSO in external solution remained below 0.1%, and the total amount of ethanol in the bath was maintained below 0.05%. The chemicals for ACSF were purchased from Fisher Scientific (Pittsburgh, PA), TTX from Ascent Scientific (Princeton, NJ), riluzole and ranolazine from Tocris (Ellisville, MO), and the other chemicals from Sigma-Aldrich (St. Louis, MO).

Data collection and analysis. Data acquisition, stimulus generation, and analysis were performed with an Instrutech ITC-18 DA/AD converter (HEKA Instruments, Bellmore, NY) controlled by IGOR Pro (WaveMetrics, Portland, OR) software on an Apple computer (Cupertino, CA) using locally written procedures. Amplified signals were filtered up to 20 kHz by the low-pass filter built into the amplifier or an external low-pass filter (model 902, Frequency Devices, Ottawa, IL). All Na⁺ current traces were averaged from three to seven individual traces. For nucleated-patch recordings, I_{Na Steph} and I_{Na CT} were quantified with activation and inactivation curves that were fitted by a Boltzmann equation, f(x) = base + max/[1 + exp(-(x - V_{1/2}(k))/k)], resulting in half-activation and half-inactivation voltage (V_{1/2}(k)) and k values for I_{Na Steph} and I_{Na CT}. The activation kinetics of I_{Na CT} was fitted by a cubic exponential function, f(x) = A × [1 - exp(-(x - x0)/τ_{fast})]³, and the kinetics of inactivation and deactivation were obtained by fitting the corresponding graphs to a single-exponential {f(x) = f0 + A × exp(-(x - x0)/τ)} or double-exponential {f(x) = f0 + A1 × exp(-(x - x0)/τ_{fast}) + A2 × exp(-(x - x0)/τ_{slow})} function, depending on the testing potentials (Engel and Jonas 2005; Martina and Jonas 1997). Exponential fits were made to the data with DISCRETE (Provencher 1976). For current-clamp recording, a frequency vs. current injection (F-I) curve was made by counting the number of spikes evoked by step current injections from 0 to 180 pA in 30-pA increments. Currents simulated by an α-function, A(α)α^(-α*t), where A is the amplitude of injected current, t is time, and 1/α is the time to peak, were injected to mimic EPSPs (α-EPSP injection). With this shape of current injection, temporal summation (α-EPSP summation) of the neuron was quantified by counting the number of spikes generated by α-EPSP injections at 25 Hz. Statistical tests performed were Mann-Whitney test (for 2 groups, unpaired data), unpaired and paired t-test (for 2 groups, unpaired and paired data), Kruskal-Wallis test and post hoc Dunn’s test (for >3 groups, unpaired data), and linear regression/correlation test. Markers and error bars in Figs. 1 and 3–8 indicate means ± SE.

RESULTS

Our initial interest was in the role of the persistent Na⁺ current in intrinsic plasticity of CA1 pyramidal cells, so we first confirmed the existence of I_{Na P} in hippocampal CA1 pyramidal neurons (Fig. 1A, top). Using techniques similar to those of others (Fleidervish and Gutnick 1996; Magistretti and Alonso 1999; Wu et al. 2005), we used ramp voltage commands with four different velocities: 400, 100, 40, and 10 mV/s. The slowest ramp voltage command of 10 mV/s reliably inactivated transient Na⁺ currents, leaving inward Na⁺ current identified as I_{Na P} (Fig. 1A, middle), but faster ramp velocities evoked Na⁺ currents contaminated by uncontrolled, escape Na⁺ spikes (data not shown). The slow inactivation of I_{Na P} was obvious from the inward current during the downslope of triangular voltage commands (Fig. 1A, bottom). When the apical dendrites of CA1 pyramidal neurons were cut in an attempt to improve space-clamp conditions (Fig. 1B, top), the amplitude of I_{Na P} was smaller than that of the intact neuron (Fig. 1B, middle and bottom), suggesting the presence of I_{Na P} in dendrites. Compared with the intact neurons, the dendrotoxinized neurons showed a significant increase in R_{N} (147.7 ± 8.6 vs. 429.6 ± 62.9 MΩ; P < 0.0001, Mann-Whitney test; Fig. 1C) and a decrease in cell capacitance (254.0 ± 10.0 vs. 152.8 ± 17.7 pF; P < 0.0001, Mann-Whitney test; Fig. 1D) and I_{Na P} amplitude (−477.6 ± 26.0 vs. −91.0 ± 12.4 pA; P < 0.0001, Mann-Whitney test; Fig. 1E), suggesting rescaling of the cut ends.

Because of the well-known lack of space clamp in pyramidal neurons from somatic whole cell voltage clamping and the possible effects of poor clamp on activation/inactivation kinetics, we decided to utilize nucleated patches for our analysis (see MATERIALS AND METHODS) (Fig. 2A) (Martina and Jonas 1997). Surprisingly, a 10 mV/s ramp voltage current was evoked by a slow (10 mV/s) ramp voltage command (middle) and partially inactivated in seconds in response to a triangle command (bottom). A neuron with its dendrite cut (top) showed reduced peak amplitude of persistent Na⁺ current (middle) and similar slow inactivation (bottom). C–E: summary of differences in input resistance (R_{N}), cell capacitance (D), and peak amplitude of persistent Na⁺ current (I_{Na P}, E) between intact and dendrotoxinized neurons.

in dendrites.
Despite this lack of persistence, the ramp current showed a voltage-dependent activation similar to that of $I_{\text{NaP}}$. We empirically compared current-voltage ($I-V$) curves from step and ramp voltage commands to determine whether the slowly inactivating inward $\text{Na}^+$ currents elicited by 400 mV/s ramps corresponded to the late $\text{Na}^+$ currents from step commands. $I_{\text{Na}}$ (leak-corrected by linear subtraction) was measured at four different time points after the onset of the step voltage command (Fig. 2C). $I-V$ curves were then constructed at each time point by measuring the current amplitude during the command (Fig. 2D). As time increased, there was a decrease in peak amplitude and a leftward shift of the peak of the $I-V$ curves revealing an inward current with small amplitude at later time points. Ramp command voltages of much faster velocities (400, 800, 4,000 and 8,000 mV/s; Fig. 2E) were applied to determine which produced the best separation of the fast, transient current from the slowly inactivating inward $\text{Na}^+$ current. As the ramp velocity decreased, the peak current amplitude also decreased and the peak current shifted to more negative potentials ($n = 4$). The ramp current with a velocity of 400 mV/s most closely matched the late (20 ms) current recorded from step commands. Therefore, this slowly inactivating component of $\text{Na}^+$ current evoked by 400 mV/s ramp voltage command was measured in subsequent experiments. Our working hypothesis is that the fastest ramp velocity (4,000–8,000 mV/s) elicits the fast $I_{\text{NaT}}$ and slower velocities.
unveil the slower components of Na\(^+\) current, with its peak occurring with a ramp velocity of \(-400\) mV/s. We called the slow component evoked by the 400 mV/s ramp voltage command \(I_{NaSS}\), a previous notation for the Na\(^+\) current with similar properties in SCN neurons (Kononenko et al. 2004).

Na\(^+\) currents in nucleated-patch recordings. We performed nucleated-patch experiments to measure \(I_{NaSS}\) and compare this to the components of Na\(^+\) current with slower activation kinetics \((I_{NaSL})\). The voltage-dependent activation of \(I_{NaSL}\) was determined by an activation protocol (see MATERIALS AND METHODS), and the resulting activation G-V curve showed half-activation voltage \(V_{1/2} = -21.0 \pm 0.7\) mV and slope factor \(k = 6.3 \pm 0.1\) (Fig. 3, A and C). The voltage dependence of activation for \(I_{NaSL}\) was determined by an inactivation protocol, and the availability curve showed \(V_{1/2} = -56.3 \pm 1.2\) mV and \(k = 5.4 \pm 0.1\) (Fig. 3, B and C). We also characterized the activation kinetics \((\tau_{act})\) of \(I_{NaSL}\) over the range of -30 to +40 mV (Fig. 3D). The rapid rise of \(I_{NaSL}\) activation was fit with a cubic exponential function with a small delay. As the test voltage increased, \(\tau_{act}\) became smaller. With a wideband low-pass filter (20 kHz) and high sampling frequency (200 kHz), we were able to measure \(\tau_{act}\) (22.2 \(\pm 2.3\) \(\mu\)s at 40 mV) (Fig. 3F, top). The time course of \(I_{NaSL}\) deactivation was also investigated (Fig. 3E). The deactivation time constant \((\tau_{deact})\) was obtained by fitting the trace with a single-exponential function at most test potentials, reaching 38.0 \(\pm 3.5\) \(\mu\)s at -100 mV (Fig. 3F, bottom), but the trace evoked by the -40 mV test potential was best fit by a double exponential.

The time constant of inactivation \((\tau_{inact})\) was measured from the decay of the current evoked by test voltage command of 100-ms duration at potentials from -30 to +40 mV. The current traces were leak subtracted with TTX (at 50-kHz sampling and 5-kHz low-pass filtering), and were best fit with a double exponential, generating a negligible residual compared with a single-exponential fit (Fig. 4A). Both \(\tau_{inact,fast}\) and \(\tau_{inact,slow}\) decreased with increasing test voltage (Fig. 4B, top), and the relative amplitudes of the fast \((\tau_{inact,fast})\) and slow \((\tau_{inact,slow})\) components are shown in Fig. 4B, bottom. The increase in the duration of inactivation prepulse shifted the availability curve to more hyperpolarized potentials (Fig. 4C). We measured the kinetics of the onset of inactivation with a prepulse protocol with increasing duration to further examine the inactivation occurring at subthreshold voltage ranges (Fig. 4D). The peak Na\(^+\) currents evoked after prepulses of various durations \((\Delta\tau)\) at \(V_{test}\) were normalized to the Na\(^+\) current evoked without a prepulse and plotted against prepulse duration. The normalized Na\(^+\) peak amplitude was fit by a double-exponential function. The relative contributions of fast and slow components (in \%) were 53.7:46.3 (-60 mV), 50.9:49.1 (-50 mV), 29.9:70.1 (-40 mV), 37.6:62.4 (-30 mV), 48.6: 51.4 (-20 mV), and 54.7:45.3 (-10 mV) at respective test voltages. The time course of development of inactivation

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*Fig. 3. Characterization of transient Na\(^+\) current \((I_{NaSS})\) in nucleated patch recordings. A and B: example traces of \(I_{NaSS}\) generated by the activation \((A)\) and inactivation \((B)\) protocols (see MATERIALS AND METHODS). C: normalized steady-state inactivation (gray) and activation (black) conductance \((G/G_{max})\) curves of \(I_{NaSS}\). Curves were fit to a single Boltzmann function. The inactivation curve gave -56.3 \(\pm 1.2\) mV for \(V_{1/2}\) and 5.4 \(\pm 0.1\) for slope factor \(k. G/G_{max}\), gave \(V_{1/2}\) = -21.0 \(\pm 0.7\) mV and \(k = 6.3 \pm 0.1\). D: kinetics of onset of \(I_{NaSS}\) activation. Currents were fit by a cubic exponential function to determine the activation time constant, \(\tau_{act}\) (see MATERIALS AND METHODS) Black lines overlie the raw curves (gray). Time constants \((\tau_{act})\) were 84.0, 55.9, and 23.1 \(\mu\)s at -10, +10, and +40 mV, respectively. E: time course of \(I_{NaSL}\) deactivation \((\tau_{deact})\) was measured by fitting \(I_{NaSL}\) tail currents with a single- or double-exponential function. The \(I_{NaSL}\) tail current evoked by -40 mV test pulse showed a biexponential time course \((\tau_{deact,fast} = 112 \mu\)s and \(\tau_{deact,slow} = 2.99\) ms), while more negative test commands showed a single-exponential time course \((\tau_{deact} = 57.6 \mu\)s at -60 mV and 35.1 \(\mu\)s at -100 mV test pulse). F: summary plots: \(\tau_{act}\) from -30 to +40 mV (top) and \(\tau_{deact}\) at voltages from -40 to -100 mV (bottom). All traces were corrected by P/I leak subtraction.*

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measured by the prepulse protocol was similar to the decaying kinetics of the Na\(^+\) currents at −30 mV (P = 0.447 for $\tau_{\text{inact, fast}}$ and 0.828 for $\tau_{\text{inact, slow}}$; unpaired t-test) but significantly faster at −20 and −10 mV (P < 0.05). The kinetics of recovery from inactivation was studied with a double-pulse protocol (Fig. 4E). It consisted of a 500-ms prepulse to 0 mV and a second pulse to 0 mV for 30 ms with variable time intervals ($\Delta t$) at several membrane potentials for the recovery ($V_{\text{rec}}$). The peak Na\(^+\) currents evoked by the second pulse were normalized by the peak Na\(^+\) current of the first pulse and
plotted against $\Delta t$. The normalized Na$^+$ peak amplitude showed a double-exponential component at recovery voltages between $-60$ and $-80$ mV. The relative contributions of fast and slow components (in %) were 50.2:49.8 at $-60$ mV, 63.5:36.5 at $-70$ mV, and 73.9:26.1 at $-80$ mV, respectively.

Using the fast and slow inactivation time constants obtained above (Fig. 4), we reconstructed a step $I-V$ curve at different time points for different test potentials for the same data set in Fig. 2, C-E ($n = 4$). Assuming $\tau_{\text{inact,slow}} = 4 \times \tau_{\text{inact,fast}}$ we chose $t = 3 \times \tau_{\text{inact,fast}}$ where the fast component was inactivated by $-95\%$ and the slow component inactivated $-50\%$. The resulting Fig. 5A allowed us to visualize how the voltage ramp protocol, where both time and voltage are changing, can cause significant voltage inactivation at higher depolarization potentials. However, the initial phase of increasing ramp current at hyperpolarized potentials was closely matched to the step $I-V$ at corresponding voltages, and it enabled us to use the ramp protocol as an effective method to assess $I_{\text{Na}}$. When we recorded both $I_{\text{NaS}}$ and $I_{\text{NaT}}$ ($n = 18$) from the same patches, we found that $G_{\text{NaS}}$ calculated in the same manner as $G_{\text{NaT}}$ showed a more negative $V_{1/2}$ ($-37.7$ vs. $-21.0$ mV) and smaller $k$ ($3.7$ vs. $6.3$) than $G_{\text{NaT}}$ (Fig. 5B). We examined the relationship between $I_{\text{NaS}}$ and $I_{\text{NaT}}$ recorded from the same nucleated patches ($n = 110$). The peak amplitude of $I_{\text{NaS}}$ occurred around $-40$ to $-30$ mV with a 400 mV/s ramp command and averaged $-5.16 \pm 0.22$ pA, while that of $I_{\text{NaT}}$, measured from step commands, peaked at $-10$ to $+10$ mV with an amplitude of $-174.6 \pm 7.8$ pA. The relationship of peak amplitudes of $I_{\text{NaS}}$ to $I_{\text{NaT}}$ was linear and the ratio $I_{\text{NaS}}/I_{\text{NaT}}$ was 0.0238 (Pearson’s correlation test, $r = 0.839$; $P < 0.0001$; $n = 110$), indicating that the amplitude of $I_{\text{NaS}}$ is $-2.38\%$ of $I_{\text{NaT}}$ from nucleated patches (Fig. 5C).

Effects of pharmacological blockers on $I_{\text{NaS}}$ and $I_{\text{NaT}}$. We tested several known Na$^+$ channel blockers for their ability to block $I_{\text{NaS}}$. We have shown in earlier experiments that TTX fully blocks all $I_{\text{Na}}$. Hexanol, one of the $n$-alcohols known to block $I_{\text{NaT}}$ (Horishita and Harris 2008; Shiraiishi and Harris 2004) and to have anesthetic effects (Alifimoff et al. 1989), was chosen because of its strong potency with relatively low concentration compared with other alcohols with a shorter carbon chain such as ethanol and butanol. Hexanol at 2.8 mM (a moderate anesthetic concentration) suppressed both $I_{\text{NaS}}$ and $I_{\text{NaT}}$, but to different degrees. Puff application of hexanol on the nucleated patch reduced the amplitude of $I_{\text{NaS}}$ by 64.7% (Fig. 6A) and of $I_{\text{NaT}}$ by 31.1% (Fig. 6B). The maximum conductance of $I_{\text{NaS}}$ elicited by a 400 mV/s ramp voltage command was reduced significantly in the presence of hexanol (Fig. 6C).

We further investigated this differential block of $I_{\text{NaS}}$ and $I_{\text{NaT}}$ with two additional drugs, riluzole (10 $\mu$M) and ranolazine (30 $\mu$M), both previously reported to block persistent Na$^+$ current (Benoit and Escande 1991; Rajamani et al. 2009). None of the three drugs caused a significant change in $V_{1/2}$ of activation (Fig. 6D). However, hexanol and riluzole, but not ranolazine, caused a significant reduction in $G_{\text{NaS,\text{max}}} (\text{drug} - \text{saline} = -49.7 \pm 3.3\%$ and $-52.6 \pm 3.4\%$, respectively; $P < 0.001$; Kruskal-Wallis test and post hoc Dunn’s test). The results from the pharmacological experiments are summarized in Table 1.

We tested the effects of hexanol, riluzole, ranolazine, and saline on the activation $G-V$ curve of $I_{\text{NaT}}$. Figure 6E illustrates the suppression of maximum conductance of $I_{\text{NaT}}$ in the activation $G-V$ curve by hexanol. No drugs revealed a significant change in voltage dependence of the channel activation (Fig. 6F). Hexanol and riluzole, but not ranolazine, decreased the maximum conductance of $I_{\text{NaT}}$ to a lesser degree than that of $I_{\text{NaS}}$ (drug – saline $= -24.9 \pm 8.0\%$ and $-22.4 \pm 6.4\%$, respectively; $P < 0.05$; Table 1).

The effects of the drugs on steady-state inactivation of $I_{\text{NaT}}$ were then tested. Hexanol application caused a decrease in the maximum amplitude of $I_{\text{NaT}}$ (drug – saline $= -21.1 \pm 3.7\%$; Fig. 6G). We then tested riluzole and ranolazine and found that riluzole also decreased the maximum amplitude of $I_{\text{NaT}}$ ($-24.9 \pm 3.3\%$). Ranolazine, however, had no significant effect on Na$^+$ current (Table 1). Among the three drugs, hexanol was the only one that produced a statistically significant left shift of $V_{1/2}$ of the steady-state inactivation curve ($-9.2 \pm 1.4$ mV; $n = 4$; $P < 0.05$; Fig. 6H and Table 1). There was no significant change in the slope factor for any of the drugs.

Fig. 5. A: amplitude of Na$^+$ currents evoked by step protocol was measured at $t = 3 \times \tau_{\text{inact,fast}}$ after activation of the current and overlaid onto the average current generated by 400 mV/s ramp protocol (the same data set as in Fig. 2, C-E). B: comparison of voltage dependence of $I_{\text{NaS}}$ and $I_{\text{NaT}}$ activation. Normalized conductance of $I_{\text{NaS}}$ ($G/G_{\text{NaS,\text{max}}}$) was plotted with the activation $G-V$ curve from recordings of $I_{\text{NaS}}$ (copied from Fig. 5C) for the same patch. The activation $G-V$ curve of $I_{\text{NaS}}$ gave $V_{1/2} = -37.7 \pm 1.0$ mV and $k = 3.7 \pm 0.2$ compared with $V_{1/2} = -21.0 \pm 0.7$ mV and $k = 6.3 \pm 0.1$ for $I_{\text{NaT}}$. C: relationship between the peak amplitudes of $I_{\text{NaS}}$ and $I_{\text{NaT}}$; both $I_{\text{NaS}}$ and $I_{\text{NaT}}$ recorded from the same patch showed a linear correlation (Pearson’s $r = 0.839$). The peak amplitude of $I_{\text{NaS}}$ was 2.38% of the peak $I_{\text{NaT}}$.
In a different set of experiments, we tested the blocking of $I_{Na,S}$ in the presence of hexanol at a lower concentration (1.4 mM). We found that the lower concentration of hexanol caused a smaller blocking effect on peak $I_{Na,T}$ compared with 2.8 mM hexanol ($-18.4 \pm 2.3\%$; $P = 0.0002$, 1-sample r-test). We also tested the effect of hexanol on both fast and slowly inactivating components of $Na^+$ current in the nucleated patch (Fig. 7). In the presence of 2.8 mM hexanol, fast inactivation in the example traces shown in Fig. 7A was enhanced and the slowly inactivating component was blocked. Overall, the drug accelerated fast inactivation prominently at most test voltages and made the slow component less voltage sensitive (Fig. 7B, bottom). The reduction of fractional amplitude of the second component was statistically significant at $-30 \text{ mV}$ (Fig. 7B, top; $P = 0.018$).

$I_{Na,S}$ and neuronal excitability. We next investigated the physiological consequences of suppressing $I_{Na,S}$ with hexanol in current-clamp recordings from in situ hippocampal CA1 neurons.

<table>
<thead>
<tr>
<th>Hexanol</th>
<th>$G_{Na,S, max}$ change, %</th>
<th>$\Delta V_{1/2}$, mV</th>
<th>$\Delta k$</th>
<th>Hexanol</th>
<th>$G_{Na,T, max}$ change, %</th>
<th>$\Delta V_{1/2}$, mV</th>
<th>$\Delta k$</th>
<th>Hexanol</th>
<th>$I_{Na,T, max}$ change, %</th>
<th>$\Delta V_{1/2}$, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>$-71.1 \pm 3.2%$</td>
<td>$-3.01 \pm 0.70$</td>
<td>$-0.45 \pm 0.35$</td>
<td>5</td>
<td>$-35.3 \pm 8.0%$</td>
<td>$2.05 \pm 1.01$</td>
<td>$0.19 \pm 0.14$</td>
<td>4</td>
<td>$-23.3 \pm 4.1%$</td>
<td>$-6.10 \pm 1.03$</td>
</tr>
<tr>
<td>7</td>
<td>$-73.9 \pm 3.4%$</td>
<td>$-4.48 \pm 1.48$</td>
<td>$0.28 \pm 0.83$</td>
<td>5</td>
<td>$-32.8 \pm 6.4%$</td>
<td>$1.89 \pm 0.73$</td>
<td>$0.41 \pm 0.46$</td>
<td>4</td>
<td>$-27.1 \pm 3.3%$</td>
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<tr>
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<td>$0.55 \pm 0.70$</td>
<td>$0.88 \pm 0.22$</td>
<td>7</td>
<td>$-25.0 \pm 1.5$</td>
<td>$1.84 \pm 0.48$</td>
<td>$0.10 \pm 0.25$</td>
<td>7</td>
<td>$-14.2 \pm 1.6$</td>
<td>$-3.34 \pm 0.47$</td>
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<td>$-0.10 \pm 0.30$</td>
<td>5</td>
<td>$-10.4 \pm 2.3$</td>
<td>$-0.55 \pm 0.65$</td>
<td>$0.07 \pm 0.49$</td>
<td>5</td>
<td>$-2.20 \pm 3.1$</td>
<td>$-3.84 \pm 0.34$</td>
</tr>
</tbody>
</table>

Values are means ± SE. The effect of each drug was compared with the saline condition. Maximum conductance $G_{max}$ (or current amplitude $I_{max}$) change was calculated by ($G_{max, Drug} - G_{max, Baseline}$)/$G_{max, Baseline} \times 100$; $\Delta V_{1/2}$ (or $\Delta k$) was calculated by $V_{1/2, Drug} - V_{1/2, Baseline}$ (or $k_{Drug} - k_{Baseline}$). Kruskal-Wallis test and post hoc Dunn’s test: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.
Fig. 7. Effects of hexanol on slowly inactivating component of Na⁺ current. A: Na⁺ current traces evoked by a voltage step from −80 to −20 mV in the presence and absence of 2.8 mM hexanol. The amplitudes of both currents were normalized and fit by a single-exponential function to elucidate their decaying kinetics. Scale bar in residual current traces (bottom) indicates 7% in normalized amplitude scale. B: group data showed that there was a significant reduction of the fractional amplitude of the slow component at −30 mV in the presence of the drug (top). The enhancement of fast inactivation and decrease in voltage dependence of slow component were caused by hexanol application (bottom). Paired t-test: *P < 0.05; **P < 0.01.

pyramidal neurons in slices. After neurons were isolated pharmacologically from the network (see MATERIALS AND METHODS), we first measured $R_N$ by injecting a family of current steps (700-ms duration, from −50 to +20 pA by 10-pA increments) (Fig. 8A). $R_N$ was measured by fitting a line to the steady-state voltage responses from a series of 700-ms current injections for baseline and after bath application of hexanol (1.4 mM). Possible effects on the population of ionic currents active at rest were investigated by injection of DC current to change the resting potentials, and normalized $R_N$ vs. membrane potential is plotted in Fig. 8B.

We examined the effects of hexanol on the voltage threshold of an action potential evoked with current injections (120–150 pA) of 700-ms duration. Current was injected to the neuron both in the absence and presence of hexanol, and the voltage thresholds (membrane voltage of $dV/dt = 20$ mV/ms) for both conditions were compared. The amplitude of injected current was adjusted to evoke the first spike at the same latency for both conditions. There was a significant increase in voltage threshold of the first spike ($−43.27 ± 1.54 \text{ vs. } −38.68 ± 2.18$ mV; $n = 5; P = 0.016$; Table 2), which was reversible on washout of hexanol (Fig. 8C1). In the phase plane plot of the same voltage traces, the difference in voltage threshold for both baseline and hexanol conditions can be identified (Fig. 8C2). There was, however, no significant change in other spike parameters such as peak $dV/dt$, spike amplitude, and spike half-width ($P > 0.05$; Table 2).

We also looked at the effect of hexanol on other physiological parameters: resting membrane potential, neuronal firing rate with given current injection, first spike latency, and temporal summation. In the presence of 1.4 mM hexanol, there was a hyperpolarization of the resting membrane potential of the neuron by $−2$–$3$ mV soon after hexanol was washed in to the bath. In a separate series of experiments ($n = 5$), we investigated whether TTX and TTX with hexanol caused a similar hyperpolarization of the resting potential. TTX application caused an average hyperpolarization of $0.77 ± 0.35$ mV, and TTX + hexanol caused an additional $1.37 ± 0.56$ mV hyperpolarization. The voltage drop was compensated with $20–30$ pA of current injection to maintain the same membrane potential as that of the baseline. With the same amplitude of sustained current injection for baseline and hexanol conditions, there was also a significant delay in the latency to the first spike from $171.4 ± 22.6$ ms to $392.1 ± 71.3$ ms ($n = 5; P = 0.016$), which was reversible by washout (Fig. 8D). A series of 700-ms-duration currents were injected from 30 to 180 pA by 30-pA steps, and a plot of the number of spikes vs. amplitude of current injection (F-I plot) showed a significant reversible rightward shift in the presence of hexanol, suggesting suppression of neuronal excitability by hexanol (Fig. 8E). Finally, the number of spikes generated by the same amplitude of α-EPSP current injections (to measure suprathreshold temporal summation) in both conditions was decreased in the presence of hexanol ($2.9 ± 0.1$ vs. $1.1 ± 0.4$ spikes; $n = 5; P = 0.0077$) (Fig. 8, F and G).

**DISCUSSION**

*Properties of $I_{NaT}$* The biophysical properties of $I_{NaT}$ reported in this study are largely consistent with those reported previously for hippocampal CA1 neurons (Kuo and Bean 1994; Magee and Johnston 1995; Martina and Jonas 1997; Sah et al. 1988; Yau et al. 2010). The half-activation voltage of $−21$ mV was placed in the range reported from $−31$ mV (Sah et al. 1988) to $−12.5$ mV (Yau et al. 2010). The midpoint voltage of steady-state inactivation ($−56.3$ mV) was also within the range of previous studies ($−89.5$ to $−55.4$ mV). Different experimental conditions such as dissociated versus in vitro prepara-
channel study reported an intermediate duration (~28 ms) of burst opening of Na\textsuperscript{+} channel in a cortical neuron in a voltage range between ~40 and 0 mV, which constitutes one of three burst opening kinetic components underlying \( I_{\text{NaP}} \) (Magistretti and Alonso 2002). \( I_{\text{NaS}} \) in the present study may reflect a similar relatively short open time of burst activity of Na\textsuperscript{+} channel in hippocampal neurons at macroscopic current level. However, voltage independence of the medium-duration burst opening contrasted with the voltage dependence of the slow component recorded in our preparation (Fig. 4, B and F). In addition, Martina and Jonas previously reported two exponential components of Na\textsuperscript{+} current decay kinetics in one-third of their nucleated-patch recordings in younger animals (Martina and Jonas 1997), which is similar to our results.

The decay kinetics and onset of inactivation that we observe were best fit with the sum of two exponentials where \( \tau_{\text{inact,slow}} \) ranged from 4 to 125 ms in most of our nucleated patches, and this observation suggests that an additional slower (4–10 times slower) inactivating process is present during the onset of Na\textsuperscript{+} channel inactivation. Its larger fractional amplitude around ~40 to ~30 mV also indicates its prominent role in that voltage range. The double-exponential components in recovery from inactivation (Fig. 4E), similarly reported in other studies (Martina and Jonas 1997; Yau et al. 2010), further support the presence of a second inactivation process. The second component in the deactivation kinetics at ~40 mV in Fig. 3E, however, indicates a mixture of deactivation and inactivation of Na\textsuperscript{+} channel at this voltage (Engel and Jonas 2005; Oxford 1981). The relative weights of the slow component \( (I_{\text{NaS}}) \) of inactivation during commands to ~20 and ~10 mV were different between measurements of the current decay phase (Fig. 4B) and that measured with a variable-duration prepulse protocol (Fig. 4C). In earlier experiments on squid axons (Gillespie and Meves 1980), the peak Na\textsuperscript{+} current was increased by a sufficiently strong conditioning pulse in double pulse that had no gap between two pulses. Considering the lower activation voltage of \( I_{\text{NaS}} \) than \( I_{\text{NaP}} \) (Fig. 5C), the conditioning pulse at ~20 and ~10 mV might be strong enough to cause this effect on \( I_{\text{NaS}} \), but not on \( I_{\text{NaP}} \). This change in peak \( I_{\text{NaS}} \) would contribute to this discrepancy.

Comparison of whole cell and nucleated-patch measurements of \( I_{\text{NaP}} \). In whole cell somatic voltage-clamp recordings of hippocampal CA1 pyramidal neurons (Fig. 1A), we measured 427 pA of peak \( I_{\text{NaP}} \) amplitude (426.6 ± 62.9 MΩ; \( n = 30 \)) with 254 pF whole cell capacitance (254.0 ± 10.0 pF; \( n = 30 \)), using a slow (10 mV/s) ramp voltage command. The calculated \( I_{\text{NaP}} \) density current was 1.93 pA/pF (1.93 ± 0.10 pF; \( n = 30 \)), which was similar to a previous report of 1.9 pA/pF peak amplitude of \( I_{\text{NaP}} \) in whole cell voltage-clamp recordings of neocortical neurons from acute slices at near-physiological temperature (Astman et al. 2006). The slow inactivation of the \( I_{\text{NaP}} \) was consistent with that seen on the 2- to 6-s timescale in other studies (Fleidervish and Gutnick 1996; Magistretti and Alonso 1999; Wu et al. 2005). If \( I_{\text{NaP}} \) were distributed evenly on the membrane, we could expect ~6.8 pA of \( I_{\text{NaP}} \) in the corresponding nucleated patch that has an average nucleated patch size of 3.5 ± 0.04 pF (\( n = 141 \)). However, the Na\textsuperscript{+} currents evoked by the same slow ramp voltage command in the peri-somatic nucleated patches did not confirm the existence of that amount of \( I_{\text{NaP}} \) (Fig. 2B). Our whole cell voltage-clamp recording on the dendroto
neurons suggested rather that there could exist a significant amount of $I_{\text{NaP}}$ along the apical dendrite as well as basal dendrite and axonal area.

The steady-state inactivation of Na$^+$ channels in dissociated hippocampal CA1 neurons has been shown to be nearly complete, generating little steady-state current (<0.5%) considered as $I_{\text{NaS}}$ (Kuo and Bean 1994; Taddei and Bean 2002). In experiments in which we measured $I_{\text{NaST}}$, the average peak $I_{\text{NaST}}$ amplitude was 175 pA (174.6 ± 7.8; $n = 110$). If we use 0.5% proportion of $I_{\text{NaS}}$ to $I_{\text{NaST}}$ (Kuo and Bean 1994), the peak ramp current would be <1 pA, which might be difficult to resolve (Fig. 2B1).

**Measurement and properties of $I_{\text{NaS}}$.** The lack of a persistent sodium current in the nucleated patches, however, led us to try other ramp velocities, and we found a component of the macroscopic Na$^+$ current in response to 400 mV/s ramp commands with a peak amplitude around 7 pA (Fig. 2B1, bottom) that inactivated faster than has been described for $I_{\text{NaS}}$ (Fleidervish and Gutnick 1996; Wu et al. 2005). The slow component of the macroscopic Na$^+$ current activated at more negative potentials and was kinetically and pharmacologically distinct from the fast inactivating Na$^+$ current, $I_{\text{NaS}}$. We have utilized the previous notation (Kononenko et al. 2004), $I_{\text{NaS}}$, to indicate its slower kinetics.

We pursued $I_{\text{NaS}}$ measured from nucleated patches. A simple and efficient demonstration of acceptable voltage control in the nucleated patches was apparent in the currents measured in response to the ramp voltage commands of very fast velocities ranging from 800 to 8,000 mV/s (Fig. 2E). Those ramp voltage commands generated no unclamped Na$^+$ current that would be inevitable with whole cell voltage-clamp recording. We chose a velocity of 400 mV/s for inactivating $I_{\text{NaST}}$ and allowing $I_{\text{NaS}}$ to be measured because that gave us the largest $I_{\text{NaS}}$ current and generated a reasonable match between the initial phase of increasing currents evoked by ramp and step protocols (Fig. 5A). Therefore, our ramp voltage protocol could be used to study $I_{\text{NaS}}$ with a approach similar to that previously utilized in a Ca$^{2+}$ current study (Corey et al. 1984). The average peak amplitude of $I_{\text{NaS}}$ evoked by voltage ramps was 5.06 ± 0.23 pA ($n = 141$) and the current density was 1.44 ± 0.06 pA/pF, assuming 1 μF/cm$^2$ specific membrane capacitance. $I_{\text{NaS}}$ showed 16.7 mV more negative $V_{1/2}$ of activation and 41.3% smaller slope factor compared with those of $I_{\text{NaST}}$, and its peak amplitude was linearly proportional to that of $I_{\text{NaST}}$.

A component of $I_{\text{NaS}}$ similar to what we call $I_{\text{NaS}}$ was found in measurements from SCN neurons with small size, short and few processes, and high $R_m$ (>1 GΩ), comparable to that of the nucleated patches, −3 GΩ (Jackson et al. 2004; Kononenko et al. 2004; Pennartz et al. 1997). Whole cell voltage clamp of intact neurons with insufficient voltage control complicates the situation because of unclamped Na$^+$ current elicited by the ramp voltage commands.

A component of Na$^+$ current with a more negative $V_{1/2}$ of activation of −62 mV in response to very slow ramps (10 mV/s) has been described in Purkinje and CA1 neurons that is able to boost synaptic events (Carter et al. 2012). These measurements were made in acutely isolated neurons with truncated processes that increased the quality of space clamp. The authors suggest that a single population of Na$^+$ channels with different gating modes could account for all recorded current, although they saw less slow inactivation than shown in our data and by others (Fleidervish and Gutnick 1996; Magistretti and Alonso 1999; Wu et al. 2005). The slow component of Na$^+$ current we described here could arise from a different population of Na$^+$ channels, since we recorded only from somatic nucleated patches that would not include channels in the axon initial segment or proximal dendrites, or a subset of channels that have undergone molecular change. For example, coexistence of splice variants of Na$^+$ channel with different kinetics, functional modifications of the channel by β-subunit, or existence of different inactivation states of the same channel could generate this component of Na$^+$ current (Chatelier et al. 2008; Isom et al. 1992; Payandeh et al. 2012). Although the mechanism of $I_{\text{NaS}}$ is not clear, a hint could be obtained from a link between the closed-state inactivation present (Fig. 4C) and ramp current. The ramp voltage command, as an efficient method to induce closed-state inactivation, elicits a ramp current that is a mixture of $I_{\text{NaST}}, I_{\text{NaS}},$ and $I_{\text{NaP}}$. The relative contribution of each component to the total ramp current is, therefore, dependent on the ramp voltage command velocity that determines the degree of the closed-state inactivation. Others have seen a positive correlation between the magnitude of the ramp current and the degree to which the channel undergoes slow closed-state inactivation (Cummins et al. 1998). The ratio of Na$^+$ channels in a conformation for closed-state inactivation to those not in this state would be reflected in the amplitude of $I_{\text{NaS}}$, which we found to be maximized with a ramp velocity of 400 mV/s. Closed-state inactivation of Na$^+$ channel can occur sufficiently with movements of voltage sensor in only domains 3 and 4 (Armstrong 2006; Bähring and Covarrubias 2011). The modification of a Na$^+$ channel required to generate $I_{\text{NaS}}$ might hamper the concerted conformational change of the channel resulting from the outward movement of those sensors on depolarization of...
membrane potential. Then the systematic introduction of mutations onto those voltage sensors and pore domain in a way similar to the previous studies of other Na⁺ channel modulators (Ragdale et al. 1994; Sheets and Hanck 2007) could shed more light on the molecular mechanism of $I_{\text{NaS}}$ and differential blocking of hexanol on $I_{\text{NaS}}$ and $I_{\text{NaT}}$. The relative insensitivity of ranolazine to $I_{\text{NaS}}$ in our data further suggested that the target region might reside on the nonhomologous sequences of the brain and cardiac Na⁺ channels.

**Physiological significance of $I_{\text{NaS}}$.** Hexanol, riluzole, and ranolazine were used to investigate the pharmacology of $I_{\text{NaS}}$. The $n$-alcohols are well-known $I_{\text{NaT}}$ blockers (Horishita and Harris 2008; Shiraiishi and Harris 2004), but their effect on $I_{\text{NaS}}$ is not known. In our results, the differential blocking of the drug on $I_{\text{NaT}}$ and $I_{\text{NaS}}$ (Fig. 6) and the enhanced rate of decay of Na⁺ current (Fig. 7) suggested that hexanol blocks the open channel with slow binding kinetics.

To investigate the possible physiological role of this $I_{\text{NaS}}$ hexanol was used as a partial $I_{\text{NaT}}$ blocker. When applied to a neuron in an intact hippocampal slice, hexanol caused a significant increase in action potential threshold (4.6 mV). Since more than a third of Na⁺ channels in hippocampal neuron are redundant for single action potential initiation (Madeja 2000), 50% blocking of $I_{\text{NaS}}$ by hexanol could explain the increased spike threshold better than differentially blocking $I_{\text{NaT}}$ (25%). This interpretation can be further supported by three other observations: a lower voltage activation of $I_{\text{NaS}}$, no change in voltage dependence of $I_{\text{NaT}}$ activation by hexanol, and no change in other active properties (Table 2). The change in other physiological parameters such as a significant delay in the first spike generated by a 700-ms current injection and reduction in the number of spikes generated in response to sustained current injections or $\alpha$-EPSP injections also suggested reduced neuronal excitability of hippocampal neurons in the presence of hexanol. Although hexanol may affect other intrinsic properties, the decrease in neuronal excitability remains consistent with the reduction of $I_{\text{NaS}}$.

**Contribution of persistent Na⁺ current to neuronal excitability.** This has been actively explored and, as an intrinsic ionic mechanism, the link between its alteration and human disease such as inherited epilepsy and pain syndromes has received intensive attention (Bean 2007; Crill 1996; Cummins et al. 2007; George 2005). However, the transient Na⁺ current as well as persistent current was recently shown to activate in the subthreshold voltage range during fast depolarization and augment synaptic integration (Carter et al. 2012). The slowly inactivating component of Na⁺ current in this study, which is prominent in that voltage range, might underlie at least part of such a subthreshold Na⁺ current. This slowly inactivating component of the macroscopic Na⁺ current may play an important role in determining neuronal excitability since it plays a role near the threshold for spike generation in the intact neuron. Understanding its properties may provide valuable insight into how an intrinsic mechanism can regulate neuronal activity and could be a new therapeutic target for channelopathies underlain by the Na⁺ channel.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: Y.Y.P., D.J., and R.G. conception and design of research; Y.Y.P. performed experiments; Y.Y.P. analyzed data; Y.Y.P., D.J., and R.G. interpreted results of experiments; Y.Y.P. prepared figures; Y.Y.P. and R.G. drafted manuscript; Y.Y.P., D.J., and R.G. edited and revised manuscript; Y.Y.P., D.J., and R.G. approved final version of manuscript.

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