



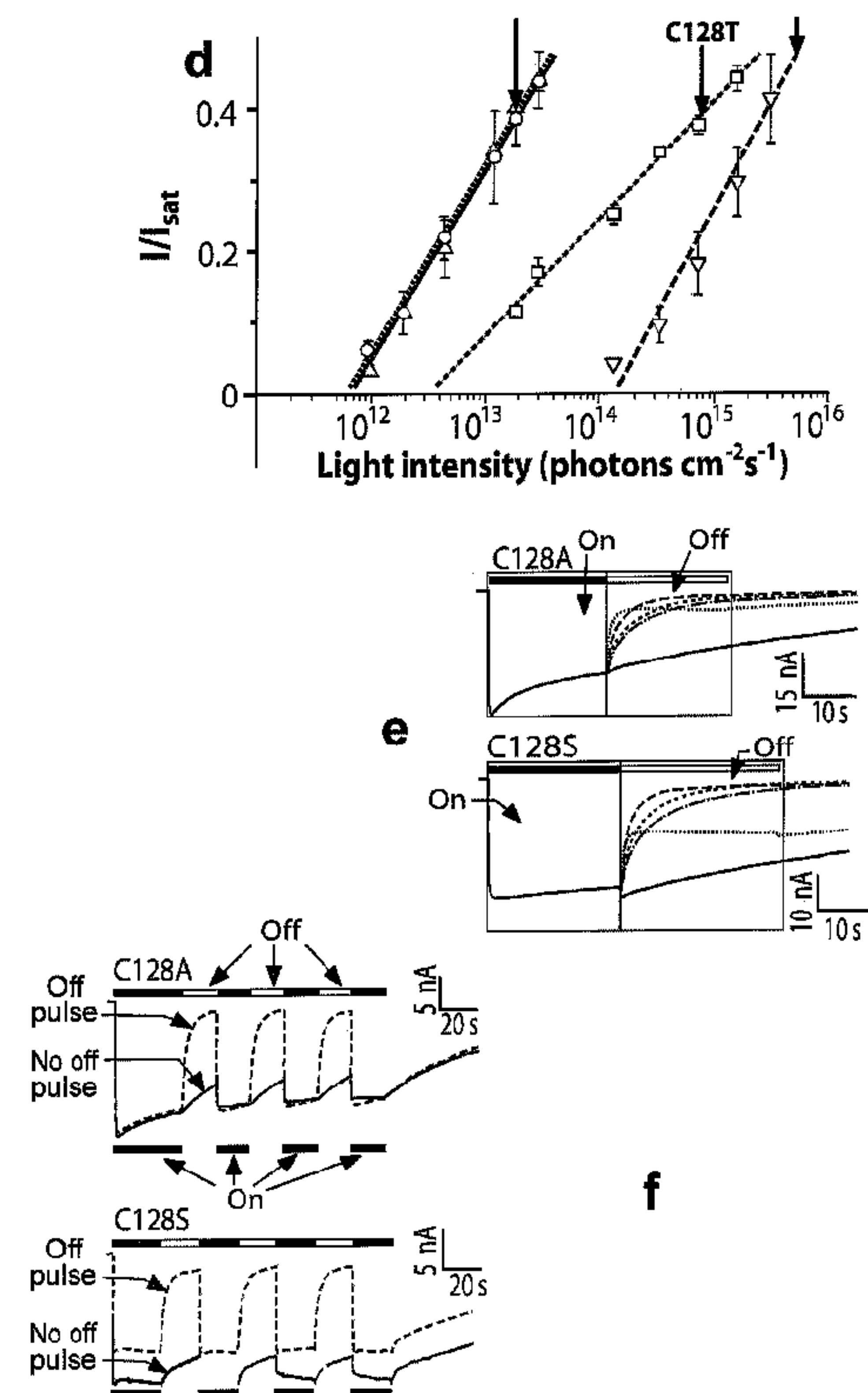
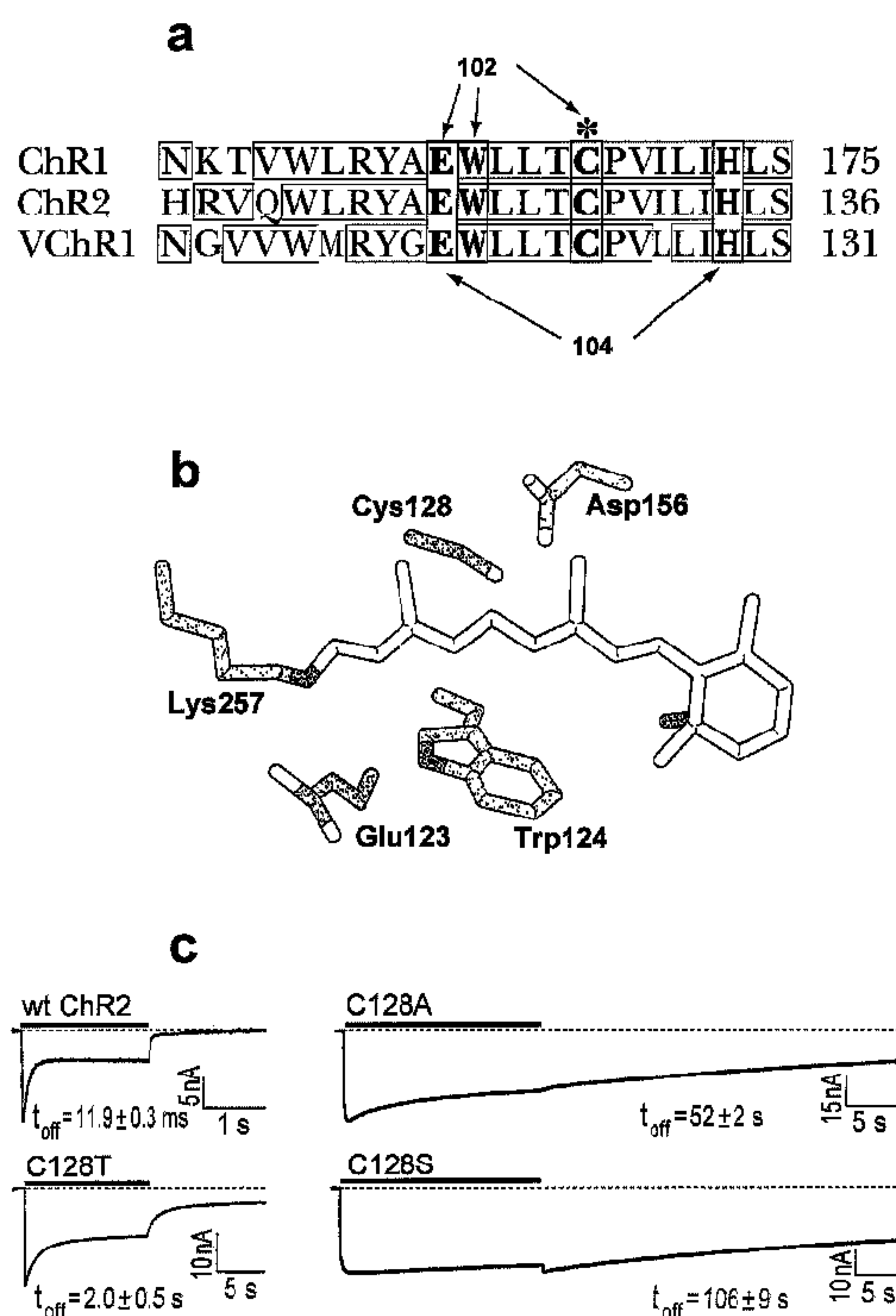
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(54) **Titre : STIMULATION OPTIQUE DE CELLULES CIBLES ET MODIFICATIONS DE CELLES-CI**
(54) **Title: OPTICALLY-BASED STIMULATION OF TARGET CELLS AND MODIFICATIONS THERETO**



(57) **Abrégé/Abstract:**

Stimulation of target cells using light, e.g., in vivo or in vitro, is implemented using a variety of methods and devices. One example involves a vector for delivering a light-activated molecule comprising a nucleic acid sequence that codes for light-activated molecule. The light-activated molecule includes a modification to a location near the all- trans retinal Schiff base, e.g., to extends the duration time of the open state. Other aspects and embodiments are directed to systems, methods, kits, compositions of matter and molecules for ion channels or pumps or for controlling currents in a cell (e.g., in in vivo and in vitro environments).

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FIG. 1a

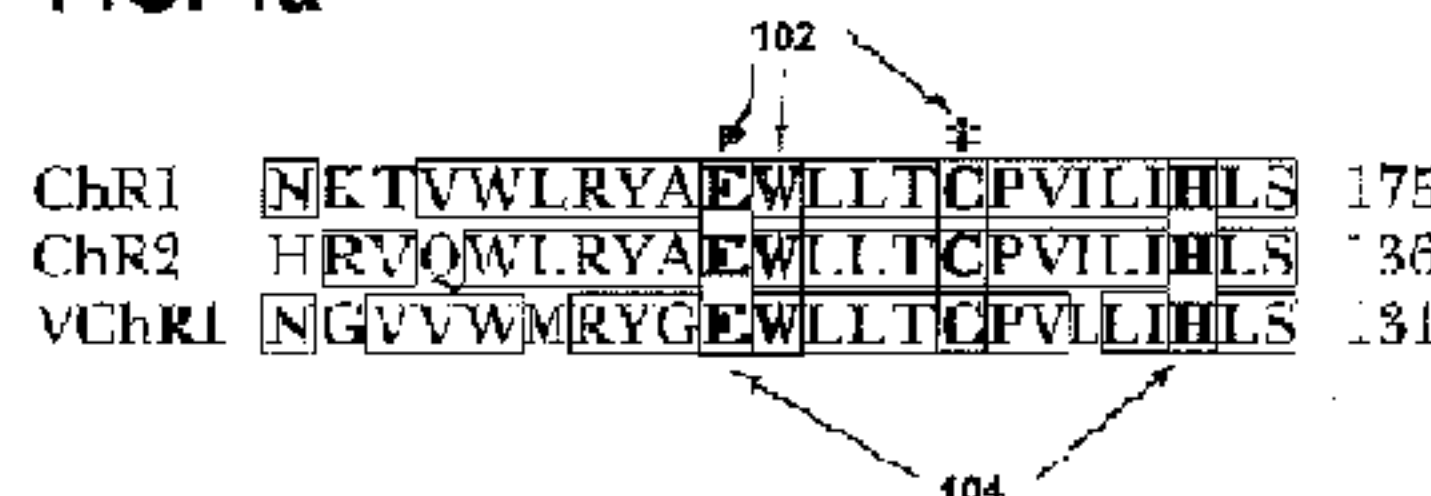


FIG. 1b

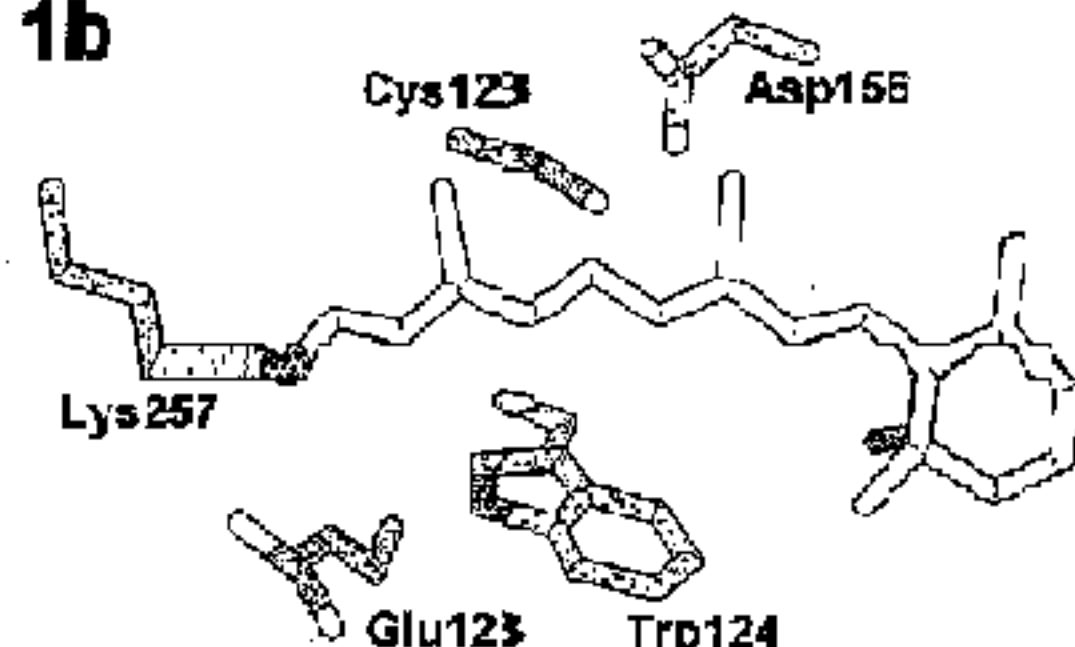


FIG. 1c

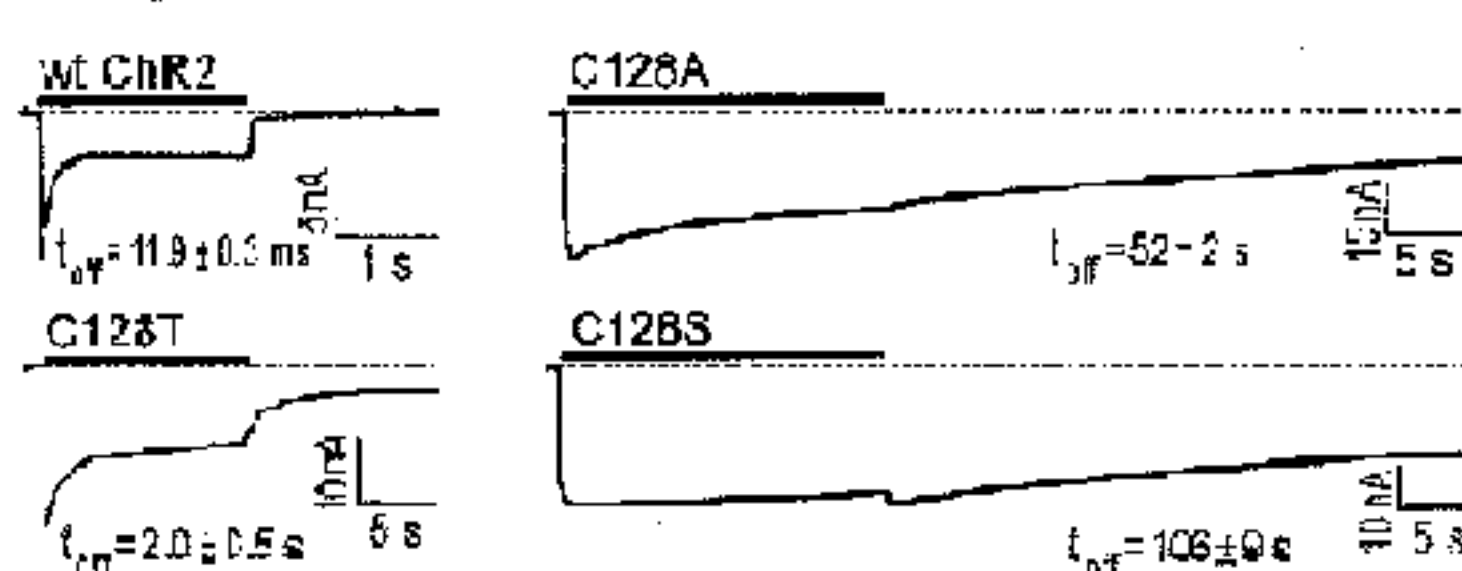


FIG. 1d

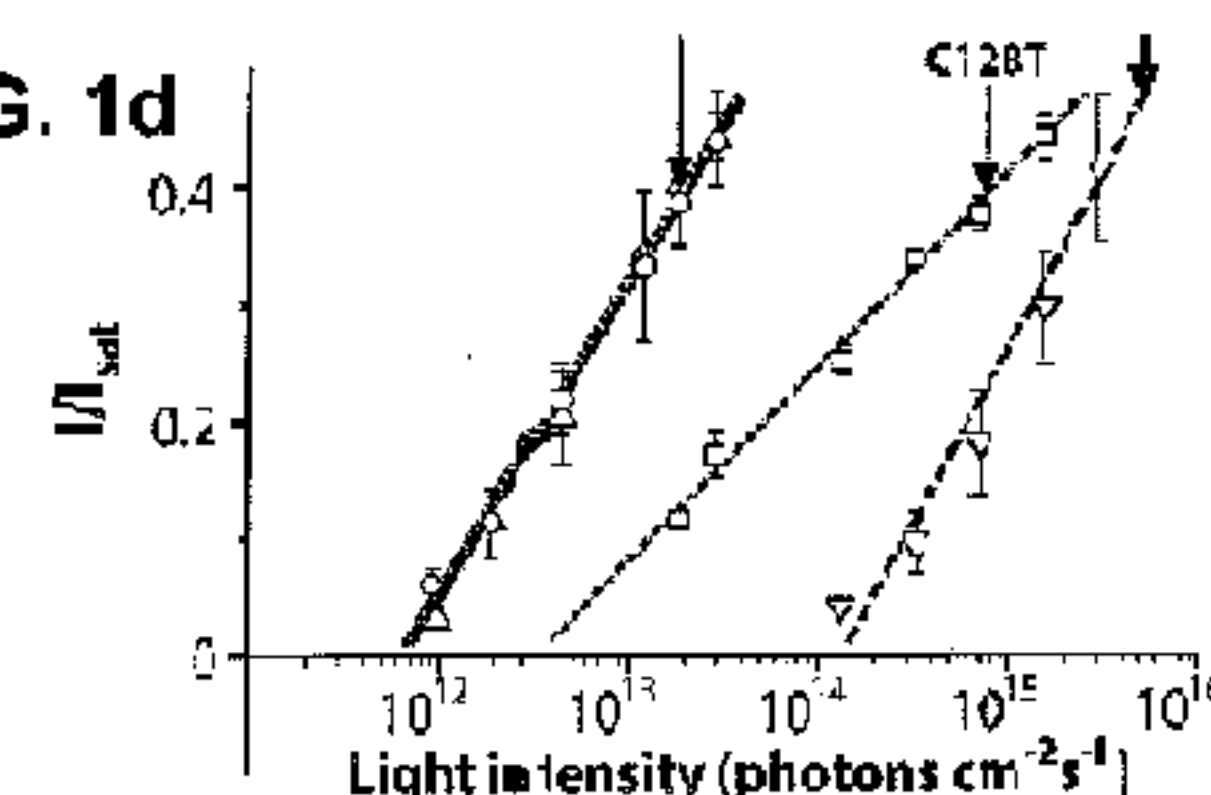


FIG. 1e

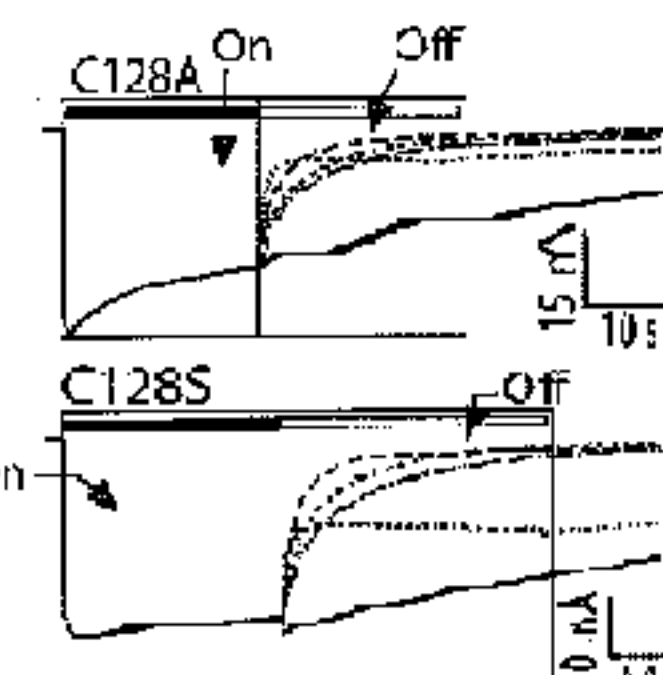
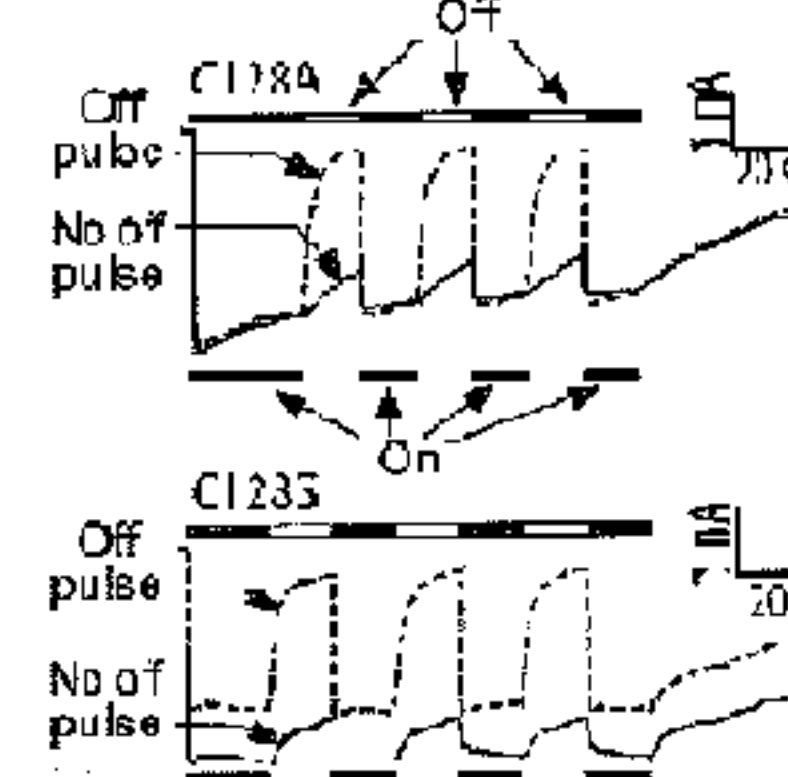


FIG. 1f



(57) **Abstract:** Stimulation of target cells using light, *e.g.*, *in vivo* or *in vitro*, is implemented using a variety of methods and devices. One example involves a vector for delivering a light-activated molecule comprising a nucleic acid sequence that codes for light-activated molecule. The light-activated molecule includes a modification to a location near the all- trans retinal Schiff base, *e.g.*, to extends the duration time of the open state. Other aspects and embodiments are directed to systems, methods, kits, compositions of matter and molecules for ion channels or pumps or for controlling currents in a cell (*e.g.*, in *in vivo* and *in vitro* environments).

**OPTICALLY-BASED STIMULATION OF TARGET CELLS AND
MODIFICATIONS THERETO**

Sequence Listing

This description contains a sequence listing in electronic form in ASCII text format. A
5 copy of the sequence listing in electronic form is available from the Canadian Intellectual
Property Office.

Field of the Invention

The present invention relates generally to systems and approaches for stimulating
target cells, and more particularly to using optics to stimulate the target cells.

20

Background and Summary

The stimulation of various cells of the body has been used to produce a number of
beneficial effects. One method of stimulation involves the use of electrodes to introduce
an externally generated signal into cells. One problem faced by electrode-based brain
25 stimulation techniques is the distributed nature of neurons responsible for a given mental
process. Conversely, different types of neurons reside close to one another such that only
certain cells in a given region of the brain are activated while performing a specific task.
Alternatively stated, not only do heterogeneous nerve tracts move in parallel through tight
spatial confines, but the cell bodies themselves may exist in mixed, sparsely embedded
30 configurations. This distributed manner of processing seems to defy the best attempts to
understand canonical order within the central nervous system (CNS), and makes
neuromodulation a difficult therapeutic endeavor. This architecture of the brain poses a
problem for electrode-based stimulation because electrodes are relatively indiscriminate
with regards to the underlying physiology of the neurons that they stimulate. Instead,
35 physical proximity of the electrode poles to the neuron is often the single largest

determining factor as to which neurons will be stimulated. Accordingly, it is generally not feasible to absolutely restrict stimulation to a single class of neuron using electrodes.

Another issue with the use of electrodes for stimulation is that because electrode placement dictates which neurons will be stimulated, mechanical stability is frequently
5 inadequate, and results in lead migration of the electrodes from the targeted area. Moreover, after a period of time within the body, electrode leads frequently become encapsulated with glial cells, raising the effective electrical resistance of the electrodes, and hence the electrical power delivery required to reach targeted cells. Compensatory
10 increases in voltage, frequency or pulse width, however, may spread the electrical current and increase the unintended stimulation of additional cells.

Another method of stimulus uses photosensitive bio-molecular structures to stimulate target cells in response to light. For instance, light activated proteins can be used to control the flow of ions through cell membranes. By facilitating or inhibiting the flow of positive or negative ions through cell membranes, the cell can be briefly
15 depolarized, depolarized and maintained in that state, or hyperpolarized. Neurons are an example of a type of cell that uses the electrical currents created by depolarization to generate communication signals (*i.e.*, nerve impulses). Other electrically excitable cells include skeletal muscle, cardiac muscle, and endocrine cells. Neurons use rapid depolarization to transmit signals throughout the body and for various purposes, such as
20 motor control (*e.g.*, muscle contractions), sensory responses (*e.g.*, touch, hearing, and other senses) and computational functions (*e.g.*, brain functions). Thus, the control of the depolarization of cells can be beneficial for a number of different purposes, including (but not limited to) psychological therapy, muscle control and sensory functions.

Depending upon the application, particular characteristics of the responsiveness of
25 the electrical stimulus and/or current flow can be important. Example characteristics include the duration the electrical current continues after light stimulus has been removed, delays between the light stimulus and the beginning of the flow of ions and the intensity or wavelength of the light necessary to cause (or inhibit) ion flow.

Summary

Various aspects of the present disclosure are directed to devices, methods and systems related light-activated proteins in a manner that addresses challenges including those discussed above.

The present disclosure is directed to step-function opsins (SFOs) that provide relatively long on-times in response to light at a first wavelength. These SFOs can also respond to light of a second wavelength by turning-off, thereby functioning as a bi-stable switch.

Consistent with certain embodiments, one or more SFOs function as light-gated membrane channels when expressed in a neuronal cell. Activation of the SFOs moves the membrane voltage/resting potential of the neuronal cell towards the action potential threshold of the cell (e.g., depolarizes the cell), thereby facilitating action potentials therein.

Other aspects of the present disclosure are directed towards use of SFOs to characterize or treat diseases associated with neurology or the central nervous system (CNS). Particular aspects relate to use of SFOs to provide targeted excitation of neural populations for treatment or characterization of diseases. Other aspects relate to characterizations of neural circuitry and, in some cases, related behavioral responses.

Other aspects of the present disclosure are directed toward mutations/substitutions of amino acids of opsins. This can include molecules coding for the mutant opsin and/or the mutant opsin itself. A particular example includes substitutions that affect the on-time and/or the on-current of the opsins. For instance, substitutions can be made to ChR2 or VChR1. In a particular implementation this can include, using ChR2 as example, substitutions at C128 and or D156. Homologous substitutions can be made to VChR1. These and other substitutions can be used alone or in combinations.

Other aspects of the present disclosure are directed toward a medicament for treatment of a neurological or CNS-based disease. The medicament is designed to introduce a mutant opsin to a patient. The introduced opsin can then be controlled through the application of light thereto as part of a treatment regimen.

Other aspects of the present disclosure are directed toward expression of multiple opsin-types within different neural populations and/or within the same cell. In one implementation, the opsins-types have respectively different responsiveness to light frequency/wavelengths, thereby allowing for individual control of each type through wavelength control of the stimulating light. In some implementations, the opsin-types have different temporal properties, different conductive properties and/or hyperpolarize or depolarize, respectively.

Other aspects of the present disclosure are directed to treatment of a disorder using SFOs or inhibitory molecules to selectively encourage or inhibit neurons. Such treatment targets a group of neurons associated with the disorder; and in this group, may include engineering an inhibitory

protein/molecule that uses an endogenous cofactor to respond to light by producing an inhibitory current to dissuade depolarization of the neurons. This aspect may include engineering SFOs in neurons, of the same group and/or of a different group. The engineered neurons are then exposed to light, thereby dissuading and/or encouraging depolarization of the neurons.

The claimed invention pertains to a light-responsive ion channel comprising an amino acid sequence having at least 90% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2, wherein the light-responsive ion channel exhibits an extended conducting or non-conducting state after exposure to a light pulse compared to that of a polypeptide comprising SEQ ID NO:1 or SEQ ID NO:2, and wherein the light-responsive ion channel comprises a Cys→Thr, a Cys→Ala, or a Cys→Ser substitution at a position corresponding to amino acid 128 of SEQ ID NO:1 or amino acid 123 of SEQ ID NO:2, and an Asp→Ala or an Asp→Asn substitution at a position corresponding to amino acid 156 of SEQ ID NO:1 or amino acid 151 of SEQ ID NO:2. The claimed invention also pertains to a nucleic acid that encodes such a light-responsive ion channel protein as well as mammalian neuronal cells comprising such a nucleic acid.

The claimed invention also pertains to a system for controlling action potential of a neuron, the system comprising: a) a delivery device for delivery of a light-responsive ion channel protein or a nucleic acid encoding the protein to the neuron, wherein the delivery device comprises said light-responsive ion channel protein or nucleic acid encoding the protein, and wherein said protein comprises an amino acid sequence having at least 90% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2, wherein the light-responsive ion channel exhibits an extended conducting state after exposure to a light pulse compared to that of a polypeptide comprising SEQ ID NO:1 or SEQ ID NO:2, and wherein the light-responsive ion channel comprises a Cys→Thr, a Cys→Ala, or a Cys→Ser substitution at a position corresponding to amino acid 128 of SEQ ID NO:1 or amino acid 123 of SEQ ID NO:2, and an Asp→Ala or an Asp→Asn substitution at a position corresponding to amino acid 156 of SEQ ID NO:1 or amino acid 151 of SEQ ID NO:2; b) a light source; and c) a control device for controlling generation of light by the light source.

The claimed invention also pertains to an *in vitro* method of stimulating a neuron, comprising exposing the neuron to light to thereby stimulate the neuron, wherein the neuron expresses a responsive ion channel or a light-responsive ion channel encoded by a nucleic acid as claimed herein.

The claimed invention also pertains to use of a light delivery device for delivering light to a target cell, wherein the target cell expresses a light-responsive ion channel as claimed herein, wherein said delivery of light effects depolarization of the target cell.

The claimed invention also pertains to use of a nucleic acid as claimed herein which encodes a light-responsive ion channel, for expressing the light-responsive ion channel in a target cell.

The above summary is not intended to describe each embodiment or every implementation of the present disclosure. The figures and detailed description that follow more particularly exemplify various embodiments.

Brief Description of the Drawings

The invention may be more completely understood in consideration of the detailed description of various embodiments of the invention that follows in connection with the accompanying drawings, in which:

FIG. 1a shows the alignment of Helix 3 of several channelrhodopsins relative to bacteriorhodopsin (BR) from *H. salinarum*. Conserved residues are shown by a highlighted background, amino acids interacting with the chromophore are indicated by 102, and the ChR2 C128 is marked by an asterisk (*). Amino acids that serve as H⁺ donor or acceptor for RSB deprotonation and reprotonation are indicated by 104.

FIG. 1b shows a ChR2 chromophore model, based on the BR X-ray structure (1KGB13), with E123, C128, and D156 of ChR2 replacing D85, T90, and D118 of BR.

FIG. 1c shows photocurrents recorded from ChR2 wild type (wt), C128T, C128A and C128S expressed in *Xenopus* oocytes at 100 mM NaCl, pH 7.4 and -50 mV in response to 450 nm light pulses, 240 mW cm⁻². Time constants shown are for the decay of current after termination of blue light stimulation (mean ± s.e.m.; n = 3 cells for each trace).

FIG. 1d shows the light dependence of the steady-state photocurrents recorded at low light intensities; cells expressing C128A and S are ~300-fold more sensitive than

those that express wt ChR2. Amplitudes were normalized to the response at saturating light (I/I_{Sat}).

FIG. 1e shows photocurrents recorded from C128A and C128S mutants. Off kinetics were accelerated when a second (off) light pulse with longer wavelength followed the excitation (on) pulse (traces: 530nm; 546nm; 570 nm; 600 nm).

FIG. 1f shows responses to alternating 450 nm and 546 nm light pulses (Off pulse) or 450 nm light pulses only (No off pulse) in oocytes expressing C128A and C128S. Bars on top and bottom indicate light stimulation protocols for alternating blue/green (On/Off) and blue-only (On-only) traces respectively.

FIG. 2a shows confocal images of cultured hippocampal neurons expressing wt ChR2, C128S, C128A and C128T under the control of the α CaMKII promoter, with intensity scaling and pixel size are identical in all images and scale bar of 25 μ m.

FIG. 2b shows a summary of photocurrents recorded from neurons expressing wt ChR2 and mutants, shown as mean $\pm$ s.e.m (n= 8, 11, 9 and 10 for wt, C128S, C128A and C128T, respectively). Cells were stimulated with a single 10ms pulse of 470nm blue light.

FIG. 2c shows depolarization induced by ChR2 mutants. Voltage recordings were made in neurons expressing C128S, A and T during an identical stimulation protocol as in FIG. 2b. Peak depolarization levels were averaged from 3, 7 and 7 cells for C128S, C128A and C128T, respectively.

FIG. 2d shows a summary of depolarization in C128A and C128S mutants in response to 470-nm light pulses of varying lengths (data are averaged from at least 3 cells for each pulse length).

FIG. 2e shows an expanded view of photocurrents evoked by a 10ms pulse of 470nm blue light in neurons expressing wt ChR2, C128S, C128A, and C128T, showing slower on-kinetics of the mutants.

Fig. 2f shows a summary of on-kinetics in response to 10ms blue light stimulation. Shown are mean time constants from exponential fits to current traces; while onset kinetics of C128S and C128A are similar in FIG. 2e traces, C128S was typically slower than C128A as summarized here.

FIG. 2g shows slower decay time constants of photocurrents in the C128 mutants. Traces are normalized to the peak photocurrent in each mutant.

FIG. 2h shows a summary of off-kinetics in C128 mutants. Mean time constants were derived from exponential fits.

FIG. 3a shows whole-cell current clamp recording from a cultured rat hippocampal neuron expressing C128S under the α CaMKII promoter. Sub-threshold depolarization was induced by a single 10ms pulse of 470 nm light (top trace; unboxed dash indicates time of stimulus) or by a series of 100Hz trains consisting of 20 5-ms pulses of 470 nm light (bottom trace, each train is indicated by an unboxed dash, boxed dashes represent green light).

FIG. 3b shows whole-cell current clamp recording from a hippocampal neuron expressing C128S stimulated with pairs of 470- and 535-nm light stimuli. The top trace shows the response to 10ms blue (unboxed dashes) and 10 ms green light (boxed dashes), and the bottom trace shows the response to 10 ms blue and 50ms green light. Stimulus pairs were given at 20s intervals, and the interval within each stimulus pair was 5s.

FIG. 3c shows magnified traces from the bottom stimulus pair in FIG. 3b showing complete inactivation with 50ms green light. Resting membrane potential is indicated by broken line.

FIG. 3d shows, on the left, whole-cell current clamp recording from a hippocampal neuron expressing C128A stimulated with a pre-recorded EPSP trace.

FIG. 4a shows excitation spectra for ChR2, ChR2 (C128A/H134R), VChR1 and VChR1 (C123S), and more particularly, FIG. 4b shows that ChR2 (C128A/H134R) maintains a shifted spectra relative to VChR1 (C123S).

FIG. 4b shows inactivation spectra for ChR2 (C128A/H134R) and VChR1 (C123S), and more particularly, FIG. 4b shows that ChR2 (C128A/H134R) maintains a shifted spectra relative to VChR1 (C123S).

FIG. 4c shows peak current size and on- and off-kinetics (*e.g.*, the time from the initial light the corresponding (de)activation of the channels) for VChR1 SFO and ChR2 gain-of-function (GF) SFO (C128A/H134R).

FIG. 5a shows current recording from a cell expressing ChR2 (C128A/H134R) and particularly a 200pA photocurrent in response to a 10ms on/blue light pulse, decaying slowly to baseline.

FIG. 5b shows a voltage recording from the same cell as in FIG. 5a, showing the response of the cell to repeated delivery of pairs of 10ms 470nm light (on/blue pulses) and 100ms 560nm light (off/green pulses).

FIG. 6 shows a current recording in a cell expressing ChR2 (C128S/D156A) showing the slow kinetics in response to an on-pulse.

While the invention is amenable to various modifications and alternative forms, specifics thereof have been shown by way of example in the drawings and will be described in detail. It should be understood, however, that the intention is not to limit the invention to the particular embodiments described. On the contrary, the intention is to cover all modifications, equivalents, and alternative falling within the scope of the invention.

Detailed Description

10 The present invention is believed to be useful for facilitating practical application of a variety of photosensitive bio-molecular structures, and the invention has been found to be particularly suited for use in arrangements and methods dealing with cellular membrane voltage control and stimulation. While the present invention is not necessarily limited to such applications, various aspects of the invention may be appreciated through
15 a discussion of various examples using this context.

Consistent with one example embodiment of the present invention, a light-responsive protein/molecule is engineered in a cell. The protein affects a flow of ions across the cell membrane in response to light. This change in ion flow creates a corresponding change in the electrical properties of the cells including, for example, the
20 voltage and current flow across the cell membrane. In one instance, the protein functions *in vivo* using an endogenous cofactor to modify ion flow across the cell membrane. In another instance, the protein changes the voltage across the cell membrane so as to dissuade action potential firing in the cell. In yet another instance, the protein is capable of changing the electrical properties of the cell within several milliseconds of the light
25 being introduced. Embodiments of the present invention relate to specific mutations of such light-activated proteins/molecules. These mutations include substitutions of one or more amino acids within the protein thereby producing surprising results as evidenced by the experimental data provided herein. These substitutions can be implemented by modifying a nucleotide sequence for coding a protein/molecule. Certain implementations
30 relate to designing the nucleotide sequence for expression in a mammalian neuronal cell.

For details on delivery of such proteins, reference may be made to U.S. Patent Publication No. 2007/0261127 entitled "Light-Activated Cation Channel and Uses Thereof".

Aspects of certain embodiments of the present invention are directed toward
5 identification and modification of specific portions of light-gated channels. These modifications involve identifying key portions of the channels. The channels can be identified using high resolution imaging of the tertiary structure of the channel. Alternatively, knowledge of the structure of similar channels can be used. The following description provides details of a specific experimental implementation and methodology.
10 The present invention is not limited to any one implementation and can be implemented for a number of different molecular modifications at various locations consistent with the teachings herein.

Specific aspects of the present invention relate to microbial opsin genes adapted for neuroscience, allowing transduction of light pulse trains into millisecond-timescale
15 membrane potential changes in specific cell types within the intact mammalian brain (*e.g.*, channelrhodopsin (ChR2), an example of which is provided as SEQ ID No. 1, Volvox channelrhodopsin (VChR1), an example of which is provided as SEQ ID No. 2, and halorhodopsin (NpHR), an example of which is provided as SEQ ID No. 3). ChR2 is a rhodopsin derived from the unicellular green alga *Chlamydomonas reinhardtii*. The
20 term "rhodopsin" as used herein is a protein that comprises at least two building blocks, an opsin protein, and a covalently bound cofactor, usually retinal (retinaldehyde). The rhodopsin ChR2 is derived from the opsin Channelopsin-2 (Chop2), originally named Chlamyopsin-4 (Cop4) in the *Chlamydomonas* genome. The temporal properties of one depolarizing channelrhodopsin, ChR2, include fast kinetics of activation and deactivation,
25 affording generation of precisely timed action potential trains. For applications seeking long timescale activation, it has been discovered that the normally fast off-kinetics of the channelrhodopsins can be slowed. For example, certain implementations of channelrhodopsins apply 1mW/mm² light for virtually the entire time in which depolarization is desired, which can be less than desirable.

30 Much of the discussion herein is directed to ChR2. Unless otherwise stated, the invention includes a number of similar variants. Examples include, but are not limited to, Chop2, ChR2-310, Chop2-310, and Volvox channelrhodopsin (VChR1), an example of which is provided as SEQ ID No. 2. For further details on VChR1 reference can be made

to “Red-shifted optogenetic excitation: a tool for fast neural control derived from *Volvox carteri*,” Nat Neurosci. June 2008, 11(6):631-3, Epub 2008 Apr 23. In other implementations similar modifications can be made to other opsin molecules. For instance, modifications/mutations can be made to ChR2 or VChR1 variants. Moreover the modified
5 variants can be used in combination with light-activated ion pumps including, but not limited to, molecules corresponding to sequences SEQ ID Nos. 3-13.

Embodiments of the present invention include relatively minor amino acid variants of the naturally occurring sequences. In one instance, the variants are greater
10 than about 75% homologous to the protein sequence of the naturally occurring sequences. In other variants, the homology is greater than about 80%. Yet other variants have homology greater than about 85%, greater than 90%, or even as high as about 93% to about 95% or about 98%. Homology in this context means sequence similarity or identity, with identity being preferred. This homology can be determined using standard
15 techniques known in the art. The compositions of embodiments of the present invention include the protein and nucleic acid sequences provided herein including variants which are more than about 50% homologous to the provided sequence, more than about 55% homologous to the provided sequence, more than about 60% homologous to the provided sequence, more than about 65% homologous to the provided sequence, more than about
20 70% homologous to the provided sequence, more than about 75% homologous to the provided sequence, more than about 80% homologous to the provided sequence, more than about 85% homologous to the provided sequence, more than about 90% homologous to the provided sequence, or more than about 95% homologous to the provided sequence.

As used herein, stimulation of a target cell is generally used to describe
25 modification of properties of the cell. For instance, the stimulus of a target cell may result in a change in the properties of the cell membrane that can lead to the depolarization or polarization of the target cell. In a particular instance, the target cell is a neuron and the stimulus affects the transmission of impulses by facilitating or inhibiting the generation of impulses (action potentials) by the neuron.

30 A specific embodiment of the present invention relates to the generation of bi-stable (*e.g.*, having extended conducting and non-conducting states in the absence of optical stimulus) channelrhodopsins that are gated into the active state with a single brief pulse of light while remaining active for a duration significantly longer than the light

pulse. Such channelrhodopsins effectively process the delta function of light into a step function of membrane potential. These and other characteristics can be particularly useful for long-timescale, neuromodulatory, developmental, and preclinical/clinical applications including those where an exogenous chemical cofactor is not desirable (*e.g.*,
5 in vivo applications).

Aspects of certain embodiments of the present invention are directed toward controlled termination of the resulting stable depolarization at a specified time, particularly where the offset of termination of the depolarization is significantly delayed from the end of the triggering light pulse. For instance, the activation of opsins can
10 temporarily shift the membrane resting potential of a neuron toward the action potential threshold voltage, thereby increasing action potentials therein. Deactivation of the same opsins restores the action potential to the “normal” resting potential. This deactivation can be implemented through optical stimulation of the appropriate frequency and intensity.

15 Embodiments of the present invention relate to one or more modifications of ChR2 to thereby affect the protein residues in manners that affect the channel kinetics. Embodiments of the present invention provide a mechanism for generating a host of modifications to light gated channels and pumps. Sequence comparisons to similar channels/pumps, such as the prokaryotic proton pump bacteriorhodopsin (BR), for which
20 the tertiary structure is available at high resolution, are used to identify locations for modification. For example, structural inferences from BR, of the seven putative transmembrane helices in microbial rhodopsins, indicated that helix 3 is likely to contain the most amino acids likely to interact with the all-*trans* retinal Schiff base (RSB) chromophore and thus govern channel gating. Many of these amino acids are conserved
25 in channelrhodopsins (Fig. 1a), suggesting that the RSB switch that governs interconversion of non-conducting and conducting states is also highly conserved. Mutations that interfere with the RSB therefore are potential candidates, not only for color tuning but also for altered kinetics and accumulation of the conducting state. Among the amino acids that interact with the RSB, the most notable sequence difference
30 between BR and ChR2 is the Cys128 residue of ChR2, corresponding to Thr90 in BR. High resolution X-ray crystallography has shown that Thr90 in BR is located close to the C11/ C12 carbons of the protonated RSB (Fig. 1b). Mutation of Thr90 to Ala or Val in BR results in a slowing of channel kinetics and accumulation of the M and O photocycle

states. Thus, embodiments of the present invention relate to a modification of C128 to control channel kinetics.

In an example implementation, channelrhodopsins was modified by replacing C128 by Thr, Ala, or Ser. ChR2-C128T, ChR2-C128A, and ChR2-C128S were
5 expressed in *Xenopus* oocytes and recorded photocurrents in response to pulses of 470-nm blue light. Surprisingly, these modifications resulted in dramatic slowing (three to four orders of magnitude) in the closing of the channel after light stimulus was ended. Accordingly, these genes are hereafter referred to as step function opsin (SFO) genes. Compared with the closing time constant of 11.9 ± 0.3 ms for wild-type (wt) ChR2, the
10 time constant for closure after removal of light was measured at 2.0 ± 0.5 s, 52 ± 2 s, and 106 ± 9 s for C128T, C128A, and C128S mutants respectively, revealing vastly extended lifetime of the conducting state (Fig. 1c). As photocurrent amplitudes at a given light intensity are set by a balance between recruitment of new open states and transitions to the closed state, the increased accumulation of the open state was tested for an effectively
15 increased responsiveness at lower light levels. The light intensity dependence of stationary photocurrents was determined by recording responses to light pulses of increasing intensity. The results were normalized to the response at saturating light power (Fig. 1d). Cells expressing C128S and C128A were responsive to light at least 300-fold lower in intensity than those expressing wt ChR2, revealing another surprising
20 property of these SFOs.

Aspects of the present invention include a temporally-precise method of terminating SFO currents. The ChR2 spectral intermediate that reflects the channel open state absorbs maximally at near 520nm (P520), which is red-shifted relative to the dark state P470. This photo-intermediate can undergo a photoreaction; brief flashes of green
25 light applied during the open state prematurely close the channel. While this photo-intermediate is normally so short-lived that the photochemical back-reaction cannot be efficiently exploited, the extended lifetime of P520 in these C128 mutants allows for the use of green light to flip off the bi-stable switch. Indeed, the inactivation dynamics of C128A and C128S were greatly accelerated when a second light pulse of longer
30 wavelength followed the excitation pulse (Fig. 1e,f). 530 nm light showed highest acceleration of the “off” kinetics, but the current declined to a level far above zero due to significant absorption of this wavelength by the dark state (Fig. 1e). Light of longer wavelength showed a slower but more complete inactivation due to lower absorption by

P480 (improved $\sum_{P520}$ to $\sum_{P480}$ ratio), and pulses of 546nm light were found optimal for rapid, complete inactivation. Alternating 450nm and 546nm light allowed reversible ON-OFF switching without rundown (Fig. 1f), thereby defining a fast bi-stable switching mechanism for SFOs.

5 This constellation of novel properties represents orders of magnitude advancements in the evolution of the quantitative properties of these channels across multiple dimensions. Many opsins (*e.g.*, Channelopsin-1 or ChR1) do not express in neurons; surprisingly, however, the three SFO genes (C128T, C128A and C128S) were successfully expressed as EYFP-fusions in hippocampal neurons using lentiviral vectors
 10 driven by the CaMKII α promoter. Neurons expressing the three mutants showed sub-cellular distributions similar to that of ChR2-EYFP (Fig. 2a), with C128A and C128S appearing to express at quantitatively reduced levels. Photocurrents evoked by 10ms pulses of 470nm light were recorded. Peak photocurrents recorded in C128T were similar to those of wild-type ChR2 (184 ± 34 pA and 240 ± 59 pA respectively; $n=10$ and 8 cells, respectively; Fig. 2b), whereas C128A and C128S indeed showed smaller photocurrent
 15 amplitudes (74 ± 17 pA and 61 ± 9 pA respectively; $n=11$ and 9 cells, respectively; Fig. 2b). However, brief flashes of up to 10 ms evoked near-maximal currents (Fig. 2b) and voltage changes (Fig. 2c,d) for C128A/S, in all cases, suggesting that the equilibrium of dark state and conducting state is reached within a few milliseconds at a given light
 20 intensity. On-kinetics (the response time of channels after a first application of light) in all three mutants remained fast, only slightly slower than wt ChR2 ($\tau_{on} = 1.7 \pm 0.1$ ms, 11.6 ± 1.5 ms, 7.2 ± 0.8 ms and 20 ± 1.4 ms for ChR2, C128T, C128A and C128S, respectively; Fig. 2f). Corresponding to the oocyte data, mutant photocurrents decayed with up to 4 orders of magnitude slower kinetics after removal of light ($\tau_{off} = 10 \pm 0.8$ ms, 1.8 ± 0.3 s, 49 ± 3.5 s and 108 ± 42 s, for wt ChR2, C128T, C128A, and C128S, respectively;
 25 Fig. 2h). These results show that step-function properties were preserved in neurons.

The capacity of the mutant channels in neurons to elicit prolonged and reversible membrane depolarization in response to short light pulses was tested and the results are depicted in FIG. 3. In neurons expressing C128S, one 10ms flash of blue light (470 nm)
 30 was able to evoke markedly prolonged sub-threshold depolarization (Fig. 3a, top trace), and chronic stimulation protocols consisting of just one 10 ms light pulse every 15 s enabled sustained stable depolarization over minutes that could be rapidly terminated with a single pulse of 535nm light (Fig. 3a, bottom trace). Indeed, multiple precise steps

could be reliably delivered and terminated in the same neurons using pairs of blue and green stimuli (Fig. 3b). Optimal inactivation was found to occur with a 50ms pulse of 535nm light (compare top and bottom traces in Fig. 3b and 3c, consistent with a reduced quantum efficiency for the P520 to P480 transition). Together these data demonstrate bi-stable switching behavior in neurons.

The stable sub-threshold depolarization evoked by photo-stimulation of C128A or C128S-expressing neurons can be particularly useful for driving precisely timed spike trains (as with WT ChR2), and also for delivering chronically increased excitability, mimicking modulated or UP states (sub-threshold 5-10 mV step-like depolarizations that modulate excitability and information throughput), and for effectively sensitizing genetically-targeted neurons to native, endogenous synaptic inputs. In certain implementations these properties facilitate testing of the causal significance of a neuron type, as neuroscientists often do not know the neural spike code for a particular cell type in executing its function, but could test the causal sufficiency of the cell type by expressing an SFO gene to stably and reversibly enhance natural/intrinsic patterns of information flow through those cells, as illustrated in Fig. 3d.

FIG. 3d shows, on the left, whole-cell current clamp recording from a hippocampal neuron expressing C128A. Native excitatory postsynaptic potential (EPSP) trains were collected with current-clamp recordings in non-transduced hippocampal pyramidal neurons, and the EPSP trains were replayed into cells expressing C128A or C128S before, during and after 20s "UP states" elicited by a blue light pulse (10ms, 470nm) and terminated by a green light pulse (50ms, 535nm; Fig. 3d). Before or after the UP states, EPSP trains produced little spiking (3 ± 1.1 spikes over 20s), while within UP states the same EPSP train elicited greatly increased spiking (17 ± 3.5 spikes during the 20s period; 9/9 cells increased spiking; $p=0.0006$, paired t-test). This showed that the SFO genes can be used for neuromodulatory or UP-state-like photo stimulation that sensitizes neurons to ongoing synaptic activity on long timescales that can be precisely defined by an experimenter.

More particularly, the recorded EPSP trace was delivered in 5 identical blocks (lowest two lines). During blocks 1, 3, and 5, pairs of 470 (on pulse)-nm and 535 (off pulse)-nm light stimuli (10ms and 50ms, respectively; indicated by boxed and unboxed dashes, respectively) were delivered to induce sub-threshold depolarization. During blocks 2 and 4, no light was delivered. On the right, FIG. 3d shows a magnification of

response to EPSPs with and without light (overlay of dashed black boxes) shows light-induced increase in spiking to EPSP stimuli (bottom trace).

The C128A and C128S probes provide properties useful for manipulating neuronal circuits. In addition to allowing novel basic science applications, reduced light requirements are particularly useful with regard to optical hardware requirements in preclinical and clinical experiments, reducing power draw, heating, and risks for long-term photo toxicity. Additional enhancements include red-shifted VChR1 versions for recruiting larger volumes of tissue with lower-energy photons, and molecular modifications to increase membrane trafficking as with eNpHR. Multiple orders of magnitude improvement in both stability and light responsiveness, coupled with precise on/off switching and the chemical cofactor independence of channelrhodopsins, together offer a constellation of key properties for both basic and preclinical/clinical research into mammalian neural circuitry.

Embodiments of the present invention include modifications of other portions of ChR2. Characterization of the properties can be carried out as discussed above. For example, modifications made in the vicinity of C128 are within the scope of the present invention. Other possibilities include, but are not limited to, modifications to, or in the vicinity of, E123 and H134 alone or in combination with modifications at or near C128.

A particular implementation relates to a mutation H134R with the mutations C128A or C128T. It has been discovered that these mutations enhance the conductance, while also providing time constants consistent with those mentioned herein mutants (42 seconds for C128A and 2.5 seconds for C128T). As shown in FIG. 4c, the current sizes are larger and thus can be particularly useful for depolarizing neurons past the threshold for spiking. Also depicted in FIG. 4c are turn on times (top right), turn-off times without light (bottom left), and turn-off times in response to light (bottom right).

FIG. 5 shows sample currents recorded from cells expressing ChR2 (C128A/H134R), also showing spiking in response to activation of this mutant. FIG. 5a shows current recording from a cell expressing ChR2 (C128A/H134R) and particularly a 200pA photocurrent in response to a 10ms on/blue light pulse, decaying slowly to baseline. FIG. 5b shows a voltage recording from the same cell as in FIG. 5a, showing the response of the cell to repeated delivery of pairs of 10ms 470nm light (on/blue pulses) and 100ms 560nm light (off/green pulses).

Embodiments of the present invention are directed to modifications of ChR1 or VChR1. As shown in FIG. 1a, the modifications can be made to locations that are homologous to those discussed in connection with ChR2. For instance, a modification is made to C123 of VChR1 that includes substitution by one of with Thr, Ala or Ser.

5 Consistent with another embodiment of the present invention, modifications/substitutions are made to ChR2 at or near D156. For instance, experimental results have shown that double mutant C128S/D156A has slow closure kinetics. In recordings from cells in culture, the current triggered by a single 10ms flash of blue light only decayed to ~90% of its initial size after 13 minutes of recording (FIG. 10 6). The light sensitivity of this mutant is superior to all previously tested SFOs and it can respond with maximal photocurrent down to 1 μ W/mm² of light.

Other embodiments of the present invention include a similar mutation(s) to VChR1 at for creating a similarly slow, yet red-shifted, channel (*e.g.*, C123S/D151A). For instance, a C123S substitution in VChR1 results in a surprising step-function opsin 15 having a time constant of channel closure after removal of light that is around 30s (FIG. 4c) relative to unmodified VChR1, which is on the order of 120ms.

Aspects of the present invention relate to the use of the red-shifted (relative to ChR2) excitation of VChR1 (FIG. 4a). The red-shift can be particularly useful for deep tissue penetration in connection with the relatively long time constant. For instance, 20 using a step-function version of VChR1, light can be delivered at both a low intensity and repetition rate to achieve chronic activation of targeted cells. Another aspect relates to the spectral separation from ChR2 and the ChR2-based SFOs (FIG. 4a, 4b). For instance, modified VChR1 can be expressed in a first neuronal population while modified ChR2 can be expressed in another neuronal population. Different wavelengths of stimulus light 25 can thereby allow dual-channel control of excitability in the two different neuronal populations.

FIG. 6 shows long current recording of ChR2 mutant C128S/D156A, consistent with an embodiment of the present invention. As shown here, the mutant provides slow closure kinetics relative to non-mutated ChR2 and similar light-responsive channels.

30 Embodiments of the present invention lend themselves to a wide range of applications. A few exemplary applications are discussed hereafter, however, the invention is not limited to these specific examples. Instead, the examples present

examples of implementations and show that aspects of the present invention lend themselves to broad range of applications.

One such application relates to facilitation of intrinsic action potential generation in neural cells for extended periods of time and with high temporal precision. As supported by the various experimental results, certain SFOs allow for rapid on/off control with bi-stable characteristics. Intrinsic neural stimulation produces action potentials in a neuron when the stimulation is sufficient to overcome the resting potential of the neuron. A neural population engineered to express such SFOs provides optical control of this resting potential, thereby facilitating action potentials as a result of a naturally-occurring stimulus. This control can be facilitated by the recognition that certain SFOs have fast-temporal responsiveness that persists over a long-time period. For instance, activation (conductive response) of the SFOs can be on the order of milliseconds after the application of an optical stimulus, while the SFOs can also remain activated for hundreds of milliseconds or even hundreds of seconds after the optical stimulus have been removed. This can be particularly useful for precise control over SFO activation while reducing the amount of potentially-detrimental (*e.g.*, cell health, optically generated heat and/or battery power drain) optical stimulus necessary to maintain SFO activation. Moreover, various SFOs have shown relative fast temporal off-times when exposed to light of a particular wavelength. Thus, precise temporal control can be accomplished with minimal optical stimulation, while facilitating intrinsic activity of neural cells or populations.

Consistent with a more specific example embodiment of the present invention an additional molecule, such as NpHR from *Natronomonas pharaonis*, can be used for temporally-precise optical inhibition of neural activity. NpHR allows for selective inhibition of single action potentials within rapid spike trains and sustained blockade of spiking over many minutes. The action spectrum of NpHR is strongly red-shifted relative to ChannelRhodopsin-2 (ChR2) (derived from *Chlamydomonas reinhardtii*) but operates at similar light power, and NpHR functions in mammals without exogenous cofactors. In one instance, both NpHR and ChR2 can be expressed in the target cells. Likewise, NpHR and ChR2 can be targeted to *C. elegans* muscle and cholinergic motoneurons to control locomotion bi-directionally. In this regard, NpHR and ChR2 form an optogenetic system for multimodal, high-speed, genetically-targeted, all-optical interrogation of living neural circuits.

According to other example embodiments of the present invention, methods for generating an inhibitory neuron-current flow involve, in a neuron, engineering a protein that responds to light by producing an inhibitory current to dissuade depolarization of the neuron. In one such method, the protein is halorhodopsin-based and in another method
5 the protein is an inhibitory protein that uses an endogenous cofactor.

In another example embodiment, a method for controlling action potential of a neuron involves the following steps: engineering a first light responsive protein in the neuron; producing, in response to light, an inhibitory current in the neuron and from the first light responsive protein; engineering a second light responsive protein in the neuron;
10 and producing, in response to light, an excitation current in the neuron from the second light responsive protein.

Another embodiment involves method for controlling a voltage level across a cell membrane of a cell, the method includes: engineering a first light responsive protein in the cell; measuring the voltage level across the cell membrane; and producing, in
15 response to light of a first wavelength and using the first light responsive protein, a current across the cell membrane that is responsive to the measured voltage level.

Another aspect of the present invention is directed to a system for controlling an action potential of a neuron *in vivo*. The system includes a delivery device, a light source, and a control device. The delivery device introduces a light responsive protein to the
20 neuron, with the light responsive protein producing an inhibitory current. The light source generates light for stimulating the light responsive protein, and the control device controls the generation of light by the light source.

In more detailed embodiments, such a system is further adapted such that the delivery device introduces the light responsive protein by one of transfection,
25 transduction and microinjection, and/or such that the light source introduces light to the neuron via one of an implantable light generator and fiber-optics.

Specific aspects of the present invention are directed toward the use of an archaeal light-driven chloride pump, such as halorhodopsin (NpHR), from *Natronomonas pharaonis*, for temporally-precise optical inhibition of neural activity. NpHR-based
30 pumps allow both knockout of single action potentials within rapid spike trains and sustained blockade of spiking over many minutes, and operate at similar light power compared to SFOs based upon ChR2 or VChR1 but with a strongly red-shifted action spectrum. The NpHR pump also functions in mammals without exogenous cofactors.

More detailed embodiments expand on such techniques. For instance, another aspect of the present invention co-expresses NpHR and SFOs (*e.g.*, ChR2 or VChr1 variants) in the species (*e.g.*, a mouse and *C. elegans*). Also, NpHR and SFOs are integrated with calcium imaging in acute mammalian brain slices for bidirectional optical modulation and readout of neural activity. Likewise, NpHR and SFOs can be targeted to *C. elegans* muscle and cholinergic motoneurons to provide bidirectional control of locomotion. Together NpHR and SFOs can be used as a complete and complementary optogenetic system for multimodal, high-speed, genetically-targeted, all-optical interrogation of living neural circuits.

In addition to variants of NpHR, ChR2 and VChr1, there are a number of channelrhodopsins, halorhodopsins, and microbial opsins that can be engineered to optically regulate ion flux or second messengers within cells. Various embodiments of the invention include codon-optimized, mutated, truncated, fusion proteins, targeted versions, or otherwise modified versions of such ion optical regulators. Thus, ChR2 and NpHR (*e.g.*, GenBank accession number is EF474018 for the ‘mammalianized’ NpHR sequence and EF474017 for the ‘mammalianized’ ChR2(1-315) sequence), and variants, are used as representative of a number of different embodiments. Discussions specifically identifying SFOs, ChR2 and NpHR are not meant to limit the invention to such specific examples of optical regulators. For further details regarding the above mentioned sequences reference can be made to “Multimodal fast optical interrogation of neural circuitry” by Feng Zhang, *et al*, Nature (April 5, 2007) Vol. 446: 633-639. As discussed herein, these sequences can be modified accordingly to provide the desired channel kinetics.

Consistent with an example embodiment of the present invention, a method is implemented for stimulating target cells *in vivo* using gene transfer vectors (for example, viruses) capable of inducing photosensitive ion channel growth (for example, SFO/ChR2-based ion channels). The vectors can be implanted in the body.

Consistent with a particular embodiment of the present invention, a protein is introduced to one or more target cells. When introduced into a cell, the protein changes the potential of the cell in response to light having a certain frequency. This may result in a change in resting potential that can be used to control (dissuade) action potential firing. In a specific example, the protein is a halorhodopsin that acts as a membrane pump for transferring charge across the cell membrane in response to light. Membrane pumps are

energy transducers which use electromagnetic or chemical bond energy for translocation of specific ions across the membrane. For further information regarding halorhodopsin membrane pumps reference can be made to “Halorhodopsin Is a Light-driven Chloride Pump” by Brigitte Schobert, *et al*, The Journal of Biological Chemistry Vol. 257, No. 17. 5 September 10, 1982, pp. 10306-10313.

The protein dissuades firing of the action potential by moving the potential of the cell away from the action potential trigger level for the cell. In many neurons, this means that the protein increases the negative voltage seen across the cell membrane. In a specific instance, the protein acts as a chloride ion pump that actively transfers negatively 10 charged chloride ions into the cell. In this manner, the protein generates an inhibitory current across the cell membrane. More specifically, the protein responds to light by lowering the voltage across the cell thereby decreasing the probability that an action potential or depolarization will occur.

As discussed above, one embodiment of the present invention involves the use of 15 an optically responsive ion-pump that is expressed in a cell. In a particular instance, the cell is either a neural cell or a stem cell. A specific embodiment involves in vivo animal cells expressing the ion-pump. Certain aspects of the present invention are based on the identification and development of an archaeal light-driven chloride pump, such as halorhodopsin derived from *Natronomonas pharaonis* (NpHR), for temporally-precise 20 optical inhibition of neural activity. The pump allows both knockout of single action potentials within rapid spike trains and sustained blockade of spiking over many minutes, and it operates at similar light power compared to ChR2-based variants but with a strongly red-shifted action spectrum. The NpHR pump also functions in mammals without exogenous cofactors.

25 According to an example embodiment of the present invention, an optically responsive ion-pump and/or channel is expressed in one or more stem cells, progenitor cells, or progeny of stem or progenitor cells. Optical stimulation is used to activate expressed pumps/channels. The activation can be used to control the ion concentrations (*e.g.*, chloride, calcium, sodium, and potassium) in the cells. This can be particularly 30 useful for affecting the survival, proliferation, differentiation, de-differentiation, or lack of differentiation in the cells. Thus, optical stimulus is implemented to provide control over the (maturation) of stem or progenitor cells.

In a particular embodiment, optically-controlled stimulus patterns are applied to the stem or progenitor cells over a period of hours or days. For further details regarding the effects of membrane potentials and ion concentrations on such cells reference can be made to “Excitation-Neurogenesis Coupling in Adult Neural Stem/Progenitor Cells” by
5 Karl Deisseroth, *et al*, Neuron (May 27, 2004) Neuron, Vol. 42, 535–552 and to U.S. Patent Publication No. 20050267011 entitled “Couple of Excitation and Neurogenesis in neural Stem/Progenitor Cells” to Deisseroth *et al*.

In a particular embodiment, a method of driving differentiation in cells is
10 implemented. The cells are caused to express light-activated NpHR/ChR2-based proteins. The cells are exposed to light to activate the NpHR/ChR2-based protein. The activation drives differentiation of the exposed cell or the progeny of the exposed cell. In another embodiment, the cells comprise stem cells.

Other embodiments relate to aspects of the present invention that are directed to a
15 method for treatment/assessment of a disorder or circuit model. One such method uses SFOs and (possibly) inhibitory molecules to selectively encourage or inhibit neurons. The method targets a group of neurons associated with the disorder; and in this group, the method includes engineering an inhibitory protein/molecule that uses an endogenous cofactor to respond to light by producing an inhibitory current to dissuade depolarization
20 of the neurons. The method also includes engineering SFOs in neurons, of the same group and/or of a different group. The engineered neurons are then exposed to light, thereby dissuading and/or encouraging depolarization of the neurons. The putative effects of this stimulation are then monitored and assessed. Different stimulation profiles and/or targeted locations can be implemented, tested and assessed. The various properties
25 (*e.g.*, the bi-stable nature and fast responsiveness) of SFOs can be particularly useful for such applications, some of which are discussed in more detail hereafter.

Many human applications of the present invention require governmental approval prior to their use. For instance, human use of gene therapy may require such approval. However, similar gene therapies in neurons (nonproliferative cells that are non-
30 susceptible to neoplasms) are proceeding rapidly, with active, FDA-approved clinical trials already underway involving viral gene delivery to human brains thereby facilitating the use of various embodiments of the present invention for a large variety of applications.

The following is a non-exhaustive list of a few examples of such applications and embodiments.

Addiction is associated with a variety of brain functions, including reward and expectation. Additionally, the driving cause of addiction may vary between individuals.

5 According to one embodiment, addiction, for example nicotine addiction, may be treated with optogenetic stabilization of small areas on the insula. Optionally, functional brain imaging- for example cued-state PET or fMRI- may be used to locate a hypermetabolic focus in order to determine a precise target spot for the intervention on the insula surface.

Optogenetic excitation of the nucleus accumbens and septum may provide reward
10 and pleasure to a patient without need for resorting to use of substances, and hence may hold a key to addiction treatment. Conversely, optogenetic stabilization of the nucleus accumbens and septum may be used to decrease drug craving in the context of addiction. In an alternative embodiment, optogenetic stabilization of hypermetabolic activity observed at the genu of the anterior cingulate (BA32) can be used to decrease drug
15 craving. Optogenetic stabilization of cells within the arcuate nucleus of the medial hypothalamus which contain peptide products of proopiomelanocortin (POMC) and cocaine-and-amphetamine-regulating transcript (CART) can also be used to decrease drug addiction behavior. For further information in this regard, reference may be made to: Naqvi NH, Rudrauf D, Damasio H, Bechara A. "Damage to the insula disrupts addiction to cigarette smoking." Science. 2007 Jan 26;315(5811):531-534.

Optogenetic stimulation of neuroendocrine neurons of the hypothalamic periventricular nucleus that secrete somatostatin can be used to inhibit secretion of growth hormone from the anterior pituitary, for example in acromegaly. Optogenetic
25 stabilization of neuroendocrine neurons that secrete somatostatin or growth hormone can be used to increase growth and physical development. Among the changes that accompany "normal" aging, is a sharp decline in serum growth hormone levels after the 4th and 5th decades. Consequently, physical deterioration associated with aging may be lessened through optogenetic stabilization of the periventricular nucleus.

30 Optogenetic stabilization of the ventromedial nucleus of the hypothalamus, particularly the proopiomelanocortin (POMC) and cocaine-and-amphetamine-regulating transcript (CART) of the arcuate nucleus, can be used to increase appetite, and thereby

treat anorexia nervosa. Alternatively, optogenetic stimulation of the lateral nuclei of the hypothalamus can be used to increase appetite and eating behaviors.

Optogenetic excitation in the cholinergic cells of affected areas including the temporal lobe, the NBM (Nucleus basalis of Meynert) and the posterior cingulate gyrus
5 (BA 31) provides stimulation, and hence neurotrophic drive to deteriorating areas. Because the affected areas are widespread within the brain, an analogous treatment with implanted electrodes may be less feasible than an optogenetic approach.

Anxiety disorders are typically associated with increased activity in the left temporal and frontal cortex and amygdala, which trends toward normal as anxiety
10 resolves. Accordingly, the affected left temporal and frontal regions and amygdala may be treated with optogenetic stabilization, so as to dampen activity in these regions.

In normal physiology, photosensitive neural cells of the retina, which depolarize in response to the light that they receive, create a visual map of the received light pattern. Optogenetic ion channels can be used to mimic this process in many parts of the body,
15 and the eyes are no exception. In the case of visual impairment or blindness due to damaged retina, a functionally new retina can be grown, which uses natural ambient light rather than flashing light patterns from an implanted device. The artificial retina grown may be placed in the location of the original retina (where it can take advantage of the optic nerve serving as a conduit back to the visual cortex). Alternatively, the artificial
20 retina may be placed in another location, such as the forehead, provided that a conduit for the depolarization signals are transmitted to cortical tissue capable of deciphering the encoded information from the optogenetic sensor matrix. Cortical blindness could also be treated by simulating visual pathways downstream of the visual cortex. The stimulation would be based on visual data produced up stream of the visual cortex or by an artificial
25 light sensor.

Treatment of tachycardia may be accomplished with optogenetic stimulation to parasympathetic nervous system fibers including CN X or Vagus Nerve. This causes a decrease in the SA node rate, thereby decreasing the heart rate and force of contraction. Similarly, optogenetic stabilization of sympathetic nervous system fibers within spinal
30 nerves T1 through T4, serves to slow the heart. For the treatment of pathological bradycardia, optogenetic stabilization of the Vagus nerve, or optogenetic stimulation of sympathetic fibers in T1through T4 will serve to increase heart rate. Cardiac dysrhythmias resulting from aberrant electrical foci that outpace the sinoatrial node may

be suppressed by treating the aberrant electrical focus with moderate optogenetic stabilization. This decreases the intrinsic rate of firing within the treated tissue, and permits the sinoatrial node to regain its role in pacing the heart's electrical system. In a similar way, any type of cardiac arrhythmia could be treated. Degeneration of cardiac tissue that occurs in cardiomyopathy or congestive heart failure could also be treated using this invention; the remaining tissue could be excited using various embodiments of the invention.

Optogenetic excitation stimulation of brain regions including the frontal lobe, parietal lobes and hippocampi, may increase processing speed, improve memory, and stimulate growth and interconnection of neurons, including spurring development of neural progenitor cells. As an example, one such application of the present invention is directed to optogenetic excitation stimulation of targeted neurons in the thalamus for the purpose of bringing a patient out of a near-vegetative (barely-conscious) state. Growth of light-gated ion channels or pumps in the membrane of targeted thalamus neurons is effected. These modified neurons are then stimulated, e.g., via optics which may also gain access by the same passageway, by directing a flash of light thereupon so as to modulate the function of the targeted neurons and/or surrounding cells. For further information regarding appropriate modulation techniques (via electrode-based treatment) or further information regarding the associated brain regions for such patients, reference may be made to: Schiff ND, Giacino JT, Kalmar K, Victor JD, Baker K, Gerber M, Fritz B, Eisenberg B, O'Connor JO, Kobylarz EJ, Farris S, Machado A, McCagg C, Plum F, Fins JJ, Rezai AR. "Behavioral improvements with thalamic stimulation after severe traumatic brain injury." *Nature*. Vol 448. Aug 2, 2007 pp 600-604.

In an alternative embodiment, optogenetic excitation may be used to treat weakened cardiac muscle in conditions such as congestive heart failure. Electrical assistance to failing heart muscle of CHF is generally not practical, due to the thin-stretched, fragile state of the cardiac wall, and the difficulty in providing an evenly distributed electrical coupling between an electrodes and muscle. For this reason, preferred methods to date for increasing cardiac contractility have involved either pharmacological methods such as Beta agonists, and mechanical approaches such as ventricular assist devices. In this embodiment of the present invention, optogenetic excitation is delivered to weakened heart muscle via light emitting elements on the inner surface of a jacket surround the heart or otherwise against the affected heart wall. Light

may be diffused by means well known in the art, to smoothly cover large areas of muscle, prompting contraction with each light pulse.

Optogenetic stabilization in the subgenual portion of the cingulate gyrus (Cg25), yellow light may be applied with an implanted device to treat depression by suppressing target activity in manner analogous to what is taught by Mayberg HS et al., "Deep Brain Stimulation for Treatment-Resistant Depression." *Neuron*, Vol. 45, 651-660, March 3, 2005, 651-660. In an alternative embodiment, an optogenetic excitation stimulation method is to increase activity in that region in a manner analogous to what is taught by Schlaepfer et al., "Deep Brain stimulation to Reward Circuitry Alleviates Anhedonia in Refractory Major Depression." *Neuropsychopharmacology* 2007 1-10.

In yet another embodiment the left dorsolateral prefrontal cortex (LDPFC) is targeted with an optogenetic excitation stimulation method. Pacing the LDPFC at 5-20 Hz serves to increase the basal metabolic level of this structure which, via connecting circuitry, serves to decrease activity in Cg 25, improving depression in the process. Suppression of the right dorsolateral prefrontal cortex (RDLPC) is also an effective depression treatment strategy. This may be accomplished by optogenetic stabilization on the RDLPC, or suppression may also be accomplished by using optogenetic excitation stimulation, and pulsing at a slow rate- 1 Hz or less, improving depression in the process. Vagus nerve stimulation (VNS) may be improved using an optogenetic approach. Use of optogenetic excitation may be used in order to stimulate only the vagus afferents to the brain, such as the nodose ganglion and the jugular ganglion. Efferents from the brain would not receive stimulation by this approach, thus eliminating some of the side-effects of VNS including discomfort in the throat, a cough, difficulty swallowing and a hoarse voice. In an alternative embodiment, the hippocampus may be optogenetically excited, leading to increased dendritic and axonal sprouting, and overall growth of the hippocampus. Other brain regions implicated in depression that could be treated using this invention include the amygdala, accumbens, orbitofrontal and orbitomedial cortex, hippocampus, olfactory cortex, and dopaminergic, serotonergic, and noradrenergic projections. Optogenetic approaches could be used to control spread of activity through structures like the hippocampus to control depressive symptoms.

So long as there are viable alpha and beta cell populations in the pancreatic islets of Langerhans, the islets can be targeted for the treatment of diabetes. For example, when

serum glucose is high (as determined manually or by closed loop glucose detection system), optogenetic excitation may be used to cause insulin release from the beta cells of the islets of Langerhans in the pancreas, while optogenetic stabilization is used to prevent glucagon release from the alpha cells of the islets of Langerhans in the pancreas.

- 5 Conversely, when blood sugars are too low (as determined manually or by closed loop glucose detection system), optogenetic stabilization may be used to stop beta cell secretion of insulin, and optogenetic excitation may be used to increase alpha-cell secretion of glucagon.

For treatment of epilepsy, quenching or blocking epileptogenic activity is
10 amenable to optogenetic approaches. Most epilepsy patients have a stereotyped pattern of activity spread resulting from an epileptogenic focus. Optogenetic stabilization could be used to suppress the abnormal activity before it spreads or truncated it early in its course. Alternatively, activation of excitatory tissue via optogenetic excitation stimulation could be delivered in a series of deliberately asynchronous patterns to disrupt the emerging
15 seizure activity. Another alternative involves the activation of optogenetic excitation stimulation in GABAergic neurons to provide a similar result. Thalamic relays may be targeted with optogenetic stabilization triggered when an abnormal EEG pattern is detected.

Another embodiment involves the treatment of gastrointestinal disorders. The
20 digestive system has its own, semi-autonomous nervous system containing sensory neurons, motor neurons and interneurons. These neurons control movement of the GI tract, as well as trigger specific cells in the gut to release acid, digestive enzymes, and hormones including gastrin, cholecystokinin and secretin. Syndromes that include inadequate secretion of any of these cellular products may be treated with optogenetic
25 stimulation of the producing cell types, or neurons that prompt their activity. Conversely, optogenetic stabilization may be used to treat syndromes in which excessive endocrine and exocrine products are being created. Disorders of lowered intestinal motility, ranging from constipation (particularly in patients with spinal cord injury) to megacolon may be treated with optogenetic excitation of motor neurons in the intestines. Disorders of
30 intestinal hypermotility, including some forms of irritable bowel syndrome may be treated with optogenetic stabilization of neurons that control motility. Neurogenetic gastric outlet obstructions may be treated with optogenetic stabilization of neurons and musculature in the pylorus. An alternative approach to hypomotility syndromes would be

to provide optogenetic excitation to stretch-sensitive neurons in the walls of the gut, increasing the signal that the gut is full and in need of emptying.

In this same paradigm, an approach to hypermobility syndromes of the gut would be to provide optogenetic stabilization to stretch receptor neurons in the lower GI, thus providing a “false cue” that the gut was empty, and not in need of emptying. In the case of frank fecal incontinence, gaining improved control of the internal and external sphincters may be preferred to slowing the motility of the entire tract. During periods of time during which a patient needs to hold feces in, optogenetic excitation of the internal anal sphincter will provide for retention. Providing optogenetic stimulation to the external sphincter may be used to provide additional continence. When the patient is required to defecate, the internal anal sphincter, and then external anal sphincter should be relaxed, either by pausing the optogenetic stimulation, or by adding optogenetic stabilization.

Conductive hearing loss may be treated by the use of optical cochlear implants. Once the cochlea has been prepared for optogenetic stimulation, a cochlear implant that flashes light may be used. Sensorineural hearing loss may be treated through optical stimulation of downstream targets in the auditory pathway.

Another embodiment of the present invention is directed toward the treatment of blood pressure disorders, such as hypertension. Baroreceptors and chemoreceptors in regions such as the aorta (aortic bodies and paraaortic bodies) and the carotid arteries (“carotic bodies”) participate the regulation of blood pressure and respiration by sending afferents via the vagus nerve (CN X), and other pathways to the medulla and pons, particularly the solitary tract and nucleus. Optogenetic excitation of the carotid bodies, aortic bodies, paraaortic bodies, may be used to send a false message of “hypertension” to the solitary nucleus and tract, causing it to report that blood pressure should be decreased. Optogenetic excitation or stabilization directly to appropriate parts of the brainstem may also be used to lower blood pressure. The opposite modality causes the optogenetic approach to serve as a pressor, raising blood pressure. A similar effect may also be achieved via optogenetic excitation of the Vagus nerve, or by optogenetic stabilization of sympathetic fibers within spinal nerves T1-T4. In an alternative embodiment, hypertension may be treated with optogenetic stabilization of the heart, resulting in decreased cardiac output and lowered blood pressure. According to another embodiment, optogenetic stabilization of aldosterone-producing cells within the adrenal cortex may be

used to decrease blood pressure. In yet another alternative embodiment, hypertension may be treated by optogenetic stabilization of vascular smooth muscle. Activating light may be passed transcutaneously to the peripheral vascular bed.

Another example embodiment is directed toward the treatment of hypothalamic-pituitary-adrenal axis disorders. In the treatment of hypothyroidism, optogenetic
5 excitation of parvocellular neuroendocrine neurons in the paraventricular and anterior hypothalamic nuclei can be used to increase secretion of thyrotropin-releasing hormone (TRH). TRH, in turn, stimulates anterior pituitary to secrete thyroid stimulating hormone (TSH). Conversely, hyperthyroidism may be treated with optogenetic stabilization of the
10 parvocellular neuroendocrine neurons. For the treatment of adrenal insufficiency, or of Addison's disease, optogenetic excitation of parvocellular neuroendocrine neurons in the supraoptic nucleus and paraventricular nuclei may be used to increase the secretion of vasopressin, which, with the help of corticotropin-releasing hormone (CRH), stimulate anterior pituitary to secrete Adrenocorticotrophic hormone (ACTH). Cushing syndrome,
15 frequently caused by excessive ACTH secretion, may be treated with optogenetic stabilization of the parvocellular neuroendocrine neurons of supraoptic nucleus via the same physiological chain of effects described above. Neuroendocrine neurons of the arcuate nucleus produce dopamine, which inhibits secretion of prolactin from the anterior pituitary. Hyperprolactinemia can therefore be treated via optogenetic excitation, while
20 hyperprolactinemia can be treated with optogenetic stabilization of the neuroendocrine cells of the arcuate nucleus.

In the treatment of hyperautonomic states, for example anxiety disorders, optogenetic stabilization of the adrenal medulla may be used to reduce norepinephrine output. Similarly, optogenetic stimulation of the adrenal medulla may be used in persons
25 with need for adrenaline surges, for example those with severe asthma, or disorders that manifest as chronic sleepiness.

Optogenetic stimulation of the adrenal cortex will cause release of chemicals including cortisol, testosterone, and aldosterone. Unlike the adrenal medulla, the adrenal cortex receives its instructions from neuroendocrine hormones secreted from the pituitary
30 and hypothalamus, the lungs, and the kidneys. Regardless, the adrenal cortex is amenable to optogenetic stimulation. Optogenetic stimulation of the cortisol-producing cells of the adrenal cortex may be used to treat Addison's disease. Optogenetic stabilization of cortisol-producing cells of the adrenal cortex may be used to treat Cushing's disease.

Optogenetic stimulation of testosterone-producing cells may be used to treat disorders of sexual interest in women: Optogenetic stabilization of those same cells may be used to decrease facial hair in women. Optogenetic stabilization of aldosterone-producing cells within the adrenal cortex may be used to decrease blood pressure. Optogenetic excitation
5 of aldosterone-producing cells within the adrenal cortex may be used to increase blood pressure.

Optogenetic excitation stimulation of specific affected brain regions may be used to increase processing speed, and stimulate growth and interconnection of neurons, including spurring the maturation of neural progenitor cells. Such uses can be
10 particularly useful for treatment of mental retardation.

According to another embodiment of the present invention, various muscle diseases and injuries can be treated. Palsies related to muscle damage, peripheral nerve damage and to dystrophic diseases can be treated with optogenetic excitation to cause contraction, and optogenetic stabilization to cause relaxation. This latter relaxation via
15 optogenetic stabilization approach can also be used to prevent muscle wasting, maintain tone, and permit coordinated movement as opposing muscle groups are contracted. Likewise, frank spasticity can be treated via optogenetic stabilization.

In areas as diverse as peripheral nerve truncation, stroke, traumatic brain injury and spinal cord injury, there is a need to foster the growth of new neurons, and assist with
20 their integration into a functional network with other neurons and with their target tissue. Re-growth of new neuronal tracts may be encouraged via optogenetic excitation, which serves to signal stem cells to sprout axons and dendrites, and to integrate themselves with the network. Use of an optogenetic technique (as opposed to electrodes) prevents receipt of signals by intact tissue, and serves to ensure that new target tissue grows by virtue of a
25 communication set up with the developing neurons, and not with an artificial signal like current emanating from an electrode.

Obesity can be treated with optogenetic excitation to the ventromedial nucleus of the hypothalamus, particularly the proopiomelanocortin (POMC) and cocaine-and-amphetamine-regulating transcript (CART) of the arcuate nucleus. In an alternative
30 embodiment, obesity can be treated via optogenetic stabilization of the lateral nuclei of the hypothalamus. In another embodiment, optogenetic stimulation to leptin-producing cells, or to cells with leptin receptors within the hypothalamus, may be used to decrease appetite and hence treat obesity.

Destructive lesions to the anterior capsule, and analogous DBS to that region, are established means of treating severe, intractable obsessive-compulsive disorder 48 (OCD48). Such approaches may be emulated using optogenetic stabilization to the anterior limb of the internal capsule, or to regions such as BA32 and Cg24 which show
5 metabolic decrease as OCD remits.

Chronic Pain can be treated using another embodiment of the present invention. Electrical stimulation methods include local peripheral nerve stimulation, local cranial nerve stimulation and “sub-threshold” motor cortex stimulation. Reasonable optogenetic approaches include optogenetic stabilization at local painful sites. Attention to promoter
10 selection would ensure that other sensory and motor fibers would be unaffected. Selective optogenetic excitation of interneurons at the primary motor cortex also may provide effective pain relief. Also, optogenetic stabilization at the sensory thalamus, (particularly medial thalamic nuclei), periventricular grey matter, and ventral raphe nuclei, may be used to produce pain relief. In an alternative embodiment, optogenetic
15 stabilization of parvalbumin-expressing cells targeting as targeting strategy, may be used to treat pain by decreasing Substance P production. The release of endogenous opioids may be accomplished by using optogenetic excitation to increase activity in the nucleus accumbens. In an alternative embodiment, when POMC neurons of the arcuate nucleus of the medial hypothalamus are optogenetically excited, beta endorphin are increased,
20 providing viable treatment approaches for depression and for chronic pain.

Parkinson’s Disease can be treated by expressing optogenetic stabilization in the glutamatergic neurons in either the subthalamic nucleus (STN) or the globus pallidus interna (GPI) using an excitatory-specific promoter such as CaMKII α , and apply
25 optogenetic stabilization. Unlike electrical modulation in which all cell-types are affected, only glutamatergic STN neurons would be suppressed.

Certain personality disorders, including the borderline and antisocial types, demonstrate focal deficits in brain disorders including “hypofrontality.” Direct or indirect optogenetic excitation of these regions is anticipated to produce improvement of
30 symptoms. Abnormal bursts of activity in the amygdala are also known to precipitate sudden, unprompted flights into rage: a symptom of borderline personality disorder, as well as other conditions, which can benefit from optogenetic stabilization of the amygdala. Optogenetic approaches could improve communication and synchronization between

different parts of the brain, including amygdala, striatum, and frontal cortex, which could help in reducing impulsiveness and improving insight.

The amygdalo-centric model of post-traumatic-stress disorder (PTSD) proposes that it is associated with hyperarousal of the amygdala and insufficient top-down control by the medial prefrontal cortex and the hippocampus. Accordingly, PTSD may be treated with optogenetic stabilization of the amygdale or hippocampus.

Schizophrenia is characterized by abnormalities including auditory hallucinations. These might be treated by suppression of the auditory cortex using optogenetic stabilization. Hypofrontality associated with schizophrenia might be treated with optogenetic excitation in the affected frontal regions. Optogenetic approaches could improve communication and synchronization between different parts of the brain which could help in reducing misattribution of self-generated stimuli as foreign.

Optogenetic stabilization of cells within the arcuate nucleus of the medial hypothalamus, which contain peptide products of proopiomelanocortin (POMC) and cocaine-and-amphetamine-regulating transcript (CART), can be used to reduce compulsive sexual behavior. Optogenetic excitation of cells within the arcuate nucleus of the medial hypothalamus which contain peptide products of proopiomelanocortin (POMC) and cocaine-and-amphetamine-regulating transcript (CART) may be used to increase sexual interest in the treatment of cases of disorders of sexual desire. In the treatment of disorders of hypoactive sexual desire, testosterone production by the testes and the adrenal glands can be increased through optogenetic excitation of the pituitary gland. Optogenetic excitation of the nucleus accumbens can be used for the treatment of anorgasmia.

The suprachiasmatic nucleus secretes melatonin, which serves to regulate sleep/wake cycles. Optogenetic excitation to the suprachiasmatic nucleus can be used to increase melatonin production, inducing sleep, and thereby treating insomnia. Orexin (hypocretin) neurons strongly excite numerous brain nuclei in order to promote wakefulness. Optogenetic excitation of orexin-producing cell populations can be used to treat narcolepsy, and chronic daytime sleepiness.

Optogenetic stimulation of the supraoptic nucleus may be used to induce secretion of oxytocin, can be used to promote parturition during childbirth, and can be used to treat disorders of social attachment.

Like muscular palsies, the motor functions that have been de-afferented by a spinal cord injury may be treated with optogenetic excitation to cause contraction, and optogenetic stabilization to cause relaxation. This latter relaxation via optogenetic stabilization approach may also be used to prevent muscle wasting, maintain tone, and permit coordinated movement as opposing muscle groups are contracted. Likewise, frank spasticity may be treated via optogenetic stabilization. Re-growth of new spinal neuronal tracts may be encouraged via optogenetic excitation, which serves to signal stem cells to sprout axons and dendrites, and to integrate themselves with the network.

Stroke deficits include personality change, motor deficits, sensory deficits, cognitive loss, and emotional instability. One strategy for the treatment of stroke deficits is to provide optogenetic stimulation to brain and body structures that have been deafferented from excitatory connections. Similarly, optogenetic stabilization capabilities can be imparted on brain and body structures that have been deafferented from inhibitory connections.

Research indicates that the underlying pathobiology in Tourette's syndrome is a phasic dysfunction of dopamine transmission in cortical and subcortical regions, the thalamus, basal ganglia and frontal cortex. In order to provide therapy, affected areas are preferably first identified using techniques including functional brain imaging and magnetoencephalography (MEG). Whether specifically identified or not, optogenetic stabilization of candidate tracts may be used to suppress motor tics. Post-implantation empirical testing of device parameters reveals which sites of optogenetic stabilization, and which are unnecessary to continue.

In order to treat disorders of urinary or fecal incontinence optogenetic stabilization can be used to the sphincters, for example via optogenetic stabilization of the bladder detrussor smooth muscle or innervations of that muscle. When micturation is necessary, these optogenetic processes are turned off, or alternatively can be reversed, with optogenetic stabilization to the (external) urinary sphincter, and optogenetic excitation of the bladder detrussor muscle or its innervations. When a bladder has been deafferented, for example, when the sacral dorsal roots are cut or destroyed by diseases of the dorsal roots such as tabes dorsalis in humans, all reflex contractions of the bladder are abolished, and the bladder becomes distended. Optogenetic excitation of the muscle directly can be used to restore tone to the detrussor, prevent kidney damage, and to assist with the micturition process. As the bladder becomes "decentralized" and hypersensitive to

movement, and hence prone to incontinence, optogenetic stabilization to the bladder muscle can be used to minimize this reactivity of the organ.

In order to selectively excite/inhibit a given population of neurons, for example those involved in the disease state of an illness, several strategies can be used to target the
5 optogenetic proteins/molecules to specific populations.

For various embodiments of the present invention, genetic targeting may be used to express various optogenetic proteins or molecules. Such targeting involves the targeted expression of the optogenetic proteins/molecules via genetic control elements such as promoters (*e.g.*, Parvalbumin, Somatostatin, Cholecystokinin, GFAP),
10 enhancers/silencers (*e.g.*, Cytomegalovirus Immediate Early Enhancer), and other transcriptional or translational regulatory elements (*e.g.*, Woodchuck Hepatitis Virus Post-transcriptional Regulatory Element). Permutations of the promoter+enhancer+regulatory element combination can be used to restrict the expression of optogenetic probes to genetically-defined populations.

15 Various embodiments of the present invention may be implemented using spatial/anatomical targeting. Such targeting takes advantage of the fact that projection patterns of neurons, virus or other reagents carrying genetic information (DNA plasmids, fragments, *etc.*), can be focally delivered to an area where a given population of neurons project to. The genetic material will then be transported back to the bodies of the neurons
20 to mediate expression of the optogenetic probes. Alternatively, if it is desired to label cells in a focal region, viruses or genetic material may be focally delivered to the interested region to mediate localized expression.

Various gene delivery systems are useful in implementing one or more embodiments of the present invention. One such delivery system is Adeno-Associated
25 Virus (AAV). AAV can be used to deliver a promoter+optogenetic probe cassette to a specific region of interest. The choice of promoter will drive expression in a specific population of neurons. For example, using the CaMKIIa promoter will drive excitatory neuron specific expression of optogenetic probes. AAV will mediate long-term expression of the optogenetic probe for at least 1 year or more. To achieve more
30 specificity, AAV may be pseudo-typed with specific serotypes 1 to 8, with each having different tropism for different cell types. For instance, serotype 2 and 5 is known to have good neuron-specific tropism.

Another gene delivery mechanism is the use of a retrovirus. HIV or other lentivirus-based retroviral vectors may be used to deliver a promoter+optogenetic probe cassette to a specific region of interest. Retroviruses may also be pseudotyped with the Rabies virus envelope glycoprotein to achieve retrograde transport for labeling cells based on their axonal projection patterns. Retroviruses integrate into the host cell's genome, therefore are capable of mediating permanent expression of the optogenetic probes. Non-lentivirus based retroviral vectors can be used to selectively label dividing cells.

Gutless Adenovirus and Herpes Simplex Virus (HSV) are two DNA based viruses that can be used to deliver promoter+optogenetic probe cassette into specific regions of the brain as well. HSV and Adenovirus have much larger packaging capacities and therefore can accommodate much larger promoter elements and can also be used to deliver multiple optogenetic probes or other therapeutic genes along with optogenetic probes.

Focal Electroporation can also be used to transiently transfect neurons. DNA plasmids or fragments can be focally delivered into a specific region of the brain. By applying mild electrical current, surrounding local cells will receive the DNA material and expression of the optogenetic probes.

In another instance, lipofection can be used by mixing genetic material with lipid reagents and then subsequently injected into the brain to mediate transfect of the local cells.

Various embodiments involve the use of various control elements. In addition to genetic control elements, other control elements (particularly promoters and enhancers whose activities are sensitive to chemical, magnetic stimulation or infrared radiation) can be used to mediate temporally-controlled expression of the optogenetic probes. For example, a promoter whose transcriptional activity is subject to infrared radiation allows one to use focused radiation to fine tune the expression of optogenetic probes in a focal region at only the desired time.

According to one embodiment of the present invention, the invention may be used in animal models of DBS, for example in Parkinsonian rats, to identify the target cell types responsible for therapeutic effects (an area of intense debate and immense clinical importance). For instance, stimulation can be targeted to specific/small neural populations within larger populations known to provide therapeutic effects in response to

stimulus. These targeted populations can then be stimulated to quantify the source of the therapeutic effects. The targeting can be implemented using spatially controlled application of the proteins within the brain and/or by tailoring the proteins for expression in specific neural cell types. The targeting can also be implemented by controlling the
5 light delivery in terms of spatial location, wavelength, intensity and/or temporal stimulation properties. Knowledge gained from such characterization can then be used in the development of pharmacological and surgical strategies for treating human disease. Such modeling and characterization is not limited to Parkinson's and can be applied to a vast array of disease and circuit modeling.

10 According to another embodiment of the present invention, genetically-defined cell types may be linked with complex systems-level behaviors, to allow the elucidation of the precise contribution of different cell types in many different brain regions to high-level organismal functioning.

Other aspects and embodiments are directed to systems, methods, kits,
15 compositions of matter and molecules for ion pumps or for controlling inhibitory currents in a cell (*e.g.*, for *in vivo* and *in vitro* environments). As described throughout this disclosure, including the claims, such systems, methods, kits, compositions of matter are realized in manners consistent herewith. For example, in one embodiment, the present invention is directed to an assembly or kit of parts, having a product containing an NpHR-
20 based molecular variant and another opsin-based molecule (SFO/VChR1/ChR2-based and or NpHR-based) as a combined preparation for use in the treatment of disease of a neurological or CNS disorder (as a category of disorder types or a specific disorder as exemplified herein), wherein at least the NpHR-based molecular variant is useful for expressing a light-activated NpHR-based molecule that responds to light by producing an
25 inhibitory current to dissuade depolarization of a cell, and wherein a high expression of the molecule manifests a toxicity level that is less than about 75% (*e.g.*, one or more of Seq Id Nos. 4-13).

Embodiments of the present invention employ implantable arrangements for *in vivo* use. These arrangements can include a light generator, such as a light-emitting
30 diode, laser or similar light source and a biological portion that modifies target cells to facilitate stimulation of the target cells in response to light generated by the light generator.

In one embodiment of the present invention, a biological portion may be composed of target cells that have been modified to be photosensitive. In another embodiment of the present invention, a biological portion may contain biological elements such as gene transfer vectors, which cause target cells to become sensitive to light. An example of this is lentiviruses carrying the gene for SFO (ChR2/VChR1 mutants) expression. In this manner, the stimulation of target cells can be controlled by an implantable device. For example, a control circuit can be arranged to respond to an external signal by activating, or deactivating a light source, or by charging a battery that powers light source. In one instance, the external signal is electromagnetic radiation that is received by a control circuit. For example, radio frequency (RF) signals can be transmitted by an external RF transmitter and received by a control circuit. In another example, a magnetic field can be used to activate and/or power the control circuit.

Control circuits can be implemented using varying degrees of complexity. In one instance, the circuit is a simple coil that when exposed to a magnetic field generates a current. The current is then used to power a light source. Such an implementation can be particularly useful for limiting the size and complexity as well as increasing the longevity of the device. In another instance, a control circuit can include an RF antenna. Optionally, a battery or similar power source, such as a capacitive element, can be used by the control circuit. While charged, the power source allows the circuitry to continue to operate without need for concurrent energy delivery from outside the body. This can be particularly useful for providing precise control over the light emitted by a light source and for increased intensity of the emitted light.

In one embodiment of the present invention, a light source is implemented using a light-emitting-diode (LED). LEDs have been proven to be useful for low power applications and also to have a relatively fast response to electrical signals.

In another embodiment of the present invention, the biological portion includes a gelatin or similar substance that contains gene transfer vectors which genetically code the target cells for photosensitivity. In one instance, the vectors are released once implanted into the body. This can be accomplished, for example, by using a containment material that allows the vectors to be released into aqueous solution (*e.g.*, using dehydrated or water soluble materials such as gelatins). The release of the vectors results in the target cells being modified such that they are stimulated in response to light from a light source.

In another embodiment of the present invention, the biological portion includes a synthetic mesh that contains the photosensitive cells. In one instance, the cells are neurons that have been modified to be photosensitive. The synthetic mesh can be constructed so as to allow the dendrites and axons to pass through the mess without
5 allowing the entire neuron (*e.g.*, the cell body) to pass. One example of such a mesh has pores that are on the order of 3-7 microns in diameter and is made from polyethylene terephthalate. In another example embodiment, the biological portion includes an injection mechanism for targeted delivery.

In various implementations, a system is adapted such that the delivery device
10 introduces the light responsive protein by one of transfection, transduction or microinjection, and/or such that the light source introduces light to an SFO expressing neuron via one of an implantable light generator and fiber-optics.

The various embodiments described above are provided by way of illustration only and should not be construed to limit the invention. Based on the above discussion
15 and illustrations, those skilled in the art will readily recognize that various modifications and changes may be made to the present invention without strictly following the exemplary embodiments and applications illustrated and described herein. For instance, such changes may include additional modifications other than those listed herein. Such Modifications and changes do not depart from the true scope of the present invention.

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 Ile Leu Leu Val Glu Trp Ala Gln Asp Ala Lys Ala Ala Gly Thr Ala
 195 200 205
 Asp Met Phe Asn Thr Leu Lys Leu Leu Thr Val Val Met Trp Leu Gly
 210 215 220
 Tyr Pro Ile Val Trp Ala Leu Gly Val Glu Gly Ile Ala Val Leu Pro
 225 230 235 240
 Val Gly Val Thr Ser Trp Gly Tyr Ser Phe Leu Asp Ile Val Ala Lys
 245 250 255
 Tyr Ile Phe Ala Phe Leu Leu Leu Asn Tyr Leu Thr Ser Asn Glu Ser
 260 265 270
 Val Val Ser Gly Ser Ile Leu Asp Val Pro Ser Ala Ser Gly Thr Pro
 275 280 285
 Ala Asp Asp
 290

<210> 4

<211> 1599

<212> DNA

<213> Artificial Sequence

<220>

<223> Humanized NpHR: the first 873bp; EYFP: the last 717bp

<400> 4

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60

40

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aacatcgcac	ttgcaggact	gagtatactg	ctgttcgttt	ttatgacccg	aggactcgat	180
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ctgagcaaag	accccaacga	gaagcgcgat	cacatggtcc	tgctggagtt	cgtgaccgcc	1560
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<210> 5

<211> 1599

<212> DNA

<213> Artificial Sequence

<220>

<223> Non-Humanized nhNpHR - EYFP

<400> 5

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agctacaccg	gccttgcatc	ggggctcacc	atcagcgtcc	tcgagatgcc	agccggccac	300
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cttgctgggt	ctaacgccac	gaagctcttt	accgccatca	ccttcgacat	cgcgatgtgt	480
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41

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cccatcgggc	acggccccgt	gctgctgccc	gacaaccact	acctgagcta	ccagtccgcc	1500
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<210> 6
 <211> 1641
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> SP(nAChR-S) - hNpHR - EYFP

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ggcagctcag	tgatgctggg	aggagaagag	gtagatgggt	tagtcaccat	gtggggacgg	420
tatctcacct	gggcactttc	cacgcccatt	attctcctcg	ctctgggtct	cctggccgga	480
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<210> 7
 <211> 1605
 <212> DNA
 <213> artificial

<220>
 <223> hNpHR - EYFP - ETQV

<400> 7

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<210> 8

<211> 1611

<212> DNA

<213> artificial

<220>

<223> hNpHR - EYFP - actin

<400> 8

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43

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<210> 9
 <211> 1605
 <212> DNA
 <213> artificial

<220>
 <223> hNpHR - EYFP - EExport

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 <211> 1644
 <212> DNA
 <213> artificial

<220>
 <223> SP(Chr2) - hNpHR - EYFP

<400> 10

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What is claimed is:

1. A light-responsive ion channel comprising an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2, wherein the light-responsive ion channel exhibits an extended conducting or non-conducting state after exposure to a light pulse compared to that of a polypeptide comprising SEQ ID NO:1 or SEQ ID NO:2, and

wherein the light-responsive ion channel comprises: i) a Cys→Thr, a Cys→Ala, or a Cys→Ser substitution at a position corresponding to amino acid 128 of SEQ ID NO:1 or amino acid 123 of SEQ ID NO:2; and ii) an Asp→Ala or an Asp→Asn substitution at a position corresponding to amino acid 156 of SEQ ID NO:1 or amino acid 151 of SEQ ID NO:2.

2. The light-responsive ion channel according to claim 1, wherein the light pulse is 10 ms or less.

3. The light-responsive ion channel according to claim 1 or 2, wherein the light-responsive ion channel comprises: i) the Cys→Ser substitution at the position corresponding to amino acid 128 of SEQ ID NO:1 or amino acid 123 of SEQ ID NO:2; and ii) the Asp→Ala substitution at the position corresponding to amino acid 156 of SEQ ID NO:1 or amino acid 151 of SEQ ID NO:2.

4. The light-responsive ion channel according to claim 1, 2 or 3, comprising said sequence having at least 90% identity to SEQ ID NO:1 and which further comprises an amino acid substitution of Glu at a position corresponding to amino acid 123 of SEQ ID NO:1.

5. The light-responsive ion channel according to any one of claims 1 to 4, comprising said sequence having at least 90% identity to SEQ ID NO:1 and which further comprises an amino acid substitution of His at a position corresponding to amino acid 134 of SEQ ID NO:1.

6. A composition comprising the light-responsive ion channel as defined in any one of claims 1 to 5 in combination with a second light-activated ion pump.
7. A nucleic acid comprising a nucleotide sequence encoding a light-responsive ion channel as defined in any one of claims 1 to 5.
8. The nucleic acid according to claim 7, wherein the nucleotide sequence is operably linked to a control element.
9. The nucleic acid according to claim 7 or 8, wherein the nucleic acid is an expression vector.
10. The nucleic acid according to claim 9, wherein the expression vector is a lentivirus vector or an adeno-associated virus vector.
11. A mammalian neuronal cell comprising a nucleic acid as defined in any one of claims 7 to 10.
12. A system for controlling action potential of a neuron, the system comprising:
 - a) a delivery device for delivery of a light-responsive ion channel protein or a nucleic acid encoding the protein to the neuron, wherein the delivery device comprises said light-responsive ion channel protein or nucleic acid encoding the protein, and wherein said protein comprises an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2, wherein the light-responsive ion channel exhibits an extended conducting state after exposure to a light pulse compared to that of a polypeptide comprising SEQ ID NO:1 or SEQ ID NO:2, and wherein the light-responsive ion channel comprises: i) a Cys→Thr, a Cys→Ala, or a Cys→Ser substitution at a position corresponding to amino acid 128 of SEQ ID NO:1 or amino acid 123 of SEQ ID NO:2; and ii) an Asp→Ala or an Asp→Asn substitution at a position corresponding to amino acid 156 of SEQ ID NO:1 or amino acid 151 of SEQ ID NO:2;

- b) a light source; and
- c) a control device for controlling generation of light by the light source.

13. The system according to claim 12, wherein the light pulse is 10 ms or less.

14. The system according to claim 12 or 13, wherein the light-responsive ion channel comprises: i) the Cys→Ser substitution at the position corresponding to amino acid 128 of SEQ ID NO:1 or amino acid 123 of SEQ ID NO:2; and ii) the Asp→Ala substitution at the position corresponding to amino acid 156 of SEQ ID NO:1 or amino acid 151 of SEQ ID NO:2.

15. The system according to claim 12, 13 or 14, wherein the light-responsive ion channel protein comprises said amino acid sequence having at least 90% identity to SEQ ID NO:1 and further comprises an amino acid substitution of Glu at a position corresponding to amino acid 123 of SEQ ID NO:1.

16. The system according to any one of claims 12 to 15, wherein the light-responsive ion channel protein comprises said amino acid sequence having at least 90% identity to SEQ ID NO:1 and further comprises an amino acid substitution of His at a position corresponding to amino acid 134 of SEQ ID NO:1.

17. The system according to any one of claims 12 to 16, wherein the light source is a light-emitting diode or a laser.

18. The system according to any one of claims 12 to 17, wherein the delivery device comprises said nucleic acid in a gene transfer vector.

19. The system according to claim 18, wherein the gene transfer vector is a lentivirus vector.

20. The system according to any one of claims 12 to 17, wherein the delivery device comprises said nucleic acid in a dehydrated or water soluble material.

21. The system according to any one of claims 12 to 20, wherein the light source is implantable.

22. An *in vitro* method of stimulating a neuron, said method comprising exposing the neuron to light to thereby stimulate the neuron, wherein the neuron expresses a light-responsive ion channel as defined in any one of claims 1 to 5 or a light-responsive ion channel encoded by a nucleic acid as defined in any one of claims 7 to 10.

23. The method according to claim 22, wherein the stimulation comprises controlling the action potential of the neuron.

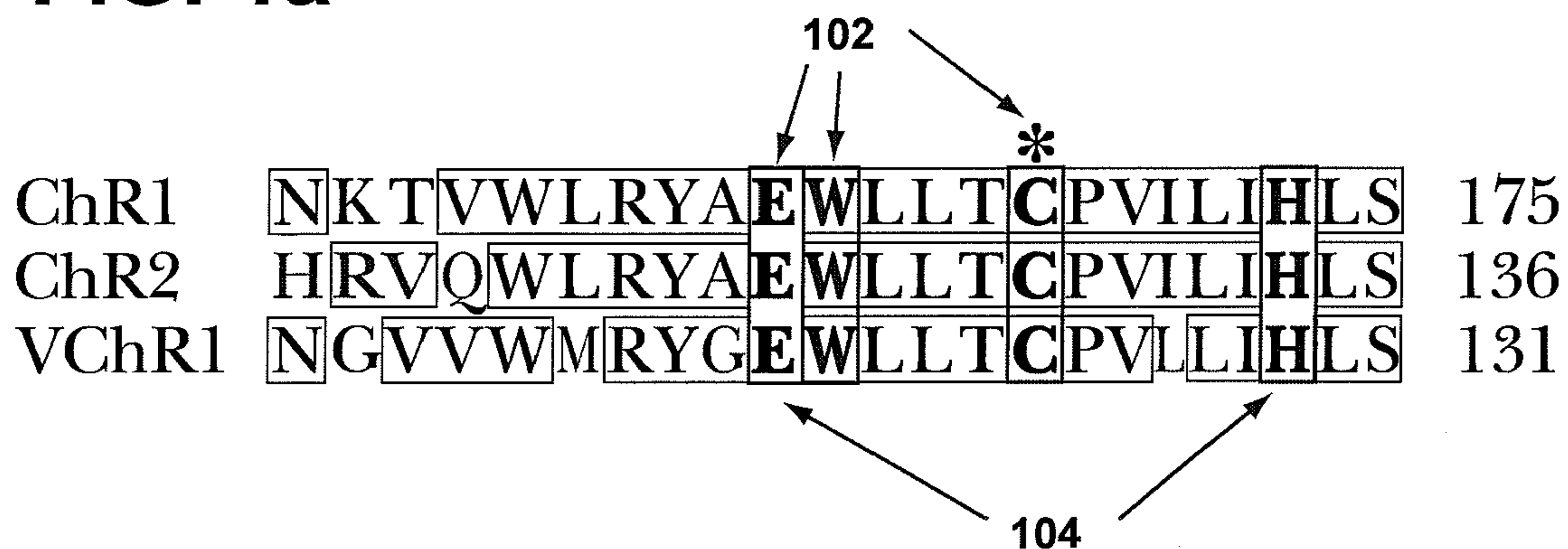
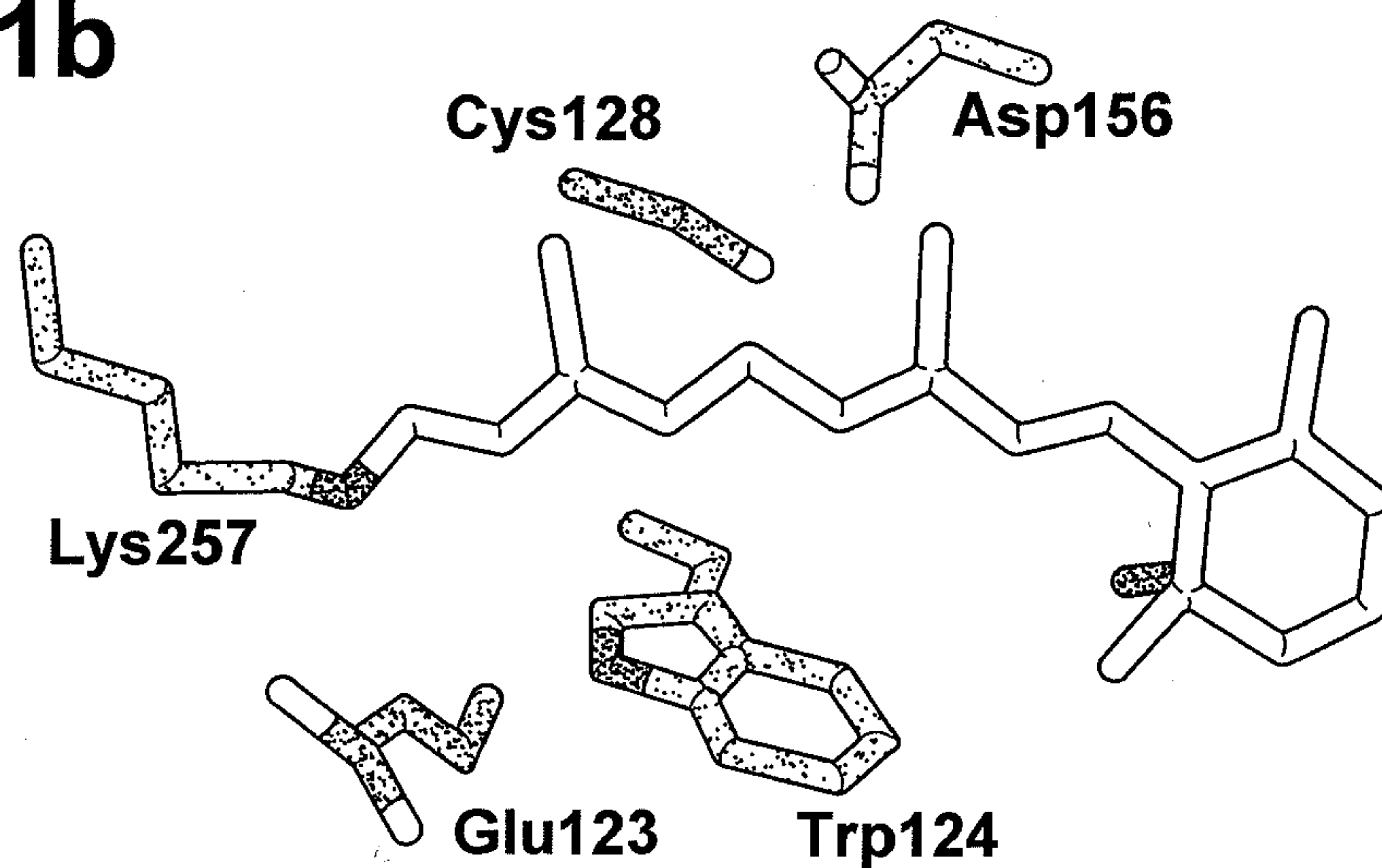
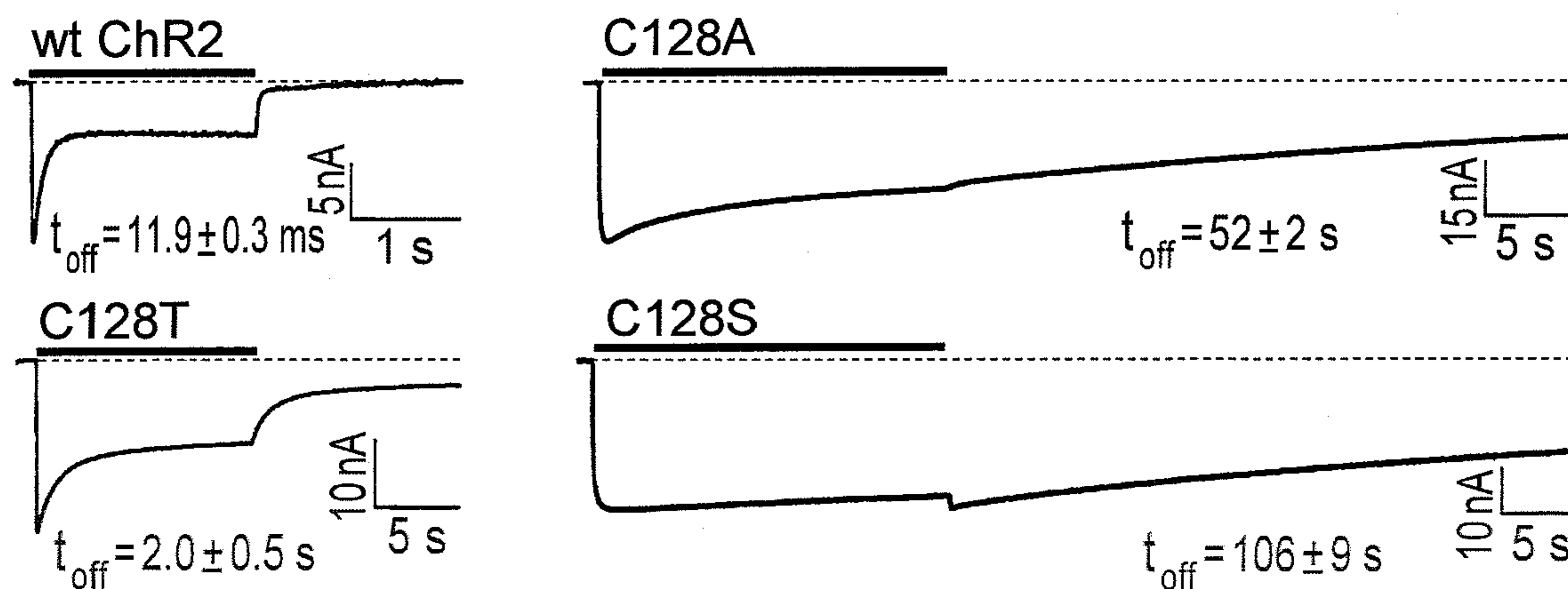
24. Use of a light delivery device for delivering light to a neuron, wherein the neuron expresses a light-responsive ion channel as defined in any one of claims 1 to 5, wherein said delivery of light effects depolarization of the neuron.

25. The use according to claim 24, wherein the light controls the action potential of the neuron.

26. Use of a nucleic acid encoding a light-responsive ion channel as defined in any one of claims 7 to 10, for expressing said light-responsive ion channel in a neuron.

27. The use of claim 26, wherein expression of the light-responsive ion channel in the neuron results in the action potential of the neuron being controllable with light.

28. The use of any one of claims 24 to 27, wherein the neuron is *in vivo*.

FIG. 1a**FIG. 1b****FIG. 1c**

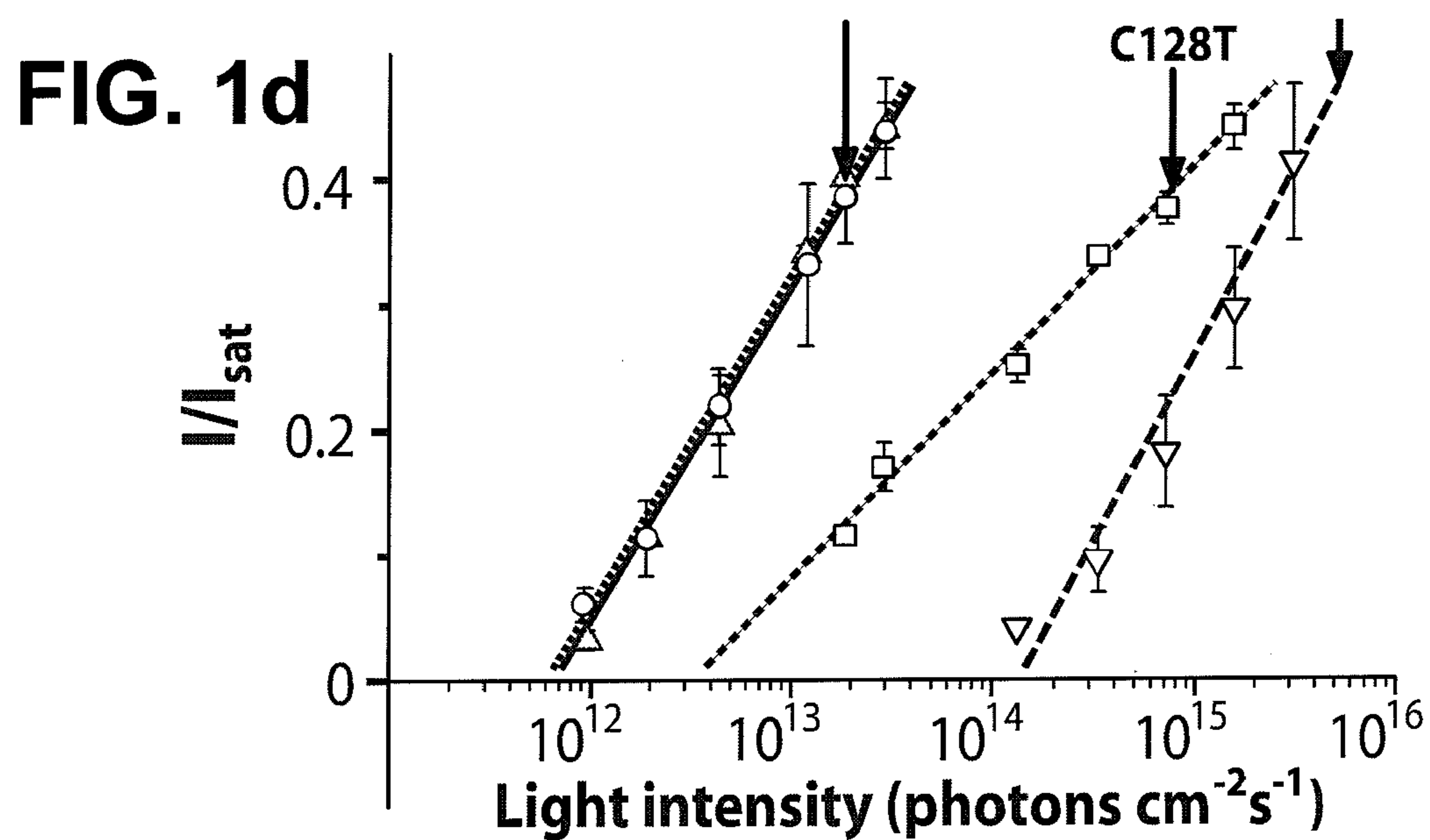
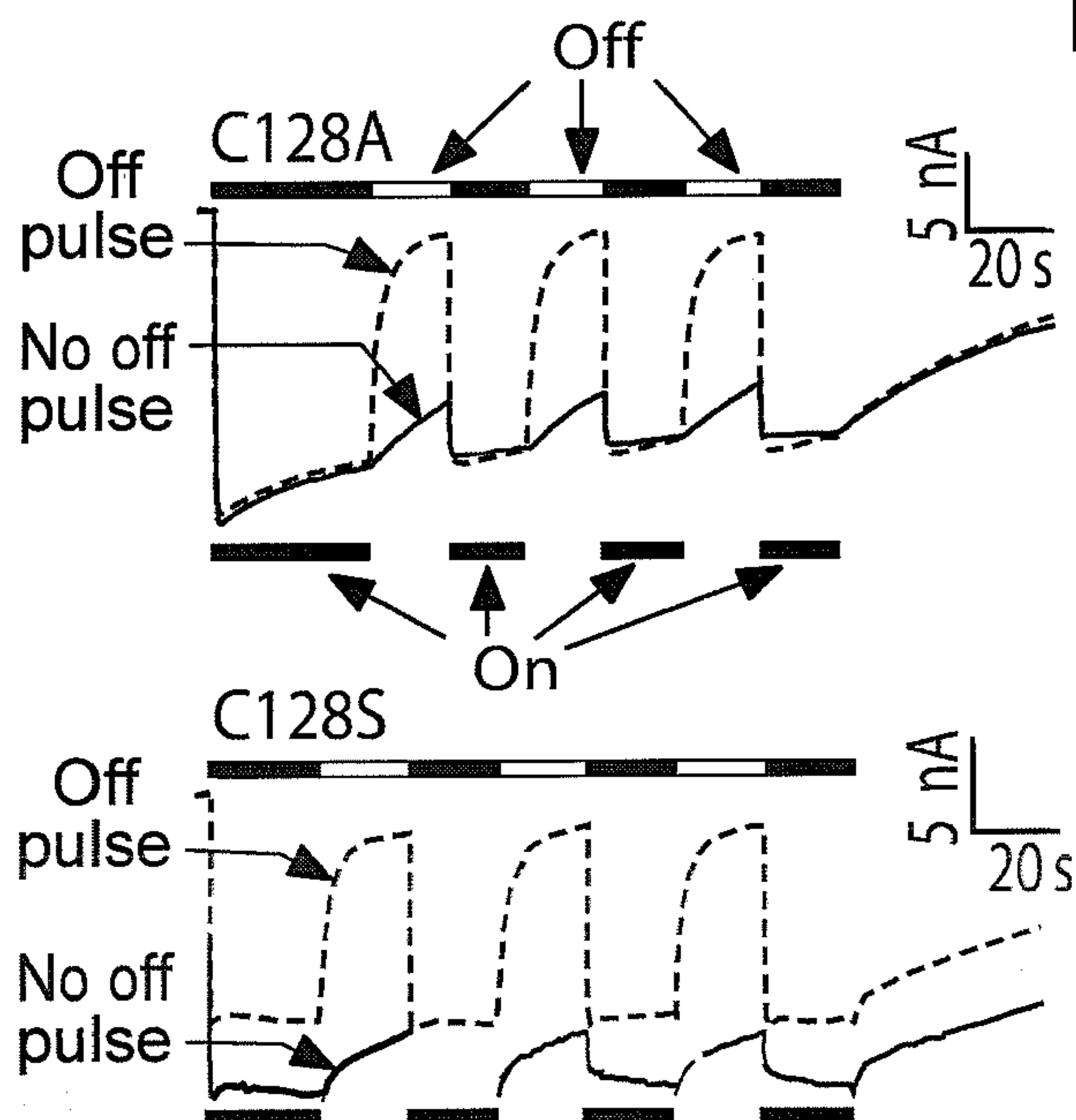
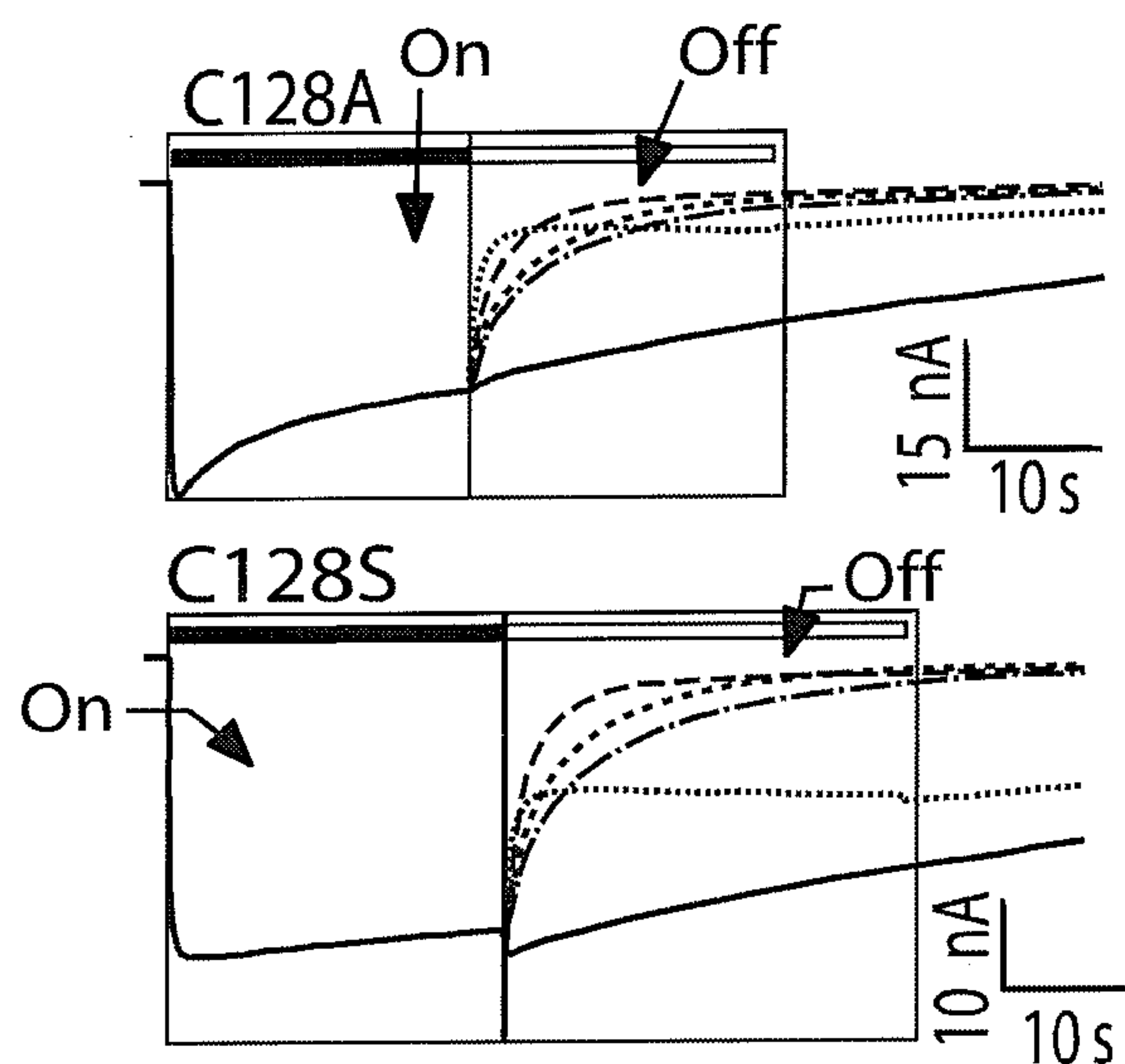
**FIG. 1e****FIG. 1f**

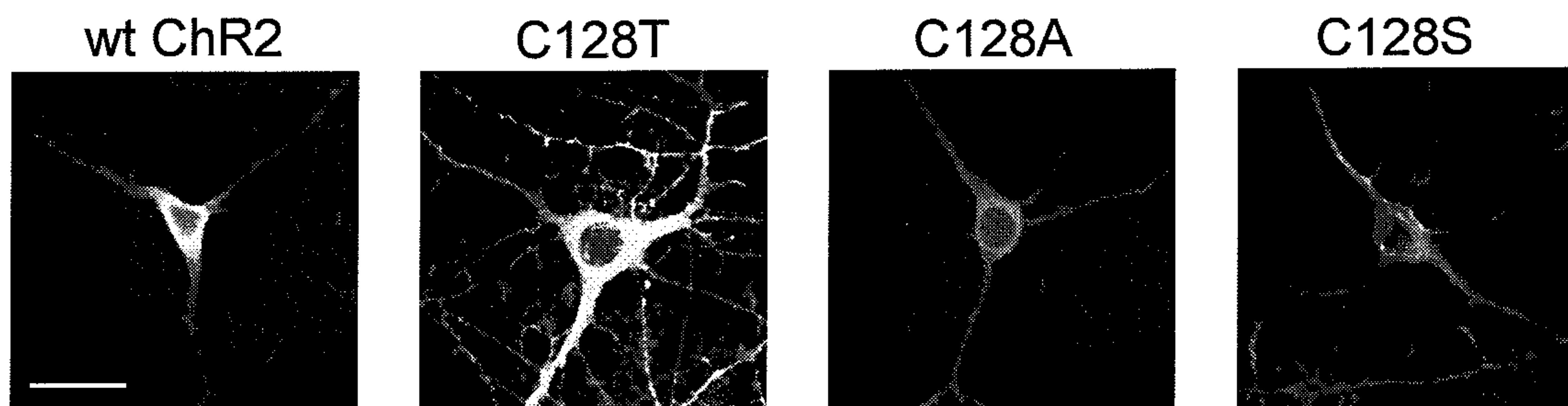
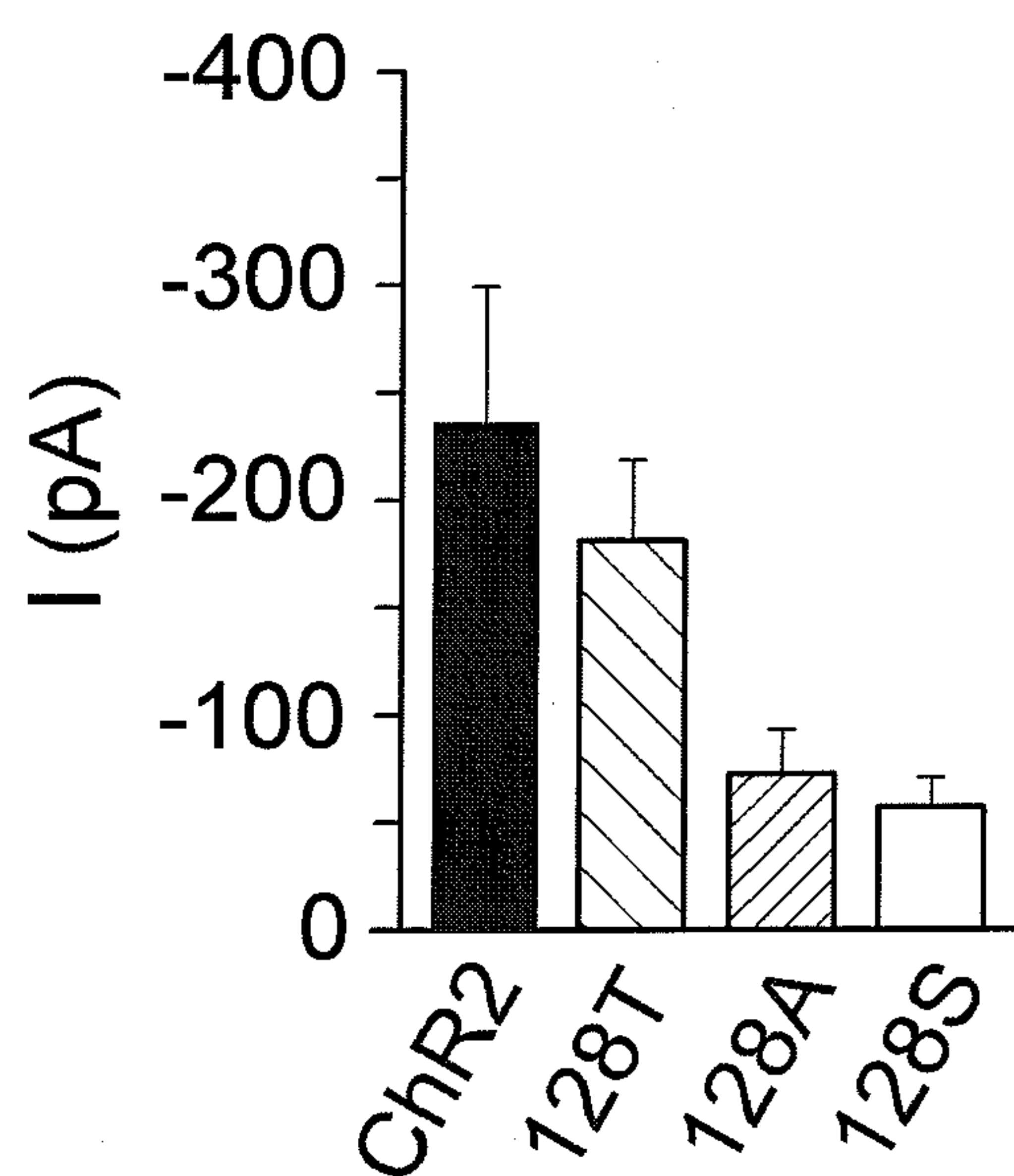
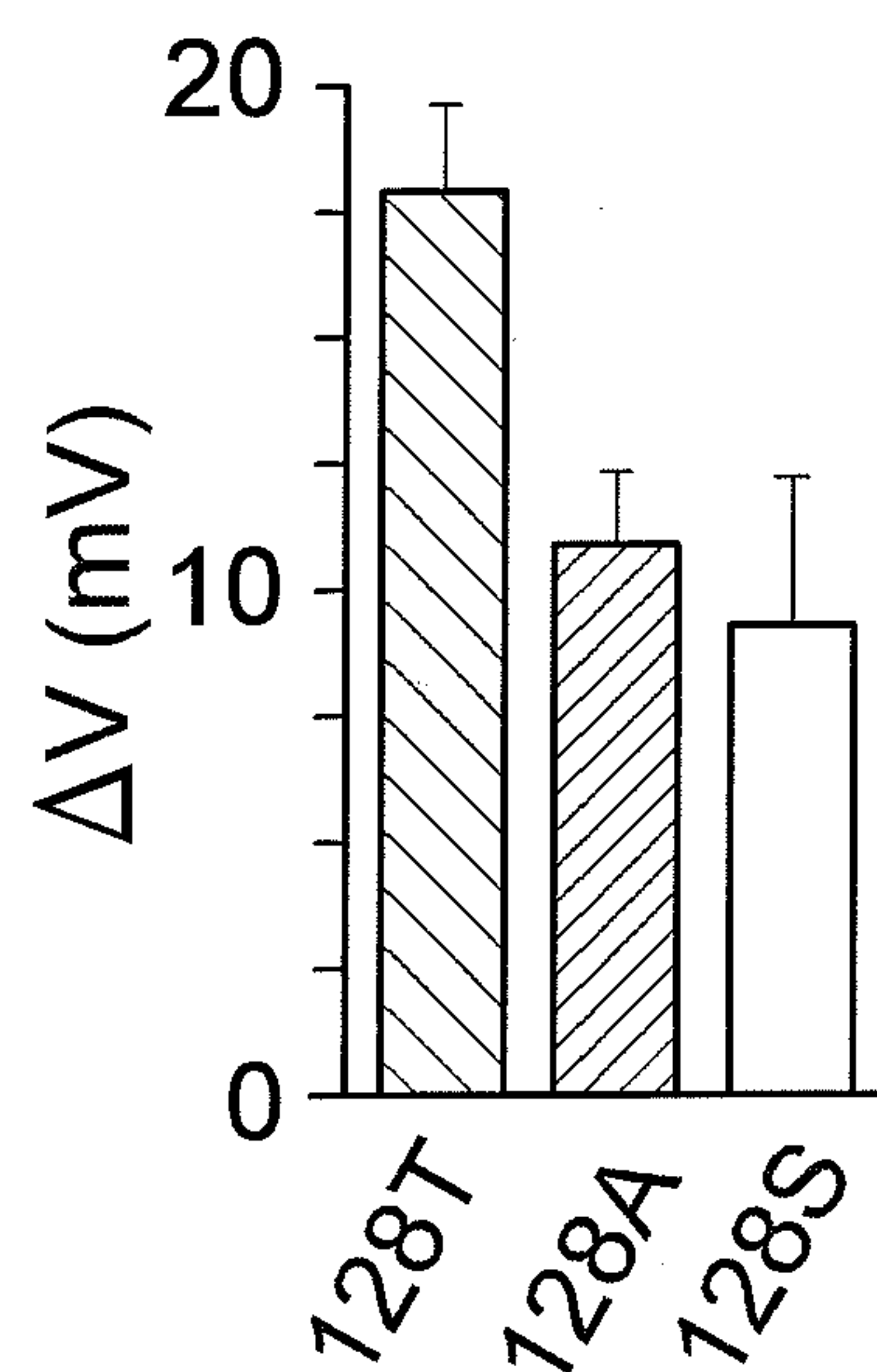
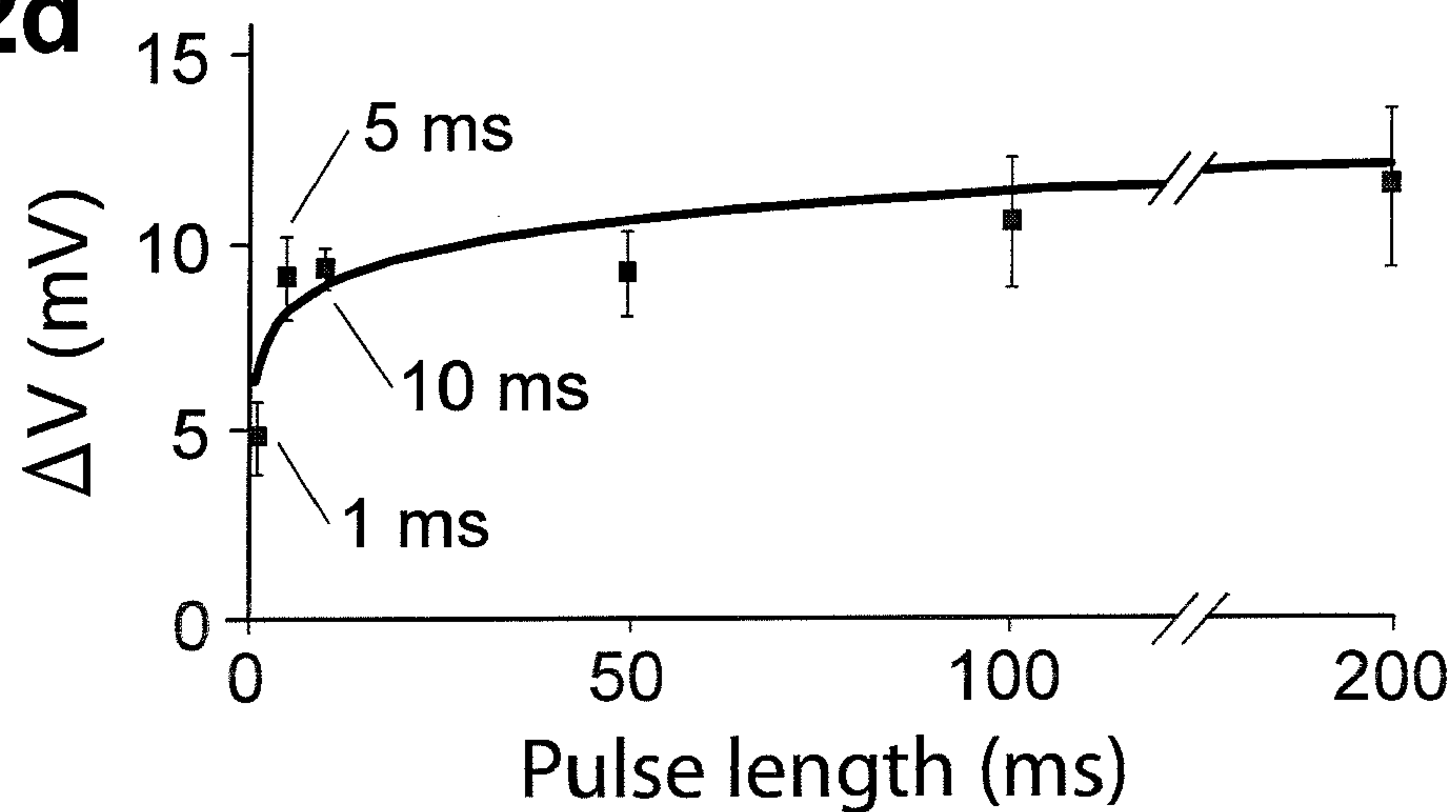
FIG. 2a**FIG. 2b****FIG. 2c****FIG. 2d**

FIG. 2e

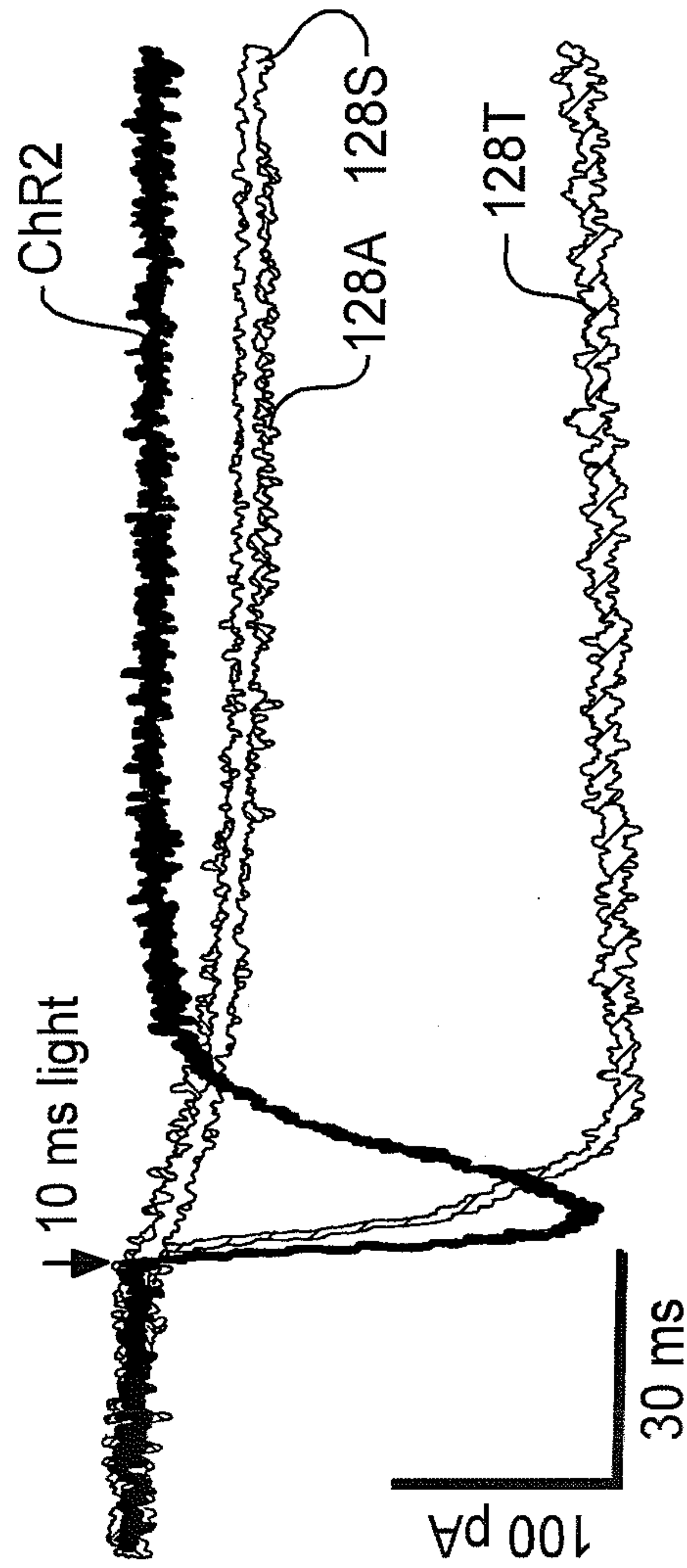


FIG. 2f

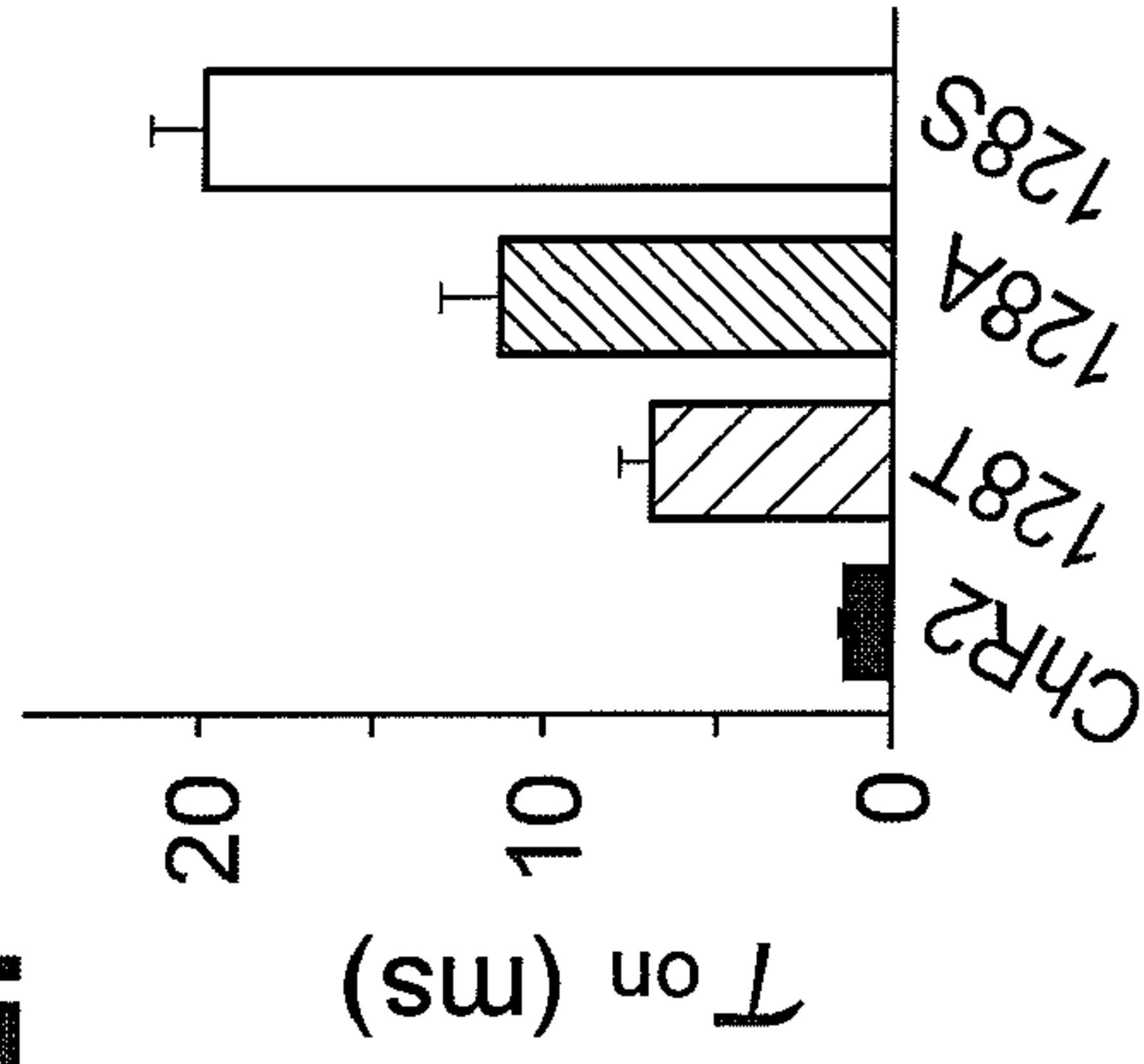


FIG. 2g

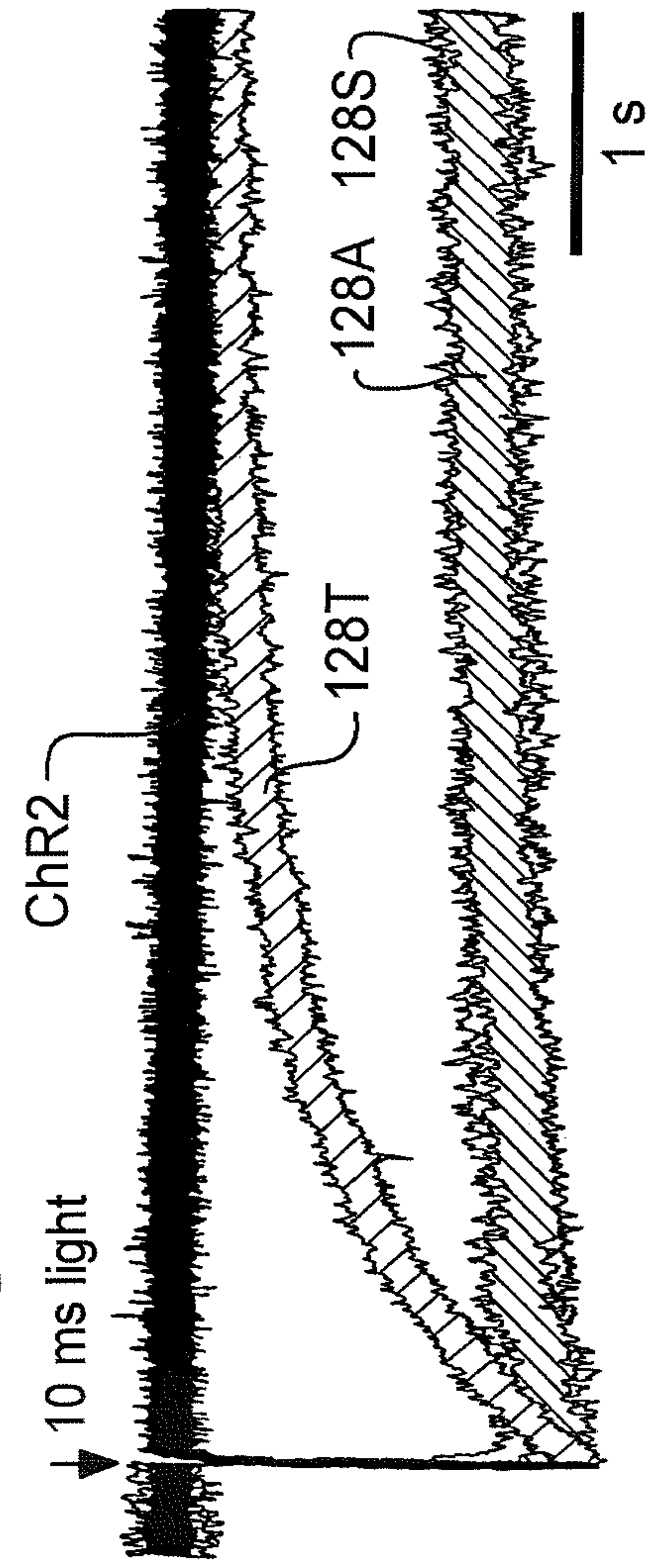


FIG. 2h

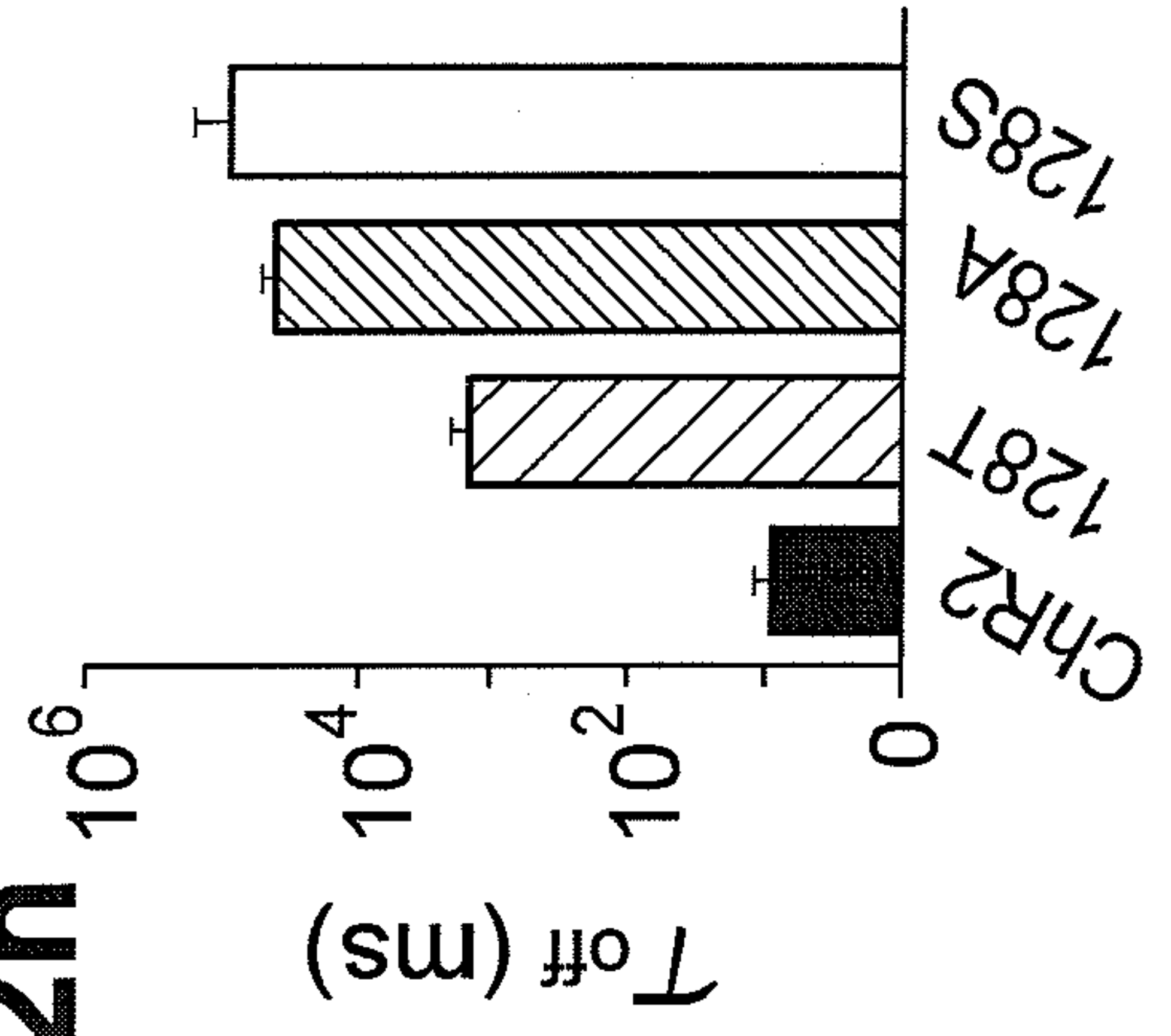


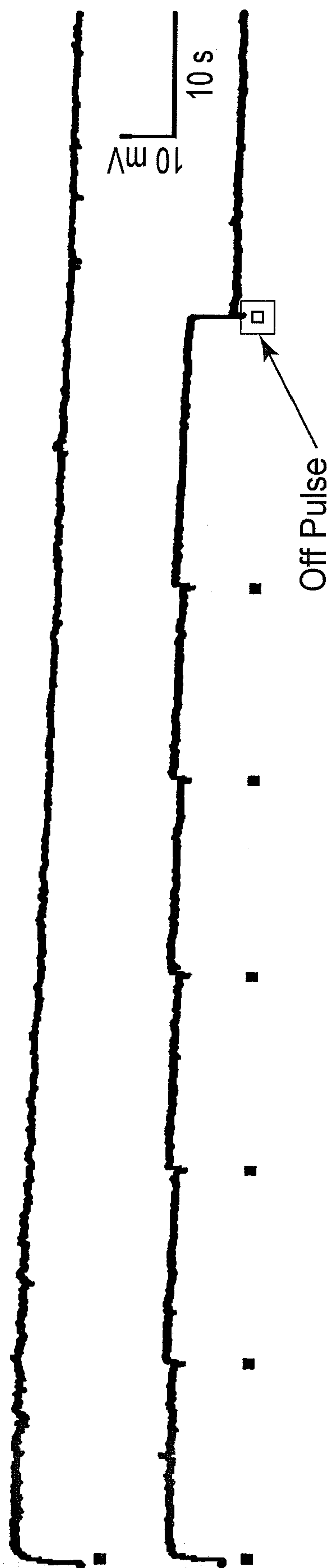
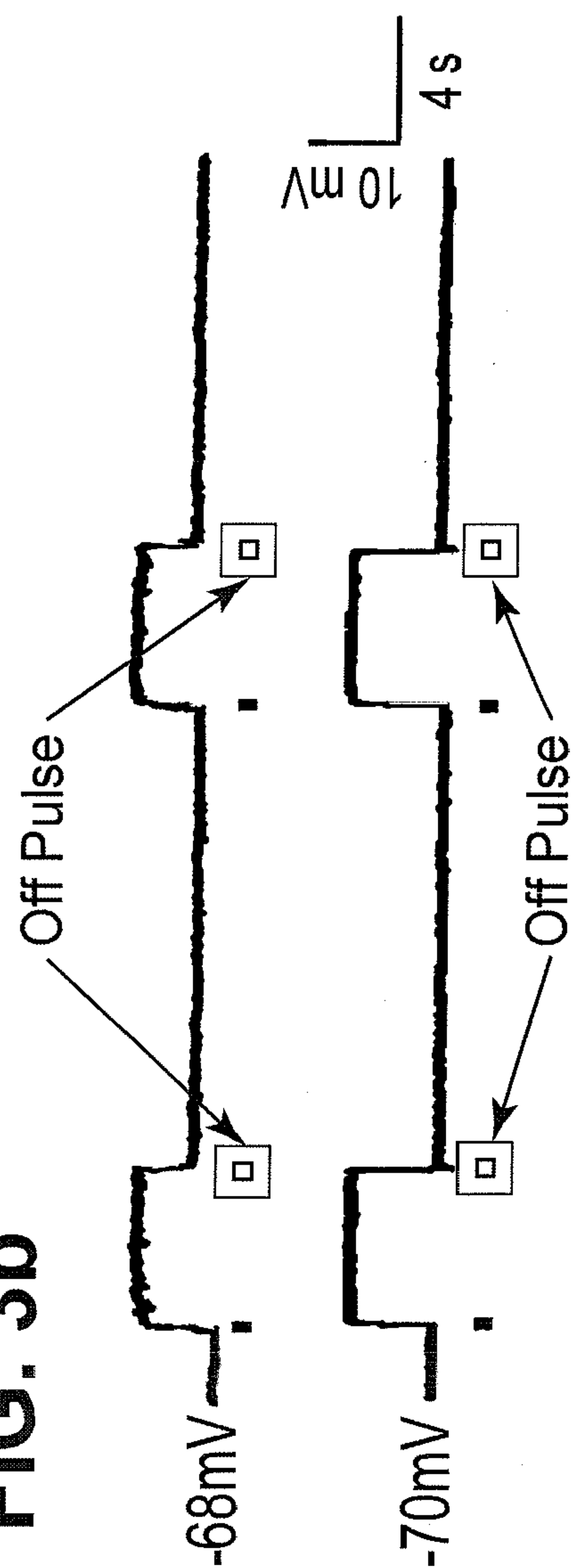
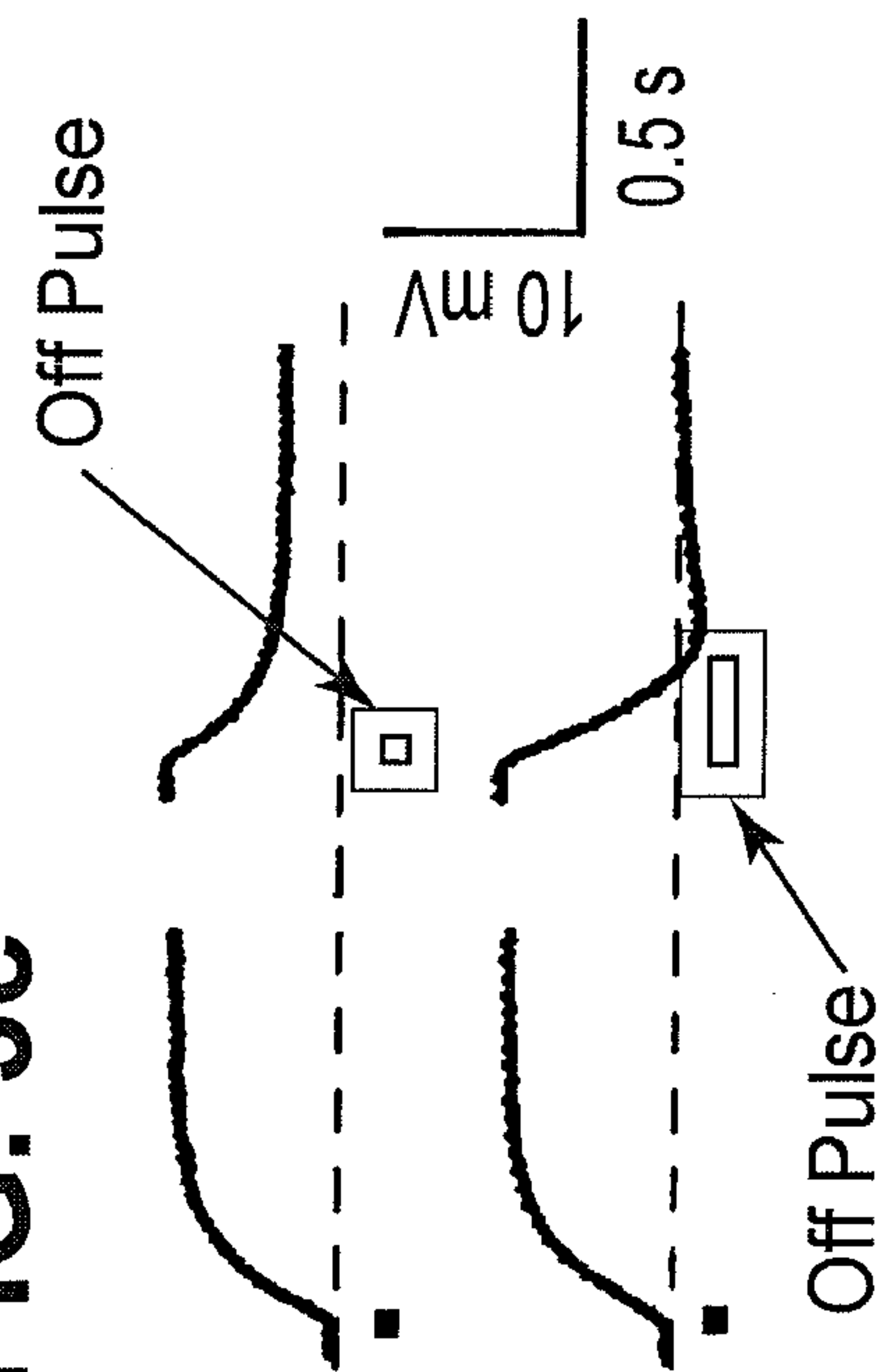
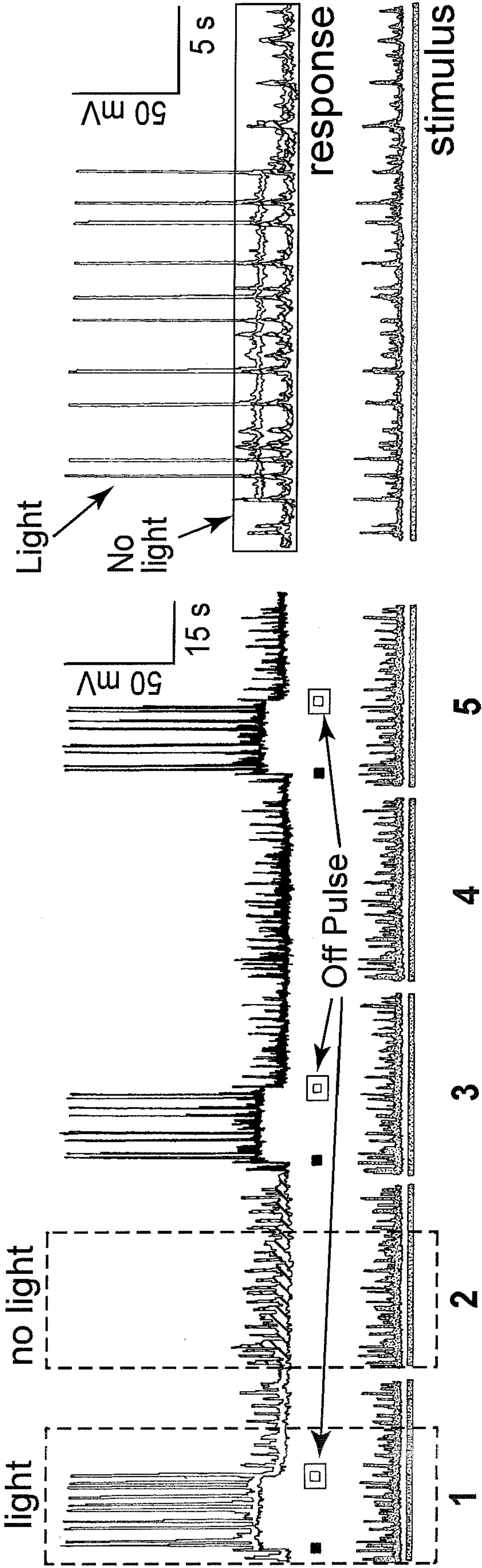
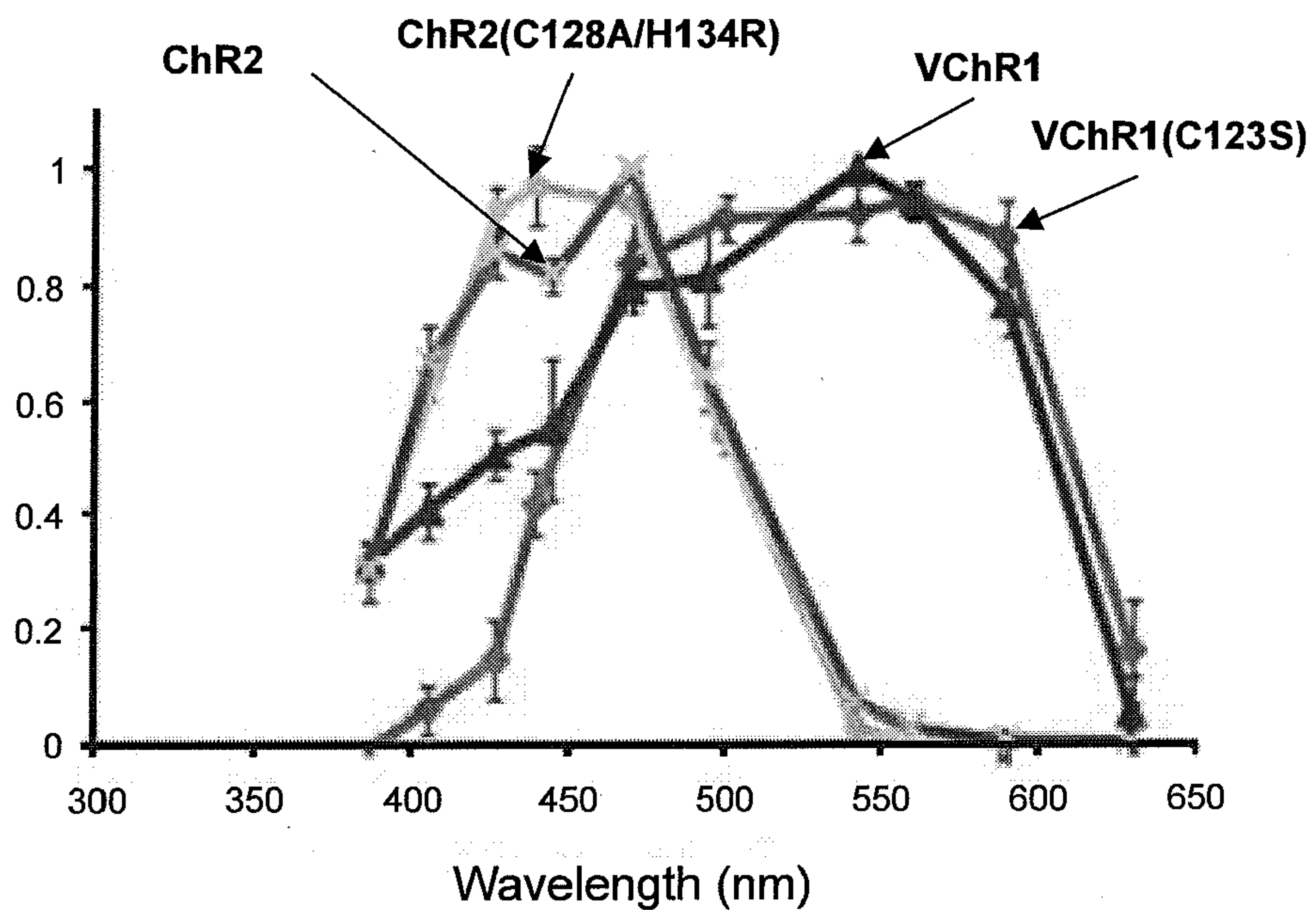
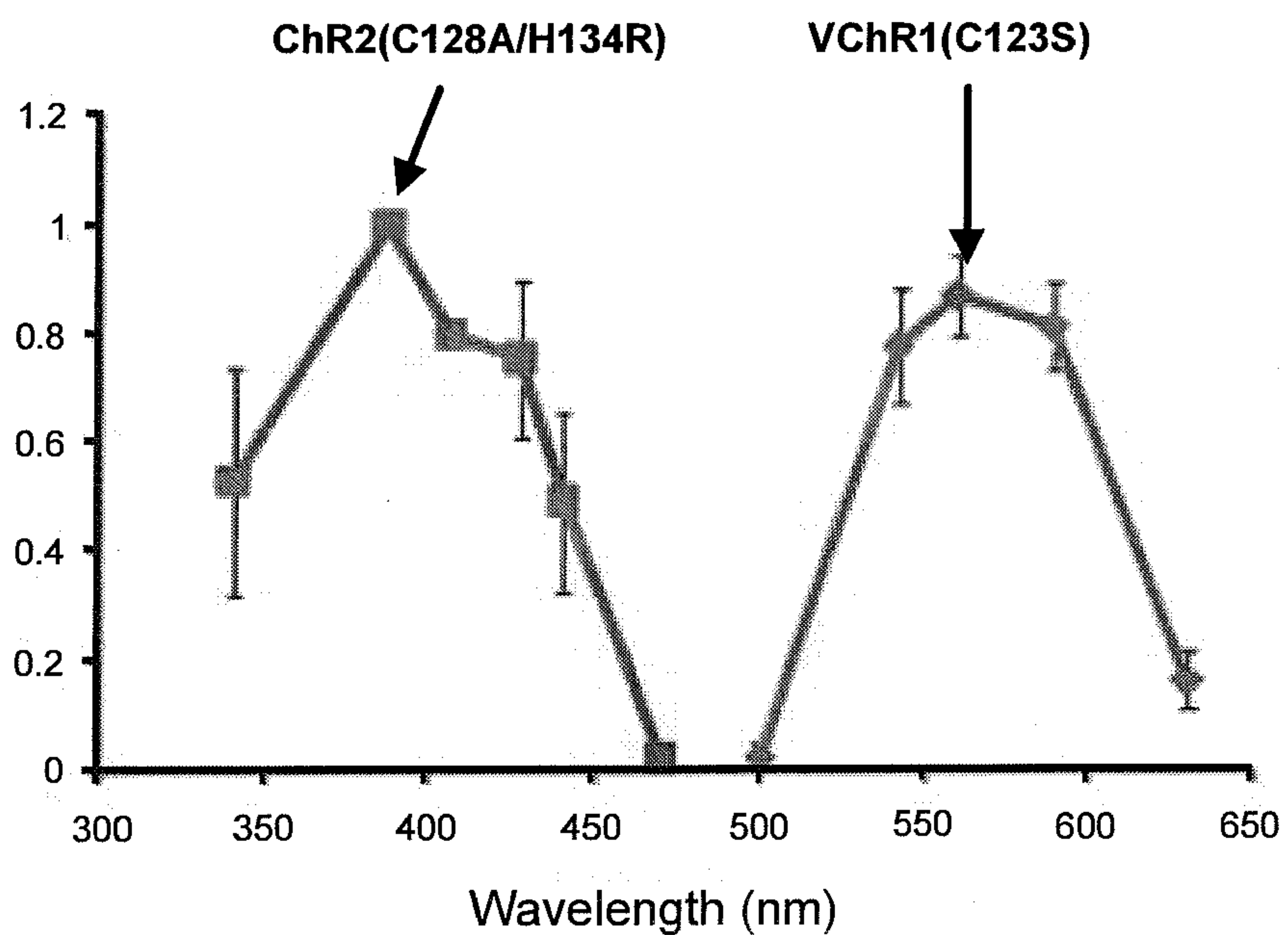
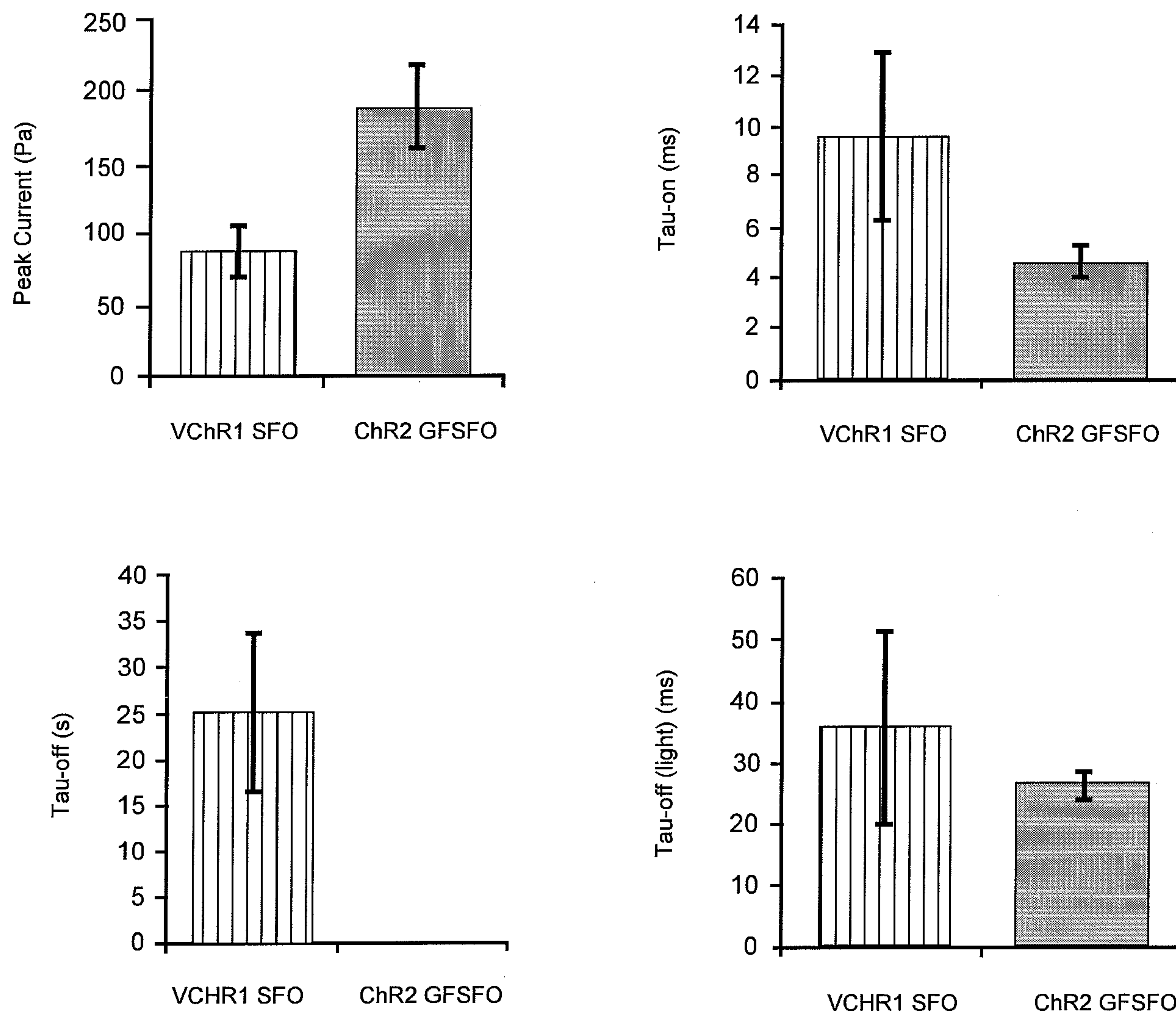
FIG. 3a**FIG. 3b****FIG. 3c**

FIG. 3d



**FIG. 4a****FIG. 4b**

**FIG. 4c**

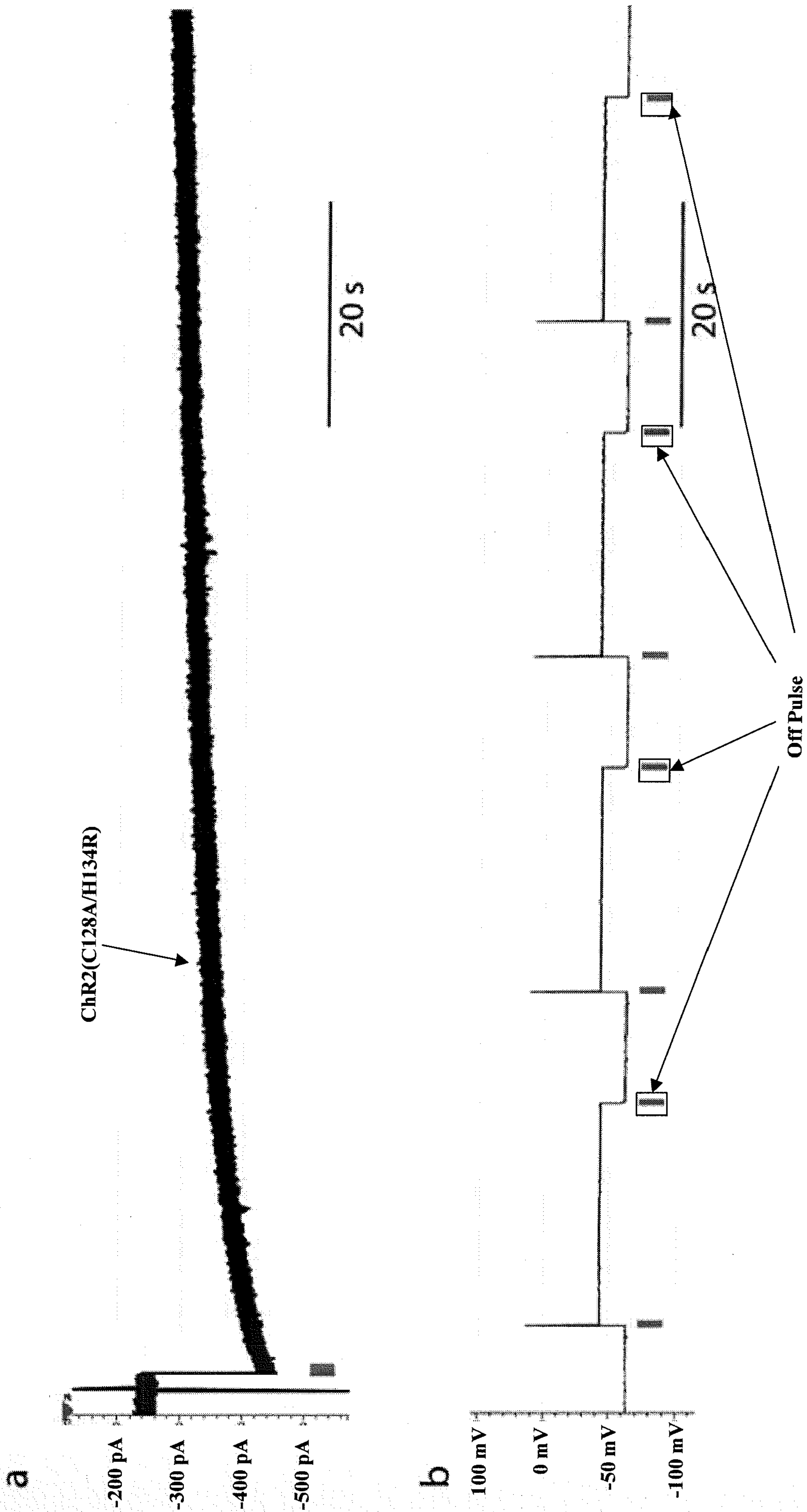
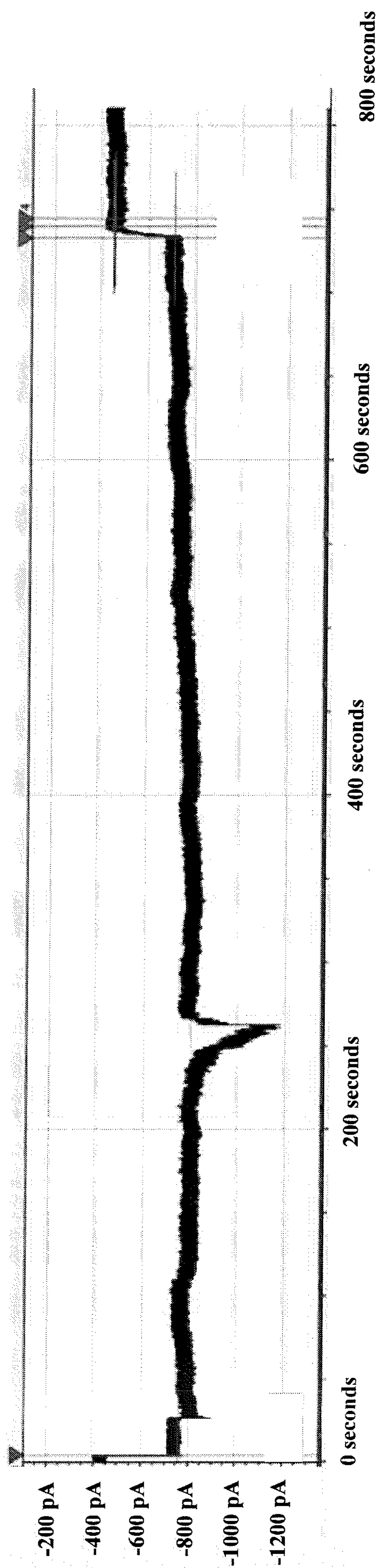


FIG. 5

**FIG. 6**

