

# Temperature-dependent Activation of Neurons by Continuous Near-infrared Laser

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**Abstract** Optical control of neuronal activity has a number of advantages over electrical methods and can be conveniently applied to intact individual neurons *in vivo*. In this study, we demonstrated an experimental approach in which a focused continuous near-infrared (CNI) laser beam was used to activate single rat hippocampal neurons by transiently elevating the local temperature. Reversible changes in the amplitude and kinetics of neuronal voltage-gated Na and K channel currents were recorded following irradiation with a single-mode 980 nm CNI-laser. Using single-channel recordings under controlled temperatures as a means of calibration, it was estimated that temperature at the neuron rose by 14°C in 500 ms. Computer simulation confirmed that small temperature changes of about 5°C were sufficient to produce significant changes in neuronal excitability. The method should be broadly applicable to studies of neuronal activity under physiological conditions,

in particular studies of temperature-sensing neurons expressing thermoTRP channels.

**Keywords** Voltage-dependent ion channels · Activation · Inactivation · Action potential · Laser · Temperature

## Introduction

The ability to control neuronal activity both *in vitro* and *in vivo* is crucial for the study of nervous system functions. Traditionally, this is achieved electrically through current injection into neurons via an electrode, such as a sharp glass electrode and a patch pipette [1]. However, electrical excitation methods have a number of drawbacks, including irreversible damage to neuronal cells and their surrounding tissues, limitation to exposed neurons, and limitation to the number of neurons that can be selectively and simultaneously excited. Optical excitation has recently emerged as an alternative approach that easily overcomes these drawbacks [2, 3]. The emergence of laser technologies has provided an energy source with sufficient power density and easy manipulation [4]. Nonetheless, reports of its usage as a means to control neuronal activities have been scarce.

Effects of light on biological tissues can be photochemical, photothermal, photomechanical, and photoelectrical, depending on the size and nature of the sample as well as the wavelength of the light [5]. The last two categories generally apply to small molecules. Photochemical and photothermal effects are both applicable to cells and biomacromolecules, but with important differences. Photochemical reactions irreversibly modify biomolecules and require higher energy levels that are generally restricted to short-wavelength light in the ultraviolet (UV) range. Photons in this wavelength range carry the right amount of energy to break chemical

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bonds, especially those of aromatic amino acids such as tryptophan, tyrosine and phenylalanine, or to catalyze cross-linking of DNA molecules [6]. Irradiation by UV-laser has been shown to quickly destroy tryptophan residues of cyclic nucleotide-gated channels in cell-free membrane patches, leading to irreversible reduction of ligand-induced channel currents [7, 8]. UV-laser irradiation can also reduce membrane excitability of myelinated nerve fibers, likely due to both a loss of Na permeability and Na channel inactivation [9].

Photothermal effects on cells can be fully reversible and easily controlled for exciting neurons. Irradiation of neurons leads to an elevated temperature local to the targeted cells, which transiently changes the membrane excitability. Opposite to photochemical effects, heat generation by light is more efficient in the long wavelength range, especially by infrared light [5]. Extended exposure to high-intensity infrared light can dramatically increase local temperature and cause tissue damage [10]. Limited exposure leads to less heat generation and generally reversible effects on neuronal activities. In 2002, Hirase et al. [11] successfully used high intensity mode-locked infrared laser to elicit action potentials in pyramidal neurons. Later, Wells et al. [12, 13] used a lower intensity-pulsed mid-infrared light (2–4  $\mu\text{m}$  in wavelength) to trigger action potentials in the sciatic nerve of leopard frog. In both cases, near-infrared laser was ruled out as a viable means to fully excite neuronal cells.

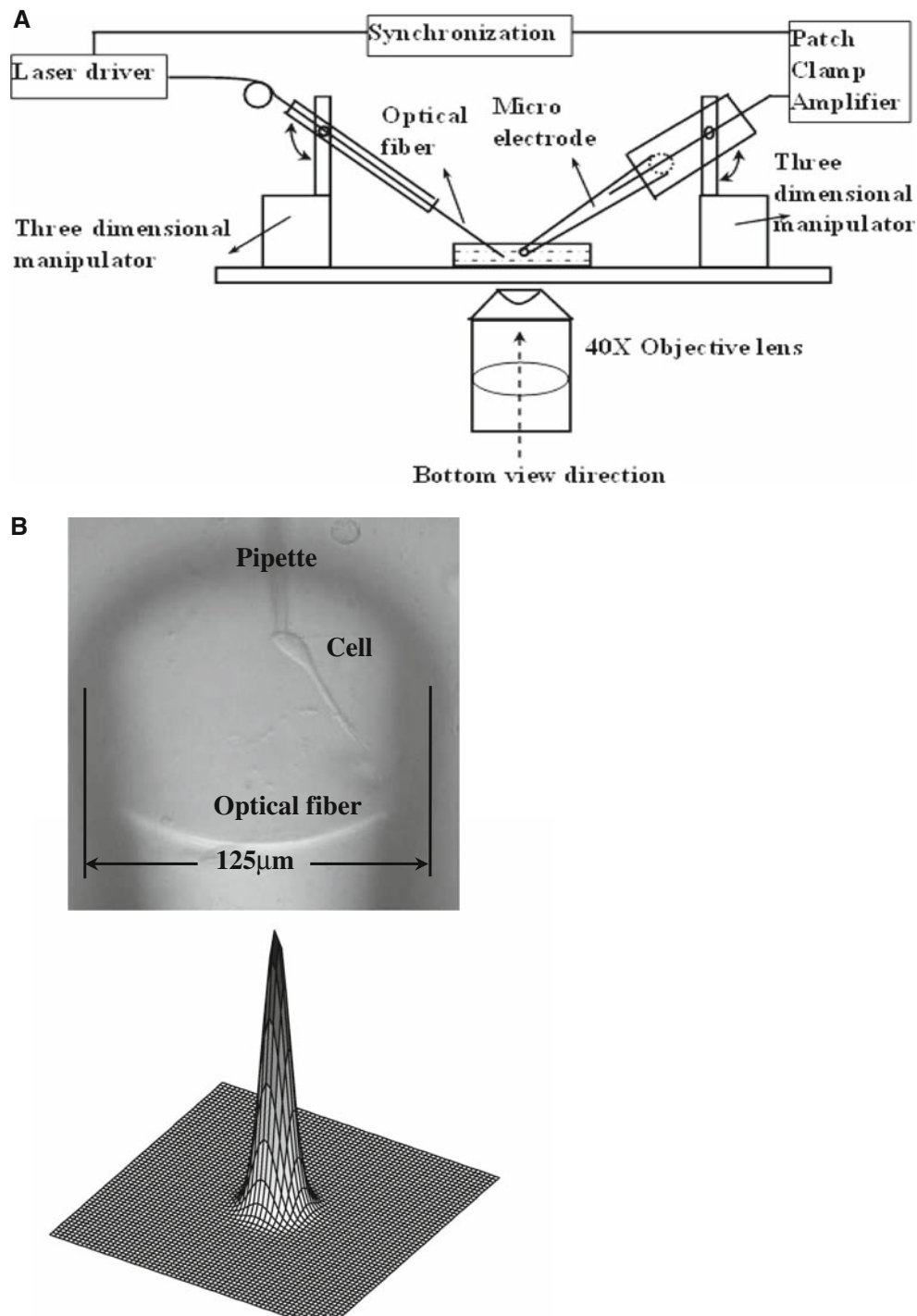
In this study, we used acutely isolated rat hippocampal neurons to assess thermal excitation effects by low-power continuous near-infrared (CNI) laser. We demonstrated that voltage-activated Na and K channel currents could be strongly and reversibly changed by irradiation as short as 500 ms. We further identified that changes in neuronal currents were originated from temperature-induced shifts in voltage-dependent activation and inactivation of Na and K channels. The results not only provided a molecular basis for laser excitation of neurons, but also demonstrated that low-power CNI-laser can be used to non-invasively manipulate neuronal activity. We propose that CNI-lasers can be especially useful in controlling temperature-sensing neurons expressing thermoTRP channels [14], which have much higher thermal sensitivity.

## Materials and Methods

Acutely dissociated hippocampal neurons were collected from 7- to 10-day-old Sprague–Dawley rats (kindly provided by the Animal Center of Dalian Medical University). The procedure for surgery and neuron collection followed that of Kay and Wong [15]. Briefly, the brain was separated and the hippocampus dissected out in cold (5°C) saline that contained: 150 mM NaCl, 5 mM KCl, 1.2 mM  $\text{KH}_2\text{PO}_4$ ,

1.3 mM  $\text{MgSO}_4$ , 2.4 mM  $\text{CaCl}_2$ , 26 mM  $\text{NaHCO}_3$ , and 10 mM Glucose. The tissue was cut into small pieces of about 0.5-mm thickness. After incubation at 32°C for 90 min, Pronase E was added at a concentration of 1.5 g/l to the tissue for 20 min. The tissue was then washed thrice with a Ca-free solution in which  $\text{CaCl}_2$  of the above-mentioned solution was replaced with 2 mM EGTA, followed by washing with the Ca-containing solution thrice. Individual neurons were separated mechanically by lightly beating with a pasteurized glass pipette. All animal experiments were conducted in the Lab of Biomedical Optics, Dalian University of Technology, in accordance with the standards set by the Institutional Animal Care and Use Committee.

The experimental setup is illustrated in Fig. 1a. Electrophysiological recording was done in the whole-cell mode with an EPC-10 amplifier driven by PULSE software (HEKA, Germany). The patch pipette was controlled by a three-dimensional MX7600 motorized micromanipulator with a MC1000e controller (SD Company). The bath solution contained: 150 mM NaCl, 5 mM KCl, 1.1 mM  $\text{MgCl}_2$ , 2.6 mM  $\text{CaCl}_2$ , 10 mM HEPES, and 10 mM Glucose (pH 7.4). The pipette solution contained: 65 mM KCl, 80 mM KF, 5 mM KOH, 2 mM  $\text{Na}_2\text{ATP}$ , 10 mM EGTA, and 10 mM HEPES (pH 7.4). The temperature of the solution in the recording chamber, about 1.5 ml in volume, was controlled by a temperature controller (TC-324B, Warner); variation of the bulk solution temperature was within 0.1°C. The neuron was housed in a recording chamber mounted on top of an Olympus IX 71 inverted microscope. Laser light of 980 nm was generated by a laser unit (LU0980M180, Lumics, Germany) driven by a Laser Diode Combi Controller (ITC510, Thorlabs). The controller was synchronized with the patch-clamp amplifier at a time accuracy level of 100  $\mu\text{s}$ . The CNI-laser light was transmitted to the cell surface through a single-mode optical fiber (125- $\mu\text{m}$  outer diameter, 10- $\mu\text{m}$  core diameter) (Fig. 1b, top panel). The intensity of light output from the fiber tip followed a Gaussian distribution, with most of the light intensity focused within a couple of micrometers in the center [16] (Fig. 1b, bottom panel). In order to make the tip of the optical fiber observable, the cladding of the fiber tip was removed with an optical fiber peeling knife. The optical fiber was mounted on an angle-adjustable universal joint that was controlled by a motorized manipulator (MS314, WPI). Visible light through the same fiber was used to guide the focusing of light onto the neuron. The laser worked in a modulated mode. The modulation signal was the output from the EPC-10 amplifier and the duration of the modulation adjusted by the PULSE software. The total light output was measured by a power meter (PM100 with S140A detector, Thorlabs). A range of 10 to 80 mW was used in these experiments.



**Fig. 1** Simultaneous laser excitation and electrical recording. **a** Schematic illustration of the experimental setup. **b** (top) An image of the fiber tip and a hippocampal neuron recorded with a patch pipette. (bottom) Spatial distribution of the optical power density

Temperature-sensitive transient receptor potential (TRP) channel TRPV1 [17] was expressed in HEK 293 cells using Lipofectamine™ 2000 (GIBCO BRL). Patch-clamp recordings in the cell-free inside-out configuration were done as previously described [18]. Temperature changes were achieved by a temperature controller unit (CL-100,

Warner). A miniature thermistor placed next to the patch pipette tip was used to monitor the temperature changes.

Single-channel analysis was done with the QuB software (<http://www.qub.buffalo.edu/>). Current amplitudes were estimated from amplitude histograms. The mean closed and open times were estimated from the event

distributions using a method developed by Sigworth and Sine [19].  $Q_{10}$  values, defined as the folds change in the reaction rate in response to a 10°C change in temperature, were calculated as  $Q_{10} = (X_2/X_1)^{10/(T_2-T_1)}$ , where  $X_1$  and  $X_2$  are the parameter values at temperature  $T_1$  and  $T_2$ , respectively.

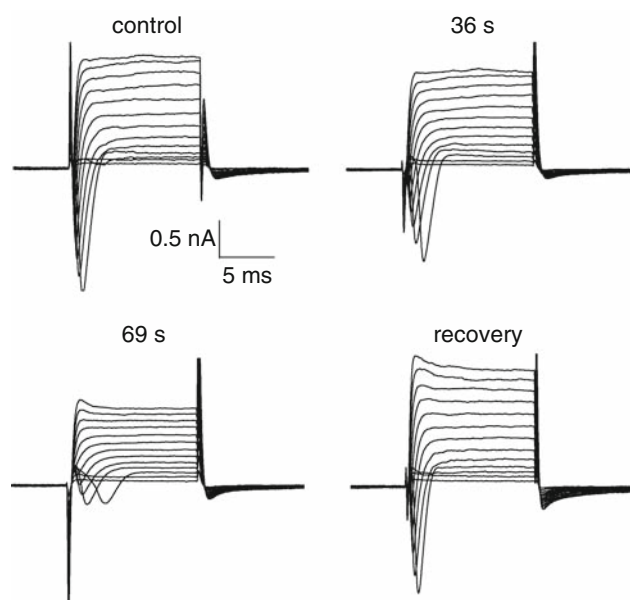
For computer simulation of model ion channels, we chose to use the standard Hodgkin-Huxley model in which the voltage-dependent Na channel undergoes a series of three activation transitions to reach the open state, followed by a single transition to the inactivated state [20]. The rates of these transitions were single-exponential functions of the rate constant for the respective transitions, with the rate constants set to generate current responses that closely reassembled experimental data. For tests of higher temperature sensitivities, the initial rates were set at much reduced levels to allow easy comparison of the channel behavior at different temperatures. All simulations were done using a customized software package generated in the Bers lab [21].

All statistical values are presented as mean  $\pm$  SEM. Statistical significance was defined as  $P < 0.05$  and determined by Student's *t*-test.

## Results

### Brief CNI-laser Irradiation Dramatically Alters Voltage-dependent Na and K Currents in Hippocampal Neurons

In order to test whether a low-power CNI-laser could generate sufficient thermal energy to manipulate neuronal activity, individual hippocampal neurons were irradiated by a 980-nm 30 mW CNI-laser while whole-cell currents elicited by depolarizing voltage steps were monitored. The laser output power density, measured at the fiber tip, was  $10^5$  W/cm<sup>2</sup>. As shown in Fig. 2, both the transient inward voltage-dependent Na and the outward voltage-dependent K currents were clearly observed from these neurons. Upon laser irradiation, the amplitude and kinetics of these currents were changed quickly. Continuous irradiation for 1–2 min almost completely eliminated the Na current and, at the same time, greatly reduced the K current. Upon termination of laser irradiation, the currents recovered close to their initial levels. Similar observations were made in seven out of nine trials. As the laser beam was very focused (Fig. 1b), resulting in a small heated spot of a couple of micrometers in size with an extremely steep temperature gradient, the two failure trials were most likely due to the failure of accurately focusing the laser light on the neuron. Purposely moving the laser beam a few micrometers away from the neuron always eliminated the



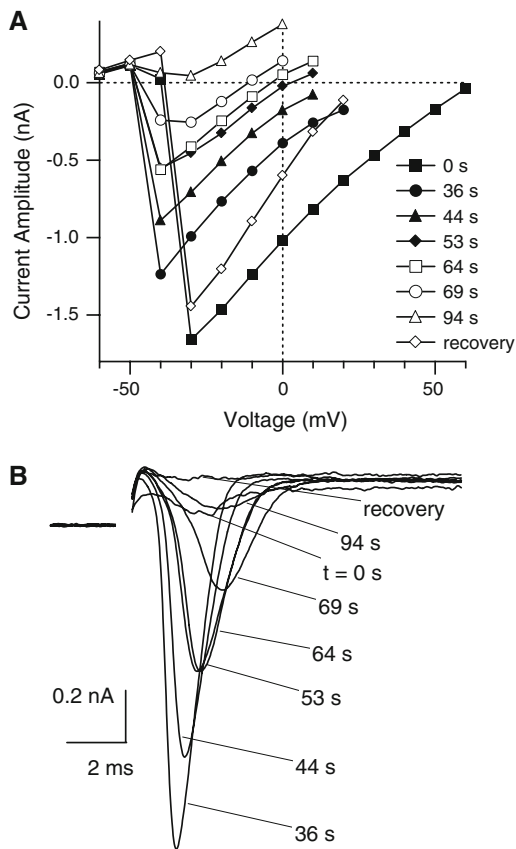
**Fig. 2** Laser irradiation alters voltage-dependent Na and K currents. Whole-cell Na and K currents of a hippocampal neuron in response to voltage steps from a  $-70$  mV holding potential to  $-60$  mV up to  $+50$  mV in 10 mV steps. The duration of laser irradiation is labeled on top of current traces. For this experiment, irradiation lasted for 120 s, and the recovery traces were recorded 130 s after the termination of irradiation

change in currents, confirming that laser-induced heat generation was indeed very localized.

Closer inspection revealed that at least three thermal effects were induced by laser irradiation. First, while the Na current was barely visible at  $-40$  mV at the control condition, it already reached peak at the same voltage after 36 s of irradiation. This shift in the activation voltage can be clearly seen in the Na current *I*-*V* curves (Fig. 3a). It indicates that the activation threshold for Na channels was noticeably shifted. Second, the amplitude of Na current initially increased but quickly became progressively lower upon continuous irradiation. Third, the amplitude of the outward K current also became lower upon prolonged irradiation. These experiments demonstrated that irradiation by a low-power CNI-laser is sufficient to alter neuronal activity.

#### Amplitude Changes in Voltage-dependent Na Currents

Time-dependent changes in the Na current amplitude are shown in Fig. 3b. Upon laser irradiation, the peak current amplitude first increased and then gradually declined. The initial increase may be due to a combination of three potential sources. First, the single-channel conductance of Na channels might increase with the increase in temperature. Second, the channel open probability also might increase with temperature. Third, the voltage dependence



**Fig. 3** Changes in Na currents in response to continuous laser irradiation. **a** Example  $I$ - $V$  relationships of the Na currents before ( $t = 0$  s) and during irradiation, as well as after recovery. The durations of irradiation are shown. **b** Time courses of Na current activation and inactivation at  $-40$  mV are shown at various durations of irradiation

of Na channel activation was shifted to more hyperpolarized voltages, as can be seen in the  $I$ - $V$  relationships (Fig. 3a). The relative contributions of each source could not be accurately determined. Nonetheless, it seemed the shift in voltage dependence played a major role (see below). The subsequent decline in the Na current amplitude occurred at all voltages. It was most likely due to the accumulation of Na channel inactivation upon extended activation.

#### Kinetic Changes in Voltage-dependent Na Currents

In addition to amplitude changes, the kinetics of Na current was also altered by laser irradiation. As can be seen in Fig. 3b, the delay in the onset of Na current, determined as the time between the beginning of depolarization and the peak current amplitude, was initially shortened during the time course when the amplitude increased. Upon longer irradiation, the delay became progressively lengthened. The rates of the raising phase and the decline phase of Na

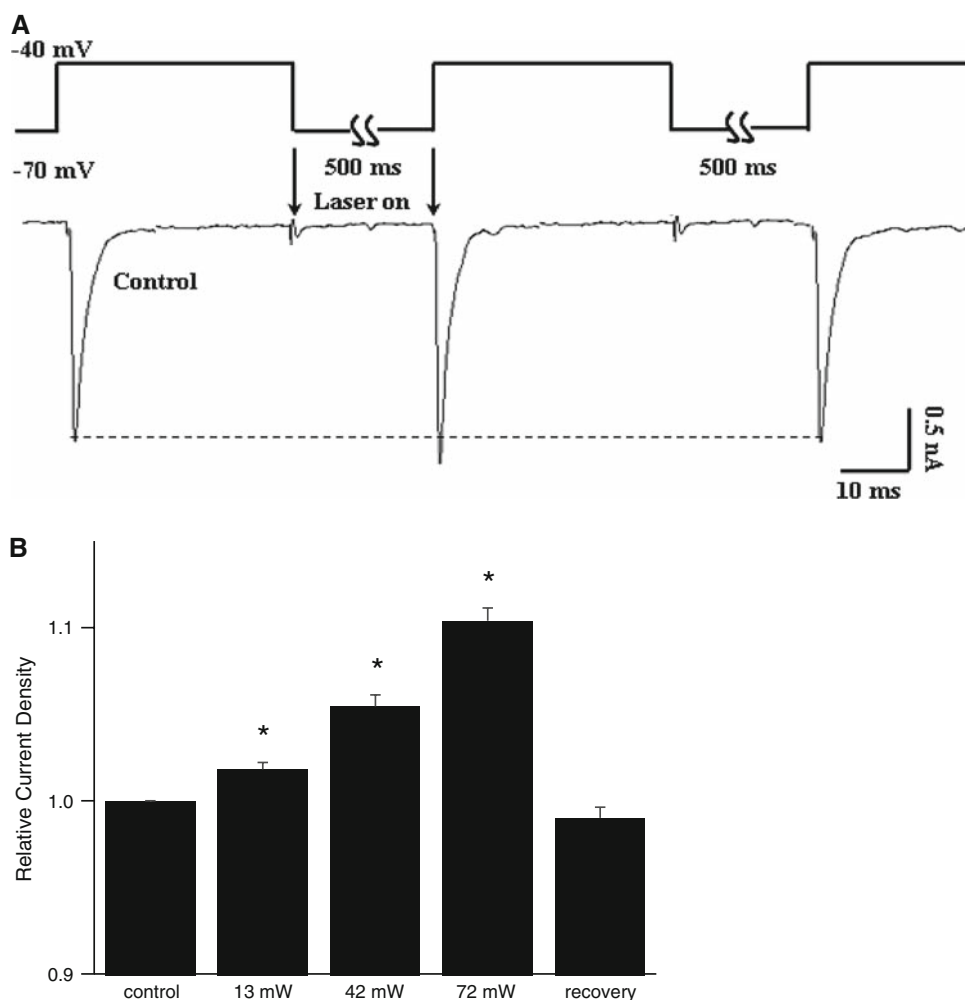
current, judged by the slope of current changes, also exhibited similar patterns, increasing first but then gradually decreasing. These results suggest that both the rates of Na channel activation and inactivation were dramatically affected by the increase in temperature.

To better understand the transient effects of laser irradiation on the Na current, we used tetraethylammonium (TEA) to block voltage-dependent K channels and studied voltage-dependent Na channels with brief laser irradiation. The further TTX block experiment proved that the inward current was TTX-sensitive sodium current. The method was shown at the end of the caption in Fig. 6. As shown in Fig. 4a, a short 500 ms laser irradiation significantly ( $P < 0.05$ ) increased the Na current amplitude. The effect was fully reversible upon termination of the laser irradiation. The mean whole-cell current density before, during, and after laser irradiation was  $180.6 \pm 7.1$ ,  $198.7 \pm 6.8$ , and  $178.2 \pm 6.7$  pA/pF ( $n = 8$ ), respectively. In addition, the increase in current amplitude was dependent on the laser power (Fig. 4b). While the lower power laser light, at 13 mW, increased the Na current amplitude noticeably at the end of the 500 ms irradiation period ( $P < 0.05$ ;  $n = 4$ ), irradiation with higher power laser lights at 42 mW ( $n = 5$ ) and 72 mW ( $n = 4$ ) clearly increased the Na current amplitude much more. The effect was fully reversible even at the highest laser power ( $n = 4$ ). Brief laser irradiation also speeded up the Na current kinetics. The rate of activation, quantified by the time spent between the beginning of depolarization and the peak amplitude, increased by  $0.8 \pm 0.2\%$  ( $P < 0.05$ ;  $n = 4$ ). The rate of inactivation, quantified by fits of the decline phase of the Na currents to a single-exponential function, increased more dramatically, by  $12.1 \pm 5.1\%$  ( $P < 0.05$ ;  $n = 4$ ). These results indicate that brief irradiation with the low-power CNI-laser could generate sufficient heat to increase the activity of Na channels. Sustained activity upon prolonged laser irradiation is expected to lead to the accumulation of channel inactivation and a gradual decrease in Na current, as seen in Figs. 2 and 3.

#### Amplitude Changes in Voltage-dependent K Currents

The voltage-dependent K current was also affected by laser irradiation. As shown by representative  $I$ - $V$  curves (Fig. 5a), the amplitude of K current decreased significantly in the first minute. The decrease, however, slowed at extended laser irradiation (Fig. 5b). In addition, the voltage-dependence of K channel activation appeared to have also changed in response to laser irradiation, as can be seen in the different shapes of the  $I$ - $V$  curves between the control and the subsequent testing sets (Fig. 5a) and the different time courses of current amplitude change at different depolarization levels (Fig. 5b).

**Fig. 4** Changes in Na currents in response to brief laser irradiation. **a** A short 500 ms period of laser irradiation at 72 mW (with the beginning and end of irradiation marked by arrows) reversibly increased the Na current amplitude. Whole-cell Na currents were recorded after K channels were blocked by TEA. **b** Laser power dependence of Na current density increases. An asterisk indicates statistically different from the control ( $P < 0.05$ ; Student's *t*-test)



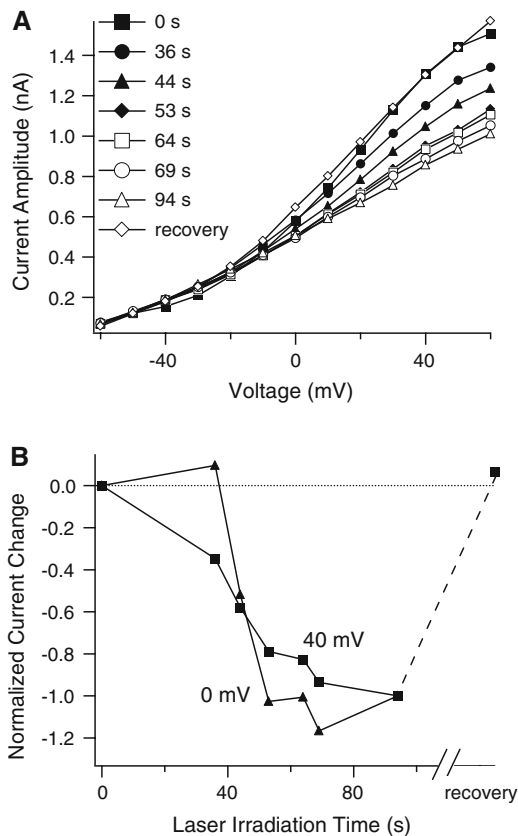
#### Temperature-dependent Changes in Channel Activity

Were changes in macroscopic currents observed in these experiments indeed caused by heat generated by laser irradiation, or by other possible side effects such as photochemical or photomechanical effects? To answer this question, we turned to single-channel recordings. As voltage-dependent Na channels inactivate quickly, yielding mostly a single transient current event upon depolarization, we chose to record from voltage-independent cation channels that are also known to be present in these neurons. Figure 6a shows an example recording trace from a channel with stable persistent activities. By changing the Na ion concentration, the channel was identified as a Na-permeable cation channel. Brief laser irradiation of 500 ms significantly increased the firing rate, which was reversible upon withdrawal of irradiation ( $n = 15$ ). The average channel open probability during irradiation increased by 180%, from  $0.088 \pm 0.010$  to  $0.248 \pm 0.035$  ( $P < 0.05$ ;  $n = 10$ ). Partial recovery was already observed during the first 500 ms period following the termination of laser irradiation ( $0.180 \pm 0.026$ ,  $n = 10$ ).

If the increase in channel activity was indeed due to heat generation by laser irradiation, raising the temperature of the solution that bathing the channel should give rise to a similar increase. We recorded single-channel activities at temperatures ranging from 15 to 29°C. As shown in Fig. 6b, channel opening was strongly enhanced by raising the temperature. Single-channel analysis revealed that both the mean open time and the mean closed time were shortened at higher temperatures (Fig. 6c). Nonetheless, the mean open probability increased at higher temperatures, due to more frequent firing (Fig. 6d). The  $Q_{10}$  value for the mean open probability was estimated to be 1.9. Using this  $Q_{10}$  value, we estimated that the temperature increase at the single neuron during 500 ms laser irradiation was about 14°C. In addition, we noticed that the single-channel amplitude also increased at higher temperatures (Fig. 6b), with a  $Q_{10}$  value of 1.7 (Fig. 6e).

#### Computer Simulation

We used a computer simulation approach to further confirm that raising the temperature of this amplitude could



**Fig. 5** Changes in K currents in response to continuous laser irradiation. **a** Example  $I$ - $V$  relationships of K currents before ( $t = 0$  s) and during irradiation, as well as after recovery. The durations of irradiation are shown. **b** Time course of the current change at two voltages

indeed generate changes in channel activity as we observed in hippocampal neurons. For this purpose, a standard Hodgkin-Huxley model for voltage-dependent Na channel was used [20]. We assumed the values of  $Q_{10}$  for the single-channel conductance, activation, and inactivation of the voltage-dependent Na channel to be 1.35, 2.3, and 3.4, respectively, as previous studies of related channels have suggested [22]. Raising the temperature by a modest amount of 5°C, from 25 to 30°C, produced a noticeable increase in the Na channel amplitude and significantly faster channel activation and inactivation time courses (Fig. 7a). As a consequence of these changes, the  $I$ - $V$  curves shifted to the left (Fig. 7b), as we observed from neuronal recordings. It is also obvious from the  $I$ - $V$  curves that at more depolarized voltages where the channel was fully activated, accumulation of inactivation became prominent, leading to a decline in the current amplitude.

The simulations shown in Fig. 7a and b assumed normal  $Q_{10}$  values commonly observed in many ion channel types. Sensory neurons generally have much higher temperature sensitivity. Temperature-sensitive ion channels of the TRP channel family, e.g., exhibit exceptionally high  $Q_{10}$  values

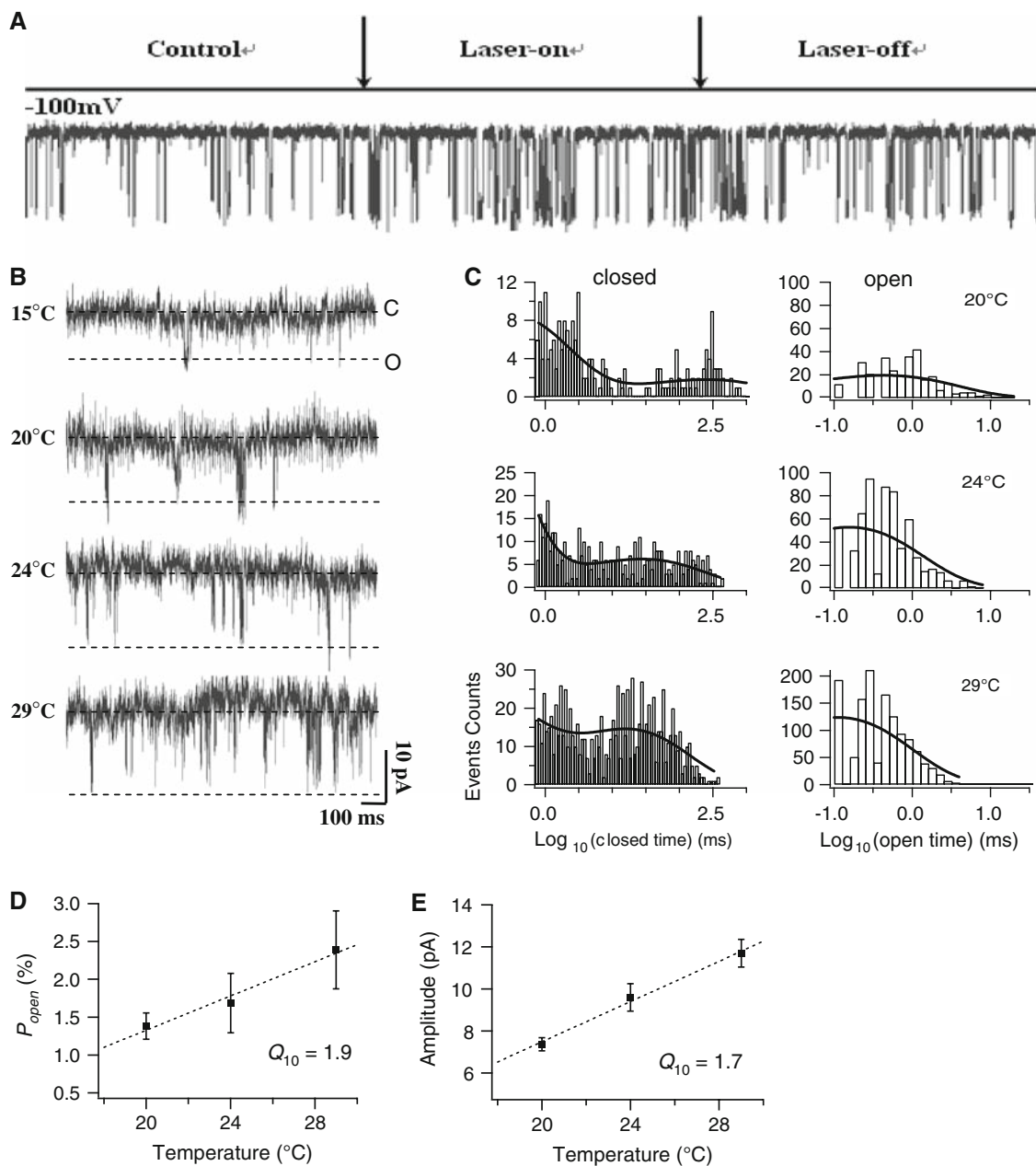
of 10 to 30 [23]. In order to predict the response of these channels to temperature increases, we used the same model channel but increased the  $Q_{10}$  values for activation and inactivation by tenfold, to 23 and 34, respectively, while keeping the  $Q_{10}$  value for conductance unchanged. As shown in Fig. 7c, the channel response to a 5°C temperature increase was indeed dramatically enhanced. In particular, the current rising and decline phases were both noticeably faster at the higher temperature. The high-temperature sensitivity is consistent with experimental data from TRPV1 channels recorded in a similar temperature range (Fig. 7d).

## Discussion

In this study, we demonstrated that brief irradiation with a low-power CNI-laser significantly increased the activity of neuronal voltage-dependent Na and K channels in a time- and intensity-dependent manner. A moderate increase in temperature of about 14°C could be achieved locally at the site of a single neuron, which resulted in transient and reversible activation of ion channels. Sustained irradiation led to gradual accumulation of channel inactivation and inhibition of neuronal activity. These results confirmed that CNI-lasers can be used as a powerful tool to non-invasively manipulate neuronal activity.

Within neuronal tissues, the major constituents responsible for capturing infrared light energy and converting it into heat are water, lipids, and to a less extent, protein molecules [24]. Although the overall absorption efficiencies of lipids and water are comparable in the near-infrared range, they differ greatly in their wavelength dependence [25]. At 920 nm, lipids have much higher absorption efficiency than water. Application of laser light at this wavelength to neuronal tissues would lead to selective heating of the cell membrane system. Water, on the other hand, has a major absorption peak at 970 nm where lipids absorption is minimal. Irradiation at this wavelength will heat water molecules without directly heating lipids and proteins. As the water temperature increases, the biomolecules bathed in water will experience a similar temperature increase. Application of CNI-laser lights at or near 970 nm to neuronal tissues is expected to result in homogenous temperature increase throughout the neuron and, at the same time, avoids non-reversible heat damage to biological macromolecules.

While the heat generation efficiency is 100–1000 times lower in the near-infrared range comparing to the mid- and far-infrared range, the resultant temperature increase is still significant for changing neuronal activity without possible advert damaging effects to biological tissues. Our results demonstrated that CNI-lasers produce sufficient heat for

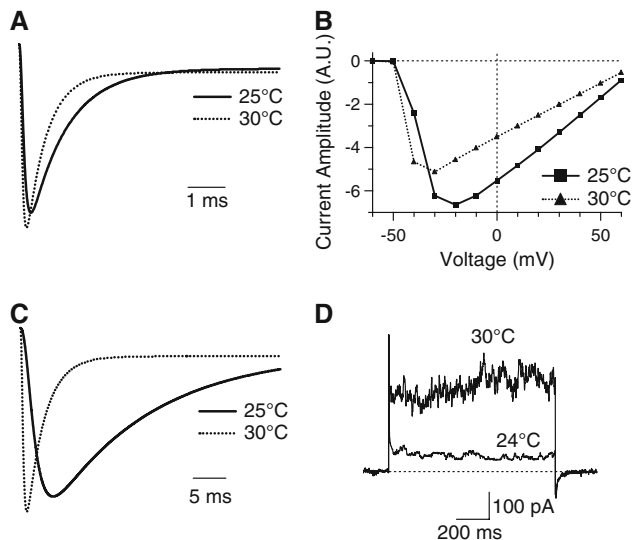


**Fig. 6** Temperature-dependent changes in channel activity. **a** A representative single-channel trace recorded at  $-100$  mV before (control), during (laser-on), and after (laser-off) irradiation. **b** Increasing single-channel open probability and current amplitude at higher temperatures. **c** Distribution of the closed (left) and open (right) times at three temperatures. **d** Temperature dependence of the mean open probability. **e** Temperature dependence of the single-

channel current amplitude. *Method:* Because TTX is an outside blocker, we added  $1 \mu\text{l}$  TTX on the top-layer of the pipette solution. It takes about 10–15 minutes to diffuse to the tip of the pipette. This provides an enough time span to examine the observed single sodium channel current in cell-attached recording model. The final concentration of TTX in the pipette was around  $10^{-5}$  M

manipulating neuronal activity. The local temperature increase achieved by the low-power CNI-laser (in the vicinity of  $10^\circ\text{C}$ ) is sufficient for reversibly manipulating neuronal activity. The lower heat conversion efficiency is not a limiting factor. It is arguably preferable for practical reasons. To selectively excite a neuron using a focused low-power CNI-laser beam, only a small, micrometer-size

spot is heated. With the steep temperature gradient associated with such a small spot, it is expected that the local temperature should quickly rise and reach the equilibrium temperature. During extended irradiation, the local temperature should remain stable at this equilibrium level as long as the continuous energy input is present. The temperature is expected to drop quickly back to the same level



**Fig. 7** Theoretical calculations suggest large changes in Na and K channel activity in response to a 5°C temperature increase. **a** Current responses of ideal Hodgkin-Huxley type Na channels upon depolarization simulated at 25°C (solid trace) and 30°C (dotted trace). **b** Na channel  $I$ - $V$  relationships at different temperature levels. **c** Current responses of model channels with ten times higher  $Q_{10}$  values. As changes in activation and inactivation were much greater at elevated  $Q_{10}$  values, the initial rate values at 25°C were set to be much lower than those used in (a). **d** Example current traces from temperature-sensitive TRPV1 channels activated by depolarization pulses to +80 mV from a -80 mV holding potential at 24 and 30°C

as the surrounding tissue and the bulk solution upon termination of laser irradiation. This is advantageous comparing to higher power lasers that would generate a large amount of heat and globally alter the tissue temperature.

Cellular processes are in general temperature sensitive. Simple thermodynamic processes such as ion diffusion and hydrogen bond formation have a  $Q_{10}$  value of 1–2 [26]. As a result, the speed of these processes about doubles when temperature is increased by 10°C. Enzymatic activities of biological molecules such as proteins have relatively higher  $Q_{10}$  values [27–29]. For example, voltage-dependent Na channels of rabbit muscle activate 2.3 times faster and inactivate 3.4–9.1 times faster upon a 10°C temperature increase in the range of 0 to 22°C [22]. Within a similar temperature range, the rates of *Myxicola* axon voltage-dependent K channel activation and inactivation were estimated to be 2.4 and 3.0 times, respectively, faster [30]. Assuming similar  $Q_{10}$  values for the rat hippocampal neuron voltage-dependent Na and K channels, we expect their activities to increase by 52–202% (for Na channel) and 55–73% (for K channel) upon 500 ms irradiation of the CNI-laser used in this study. This is consistent with our observations reported here.

Although this study demonstrated that low-power CNI-lasers are useful in manipulating activities of hippocampal neurons, the approach should be applicable to other neuron

types as well. In particular, laser irradiation may be especially powerful for the study of temperature-sensitive neurons in dorsal root ganglia (DRG). These are sensory neurons that receive information from peripheral tissues such as skin where the ambient temperature is detected. Recently it is found that a group of ion channels belonging to the TRP channel family are highly expressed in DRG temperature-sensing neurons [14, 31]. These so-called thermoTRP channels exhibit exceptionally high-temperature sensitivities, with  $Q_{10}$  values in the 10–30 range [32, 33]. Laser irradiation provides a versatile tool to selectively and reversibly excite these sensory neurons in intact preparations for the study of temperature detection and neuronal coding of temperature information.

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