US 20170157269A1

(19) **United States**

(12) **Patent Application Publication**

Deisseroth et al.

(10) **Pub. No.: US 2017/0157269 A1**

(43) **Pub. Date: Jun. 8, 2017**

(54) **OPTOGENETIC CONTROL OF REWARD-RELATED BEHAVIORS**

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(21) Appl. No.: **15/214,399**

(22) Filed: **Jul. 19, 2016**

Related U.S. Application Data

(62) Division of application No. 13/882,670, filed on Aug. 12, 2013, filed as application No. PCT/US11/59295 on Nov. 4, 2011.

(60) Provisional application No. 61/410,692, filed on Nov. 5, 2010.

Publication Classification

(51) **Int. Cl.**

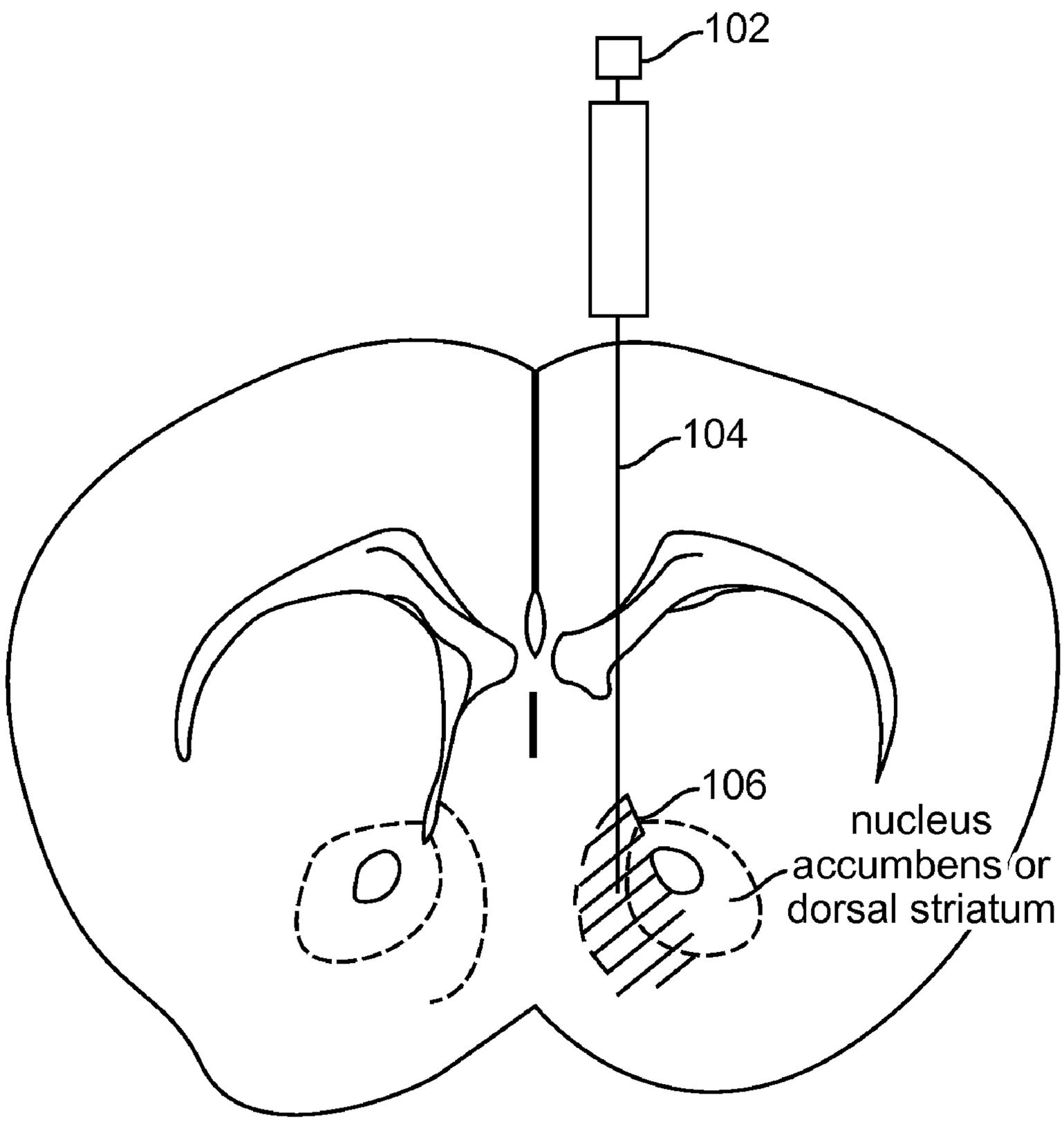
A61K 48/00	(2006.01)
C07K 14/47	(2006.01)
C12Q 1/00	(2006.01)
C12M 1/34	(2006.01)
C12M 1/00	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 48/0058*** (2013.01); ***C12M 41/32*** (2013.01); ***C12M 31/10*** (2013.01); ***C12Q 1/00*** (2013.01); ***C07K 14/47*** (2013.01); *A61K 2121/00* (2013.01)

(57) **ABSTRACT**

Provided herein are compositions and methods for disrupting at least one reward-related behavior in an individual through the use of light-responsive opsin proteins used to control the polarization state of the cholinergic intemeurons of the nucleus accumbens or the striatum.



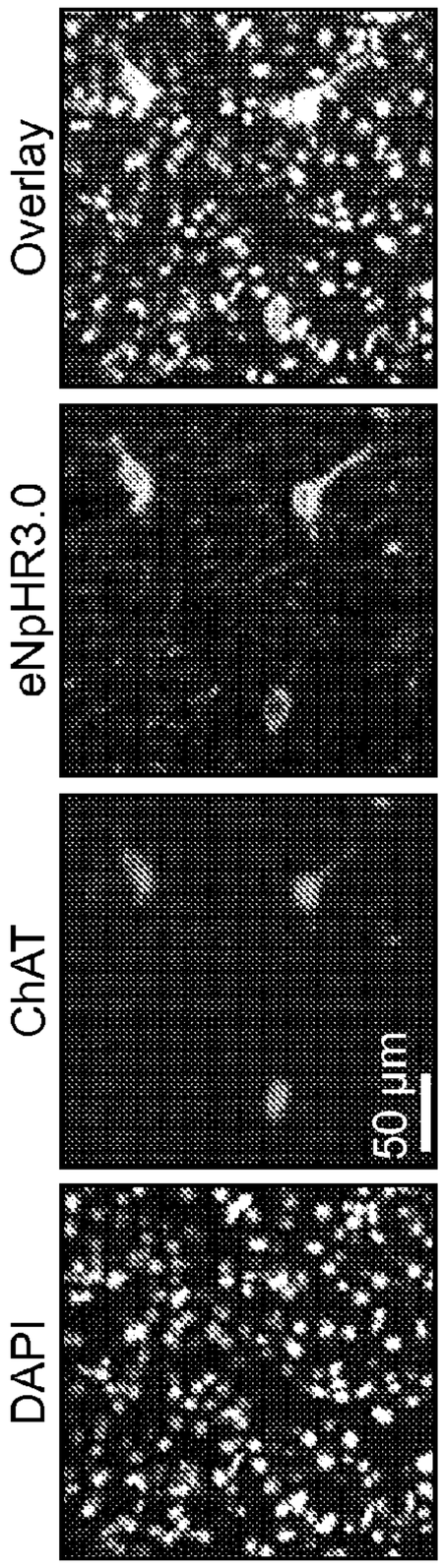


FIG. 1B

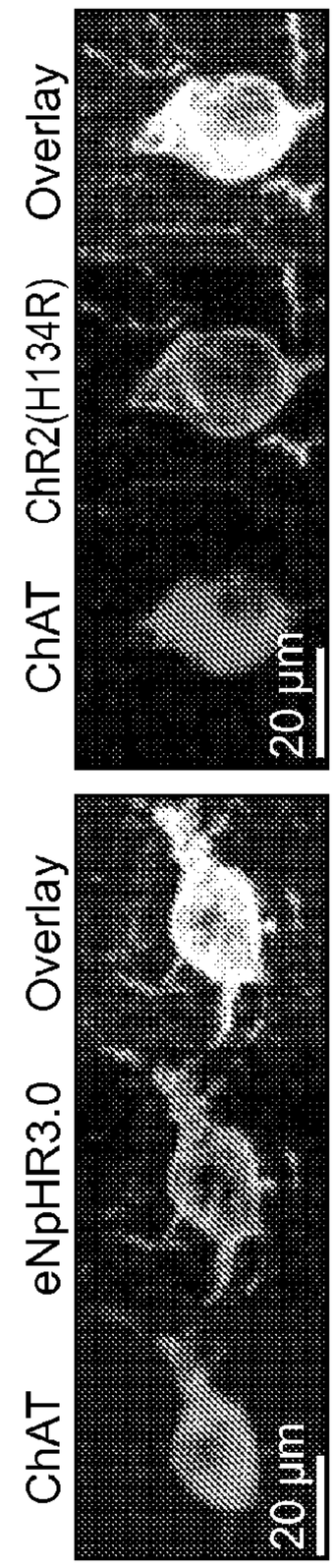


FIG. 1D

FIG. 1H

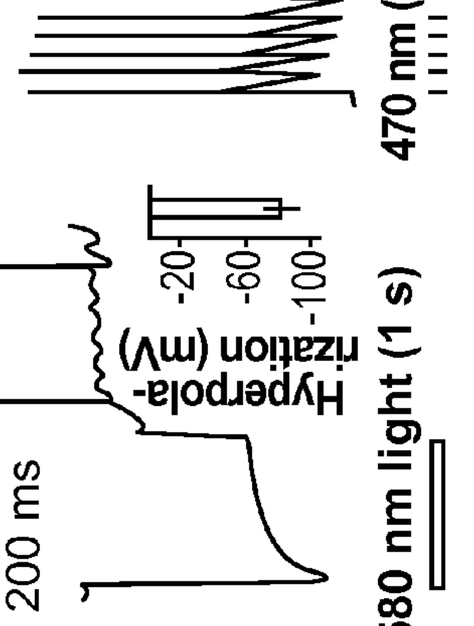


FIG. 1F

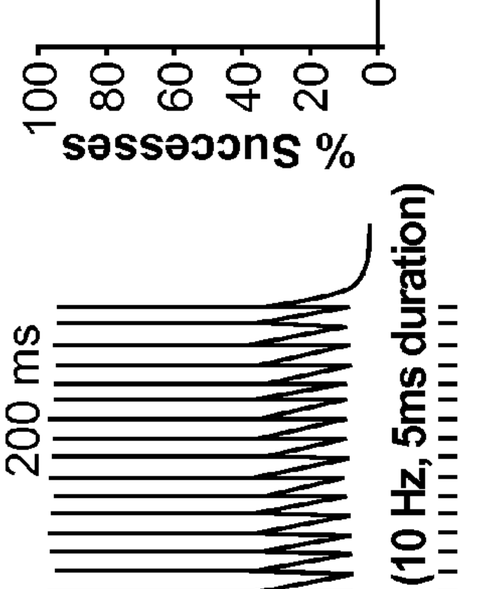


FIG. 1G

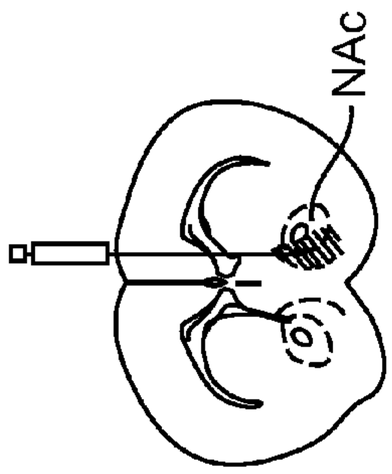


FIG. 1A

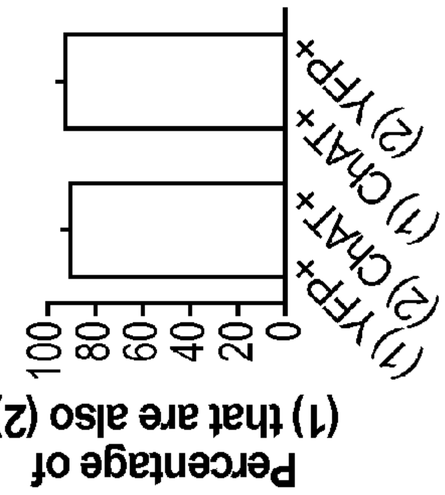


FIG. 1C

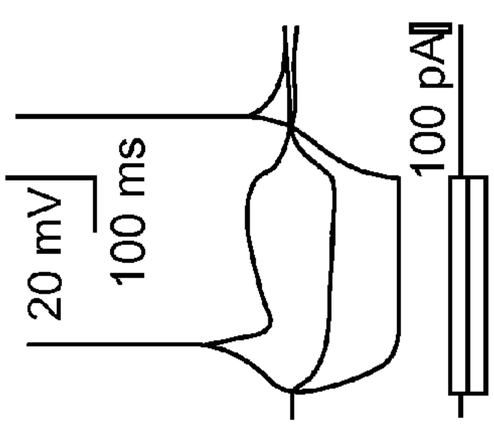


FIG. 1E

FIG. 2A

FIG. 2B

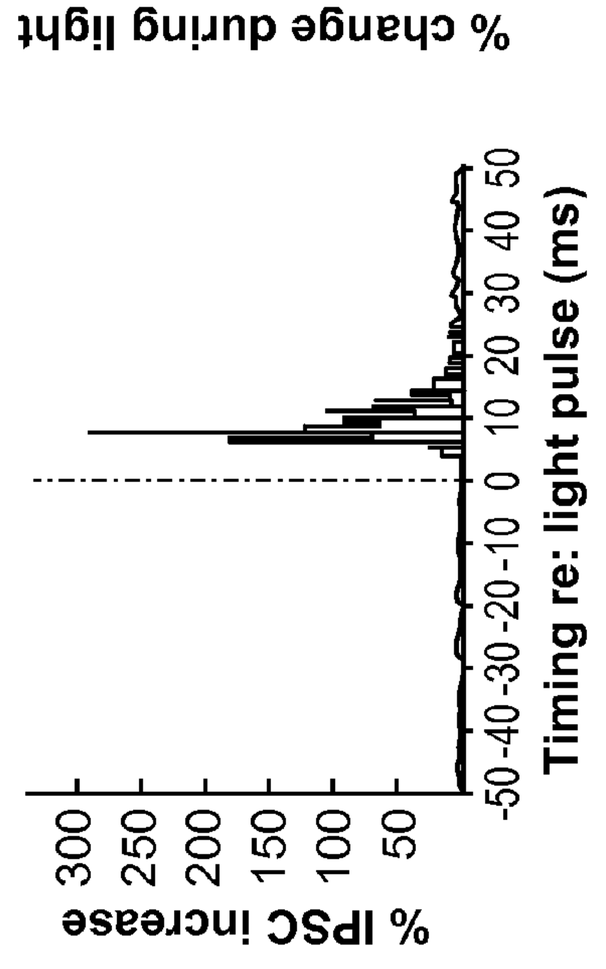


FIG. 2D

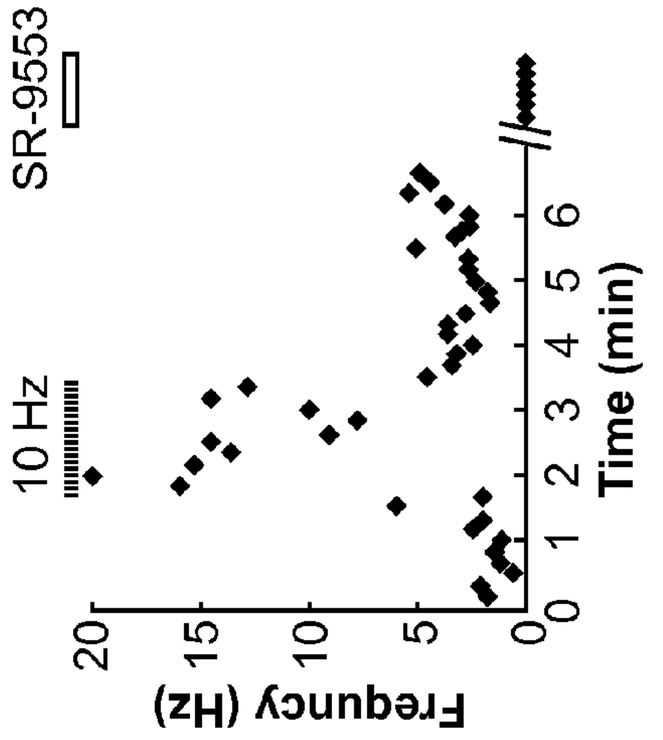


FIG. 2C

FIG. 2E

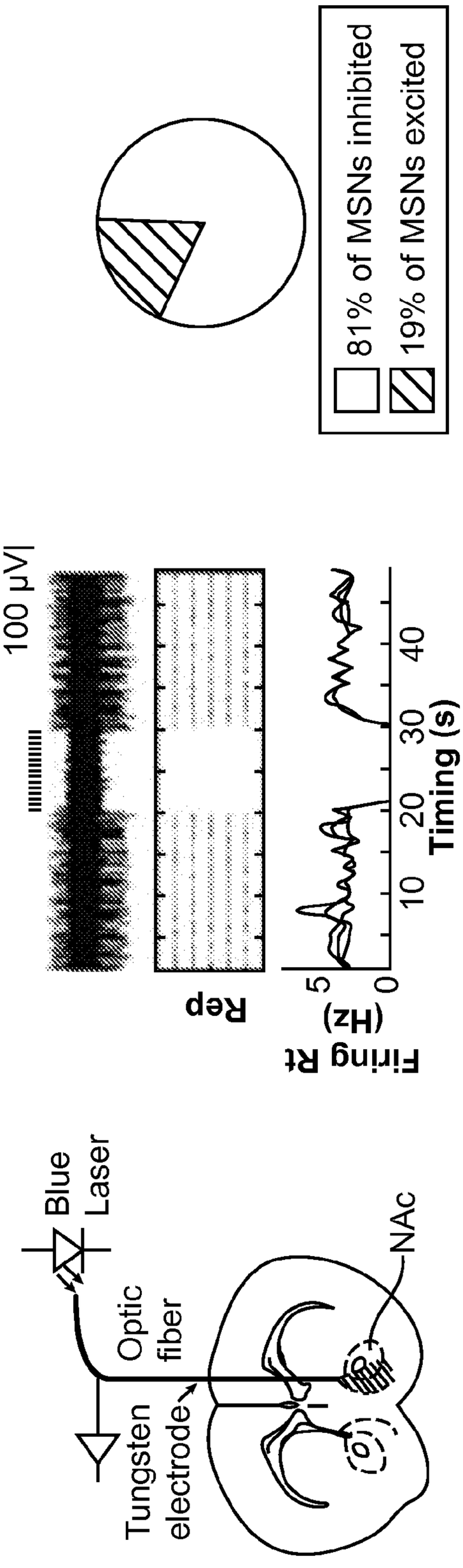


FIG. 2F

FIG. 2G

FIG. 2H

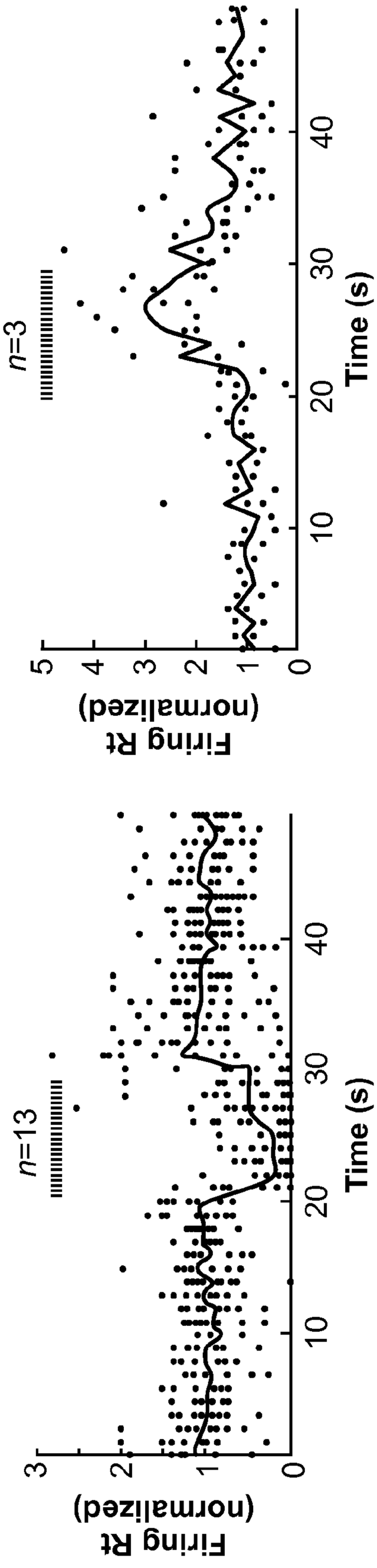


FIG. 2I

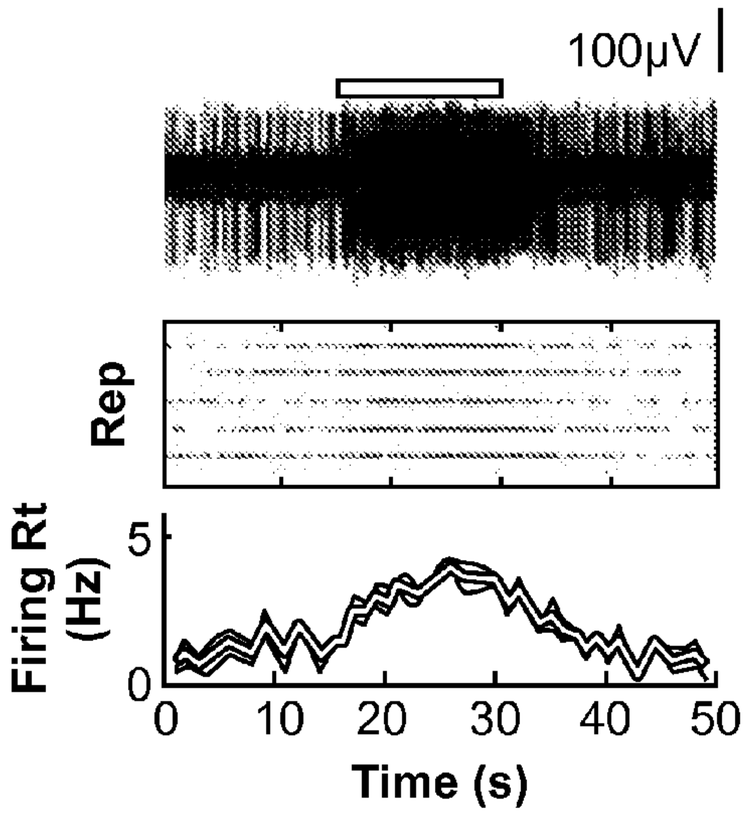


FIG. 3A

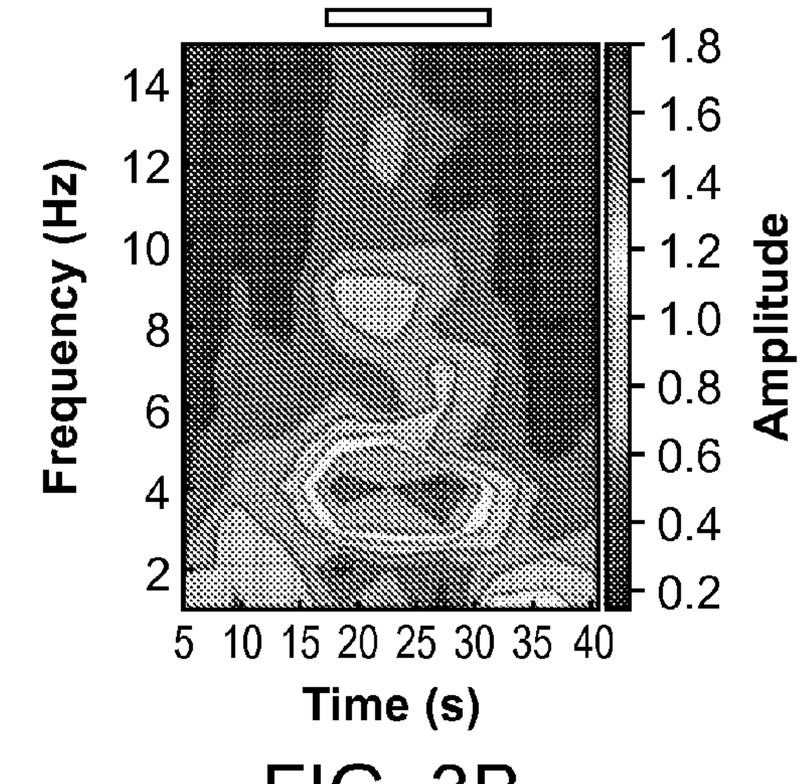


FIG. 3B

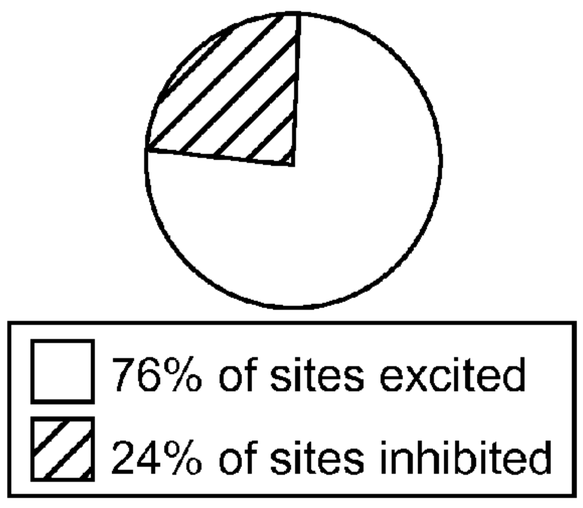


FIG. 3C

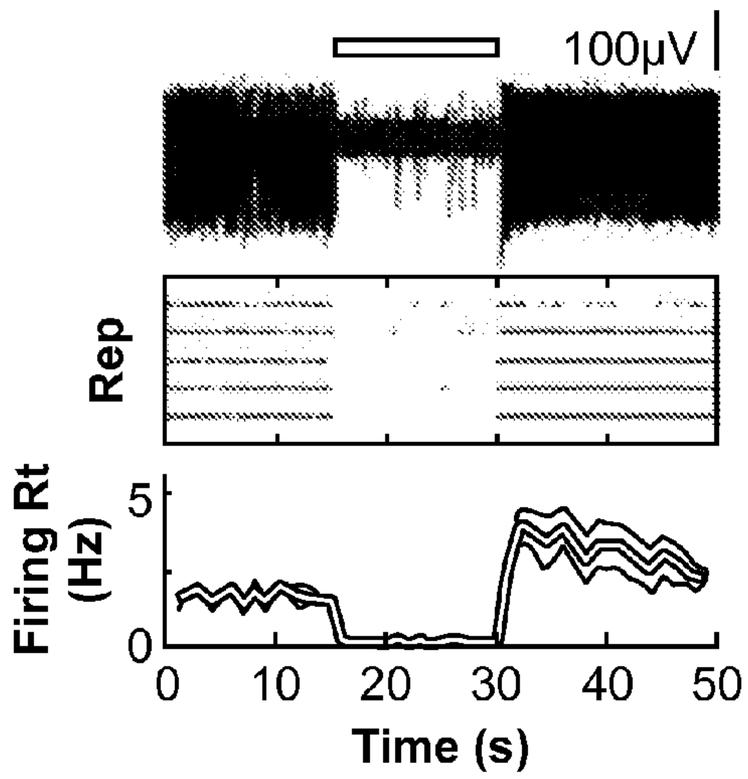


FIG. 3D

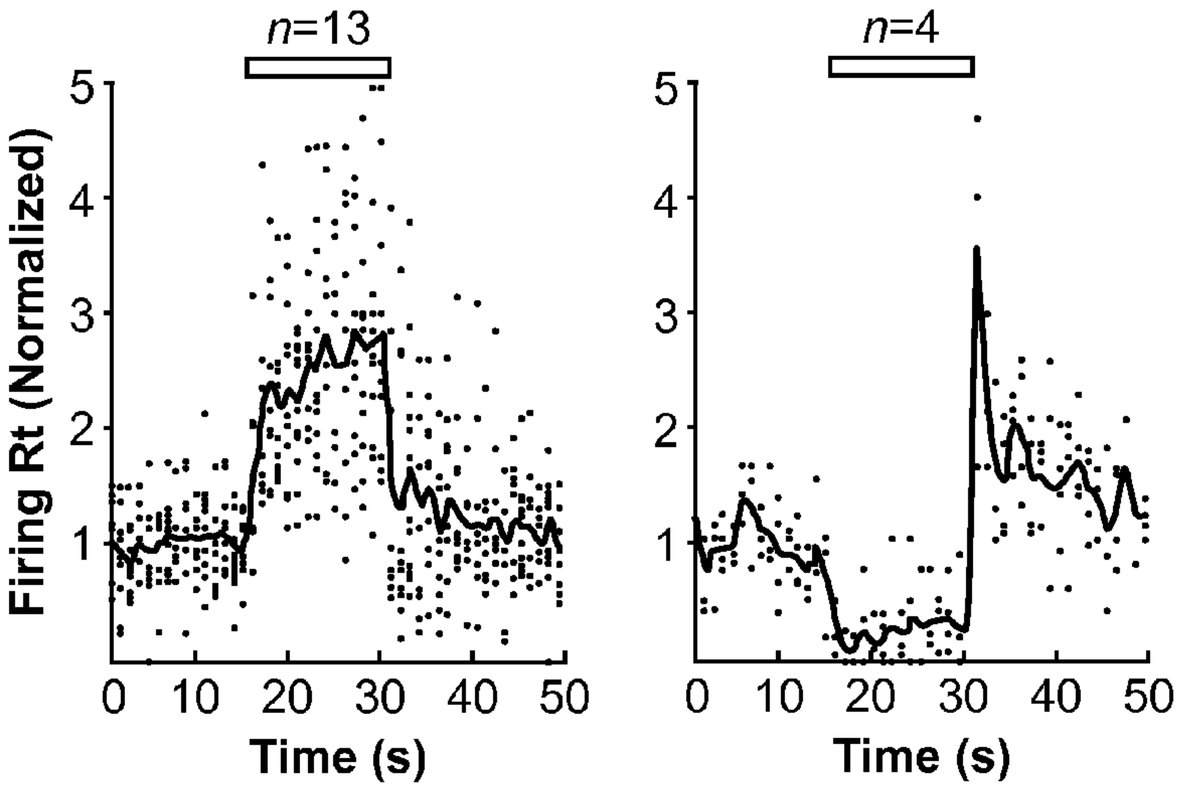


FIG. 3E

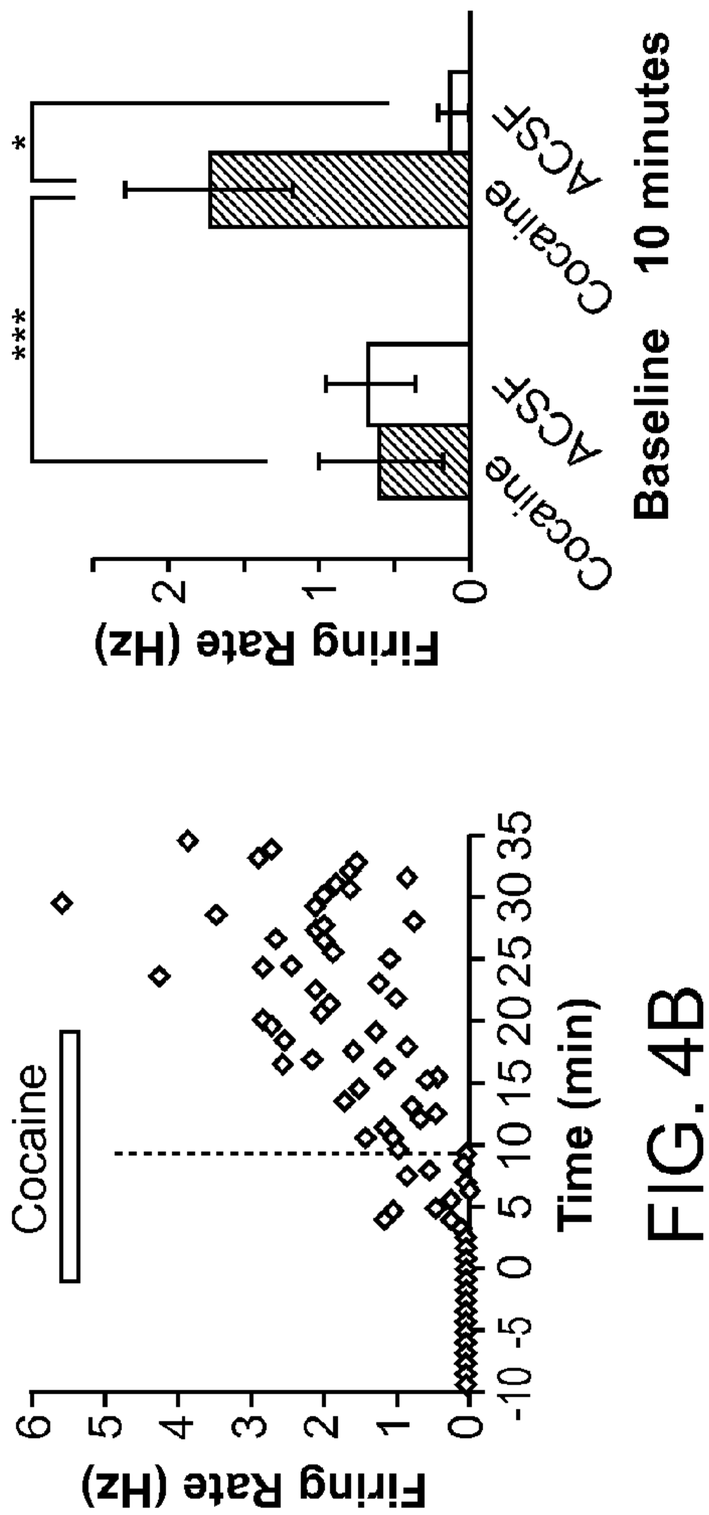


FIG. 4C

FIG. 4B

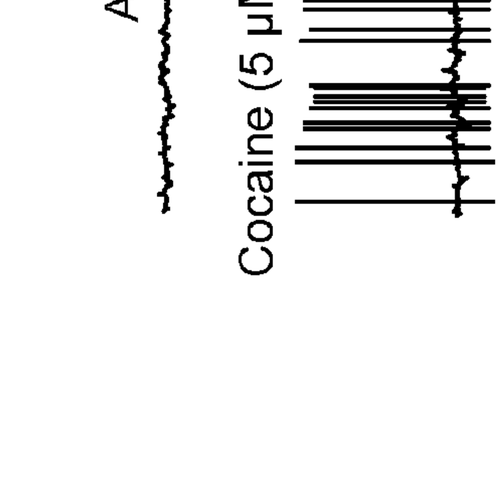


FIG. 4A

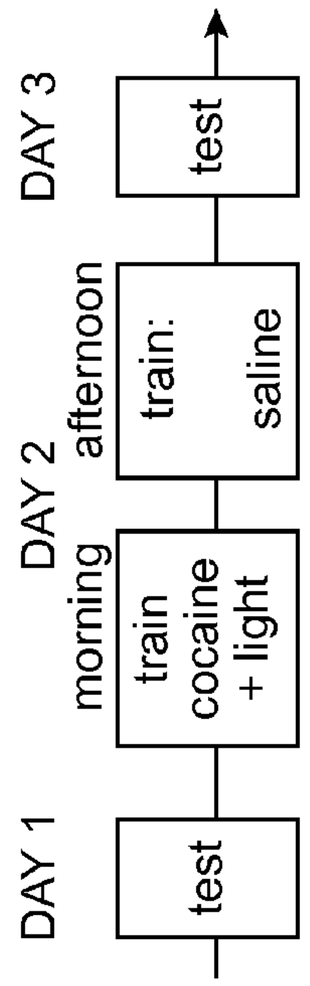


FIG. 4E

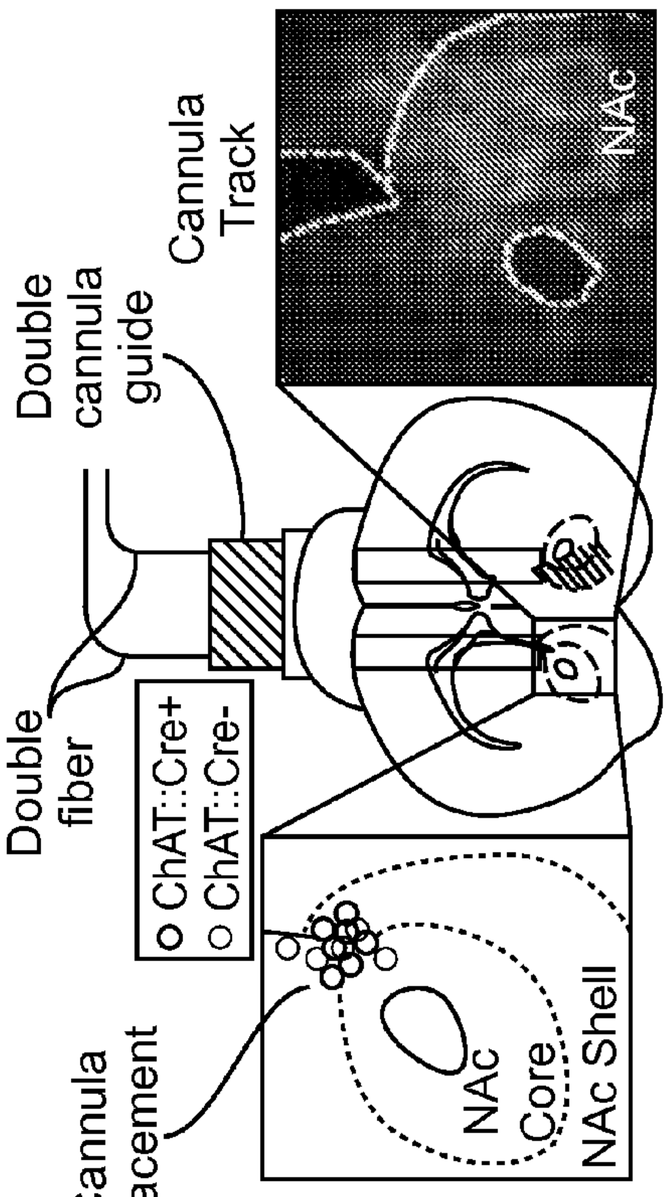


FIG. 4D

FIG. 4F

FIG. 4G

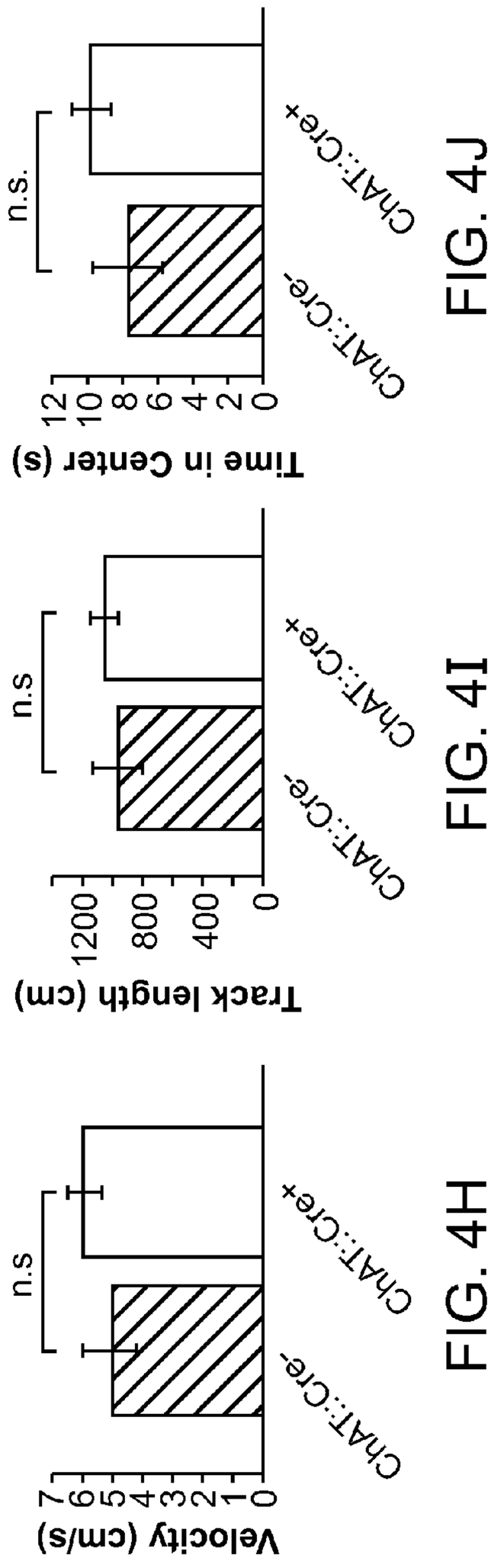


FIG. 4J

FIG. 4I

FIG. 4H

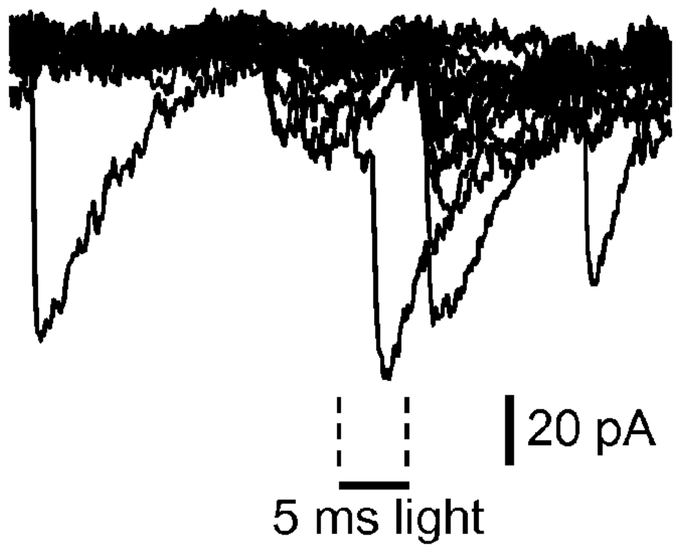


FIG. 5A

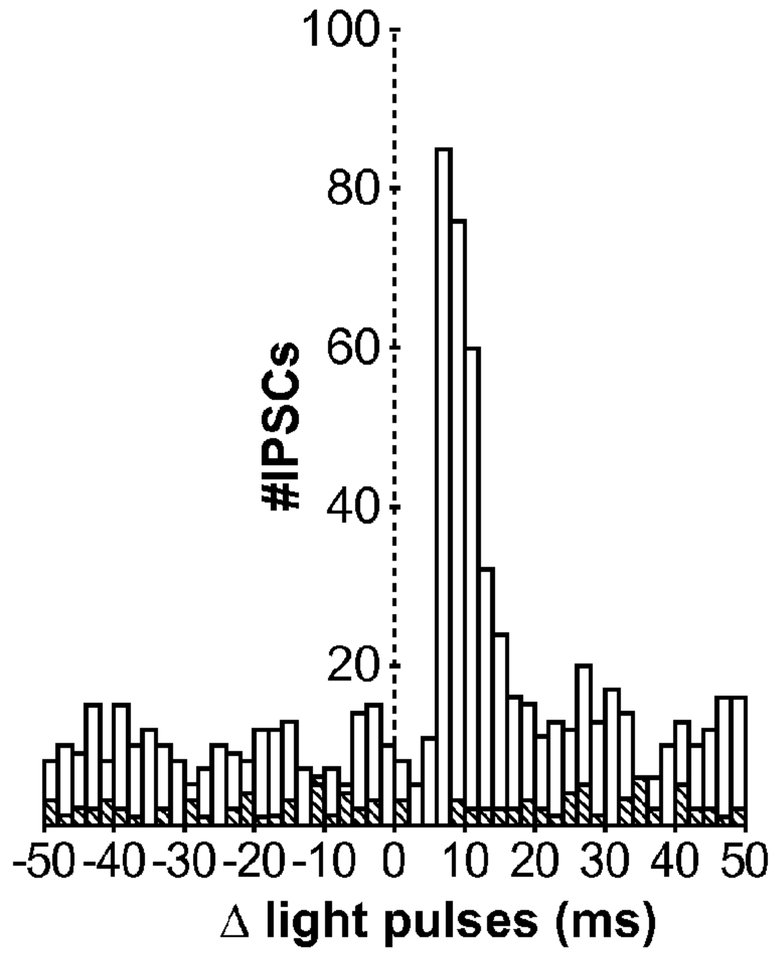


FIG. 5B

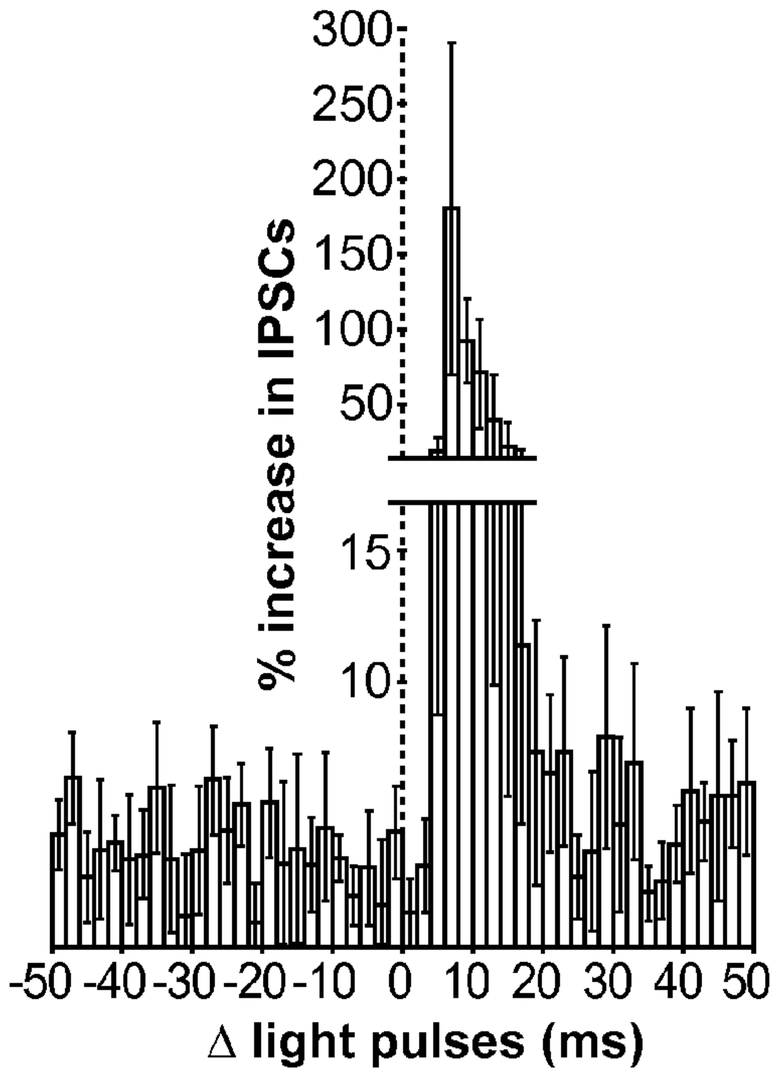


FIG. 5C

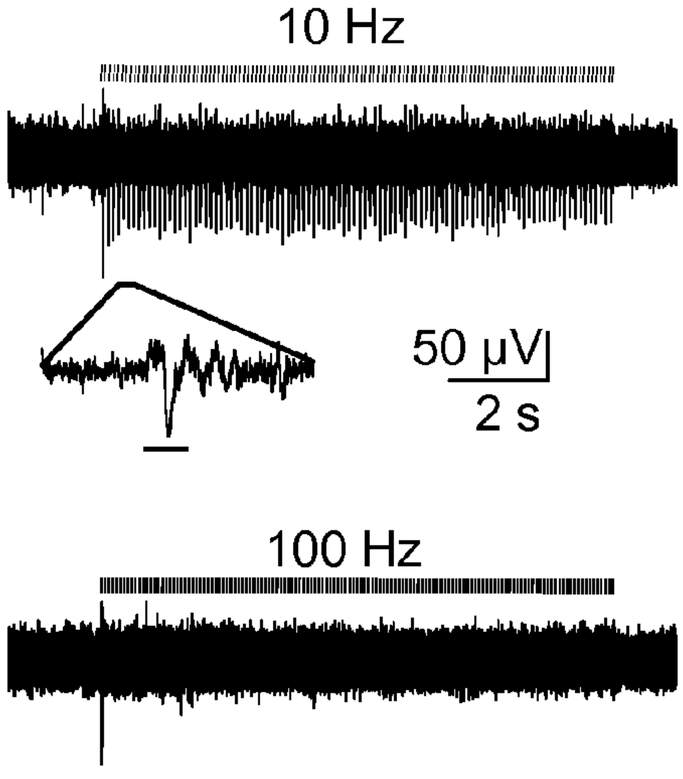


FIG. 5D

FIG. 6A

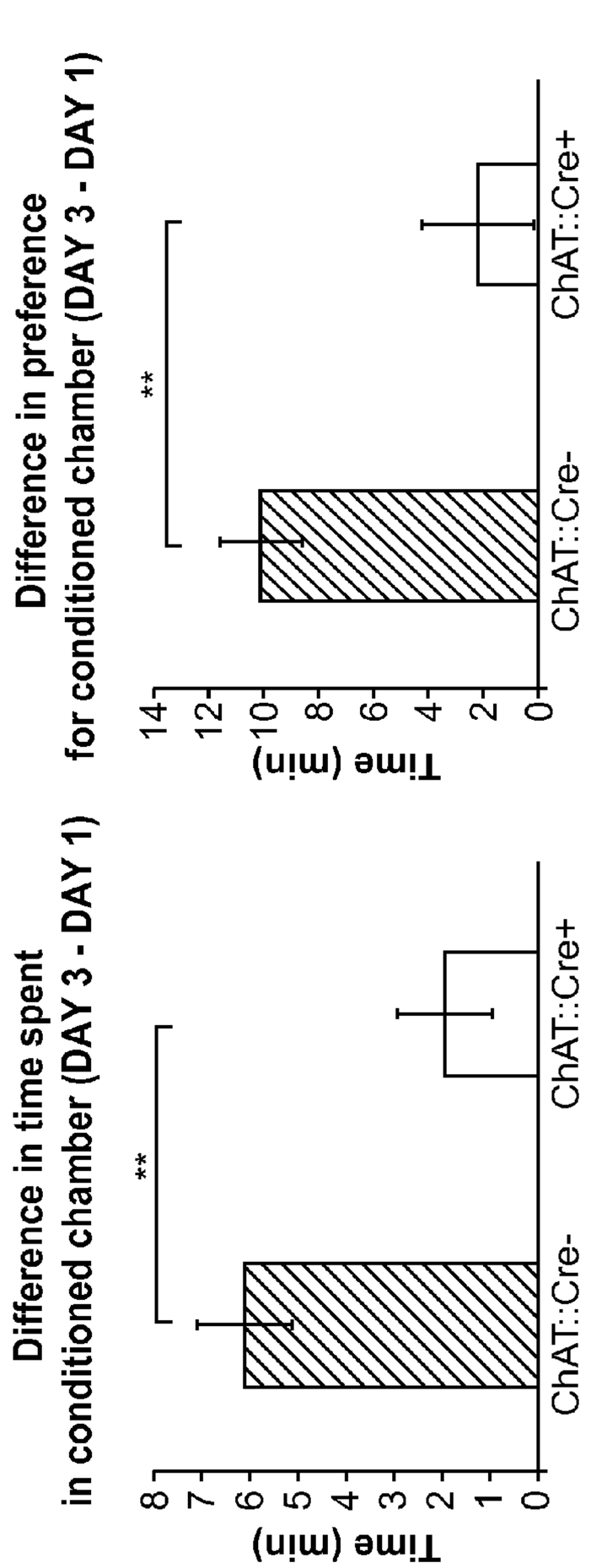
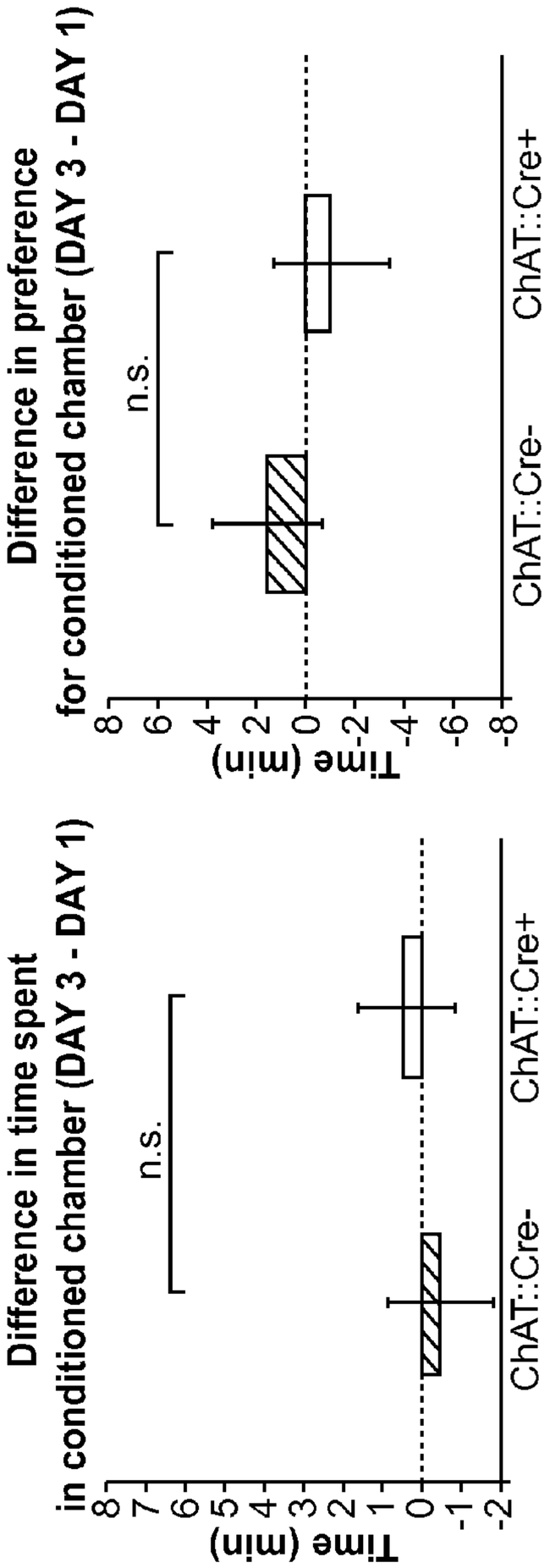

ChAT- eNpHR3.0 Cocaine CPP (20 mg/kg)
Difference in time spent in conditioned chamber (DAY 3 - DAY 1)
**
Time (min)
ChAT::Cre-
ChAT::Cre+
Difference in preference for conditioned chamber (DAY 3 - DAY 1)
**
Time (min)
ChAT::Cre-
ChAT::Cre+

FIG. 6B


ChAT- eNpHR3.0 CPP (without cocaine)
Difference in time spent in conditioned chamber (DAY 3 - DAY 1)
n.s.
Time (min)
ChAT::Cre-
ChAT::Cre+
Difference in preference for conditioned chamber (DAY 3 - DAY 1)
n.s.
Time (min)
ChAT::Cre-
ChAT::Cre+

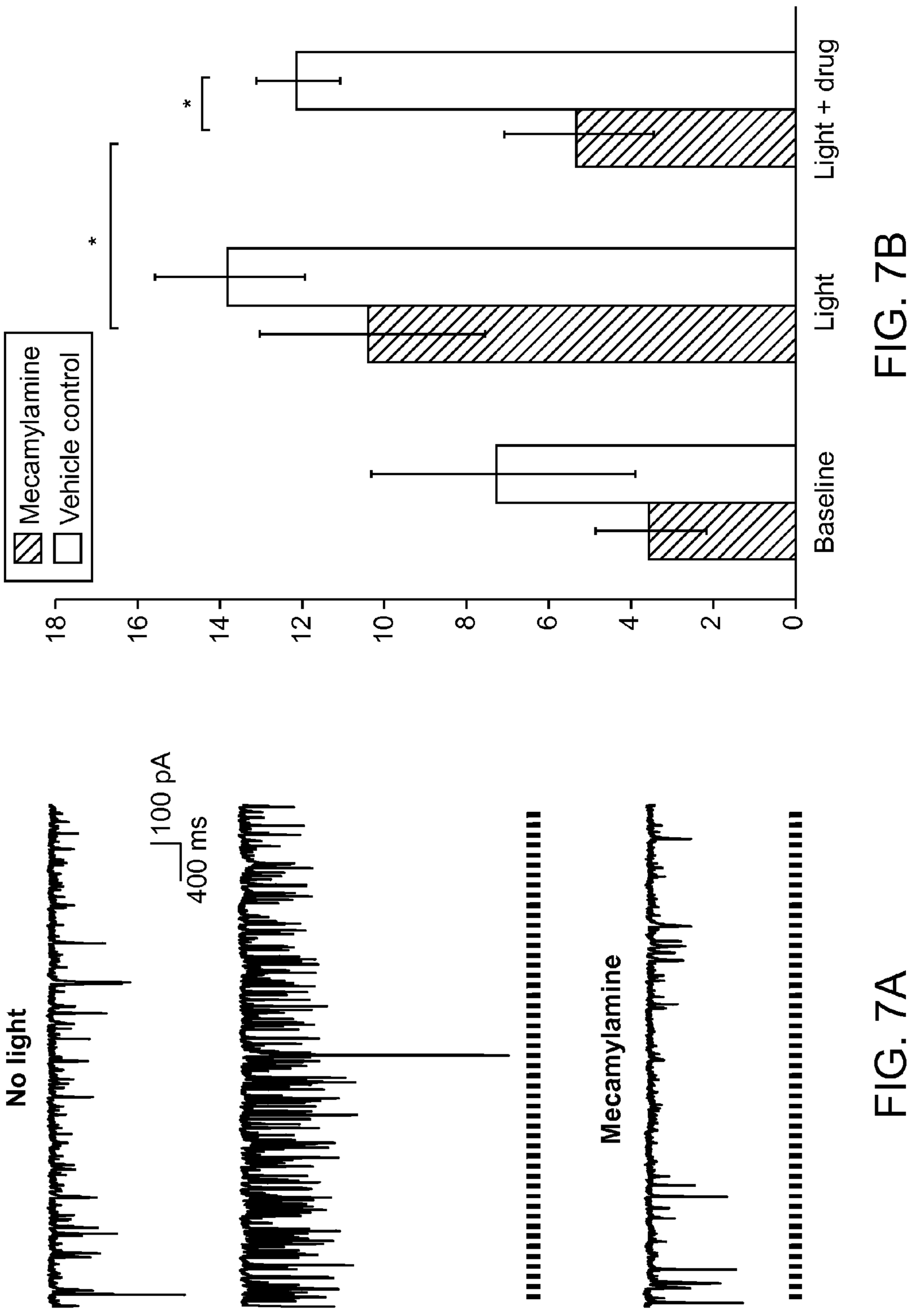

No light
100 pA
400 ms
Mecamylamine
Baseline
Light
Light + drug
Mecamylamine
Vehicle control
0
2
4
6
8
10
12
14
16
18
*
*

FIG. 7A

FIG. 7B

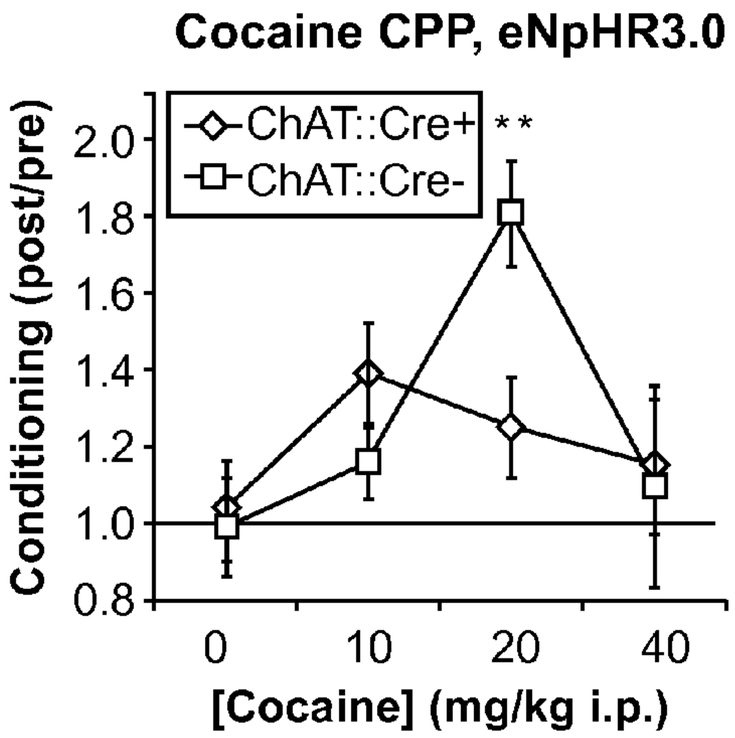


FIG. 8A

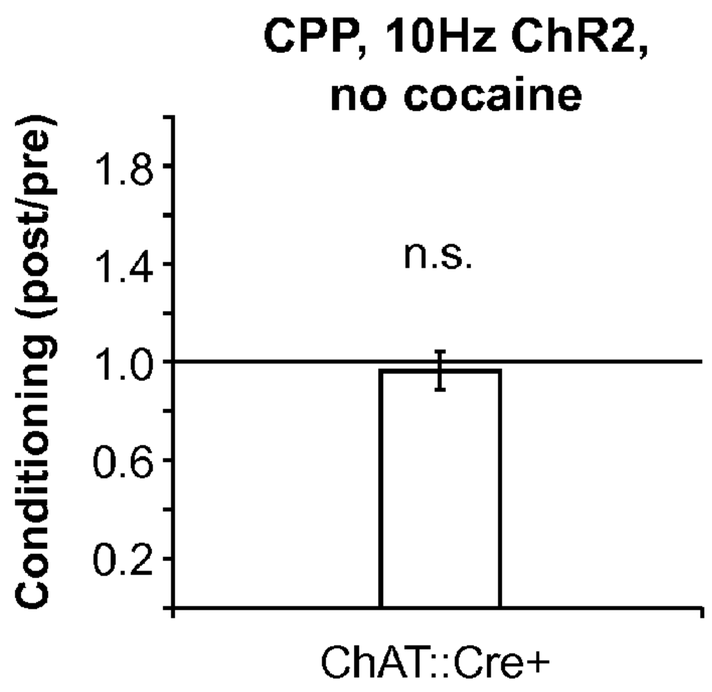


FIG. 8B

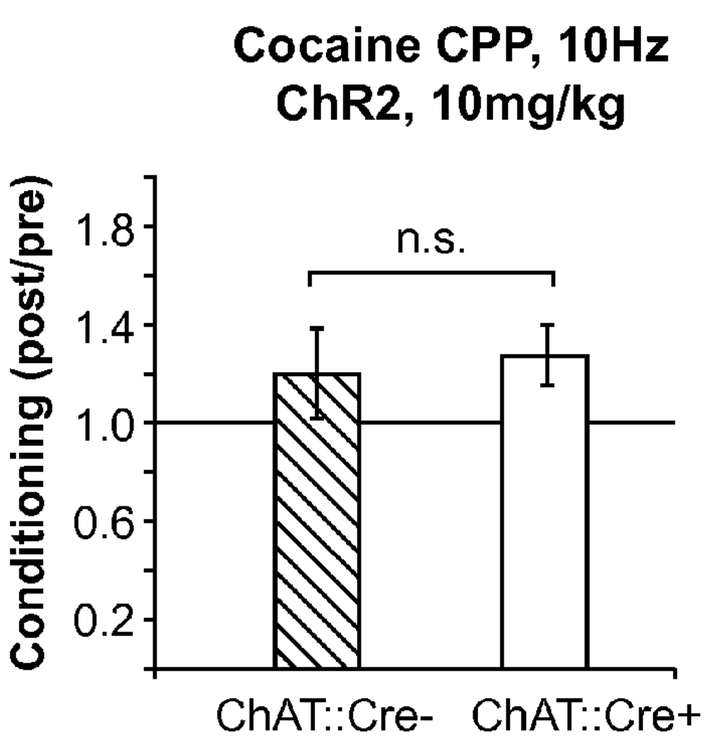


FIG. 8C

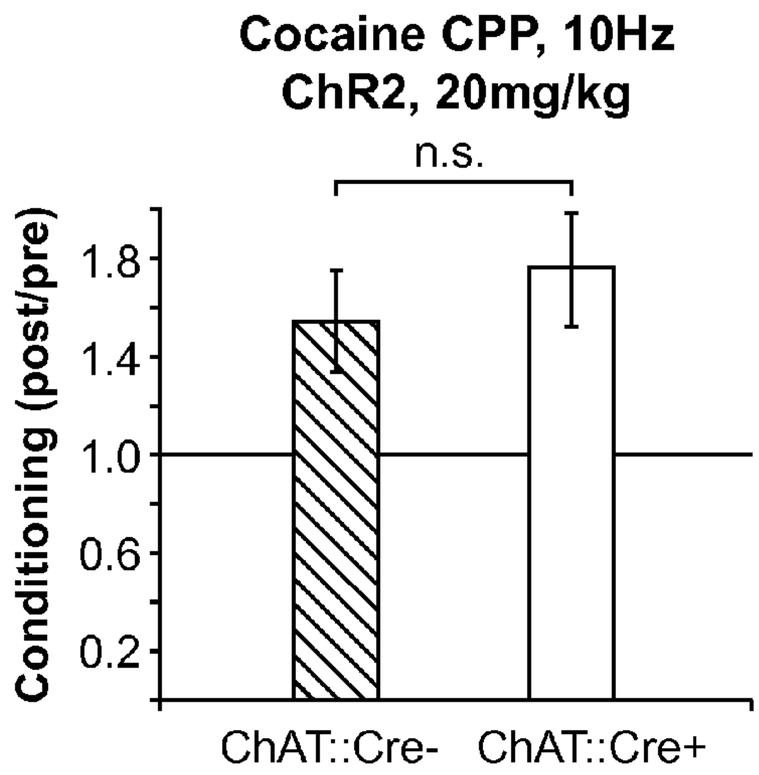


FIG. 8D

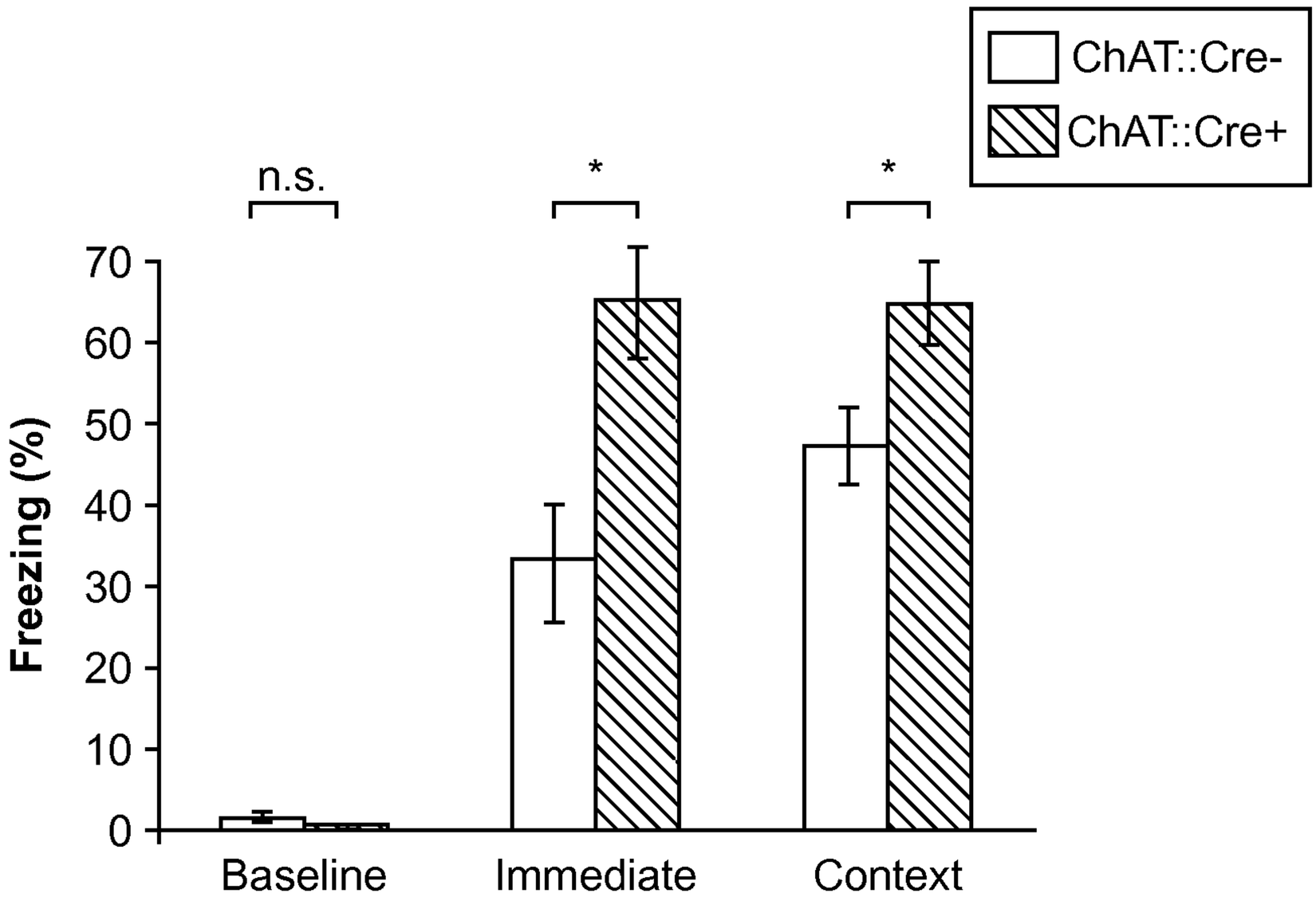

ChAT::Cre-
ChAT::Cre+
n.s.
*
*
Freezing (%)
0
10
20
30
40
50
60
70
Baseline
Immediate
Context

FIG. 9A

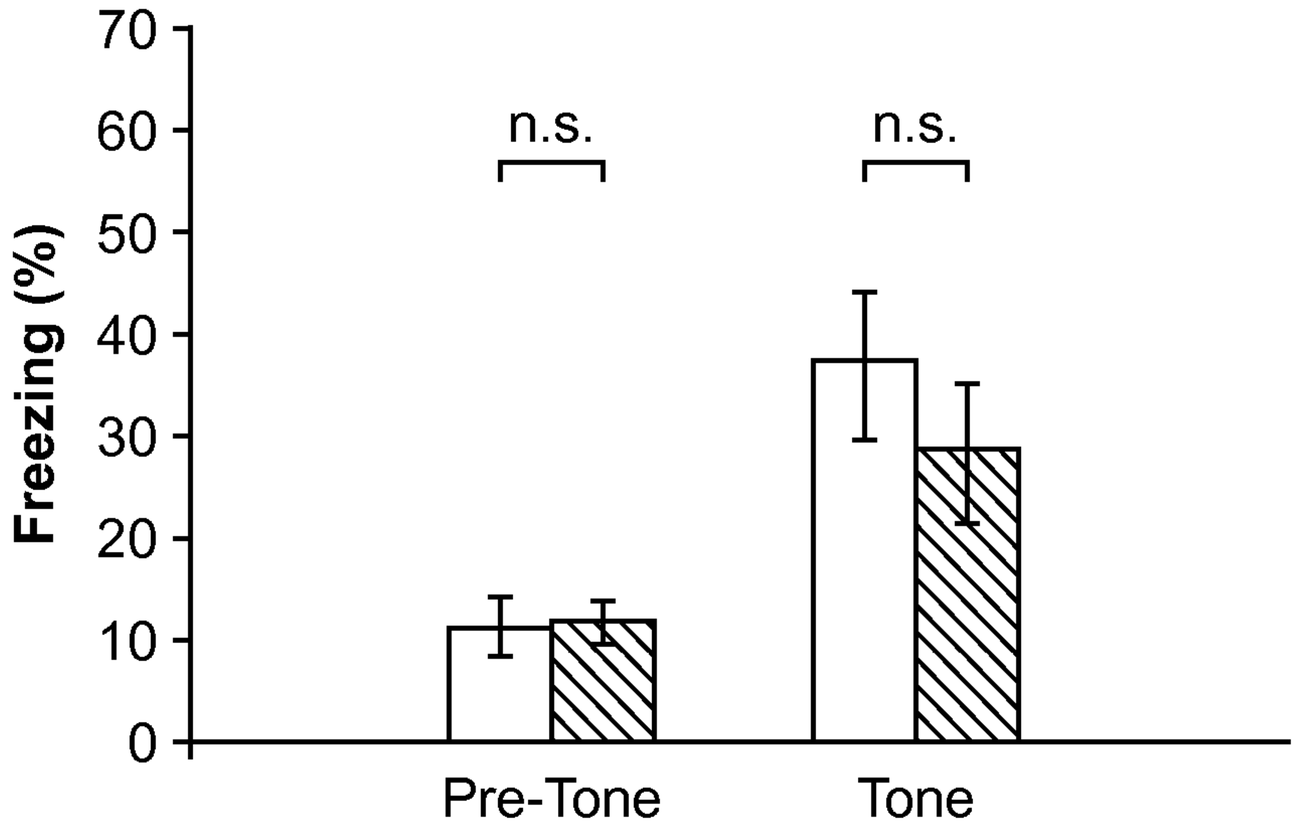

n.s.
n.s.
Freezing (%)
0
10
20
30
40
50
60
70
Pre-Tone
Tone

FIG. 9B

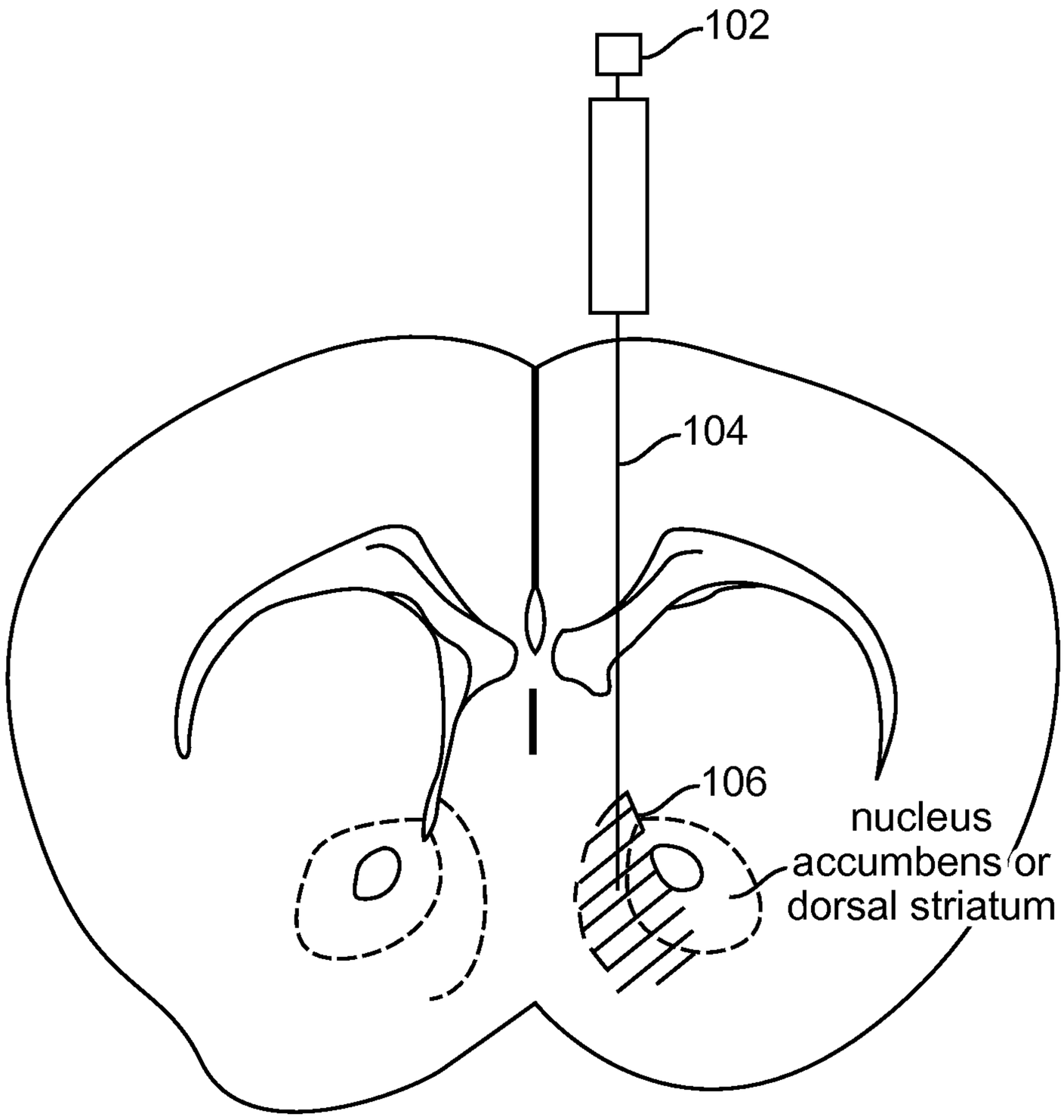


FIG. 10

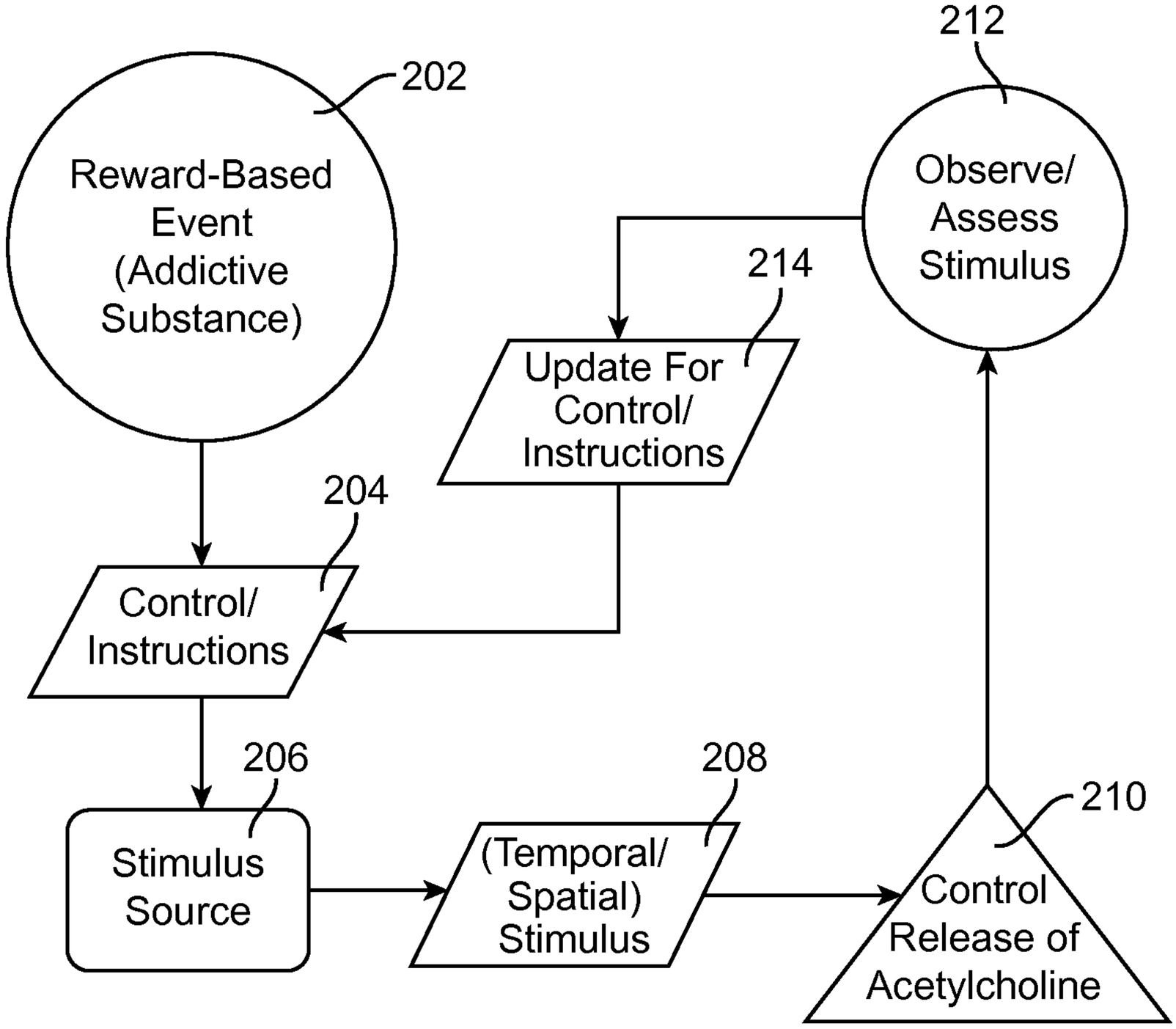

212
202
Reward-Based Event (Addictive Substance)
Observe/ Assess Stimulus
214
Update For Control/ Instructions
204
Control/ Instructions
206
Stimulus Source
208
(Temporal/ Spatial) Stimulus
210
Control Release of Acetylcholine

FIG. 11

OPTOGENETIC CONTROL OF REWARD-RELATED BEHAVIORS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application is a divisional of U.S. Ser. No. 13/882,670 filed, Aug. 12, 2013, which is a national stage filing under 35 U.S.C. 371 of PCT/US2011/059295, filed Nov. 4, 2011, which claims priority to U.S. Provisional Application No. 61/410,692 filed on Nov. 5, 2010, each of which applications is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] This application pertains to compositions comprising animal cells expressing light-responsive opsin proteins on their plasma membranes and methods of using the same to selectively hyperpolarize cholinergic interneurons residing in microcircuits of the nucleus accumbens or dorsal striatum to affect one or more behaviors associated with reward-related conditioning in the animal.

BACKGROUND

[0003] Substance abuse and dependency are important problems facing societies all over the world. According to the World Drug Report 2008, about 5% of the world population uses illicit drugs and in 0.6% of the world population, drug use is a problem. In the United States, according to the Substance Abuse and Mental Health Services Administration's (SAMHSA's) National Survey on Drug Use and Health in 2006, 23.6 million persons aged 12 or older needed treatment for an illicit drug or alcohol abuse problem (9.6 percent of the persons aged 12 or older). Of these, only 2.5 million-10.8 percent of those who needed treatment—received it at a specialty facility. Substance abuse and dependency result in enormous loss of productive manpower all over the world and imposes costs on governments and societies in terms of treatment support, insurance payouts, and spending on prevention and de-addiction programs.

[0004] Optogenetics is the combination of genetic and optical methods used to control specific events in targeted cells of living tissue, even within freely moving mammals and other animals, with the temporal precision (millisecond-timescale) needed to keep pace with functioning intact biological systems. The hallmark of optogenetics is the introduction of fast light-responsive opsin channel or pump proteins to the plasma membranes of target neuronal cells that allow temporally precise manipulation of neuronal membrane potential while maintaining cell-type resolution through the use of specific targeting mechanisms. Among the microbial opsins which can be used to investigate the function of neural systems are the halorhodopsins (NpHRs), used to promote membrane hyperpolarization when illuminated. In just a few short years, the field of optogenetics has furthered the fundamental scientific understanding of how specific cell types contribute to the function of biological tissues such as neural circuits in vivo. Moreover, on the clinical side, optogenetics-driven research has led to insights into the neurological mechanisms underlying mammalian behavior.

[0005] In spite of these advances, the neurophysiological substrates underlying complex human behaviors, such as substance abuse and dependency (addiction) remain poorly understood, despite emerging information on the role that specific areas of the brain play in these behaviors. For example, the nucleus accumbens (NAc) is a collection of neurons that forms the main part of the ventral striatum. The NAc is thought to play an important role in reward, pleasure, laughter, addiction, aggression, fear, and the placebo effect. Acetylcholine is an important and widely studied neurotransmitter, which acts on a variety of receptors and target cells. Some in vivo pharmacological approaches have shown that cholinergic transmission in the NAc is required for reward learning behaviors. Cholinergic interneurons within the NAc are particularly intriguing because they constitute less than 1% of the local neural population, yet they project throughout the NAc and provide its only known cholinergic input. Relevant cholinergic receptors are expressed locally, and nicotinic and muscarinic pharmacological agonists can exert complex influences on medium spiny neurons (MSNs, which represent >95% of the local neuronal population and constitute the output of the NAc). However, the net effect (if any) of the cholinergic interneurons on any aspect of NAc physiology or reward-related behavior is unknown.

[0006] What is needed, therefore, is a tool which would permit investigation of the causal role played by cholinergic interneurons within the NAc in reward-related behaviors such as substance dependency. Understanding the neural pathways that underlie addiction may help aid in the discovery and screening of pharmacological therapies to treat patients with such disorders as well as open up the possibility of using such tools to disrupt these behaviors in the brains of drug-addicted individuals.

[0007] Throughout this specification, references are made to publications (e.g., scientific articles), patent applications, patents, etc., all of which are herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0008] Provided herein are compositions and methods for disrupting reward-related behaviors in an individual via the use of stably expressed light-responsive opsin proteins capable of altering the membrane polarization state of the cholinergic interneurons of the nucleus accumbens or the striatum of the individual wherein the alteration of the membrane polarization state of the cholinergic interneurons of the nucleus accumbens or the striatum disrupts one or more reward-related behaviors in the animal. In some embodiments, the reward-related behavior is addiction-related behavior. In other embodiments, the addiction-related behavior is cocaine addiction.

[0009] Accordingly, in some aspects, provided herein is a non-human animal comprising a light-responsive opsin protein expressed on the cell membrane of a cholinergic interneuron in the nucleus accumbens or the striatum of the animal, wherein the protein is responsive to light and is capable of inducing membrane hyperpolarization of the interneurons when the interneurons are illuminated with the light, wherein the illumination of the opsin disrupts at least one reward-related behavior of the animal.

[0010] In other aspects, provided herein is a brain slice comprising a cross section of the nucleus accumbens or the striatum, wherein a light-responsive opsin protein is expressed on the cell membrane of cholinergic interneurons wherein the protein is responsive to light and is capable of inducing membrane hyperpolarization of the interneurons

when the interneurons are illuminated with the light, wherein the illumination of the protein disrupts reward-related brain function.

[0011] In some aspects, provided herein is a method for disrupting reward-related behavior in an individual comprising: administering a polynucleotide encoding a light-responsive opsin protein to the individual, wherein the light-responsive opsin protein is expressed on the cell membrane of cholinergic interneurons in the nucleus accumbens or the striatum of the individual, and the protein is responsive to light and is capable of inducing membrane hyperpolarization of the interneurons when the interneurons are illuminated with the light, whereby activating the protein by the light disrupts at least one reward-related behavior in the individual. In some embodiments, the polynucleotide is administered to the nucleus accumbens or the striatum of the individual.

[0012] In still other aspects, provided herein is a method for treating drug addiction in an individual comprising: administering a polynucleotide encoding a light-responsive opsin protein to the individual, wherein the light-responsive opsin protein is expressed on the cell membrane of cholinergic interneurons in the nucleus accumbens or the striatum of the individual, and the protein is responsive to light and is capable of hyperpolarizing the interneurons when the interneurons are illuminated with the light, whereby activating the protein by the light disrupts reward-related behavior in the individual, wherein the individual no longer desires to take drugs. In some embodiments, the polynucleotide is administered to the nucleus accumbens or the striatum of the individual.

[0013] Aspects of the present disclosure relate to control or characterization of reinforced behavior in living animals, as described herein. While the present disclosure is not necessarily limited in these contexts, various aspects of the invention may be appreciated through a discussion of examples using these and other contexts.

[0014] Embodiments of the present disclosure are directed toward specially-targeted circuits that are associated with hedonic and/or reinforced behavior. More particular embodiments relate to spatio-temporal control over neural circuitry to identify an association between specific circuit targets associated with and corresponding to reward memory, anhedonia, addiction and/or reinforced behavior.

[0015] Particular embodiments of the present disclosure are directed toward inhibition of targeted cells within structures involved in natural reward-related behaviors and/or for reward learning including, but not necessarily limited to, the nucleus accumbens (NAc) or the dorsal striatum. In a particular example, the targeting of specific cholinergic neurons within the NAc is particularly well suited for disrupting the release of acetylcholine by these cholinergic neurons. It has been discovered that such neural inhibition can be effective to targeted neural inhibition can reduce or eliminate undesired effects on the reinforcement of other behaviors, e.g., appetitive or aversive responses. Aspects of the present disclosure relate to stimulation that is specific to temporal, spatial and/or cell-types. In certain embodiments, this inhibition is performed using an optogenetic system that involves the expression of light-responsive opsins in the cells of the neural circuit. In other embodiments, the inhibition can be performed using direct electrical stimulus. Still other embodiments allow for the use of temporally-precise pharmaceuticals.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Various example embodiments may be more completely understood in consideration of the following description and the accompanying drawings, in which:

[0017] FIG. **1**A-FIG. **1**H depicts specificity, membrane targeting, and functionality of ChR2 and eNpHR3.0 in ChAT inter-neurons of the NAc. (A) Cre-dependent AAV (expressing either eNpHR3.0-eYFP or ChR2(H134R)-eYFP) was injected into the medial portion of the NAc. (B) Confocal image of an injected slice demonstrates colocalization of eYFP expression with the ChAT antibody, costained with 4',6'-diamidino-2-phenylindole (DAPI). (C) 91.3±1.3% of neurons that expressed YFP also stained for the ChAT antibody (n=418); 93.5±2.8% of neurons that stained for the ChAT antibody also expressed YFP (n=413). Error bars indicate SEM. (D) High-magnification view reveals membrane localization of eNphR3.0-eYFP (left) and ChR2-eYFP (right), costained with ChAT antibody. (E) Membrane potential changes induced by current injection in a ChR2-eYFP-expressing ChAT neuron. V_M=−48 mV. Current steps: −60, −20, +20 pA. (F) Membrane potential changes induced by 1 s of 580-nm light in an eNpHR3.0-eYFP-expressing ChAT neuron (peak hyperpolarization: −103 mV). V_M=−49 mV. (Inset) Population-averaged peak hyperpolarization (mean±SEM: −83.8±11.9 mV; n=4). (G) Consecutive action potentials in a ChR2-eYFP-expressing ChAT neuron evoked by a 470-nm pulse train (5 ms pulse width; 10 Hz). (H) Average success probability for generating action potentials in ChR2-eYFP-expressing ChAT neurons at different stimulation frequencies (n=4; mean±SEM; 470-nm pulse train, 5-ms pulse width).

[0018] FIG. **2**A-FIG. **2**I depicts optogenetic photoactivation of ChAT interneurons increases frequency of inhibitory currents and suppresses MSN spiking. (A) ChAT neurons transduced with ChR2-eYFP were activated with blue light (470 nm) in brain slices, and nearby MSNs ($eYFP^-$ cells) were whole-cell patch-clamped. (B) (Left) Spontaneous synaptic currents were observed in an MSN in a slice expressing ChR2-eYFP in ChAT neurons. (Middle) Synaptic currents increased in frequency in response to 470-nm light pulses (5-ms pulse width; 10 Hz). (Right) These currents were blocked by $GABA_A$ receptor antagonist SR-95531 (5 mM) and are thus considered IPSCs. 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline-2,3-dione (NBQX) (5 mM) and (RS)-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (RS-CPP) (5 μM) were present in all experiments. (C) Time course of IPSC frequencies for this MSN, showing the effect of light pulses (blue dashed bars) and SR-95531 (black bar). (D) Average percentage increase in IPSC frequency during the light-on periods (normalized to that of light-off periods) as a function of time relative to light pulses (n=6). The blue dashed line indicates the onset of light pulses; error bars denote SEM. (E) Light pulses increased the frequency of IPSCs by 525.8±154.3% (n=6, P=0.01, paired two-tailed t test), whereas the average amplitudes of spontaneous IPSCs were changed by 21.3±28.9% (P>0.05). (F) An optrode (optical fiber attached to a tungsten electrode) was stereotaxically positioned in vivo into a NAc that expressed ChR2-eYFP in ChAT cells. (G) (Top) Voltage trace of an isolated unit that is inhibited by blue light stimulation. (Middle) Raster plot displaying the response of the same unit to five repetitions of the light stimulation, with each action potential represented by a dot. (Bottom) Average and SEM of the firing rate over time for the same unit. (H)

Fraction of sites that were inhibited versus excited by light stimulation. (I) Population summary of the time course of response to light stimulation for sites that were inhibited (left; n=13 of 16) or excited (right; n=3 of 16) by light. Solid lines represent average firing rate across sites as a function of time; each dot represents the average firing rate of an individual site. All firing rates are normalized to the mean rate before light stimulation. (F to I) Duration of photostimulation, 10 s; pulse duration, 5 ms; wavelength, 470 nm; frequency, 10 Hz. Epochs of light stimulation are represented by dashed lines.

[0019] FIG. **3**A-FIG. **3**E depicts optogenetic photoinhibition of ChAT interneurons enhances MSN spiking in vivo. (A) (Top) Voltage trace of an isolated unit (recorded from the NAc in vivo) that was excited by optogenetic photoinhibition of the ChAT interneurons with eNpHR3.0. (Middle) Raster plot displaying the response of the same unit to five repetitions of the light stimulation, with each action potential represented by a dot. (Bottom) Average and SEM of the firing rate over time for the same unit. (B) Wavelet analysis reveals power of spiking as a function of frequency and time (average across five repetitions) for the same unit as in (A). (C) Fraction of sites that were inhibited versus excited by light stimulation. (D) Same as (A), for a unit that was inhibited by light stimulation. (E) Population summary of the time course of response to light stimulation for sites that were inhibited (left; n=13 of 17) or excited (right; n=4 of 17) by light. Solid lines represent the average firing rate across sites as a function of time; each dot represents the average firing rate of an individual site. All firing rates are normalized to the mean value before light stimulation. (A to E) Duration of photostimulation, 15 s (constant illumination); wavelength, 560 nm. Epochs of light stimulation are represented by bars.

[0020] FIG. **4**A-FIG. **4**J depicts ChAT interneurons can be activated by cocaine in slice and required for cocaine conditioning in vivo. (A) The frequency of spontaneous action potentials in a ChAT neuron increased 10 min after bath application of cocaine (5 μM). ACSF, artificial cerebrospinal fluid. (B) Firing rate over time for this ChAT neuron. Horizontal gray bar, application of cocaine; vertical dotted line, 10 min after cocaine application, the time point illustrated in detail in (A) and (C). (C) Population data illustrating the cocaine-induced increase in firing in ChAT neurons, comparing the baseline firing rate (averaged over the 2.5 min before cocaine application) with the rate after cocaine infusion (averaged between 10 and 12.5 min after onset of cocaine application; gray bars, cells receiving cocaine; white bars, control cells receiving only ACSF; $P<0.005$, paired two-tailed t test for cocaine-treated group before versus after cocaine; $P<0.05$ unpaired two-tailed t test comparing cocaine versus control cells after cocaine or vehicle). (D) Schematic illustration of a bilateral cannula system with double fibers inserted to illuminate the medial portion of the NAc. (Left inset) Endpoint of cannula track for all mice used in (H). (Right inset) eYFP expression in NAc of a ChAT::Cre$^+$ mouse injected with Cre-dependent eNpHR3.0-eYFP. (E) Conditioning paradigm for cocaine CPP (H). Mice were conditioned with ip cocaine (20 mg/kg), along with ChAT cell inhibition with eNpHR3.0 (wavelength: 590 nm). (F) Tracking data from representative ChAT::Cre$^+$ and ChAT::Cre$^-$ mice on the testing day after cocaine conditioning (day 3). On the previous day (day 2), the mice had received cocaine and light in one left chamber, whereas in the other they received saline. The ChAT::Cre$^-$ mouse (but not the ChAT::Cre$^+$ mouse) exhibited a preference for the conditioned chamber. (G) (Left) Fold change in time in conditioned chamber during day 3 versus day 1 of cocaine CPP (conditioning with cocaine and light). Comparison of ChAT::Cre$^+$ and ChAT::Cre$^-$ littermates; in both cases injected with Cre-dependent eNpHR3.0 (n=10 ChAT::Cre$^+$, n=12 ChAT::Cre$^-$; $P<0.01$ for two-tailed t test; three cohorts). (Right) Fold change in time in conditioned chamber during day 3 versus day 1 for conditioning with light alone (no cocaine; n=9 ChAT::Cre$^+$, n=7 ChAT::Cre; $P>0.05$ for two-tailed t test; three cohorts). Error bars indicate SEM. n.s., not significant. (H) Velocity of virus-injected (Cre-dependent eNpHR3.0) and photostimulated ChAT::Cre$^+$ and ChAT::Cre$^-$ mice in the open field (n=10 ChAT::Cre$^+$, n=10 ChAT::Cre$^-$; $P>0.05$ for two-tailed t test; three cohorts). (I) Same as (H) for track length in open field (n=10 ChAT::Cre$^+$, n=10 ChAT::Cre$^-$; $P>0.05$ for two-tailed t test; three cohorts). (J) Same as (H) for time in center of open field (n=10 ChAT::Cre$^+$, n=10 ChAT::Cre; $P>0.05$ for two-tailed t test; three cohorts). (A to J) *$P<0.05$; **$P<0.01$;***$P<0.005$.

[0021] FIG. **5**A-FIG. **5**D depicts optogenetic photoactivation of ChAT interneurons in slice and in vivo. (A): Overlay of 15 current traces for the same MSN as in FIG. **2**B, with each trace aligned to the light pulse. Some IPSCs are not time locked to the light pulses, whereas many are time locked with latency of ~8 ms after light pulse onset. (B): IPSC occurrence as a function of time relative to light pulse for the same neuron. Open bars correspond to the number of IPSCs recorded during light stimulation; grey bars correspond to the number of IPSCs recorded during baseline (before light stimulation) using the same temporal alignment. For this neuron, an asynchronous enhancement in IPSC frequency is evident, in addition to the more prominent synchronous increase. (C): A rescaled presentation of FIG. **2**D, displaying population-averaged percentage increase in IPSC frequency as a function of time relative to light pulses during the light on relative to light-off period (n=6). Across the population, an asynchronous enhancement in IPSC frequency is evident, in addition to the more prominent synchronous increase. Pulse parameters for panels A-C: 470 nm, 5 ms pulse duration, 10 Hz. (D): Voltage traces from in vivo recordings showing population spikes (presumably generated by ChAT cells expressing ChR2) that track pulsed blue light stimulation at 10 Hz (top) but not 100 Hz (bottom; 470 nm light; 10 sec total stimulation duration).

[0022] FIG. **6**A-FIG. **6**B depicts ChAT neuron inhibition disrupts cocaine CPP without affecting CPP in the absence of cocaine. (A): Cocaine CPP, same data as FIG. **4**G (left panel) but plotted as difference rather than fold-change. Left: Difference in time in cocaine-conditioned chamber after conditioning versus before conditioning. (n=10 ChAT::Cre+, n=12 ChAT::Cre–; $p<0.01$ for two-tailed t test; 3 cohorts). Right: Difference in preference for cocaine-conditioned chamber after versus before conditioning, where preference is defined as the difference in time spent in conditioned chamber versus the unconditioned chamber (n=10 ChAT::Cre+, n=12 ChAT::Cre–; $p<0.01$ for two-tailed t-test; 3 cohorts). (B): CPP without cocaine, same data as FIG. **4**G (right panel) and same data presentation as A. (For both panels, n=9 ChAT::Cre+, n=7 ChAT::Cre–; $p>0.05$ for two-tailed t-test; 3 cohorts).

[0023] FIG. **7**A-FIG. **7**B depicts nicotinic receptor antagonism decreases ChAT interneuron-evoked IPSCs recorded in MSNs. (A): Representative IPSC sweeps from a typical MSN in the acute slice preparation under the conditions of no light, light pulses (470 nm, 10 Hz, 5 ms pulse width), and identical light pulses with 10 μM mecamylamine (B): Summary graph of IPSCs recorded as in A from a population of MSNS before light presentation, with light presentation, and with light and either mecamylamine or vehicle. Light stably increased IPSC frequency from 3.4+/−1.3 Hz to 10.1+/−1.2 Hz ($p<0.05$; n=7, paired t-test), while mecamylamine reduced this increase to 5.1+/−1.8 Hz ($p<0.05$ compared to light-alone within the same cells, paired t-test; $p<0.05$ compared to the vehicle control, n=5, unpaired t-test).

[0024] FIG. **8**A-FIG. **8**D depicts modulation of ChAT interneurons over a range of cocaine-CPP parameters. (A): Dose-response curve for cocaine CPP during eNpHR3.0-mediated inhibition of the ChAT interneurons. Cocaine CPP is significantly decreased in ChAT::Cre+ mice for the standard rewarding dose of 20 mg/kg i.p. ($p<0.01$), but not at other concentrations thought to be anxiogenic or insufficient (590 nm light, constant illumination; see Table 2, infra). (B): Stimulation of ChAT neurons with ChR2 does not drive place preference by itself. (470 nm light, 5 ms pulse width, 10 sec of 10 Hz stimulation every 30 sec; n=4, $p>0.05$ two-tailed t-test). (C): Stimulation of ChAT neurons at 10 Hz with ChR2 does not significantly modulate cocaine place preference for i.p. 10 mg/kg cocaine. (470 nm light, 5 ms pulse width, constant 10 Hz stimulation during cocaine conditioning; ChAT::Cre+ n=6, ChAT::Cre− n=6; $p>0.05$ two-tailed t-test). (D): Stimulation of ChAT neurons at 10 Hz with ChR2 does not significantly modulate cocaine place preference for i.p. 20 mg/kg cocaine (470 nm light, 5 ms pulse width, steady 10 Hz stimulation during cocaine conditioning; ChAT::Cre+ n=4, ChAT::Cre− n=3; $p>0.05$ two-tailed t-test).

[0025] FIG. **9**A-FIG. **9**B depicts inhibition of ChAT interneurons with eNphR3.0 does not impair contextual or auditory-cued fear conditioning. (A): Percentage time spent freezing was quantified in a standard contextual fear conditioning paradigm. "Baseline" refers to the 30 seconds preceding the first toneshock pairing "Immediate" refers to the 30 seconds immediately after the second (and final) toneshock pairing. "Context" refers to freezing to the same context on the day after the conditioning session. ChAT::Cre+ mice exhibited enhanced immediate and context freezing. (n=9 ChAT::Cre+; n=8 ChAT::Cre−; two-tailed t-test; $p<0.05$ comparing ChAT::Cre+ and ChAT::Cre− for immediate and context freezing). (B): Percentage time spent freezing for the auditory-cued fear conditioning paradigm. "Pre-tone" refers to the 2.5 minutes in the new context before the onset of the tone; "Tone" refers to the 2.5 minutes during tone (n=9 ChAT::Cre+; n=8 ChAT::Cre−; two tailed t-test; $p>0.05$ comparing ChAT::Cre+ and ChAT::Cre−).

[0026] FIG. **10** depicts a system for controlling the nucleus accumbens (NAc) or the dorsal striatum, consistent with an embodiment of the present disclosure.

[0027] FIG. **11** depicts a flow diagram for controlling the release of acetylcholine, consistent with embodiments of the present disclosure.

DETAILED DESCRIPTION

[0028] This invention provides, inter alia, compositions and methods for disrupting reward-related behavior in an individual by selectively altering the electrical membrane potential of the cholinergic interneuron cells of the nucleus accumbens or the dorsal striatum. The invention is based on the inventors' discovery that selective hyperpolarization of the cholinergic interneuron cells of the nucleus accumbens with light-responsive opsin ion pump proteins disrupts reward-seeking behavior in an animal model of drug addiction.

[0029] While the present disclosure 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 present disclosure to the particular embodiments described. On the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the scope of the present disclosure including aspects defined in the claims.

[0030] General Techniques

[0031] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, cell biology, biochemistry, nucleic acid chemistry, immunology, physiology, and the pathophysiology drug addiction and reward-related behaviors which are well known to those skilled in the art. Such techniques are explained fully in the literature, such as, *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook et al., 1989) and *Molecular Cloning: A Laboratory Manual*, third edition (Sambrook and Russel, 2001), (jointly referred to herein as "Sambrook"); *Current Protocols in Molecular Biology* (F. M. Ausubel et al., eds., 1987, including supplements through 2001); *PCR: The Polymerase Chain Reaction*, (Mullis et al., eds., 1994); Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York; Harlow and Lane (1999) *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (jointly referred to herein as "Harlow and Lane"), Beaucage et al. eds., *Current Protocols in Nucleic Acid Chemistry*, John Wiley & Sons, Inc., New York, 2000), *Handbook of Experimental Immunology,* 4th edition (D. M. Weir & C. C. Blackwell, eds., Blackwell Science Inc., 1987); and *Gene Transfer Vectors for Mammalian Cells* (J. M. Miller & M. P. Calos, eds., 1987). Other useful references include Harrison's *Principles of Internal Medicine* (McGraw Hill; J. Isseleacher et al., eds.), and *Addiction Research Methods*, (Miller et al, eds., 2010; Wiley-Blackwell, United Kingdom).

DEFINITIONS

[0032] As used herein, "reward-related behavior" is a process that reinforces a behavior—something that increases the rate, probability, or intensity of a particular behavior in the form of an oftentimes pleasurable response by the delivery or emergence of a stimulus immediately or shortly after performing the behavior. Reward related behaviors can include, but are not limited to, obtaining food, sexual behaviors, gambling behaviors, and/or drug related-addictive behavior.

[0033] "Drug-related addictive behavior" is behavior resulting from compulsive substance use and is characterized by apparent dependency on the substance. Symptomatic of addiction-related behavior is (i) overwhelming involvement with the use of the drug, (ii) the securing of its supply, and (iii) a high probability of relapse after withdrawal.

[0034] As referred herein, the term "drug" or "narcotic" is meant to include opioids, such as opium and heroin, methamphetamine, cocaine (benzoylmethylecgonine), ketamine, MDMA (3,4-Methylenedioxymethamphetamine; a.k.a. "Ecstasy"), lysergic acid diethylamide (LSD), or cannabinoids. Additionally, narcotic is meant to include alcohol, nicotine, or any other controlled substance.

[0035] An "individual" is a mammal including a human Mammals include, but are not limited to, farm animals, sport animals, pets, primates, mice and rats. Individuals also include companion animals including, but not limited to, dogs and cats. In some aspects, an individual is a non-human animal, such as a mammal. In another aspect, an individual is a human.

[0036] As used herein, an "effective dosage" or "effective amount" of drug, compound, or pharmaceutical composition is an amount sufficient to effect beneficial or desired results. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0037] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including and preferably clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, delaying the progression of the disease, and/or prolonging survival of individuals.

[0038] As used herein, the singular form "a", "an", and "the" includes plural references unless indicated otherwise.

[0039] It is intended that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

[0040] The Nucleus Accumbens

[0041] The nucleus accumbens (NAc), also known as the accumbens nucleus or as the nucleus accumbens septi, is a collection of neurons that forms the main part of the ventral striatum. It is thought to play an important role in reward, pleasure, laughter, addiction, aggression, fear, and the placebo effect. The principal neuronal cell type found in the nucleus accumbens is the medium spiny neuron (MSN). The neurotransmitter produced by these neurons is gamma-aminobutyric acid (GABA), one of the main inhibitory neurotransmitters of the central nervous system. MSNs are also the main projection or output neurons of the nucleus accumbens. While 95% of the neurons in the nucleus accumbens are medium spiny GABA-ergic projection neurons, other neuronal types are also found such as large aspiny cholinergic interneurons, which comprise ~1% of the cells in this region of the brain.

[0042] Acetylcholine (ACh) was the first-discovered member of a class of biochemicals that eventually became known as neurotransmitters. In the central nervous system, ACh is important for varied bodily functions, such as sensory and motor processing, sleep, nociception, mood, stress response, attention, arousal, memory, motivation and reward. Another neurotransmitter, dopamine (DA) is also found in the NAc and its release is a critical event mediating the rewarding effects of stimulant drugs (Sofuoglu & Mooney, 2009, *CNS Drugs,* 20(11):939-952). Cholinergic interneurons release ACh in the nucleus accumbens. The activity of MSNs can be modulated by both cholinergic and dopaminergic control, which may be excitatory or inhibitory depending on the receptor subtypes that are stimulated: the D1 dopaminergic and M1 mAChR are excitatory while D2 dopaminergic and M4 mAChR are inhibitory (Calabresi et al., *Lancet Neurol.,* 2006, 5(11):974-83). Cholinergic interneurons receive dopaminergic input from the ventral tegmental area (VTA), and glutamatergic input mainly from the prefrontal cortex, hippocampus, and amygdala (Sofuoglu & Mooney, 2009, *CNS Drugs,* 20(11):939-952). Without being bound to theory, it is thought that this DA and glutamate convergence on cholinergic interneurons may provide a mechanism for DA-mediated reward to be associated with glutamate-mediated learning and contextual information (Berlanga et al., Neuroscience. 2003; 120(4): 1149-56) Accordingly, cholinergic interneurons are believed to regulate the translation of reward signals into contextually appropriate behavior.

[0043] Commonly abused drugs and natural rewards share the mutual action of altering extracellular concentrations of neurotransmitters in the NAc (Di Chiara & Imperato, 1988, PNAS, 85(14):5274-8; Phaus, *Curr Opin Neurobiol.,* 1999, 9(6):751-8). Moreover, lesions of the NAc have been shown to decrease the rewarding effects of various stimulants and opiates (Kelsey et al., *Behav Neurosci.,* 1989 103(6):1327-34). Nonetheless, it has been experiments encompassing direct microinfusion of narcotics into the NAc that have provided the most robust evidence for the role it plays in reward-related behavior rewarding states. For example, rodent models of addiction will readily self-administer narcotics such as amphetamine (a dopamine-releasing agent), cocaine (a dopamine reuptake inhibitor), and nomifensine (a dopamine reuptake inhibitor) directly into the NAc, thereby demonstrating that dopamine plays an important role in the

NAc to regulate behavior based on reward and motivation (Carlezon & Thomas, *Neuropharmacology,* 2009; 56(Suppl 1): 122-132).

[0044] Still unclear, however, are the roles played by the cells of the NAc itself in mediating these complex mammalian behaviors in response to the input of neurotransmitters like dopamine, especially with regard to reward-related behaviors such as substance abuse and dependency (addiction). Also unknown are the specific roles played by the neurotransmitters dopamine and acetylcholine in bringing about reward-related behaviors through the NAc. A reward is a process that reinforces behavior—something that, when offered, causes a behavior to increase in intensity. Reward is an operational concept for describing the positive value an individual ascribes to an object, behavioral act, or an internal physical state. Natural rewards include those that are necessary for the survival of species, such as eating, mating, and fighting. The NAc has been associated with many of these types of rewards-related behaviors, as varied as drug addiction, sex addiction, and gambling addiction.

[0045] Light-Responsive Opsin Proteins

[0046] Provided herein are optogenetic-based compositions and methods for selectively hyperpolarizing cholinergic neurons in the nucleus accumbens and striatum of individuals to disrupt at least one rewards-related behavior in the individual. Optogenetics refers to the combination of genetic and optical methods used to control specific events in targeted cells of living tissue, even within freely moving mammals and other animals, with the temporal precision (millisecond-timescale) needed to keep pace with functioning intact biological systems. Optogenetics requires the introduction of fast light-responsive channel or pump proteins to the plasma membranes of target neuronal cells that allow temporally precise manipulation of neuronal membrane potential while maintaining cell-type resolution through the use of specific targeting mechanisms.

[0047] Light-responsive opsins that may be used in the present invention includes opsins that induce hyperpolarization in neurons by light and opsins that induce depolarization in neurons by light. Examples of opsins are shown in Tables 1 and 2 below.

Table 1 shows identified opsins for inhibition of cellular activity across the visible spectrum:

Opsin Type	Biological Origin	Wavelength Sensitivity	Defined action
NpHR	*Natronomonas pharaonis*	589 nm max	Inhibition (hyperpolarization)
BR	*Halobacterium helobium*	570 nm max	Inhibition (hyperpolarization)
AR	*Acetabulaira acetabulum*	518 nm max	Inhibition (hyperpolarization)
GtR3	*Guillardia theta*	472 nm max	Inhibition (hyperpolarization)
Mac	*Leptosphaeria maculans*	470-500 nm max	Inhibition (hyperpolarization)
NpHr3.0	*Natronomonas pharaonis*	680 nm utility 589 nm max	Inhibition (hyperpolarization)
NpHR3.1	*Natronomonas pharaonis*	680 nm utility 589 nm max	Inhibition (hyperpolarization)

Table 2 shows identified opsins for excitation and modulation across the visible spectrum:

Opsin Type	Biological Origin	Wavelength Sensitivity	Defined action
VChR1	*Volvox carteri*	589 nm utility 535 nm max	Excitation (depolarization)
DChR	*Dunaliella salina*	500 nm max	Excitation (depolarization)
ChR2	*Chlamydomonas reinhardtii*	470 nm max 380-405 nm utility	Excitation (depolarization)
ChETA	*Chlamydomonas reinhardtii*	470 nm max 380-405 nm utility	Excitation (depolarization)
SFO	*Chlamydomonas reinhardtii*	470 nm max 530 nm max	Excitation (depolarization) Inactivation
SSFO	*Chlamydomonas reinhardtii*	445 nm max 590 nm; 390-400 nm	Step-like activation (depolarization) Inactivation
C1V1	*Volvox carteri* and *Chlamydomonas reinhardtii*	542 nm max	Excitation (depolarization)
C1V1 E122	*Volvox carteri* and *Chlamydomonas reinhardtii*	546 nm max	Excitation (depolarization)
C1V1 E162	*Volvox carteri* and *Chlamydomonas reinhardtii*	542 nm max	Excitation (depolarization)
C1V1 E122/ E162	*Volvox carteri* and *Chlamydomonas reinhardtii*	546 nm max	Excitation (depolarization)

[0048] As used herein, a light-responsive opsin (such as NpHR, BR, AR, GtR3, Mac, ChR2, VChR1, DChR, and ChETA) includes naturally occurring protein and functional variants, fragments, fusion proteins comprising the fragments or the full length protein. In some embodiments, the signal peptide may be removed. A variant may have an amino acid sequence at least about any of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the naturally occurring protein sequence. A functional variant may have the same or similar hyperpolarization function or depolarization function as the naturally occurring protein.

[0049] Enhanced Intracellular Transport Amino Acid Motifs

[0050] The present disclosure provides for the modification of light-responsive opsin proteins expressed in a cell by the addition of one or more amino acid sequence motifs which enhance transport to the plasma membranes of mammalian cells. Light-responsive opsin proteins having components derived from evolutionarily simpler organisms may not be expressed or tolerated by mammalian cells or may exhibit impaired subcellular localization when expressed at high levels in mammalian cells. Consequently, in some embodiments, the light-responsive opsin proteins expressed in a cell can be fused to one or more amino acid sequence motifs selected from the group consisting of a signal peptide, an endoplasmic reticulum (ER) export signal, a membrane trafficking signal, and/or an N-terminal golgi export signal. The one or more amino acid sequence motifs which enhance light-responsive opsin protein transport to the plasma membranes of mammalian cells can be fused to the N-terminus, the C-terminus, or to both the N- and C-terminal ends of the light-responsive opsin protein. Optionally, the light-responsive opsin protein and the one or more amino acid sequence motifs may be separated by a linker. In some embodiments, the light-responsive opsin protein can be modified by the addition of a trafficking signal (ts) which enhances transport of the protein to the cell plasma membrane. In some embodi-

ments, the trafficking signal can be derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In other embodiments, the trafficking signal can comprise the amino acid sequence KSRITSEGEYI-PLDQIDINV (SEQ ID NO:12).

[0051] Additional protein motifs which can enhance light-responsive opsin protein transport to the plasma membrane of a cell are described in U.S. patent application Ser. No. 12/041,628, which is incorporated herein by reference in its entirety. In some embodiments, the signal peptide sequence in the protein can be deleted or substituted with a signal peptide sequence from a different protein.

[0052] Light-Responsive Chloride Pumps

[0053] In some aspects of the methods provided herein, one or more members of the Halorhodopsin family of light-responsive chloride pumps are expressed on the plasma membranes of the cholinergic interneurons of the nucleus accumbens or the striatum.

[0054] In some aspects, said one or more light-responsive chloride pump proteins expressed on the plasma membranes of the nerve cells of the cholinergic interneurons of the nucleus accumbens or the striatum can be derived from *Natronomonas pharaonic*. In some embodiments, the light-responsive chloride pump proteins can be responsive to amber light as well as red light and can mediate a hyperpolarizing current in the interneuron when the light-responsive chloride pump proteins are illuminated with amber or red light. The wavelength of light which can activate the light-responsive chloride pumps can be between about 580 and 630 nm. In some embodiments, the light can be at a wavelength of about 590 nm or the light can have a wavelength greater than about 630 nm (e.g. less than about 740 nm). In another embodiment, the light has a wavelength of around 630 nm. In some embodiments, the light-responsive chloride pump protein can hyperpolarize a neural membrane for at least about 90 minutes when exposed to a continuous pulse of light. In some embodiments, the light-responsive chloride pump protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1. Additionally, the light-responsive chloride pump protein can comprise substitutions, deletions, and/or insertions introduced into a native amino acid sequence to increase or decrease sensitivity to light, increase or decrease sensitivity to particular wavelengths of light, and/or increase or decrease the ability of the light-responsive protein to regulate the polarization state of the plasma membrane of the cell. In some embodiments, the light-responsive chloride pump protein contains one or more conservative amino acid substitutions. In some embodiments, the light-responsive protein contains one or more non-conservative amino acid substitutions. The light-responsive protein comprising substitutions, deletions, and/or insertions introduced into the native amino acid sequence suitably retains the ability to hyperpolarize the plasma membrane of a neuronal cell in response to light.

[0055] Additionally, in other aspects, the light-responsive chloride pump protein can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1 and an endoplasmic reticulum (ER) export signal. This ER export signal can be fused to the C-terminus of the core amino acid sequence or can be fused to the N-terminus of the core amino acid sequence. In some embodiments, the ER export signal is linked to the core amino acid sequence by a linker. The linker can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments, the ER export signal can comprise the amino acid sequence FXYENE, where X can be any amino acid. In another embodiment, the ER export signal can comprise the amino acid sequence VXXSL, where X can be any amino acid. In some embodiments, the ER export signal can comprise the amino acid sequence FCYENEV (SEQ ID NO:14).

[0056] In other aspects, the light-responsive chloride pump proteins provided herein can comprise a light-responsive protein expressed on the cell membrane, wherein the protein comprises a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1 and a trafficking signal (e.g., which can enhance transport of the light-responsive chloride pump protein to the plasma membrane). The trafficking signal may be fused to the C-terminus of the core amino acid sequence or may be fused to the N-terminus of the core amino acid sequence. In some embodiments, the trafficking signal can be linked to the core amino acid sequence by a linker which can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments, the trafficking signal can be derived from the amino acid sequence of the human inward rectifier potassium channel $K_{ir}2.1$. In other embodiments, the trafficking signal can comprise the amino acid sequence KSRITSEGEYIPLDQIDINV (SEQ ID NO:12).

[0057] In some aspects, the light-responsive chloride pump protein can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1 and at least one (such as one, two, three, or more) amino acid sequence motifs which enhance transport to the plasma membranes of mammalian cells selected from the group consisting of an ER export signal, a signal peptide, and a membrane trafficking signal. In some embodiments, the light-responsive chloride pump protein comprises an N-terminal signal peptide, a C-terminal ER Export signal, and a C-terminal trafficking signal. In some embodiments, the C-terminal ER Export signal and the C-terminal trafficking signal can be linked by a linker. The linker can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker can also further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments the ER Export signal can be more C-terminally located than the trafficking signal. In other embodiments the trafficking signal is more C-terminally located than the ER Export signal. In some embodiments, the signal peptide comprises the amino acid sequence MTETLPPVTESAVALQAE (SEQ ID NO:15). In another embodiment, the light-responsive chlo-

ride pump protein comprises an amino acid sequence at least 95% identical to SEQ ID NO:2.

[0058] Moreover, in other aspects, the light-responsive chloride pump proteins can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1, wherein the N-terminal signal peptide of SEQ ID NO:1 is deleted or substituted. In some embodiments, other signal peptides (such as signal peptides from other opsins) can be used. The light-responsive protein can further comprise an ER transport signal and/or a membrane trafficking signal described herein. In some embodiments, the light-responsive chloride pump protein comprises an amino acid sequence at least 95% identical to SEQ ID NO:3.

[0059] Also provided herein are polynucleotides encoding any of the light-responsive chloride ion pump proteins described herein, such as a light-responsive protein comprising a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal. In another embodiment, the polynucleotides comprise a sequence which encodes an amino acid at least 95% identical to SEQ ID NO:2 and SEQ ID NO:3. The polynucleotides may be in an expression vector (such as, but not limited to, a viral vector described herein). The polynucleotides may be used for expression of the light-responsive chloride ion pump proteins in the cholinergic neurons of the NAc or the striatum.

[0060] In some embodiments, the light-responsive opsin protein is a NpHR opsin protein comprising an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the sequence shown in SEQ ID NO:1. In some embodiments, the NpHR opsin protein further comprises an endoplasmic reticulum (ER) export signal and/or a membrane trafficking signal. For example, the NpHR opsin protein comprises an amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1 and an endoplasmic reticulum (ER) export signal. In some embodiments, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1 is linked to the ER export signal through a linker. In some embodiments, the ER export signal comprises the amino acid sequence FXYENE (SEQ ID NO:13), where X can be any amino acid. In another embodiment, the ER export signal comprises the amino acid sequence VXXSL, where X can be any amino acid. In some embodiments, the ER export signal comprises the amino acid sequence FCYENEV (SEQ ID NO:14). In some embodiments, the NpHR opsin protein comprises an amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal. In other embodiments, the NpHR opsin protein comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, the ER export signal, and the membrane trafficking signal. In other embodiments, the NpHR opsin protein comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, the membrane trafficking signal, and the ER export signal. In some embodiments, the membrane trafficking signal is derived from the amino acid sequence of the human inward rectifier potassium channel $K_{ir}2.1$. In some embodiments, the membrane trafficking signal comprises the amino acid sequence K S R I T S E G E Y I P L D Q I D I N V (SEQ ID NO:12). In some embodiments, the membrane trafficking signal is linked to the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1 by a linker. In some embodiments, the membrane trafficking signal is linked to the ER export signal through a linker. The linker may comprise any of 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments, the light-responsive opsin protein further comprises an N-terminal signal peptide. In some embodiments, the light-responsive opsin protein comprises the amino acid sequence of SEQ ID NO:2. In some embodiments, the light-responsive opsin protein comprises the amino acid sequence of SEQ ID NO:3.

[0061] Further disclosure related to light-responsive chloride pump proteins can be found in U.S. Patent Application Publication Nos: 2009/0093403 and 2010/0145418 as well as in International Patent Application No: PCT/US2011/028893, the disclosures of each of which are hereby incorporated by reference in their entireties.

[0062] Light-Responsive Proton Pumps

[0063] In some aspects of the compositions and methods provided herein, one or more light-responsive proton pumps are expressed on the plasma membranes of the cholinergic interneurons of the nucleus accumbens or the striatum.

[0064] In some embodiments, the light-responsive proton pump protein can be responsive to blue light and can be derived from *Guillardia theta*, wherein the proton pump protein can be capable of mediating a hyperpolarizing current in the cell when the cell is illuminated with blue light. The light can have a wavelength between about 450 and about 495 nm or can have a wavelength of about 490 nm. In another embodiment, the light-responsive proton pump protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4. The light-responsive proton pump protein can additionally comprise substitutions, deletions, and/or insertions introduced into a native amino acid sequence to increase or decrease sensitivity to light, increase or decrease sensitivity to particular wavelengths of light, and/or increase or decrease the ability of the light-responsive proton pump protein to regulate the polarization state of the plasma membrane of the cell. Additionally, the light-responsive proton pump protein can contain one or more conservative amino acid substitutions and/or one or more non-conservative amino acid substitutions. The light-responsive proton pump protein comprising substitutions, deletions, and/or insertions introduced into the native amino acid sequence suitably retains the ability to hyperpolarize the plasma membrane of a neuronal cell in response to light.

[0065] In other aspects of the methods disclosed herein, the light-responsive proton pump protein can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4 and at least one (such as one, two, three, or more) amino acid sequence motifs which enhance transport to the plasma membranes of mammalian cells selected from the group consisting of a

signal peptide, an ER export signal, and a membrane trafficking signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide and a C-terminal ER export signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide and a C-terminal trafficking signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide, a C-terminal ER Export signal, and a C-terminal trafficking signal. In some embodiments, the light-responsive proton pump protein comprises a C-terminal ER Export signal and a C-terminal trafficking signal. In some embodiments, the C-terminal ER Export signal and the C-terminal trafficking signal are linked by a linker. The linker can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments the ER Export signal is more C-terminally located than the trafficking signal. In some embodiments the trafficking signal is more C-terminally located than the ER Export signal.

[0066] Also provided herein are isolated polynucleotides encoding any of the light-responsive proton pump proteins described herein, such as a light-responsive proton pump protein comprising a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4. Also provided herein are expression vectors (such as a viral vector described herein) comprising a polynucleotide encoding the proteins described herein, such as a light-responsive proton pump protein comprising a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4. The polynucleotides may be used for expression of the light-responsive opsin protein in neural cells (e.g. the cholinergic interneurons of the NAc or the striatum).

[0067] Further disclosure related to light-responsive proton pump proteins can be found in International Patent Application No. PCT/US2011/028893, the disclosure of which is hereby incorporated by reference in its entirety.

[0068] Light-Responsive Channel Proteins

[0069] In some aspects of the methods provided herein, one or more members of the Channelrhodopsin family of light-responsive ion channels are expressed on the plasma membranes of the cholinergic interneurons of the nucleus accumbens or the striatum.

[0070] In some aspects, the light-responsive cation channel protein can be derived from *Chlamydomonas reinhardtii*, wherein the cation channel protein can be capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In another embodiment, the light-responsive cation channel protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:5. The light used to activate the light-responsive cation channel protein derived from *Chlamydomonas reinhardtii* can have a wavelength between about 460 and about 495 nm or can have a wavelength of about 470 nm. Additionally, the light can have an intensity of at least about 100 Hz. In some embodiments, activation of the light-responsive cation channel derived from *Chlamydomonas reinhardtii* with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the light-responsive cation channel. The light-responsive cation channel protein can additionally comprise substitutions, deletions, and/or insertions introduced into a native amino acid sequence to increase or decrease sensitivity to light, increase or decrease sensitivity to particular wavelengths of light, and/or increase or decrease the ability of the light-responsive cation channel protein to regulate the polarization state of the plasma membrane of the cell. Additionally, the light-responsive cation channel protein can contain one or more conservative amino acid substitutions and/or one or more non-conservative amino acid substitutions. The light-responsive cation channel protein comprising substitutions, deletions, and/or insertions introduced into the native amino acid sequence suitably retains the ability to depolarize the plasma membrane of a neuronal cell in response to light.

[0071] In other embodiments, the light-responsive cation channel protein can be a step function opsin (SFO) protein or a stabilized step function opsin (SSFO) protein that can have specific amino acid substitutions at key positions throughout the retinal binding pocket of the protein. In some embodiments, the SFO protein can have a mutation at amino acid residue C128 of SEQ ID NO:5. In other embodiments, the SFO protein has a C128A mutation in SEQ ID NO:5. In other embodiments, the SFO protein has a C128S mutation in SEQ ID NO:5. In another embodiment, the SFO protein has a C128T mutation in SEQ ID NO:5. In some embodiments, the SFO protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:6 or SEQ ID NO:7.

[0072] In other embodiments, the light-responsive cation channel protein can be a C1V1 chimeric protein derived from the VChR1 protein of *Volvox carteri* and the ChR1 protein from *Chlamydomonas reinhardti*, wherein the protein comprises the amino acid sequence of VChR1 having at least the first and second transmembrane helices replaced by the first and second transmembrane helices of ChR1; is responsive to light; and is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. Additionally, in some embodiments, the invention can include polypeptides comprising substituted or mutated amino acid sequences, wherein the mutant polypeptide retains the characteristic light-responsive nature of the precursor C1V1 chimeric polypeptide but may also possess altered properties in some specific aspects. For example, the mutant light-responsive C1V1 chimeric proteins described herein can exhibit an increased level of expression both within an animal cell or on the animal cell plasma membrane; an altered responsiveness when exposed to different wavelengths of light, particularly red light; and/or a combination of traits whereby the chimeric C1V1 polypeptide possess the properties of low desensitization, fast deactivation, low violet-light activation for minimal cross-activation with other light-responsive cation channels, and/or strong expression in animal cells. In some embodiments, the C1V1 protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NOs:8, 9, 10, or 11.

[0073] Further disclosure related to light-responsive cation channel proteins can be found in U.S. Patent Application Publication No. 2007/0054319 and International Patent

Application Publication Nos. WO 2009/131837 and WO 2007/024391. Further disclosure related to SFO or SSFO proteins can be found in International Patent Application Publication No. WO 2010/056970 and U.S. Provisional Patent Application Nos. 61/410,704 and 61/511,905. Further disclosure related to C1V1 chimeric cation channels as well as mutant variants of the same can be found in U.S. Provisional Patent Application Nos. 61/410,736, 61/410,744, and 61/511,912. The disclosures of each of the aforementioned references related to specific light-responsive opsin proteins are hereby incorporated by reference in their entireties.

[0074] Polynucleotides

[0075] The disclosure also provides polynucleotides comprising a nucleotide sequence encoding a light-responsive opsin protein described herein. In some embodiments, the polynucleotide comprises an expression cassette. In some embodiments, the polynucleotide is a vector comprising the above-described nucleic acid. In some embodiments, the nucleic acid encoding a light-responsive opsin protein of the disclosure is operably linked to a promoter. Promoters are well known in the art. Any promoter that functions in a cholinergic interneuron can be used for expression of the light-responsive proteins and/or any variant thereof of the present disclosure. Initiation control regions or promoters, which are useful to drive expression of the light-responsive opsin proteins or variant thereof in a specific animal cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these nucleic acids can be used. In some embodiments, the promoter used to drive expression of the light-responsive protein can be the choline acetyltransferase (ChAT) promoter, which is capable of driving robust expression of transgenes in cholinergic interneuron (See, e.g., Gong et al., *J. Neurosci.,* 27, 9817-9823 (2007)).

[0076] Also provided herein are vectors comprising a nucleotide sequence encoding a light-responsive opsin protein or any variant thereof described herein. The vectors that can be administered according to the present invention also include vectors comprising a nucleotide sequence which encodes an RNA (e.g., an mRNA) that when transcribed from the polynucleotides of the vector will result in the accumulation of light-responsive proteins on the plasma membranes of target animal cells. Vectors which may be used, include, without limitation, lentiviral, HSV, adenoviral, and adeno-associated viral (AAV) vectors. Lentiviruses include, but are not limited to HIV-1, HIV-2, SIV, FIV and EIAV. Lentiviruses may be pseudotyped with the envelope proteins of other viruses, including, but not limited to VSV, rabies, Mo-MLV, baculovirus and Ebola. Such vectors may be prepared using standard methods in the art.

[0077] In some embodiments, the vector is a recombinant AAV vector. AAV vectors are DNA viruses of relatively small size that can integrate, in a stable and site-specific manner, into the genome of the cells that they infect. They are able to infect a wide spectrum of cells without inducing any effects on cellular growth, morphology or differentiation, and they do not appear to be involved in human pathologies. The AAV genome has been cloned, sequenced and characterized. It encompasses approximately 4700 bases and contains an inverted terminal repeat (ITR) region of approximately 145 bases at each end, which serves as an origin of replication for the virus. The remainder of the genome is divided into two essential regions that carry the encapsidation functions: the left-hand part of the genome, that contains the rep gene involved in viral replication and expression of the viral genes; and the right-hand part of the genome, that contains the cap gene encoding the capsid proteins of the virus.

[0078] AAV vectors may be prepared using standard methods in the art. Adeno-associated viruses of any serotype are suitable (see, e.g., Blacklow, pp. 165-174 of "*Parvoviruses and Human Disease*" J. R. Pattison, ed. (1988); Rose, *Comprehensive Virology* 3:1, 1974; P. Tattersall "The Evolution of Parvovirus Taxonomy" In *Parvoviruses* (J R Kerr, S F Cotmore. M E Bloom, R M Linden, C R Parrish, Eds.) p5-14, Hudder Arnold, London, U K (2006); and D E Bowles, J E Rabinowitz, R J Samulski "*The Genus Dependovirus*" (J R Kerr, S F Cotmore. M E Bloom, R M Linden, C R Parrish, Eds.) p15-23, Hudder Arnold, London, UK (2006), the disclosures of each of which are hereby incorporated by reference herein in their entireties). Methods for purifying for vectors may be found in, for example, U.S. Pat. Nos. 6,566,118, 6,989,264, and 6,995,006 and WO/1999/011764 titled "Methods for Generating High Titer Helper-free Preparation of Recombinant AAV Vectors", the disclosures of which are herein incorporated by reference in their entirety. Preparation of hybrid vectors is described in, for example, PCT Application No. PCT/US2005/027091, the disclosure of which is herein incorporated by reference in its entirety. The use of vectors derived from the AAVs for transferring genes in vitro and in vivo has been described (See e.g., International Patent Application Publication Nos.: 91/18088 and WO 93/09239; U.S. Pat. Nos. 4,797,368, 6,596,535, and 5,139,941; and European Patent No.: 0488528, all of which are hereby incorporated by reference herein in their entireties). These publications describe various AAV-derived constructs in which the rep and/or cap genes are deleted and replaced by a gene of interest, and the use of these constructs for transferring the gene of interest in vitro (into cultured cells) or in vivo (directly into an organism). The replication defective recombinant AAVs according to the invention can be prepared by co-transfecting a plasmid containing the nucleic acid sequence of interest flanked by two AAV inverted terminal repeat (ITR) regions, and a plasmid carrying the AAV encapsidation genes (rep and cap genes), into a cell line that is infected with a human helper virus (for example an adenovirus). The AAV recombinants that are produced are then purified by standard techniques.

[0079] In some embodiments, the vector(s) for use in the methods of the invention are encapsidated into a virus particle (e.g. AAV virus particle including, but not limited to, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, and AAV16). Accordingly, the invention includes a recombinant virus particle (recombinant because it contains a recombinant polynucleotide) comprising any of the vectors described herein. Methods of producing such particles are known in the art and are described in U.S. Pat. No. 6,596,535, the disclosure of which is hereby incorporated by reference in its entirety.

[0080] Delivery of Light-Responsive Opsin Proteins

[0081] In some aspects, polynucleotides encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered directly to the cholinergic interneurons of the nucleus accumbens or striatum using a needle, catheter, or related device, using neu-

rosurgical techniques known in the art, such as by stereotactic injection (See, e.g., Stein et al., *J. Virol,* 73:34243429, 1999; Davidson et al., *PNAS,* 97:3428-3432, 2000; Davidson et al., *Nat. Genet.* 3:219-223, 1993; and Alisky & Davidson, *Hum. Gene Ther.* 11:2315-2329, 2000, the contents of each of which are hereby incorporated by reference herein in their entireties) or fluoroscopy.

[0082] In other aspects, any of the light-responsive opsin proteins can be expressed in the cholinergic interneurons of the nucleus accumbens or striatum of a transgenic animal. For example, a transgenic mouse line can be employed using Cre-recombinase under control of the choline acetyltransferase (ChAT) promoter. A Cre-inducible adeno-associated virus (AAV) vector carrying the light-responsive opsin gene can then be stereotaxically injected into the NAc.

[0083] Other methods to deliver the light-responsive proteins to cholinergic interneurons can also be used, such as, but not limited to, transfection with ionic lipids or polymers, electroporation, optical transfection, impalefection, or via gene gun.

[0084] Light Sources

[0085] Any device that is capable of applying light having a wavelength to activate the light-responsive proteins expressed in a neuron may be used to depolarize and/or hyperpolarize the neuron. For example, a light-delivery device for activating ion channels and/or ionic pumps to affect the membrane voltage of one or more neurons may be used. A light-delivery device can be configured to provide optical stimulus to a target region of the brain. The light-delivery device may comprise a base, a cannula guide that is attached to the base, and one or more optical conduits attached to the base via the cannula guide. The base may comprise one or more light delivery ports that are positioned to deliver light from the optical conduits to targeted tissue regions, such as the nucleus accumbens or the striatum. The optical conduits may be optical fibers, where the proximal end of the fiber is attached to an optical light source, and the distal end is in communication with the light delivery ports. The optical light source may be capable of providing continuous light and/or pulsed light, and may be programmable to provide light in pre-determined pulse sequences. The light delivery device may have any number of optical conduits as may be desirable, e.g., 1, 2, 3, 4, 5, 10, 15, 20, etc. The optical conduits may each carry light of the same or different wavelengths. The delivered light may have a wavelength between 450 nm and 600 nm, such as yellow or green or blue light. The light delivery device may have any number of light delivery ports as may be desirable, e.g., 1, 2, 3, 4, 5, 10, 15, 20, etc. In some variations, there may be the same number of light delivery ports as optical conduits while in other variations, there may be different number of optical conduits and light delivery ports. For example, there may be a single optical conduit that conveys light to two or more light delivery ports. Alternatively or additionally, a single optical conduit may connect to a single light delivery port. The cannula guide may be configured to help secure and align the optical conduits with the light delivery ports. In some embodiments, the light delivery device is configured to deliver light to the nucleus accumbens or the striatum to disrupt at least one reward-related behavior in an individual. Light delivery devices may also comprise one or more measurement electrodes that may be configured for measuring neural activity. For example, measurement electrodes may record changes in the membrane potential (e.g., action potentials) and/or current flow across a membrane of one or more neurons as the neurons respond to a stimulus. In some variations, the measurement electrodes may measure the electrical response of one or more neurons to optical stimulation. Measurement electrodes may be extracellular or intracellular electrodes.

[0086] In other aspects, the light delivery device can be an implantable light source that does not require physical tethering to an external power source. The implantable light source can comprise an inner body, the inner body having at least one means for generating light which is configured to a power source. In some embodiments, the power source can be an internal battery for powering the light-generating means. In another embodiment, the implantable light source can comprise an external antenna for receiving wirelessly transmitted electromagnetic energy from an external source for powering the light-generating means. The wirelessly transmitted electromagnetic energy can be a radio wave, a microwave, or any other electromagnetic energy source that can be transmitted from an external source to power the light-generating means of the implantable light source. In one embodiment, the light-generating means is controlled by an integrated circuit produced using semiconductor or other processes known in the art.

[0087] In some aspects, the light means can be a light emitting diode (LED). In some embodiments, the LED can generate blue and/or green light. In other embodiments, the LED can generate amber, yellow and/or blue light. In some embodiments, several micro LEDs are embedded into the inner body of the implantable light source. In other embodiments, the light-generating means is a solid state laser diode or any other means capable of generating light. The light generating means can generate light having an intensity sufficient to activate the light-responsive proteins expressed on the plasma membrane of the nerves in proximity to the light source. In some embodiments, the intensity of the light reaching the cholinergic interneurons of the NAc or striatum produced by the light-generating means has an intensity of any of about 0.05 mW/mm^2, 0.1 mW/mm^2, 0.2 mW/mm^2, 0.3 mW/mm^2, 0.4 mW/mm^2, 0.5 mW/mm^2, about 0.6 mW/mm^2, about 0.7 mW/mm^2, about 0.8 mW/mm^2, about 0.9 mW/mm^2, about 1.0 mW/mm^2, about 1.1 mW/mm^2, about 1.2 mW/mm^2, about 1.3 mW/mm^2, about 1.4 mW/mm^2, about 1.5 mW/mm^2, about 1.6 mW/mm^2, about 1.7 mW/mm^2, about 1.8 mW/mm^2, about 1.9 mW/mm^2, about 2.0 mW/mm^2, about 2.1 mW/mm^2, about 2.2 mW/mm^2, about 2.3 mW/mm^2, about 2.4 mW/mm^2, about 2.5 mW/mm^2, about 3 mW/mm^2, about 3.5 mW/mm^2, about 4 mW/mm^2, about 4.5 mW/mm^2, about 5 mW/mm^2, about 5.5 mW/mm^2, about 6 mW/mm^2, about 7 mW/mm^2, about 8 mW/mm^2, about 9 mW/mm^2, or about 10 mW/mm^2 inclusive, including values in between these numbers.

[0088] In some aspects, the light-generating means can be externally activated by an external controller. The external controller can comprise a power generator which can be mounted to a transmitting coil. In some embodiments of the external controller, a battery can be connected to the power generator, for providing power thereto. A switch can be connected to the power generator, allowing an individual to manually activate or deactivate the power generator. In some embodiments, upon activation of the switch, the power generator can provide power to the light-generating means on the light source through electromagnetic coupling between the transmitting coil on the external controller and

the external antenna of the implantable light source. The transmitting coil can establish an electromagnetic coupling with the external antenna of the implantable light source when in proximity thereof, for supplying power to the light-generating means and for transmitting one or more control signals to the implantable light source. In some embodiments, the electromagnetic coupling between the transmitting coil of the external controller and the external antenna of the implantable light source can be radio-frequency magnetic inductance coupling. When radio-frequency magnetic inductance coupling is used, the operational frequency of the radio wave can be between about 1 and 20 MHz, inclusive, including any values in between these numbers (for example, about 1 MHz, about 2 MHz, about 3 MHz, about 4 MHz, about 5 MHz, about 6 MHz, about 7 MHz, about 8 MHz, about 9 MHz, about 10 MHz, about 11 MHz, about 12 MHz, about 13 MHz, about 14 MHz, about 15 MHz, about 16 MHz, about 17 MHz, about 18 MHz, about 19 MHz, or about 20 MHz). However, other coupling techniques may be used, such as an optical receiver, infrared, or a biomedical telemetry system (See, e.g., Kiourti, "Biomedical Telemetry: Communication between Implanted Devices and the External World, *Opticon* 1826, (8): Spring, 2010).

[0089] Examples of light stimulation devices, including light sources, can be found in International Patent Application Nos: PCT/US08/50628 and PCT/US09/49936 and in Llewellyn et al., 2010, *Nat. Med.,* 16(10):161-165, the disclosures of each of which are hereby incorporated herein in their entireties.

[0090] Light-Responsive Opsins Expressed in Cholinergic Interneurons

[0091] Provided herein are non-human animals comprising a light-responsive opsin protein expressed on the cell membrane of a cholinergic interneuron in the nucleus accumbens or the striatum of the animal, wherein the protein is responsive to light and is capable of altering the membrane polarization state of the interneurons when the interneurons are illuminated with the light, wherein the illumination of the opsin disrupts at least one reward-related behavior of the animal. In some embodiments, the light-responsive protein is selected from the group consisting of NpHR, BR, AR and GtR3 described herein. For example, any of the NpHR proteins described herein may be expressed on the cell membrane of the target neurons. In some embodiments, the reward-related behavior is drug-related addictive behavior. The drug can be any addictive drug such as, but not limited to, opioids (for example, opium and heroin), methamphetamine, cocaine, ketamine, MDMA (3,4-Methylenedioxymethamphetamine), lysergic acid diethylamide, cannabinoids, alcohol, nicotine, or any other controlled substance. In one embodiment, the drug is cocaine. In another embodiment, the reward-related behavior is cocaine addiction.

[0092] Also provided herein are brain tissue slices comprising the nucleus accumbens or striatum, wherein a light-responsive protein is expressed on the cell membrane of cholinergic interneurons of the nucleus accumbens, wherein the protein is responsive to light and is capable of altering the membrane polarization state of the interneurons when the interneurons are illuminated with the light, wherein the illumination of the protein disrupts at least one reward-related behavior. In some embodiments, the brain tissue slices are cultured tissue slices taken from the non-human animals described herein. In some embodiments, the light-responsive protein is capable of hyperpolarizing the membranes of cholinergic interneurons of the nucleus accumbens and is selected from the group consisting of NpHR, BR, AR and GtR3 described herein. For example, any of the NpHR proteins described herein may be expressed on the cell membrane of the target neurons. In other embodiments, the light-responsive protein is capable of depolarizing the membrane of cholinergic interneurons of the nucleus accumbens and is selected from the group consisting of ChR2, SFO, SSFO, and the C1V1s described herein.

[0093] Methods of the Invention

[0094] In some aspects, provided herein are methods for disrupting reward-related behavior in an individual comprising: administering a polynucleotide encoding a light-responsive opsin protein to the individual, wherein the light-responsive opsin protein is expressed on the cell membrane of cholinergic interneurons in the nucleus accumbens or the striatum of the individual, and the protein is responsive to light and is capable of inducing membrane hyperpolarization of the interneurons when the interneurons are illuminated with the light, whereby activating the protein by the light disrupts at least one reward-related behavior in the individual. In some embodiments, the polynucleotide is administered to the nucleus accumbens or the striatum of the individual. In some embodiments, the light-responsive protein is selected from the group consisting of NpHR, BR, AR and GtR3 described herein. For example, any of the NpHR proteins described herein may be expressed on the cell membrane of the target neurons. In some embodiments, the reward-related behavior is drug-related addictive behavior. The drug can be any addictive drug such as, but not limited to, opioids (for example, opium and heroin), methamphetamine, cocaine, ketamine, MDMA (3,4-Methylenedioxymethamphetamine), lysergic acid diethylamide, cannabinoids, alcohol, nicotine, or any other controlled substance. In one embodiment, the drug is cocaine. In another embodiment, the reward-related behavior is cocaine addiction. In some embodiments, the individual is a non-human animal. In some embodiments, the individual is a human In some embodiments, the polynucleotide further comprises a promoter (e.g., a ChAT promoter) operably linked to the light-responsive opsin protein. In some embodiments, the polynucleotide is a vector.

[0095] Methods for measuring disruption of reward-related behavior are many and well known in the art (See, e.g., *Addiction Research Methods*, (Miller et al., eds., 2010; Wiley-Blackwell, United Kingdom)). For example, cocaine addiction and disruption of drug-related addictive behavior can be assessed by using conditioned place preference (CPP; also known as environmental place) conditioning. CPP is a technique commonly used in animal studies to evaluate preferences for environmental stimuli that have been associated with a positive or negative reward. The technique is often used to determine the addictive potential of drugs. The procedure involves several trials where the animal is presented with the positive stimulus (e.g., food, neurotransmitters or the effects of a drug of abuse) paired with placement in a distinct environment containing various cues (e.g., tactile, visual, and/or olfactory). When later tested in the normal state, approaches and the amount of time spent in the compartments previously associated with the positive stimulus serves as an indicator of preference and a measure of reward learning.

[0096] In other aspects, provided herein is a method for treating drug addiction in an individual comprising: administering a polynucleotide encoding a light-responsive opsin protein the individual, wherein the light-responsive opsin protein is expressed on the cell membrane of cholinergic interneurons in the nucleus accumbens or the striatum of the individual, and the protein is responsive to light and is capable of hyperpolarizing the interneurons when the interneurons are illuminated with the light, whereby activating the protein by the light disrupts reward-related behavior in the individual, wherein the individual no longer desires to take drugs. In some embodiments, the polynucleotide is administered to the nucleus accumbens or the striatum of the individual. In some embodiments, the light-responsive protein is selected from the group consisting of NpHR, BR, AR and GtR3 described herein. For example, any of the NpHR proteins described herein may be expressed on the cell membrane of the target neurons. In some embodiments, the reward-related behavior is drug-related addictive behavior. The drug can be any addictive drug such as, but not limited to, opioids (for example, opium and heroin), methamphetamine, cocaine, ketamine, MDMA (3,4-Methylenedioxymethamphetamine), lysergic acid diethylamide, cannabinoids, alcohol, nicotine, or any other controlled substance. In one embodiment, the drug is cocaine. In another embodiment, the reward-related behavior is cocaine addiction. In some embodiments, the individual is a non-human animal. In another embodiment, the individual is a human. In some embodiments, the individual no longer experiences the positively reinforcing experience of using the drug. In some embodiments, the polynucleotide further comprises a promoter (e.g., a ChAT promoter) operably linked to the light-responsive opsin protein. In some embodiments, the polynucleotide is a vector.

Exemplary Embodiments

[0097] The present disclosure is believed to be useful for control or characterization of reinforced behavior in living animals. Specific applications of the present invention facilitate assessing addiction and other reinforced behaviors in living animals. As many aspects of the example embodiments disclosed herein relate to and significantly build on previous developments in this field, the following discussion summarizes such previous developments to provide a solid understanding of the foundation and underlying teachings from which implementation details and modifications might be drawn. It is in this context that the following discussion is provided and with the teachings in the references incorporated herein by reference. While the present invention is not necessarily limited to such applications, various aspects of the invention may be appreciated through a discussion of various examples using this context.

[0098] FIG. **10** depicts a system for controlling the nucleus accumbens (NAc) or the dorsal striatum, consistent with an embodiment of the present disclosure. A stimulus source **102** is linked **104** to a target location **106**. This target location can be located at or near the NAc or the dorsal striatum, e.g., the depicted location aligns generally with the NAc, but is not necessarily so limited.

[0099] Consistent with embodiments of the present disclosure, stimulus source **102** can include an optical light source. The optical light source is optically linked to the target location **106** (e.g., using fiber optics). The target location **106** is configured to include cells that respond to optical stimulus. These cells can include cells that express light-responsive opsins including, but not limited to ion pumps (e.g, NpHR and NpHR variants) and/or ion channels (e.g., ChR2/ChR1 and ChR2/ChR1 variants).

[0100] Consistent with various other embodiments of the present disclosure, stimulus source **102** can include a drug/pharmacological delivery device. The delivery device is linked to the target location (e.g., using a delivery lumen).

[0101] Certain embodiments of the present disclosure are directed toward targeting of cholinergic neurons of structures involved in natural reward-related behaviors and/or in reward learning (e.g., NAc or dorsal striatum) using a stimulus source. The stimulus source **102** provides a stimulus that controls the release of acetylcholine within the structure. In certain embodiments, this control is accomplished in a localized spatio-temporal manner that can be particularly useful for disrupting the addictive properties of substance abuse without noticeably affecting the reinforcement of other behaviors, e.g., appetitive or aversive responses. The stimulus can be provided from a number of different stimulus sources. Non-limiting examples include activating light-responsive opsins expressed in cholinergic neurons, applying an electrical pulse through one or more electrodes positioned near the cholinergic neurons, releasing a drug at a location proximate to the cholinergic neurons, applying a magnetic field to a location proximate to the cholinergic neurons and/or surgical alternations based upon this understanding.

[0102] FIG. **11** depicts a flow diagram for controlling the release of acetylcholine, consistent with embodiments of the present disclosure. A reward-based event **202** provides a basis for assessing or controlling reward-related behaviors and/or for reward learning. Although not limited thereto, the reward-based event **202** can be the introduction of an addictive substance to a patient. Control instructions **204** determine how stimulus source **206** applies a stimulus **208** as a function of a target that can be defined by one or more of temporal attributes, spatial location and/or cell-type. The stimulus **208** results in changes to the release of acetylcholine **210**. The effect of the stimulus can then be monitored **212**. The monitoring can be used to adjust the control instructions, thereby fine-tuning the stimulus for the intended result. Various embodiments discussed herein provide further examples that can be used in connection with (or in addition to) such a process.

[0103] Embodiments of the present disclosure are directed towards the assessment of the addictive properties of a substance. Control and/or monitoring of the activity of cholinergic neurons in (or derived from) specially targeted neural structures can be used to predict the addictive nature of the substance. For instance, the cholinergic neurons of a nucleus accumbens can be exposed to the substance under study. The activity of cholinergic neurons of the nucleus accumbens is then monitored after exposure to the substance. This monitoring can include, but is not limited to, electrical activity (e.g., action potentials/firing) and/or the release of acetylcholine.

[0104] Consistent with other embodiments of the present disclosure, the effects of a treatment for an addictive substance can be assessed. For instance, a potential treatment can be used in connection with the exposure of cholinergic neurons of a nucleus accumbens to the substance. The

activity of cholinergic neurons of the nucleus accumbens can be monitored in connection with the treatment to assess the effectiveness thereof.

[0105] According to embodiments of the present disclosure, the effects of a treatment for substance dependence is assessed by artificially inducing a substance dependency in an animal by exciting cholinergic neurons of a nucleus accumbens of an animal while teaching a conditioned response to the animal. The effects of the treatment are assessed by then applying the treatment, and monitoring the conditioned response of the patient.

[0106] Aspects of the present disclosure are directed toward embodiments of a system that includes a set of cholinergic neurons, a drug delivery device for providing drugs to the set of cholinergic neurons; and a monitoring device for assessing the activity of the set of cholinergic neurons in response to the drugs being provided to the set of cholinergic neurons. Consistent with certain embodiments, the set of cholinergic neurons include light-responsive opsins, and the system further includes an optical delivery system for exciting the cholinergic neurons by activating the light-responsive opsins.

[0107] Consistent with various embodiments of the present disclosure, control over the neural circuit can include inhibition or excitation, which can each include coordinated firing, and/or modified susceptibility to external circuit inputs. For instance, inhibition can be accomplished using a light-responsive opsin, such as an ion pump (e.g., NpHR and NpHR variants). Such ion pumps move the membrane potential of the neuron away from its threshold voltage to dissuade or inhibit action potentials. In another instance, excitation can be accomplished using a light responsive opsin, such as an ion channel (e.g., ChR2 and ChR2 variants). Such ion channels can cause the membrane potential to move toward and/or past the threshold voltage, thereby exciting or encouraging action potentials. Consistent with various embodiments, a light-responsive opsin can be used to (temporarily) shift the resting potential of a neuron to increase or decrease its susceptibility to external circuit inputs. These various options can also be used in combination.

[0108] Various embodiments of the present disclosure relate to an optogenetic system or method that correlates temporal control over a neural circuit with measurable metrics. For instance, a particular memory function might be associated with a neurological disorder. The optogenetic system targets a neural circuit within a patient for selective control thereof. The optogenetic system involves monitoring the patient for metrics (e.g., symptoms) associated with the neurological disorder. In this manner the optogenetic system can provide detailed information about the neural circuit, its function and/or the neurological disorder.

[0109] Embodiments of the present disclosure are directed toward combined solution(s) in which control over neural structures associated with reward-related behaviors and/or reward learning is used in combination with the disruption of memory acquisition and recall associated with the reward-related behavior. For instance, cocaine addiction can be studied and/or treated by inhibiting neural structures associated with reward-related behaviors and/or reward learning when the neural structures are exposed to cocaine. Moreover, memory acquisition associated with the cocaine use can be disrupted at the time that the cocaine is introduced to the patient. Memory recall associated with cocaine use can also be disrupted, e.g., in response to a trigger event associated with cocaine use. While cocaine is presented as an example, the application of such solution(s) is not so limited. Embodiments and experimental results relating to memory disruption are discussed in more detail hereafter.

[0110] Embodiments of the present disclosure are directed towards disrupting memory acquisition, recall and/or associations between memory and emotional responses, such as addiction-based or fear-based memories. In a particular embodiment, a specific neural circuit is targeted through the expression of light-responsive opsins therein. Function of the neural circuit is disrupted by activation of the expressed opsins, which can inhibit function of the neural circuit (e.g., using NpHR or NpHR variants). In other embodiments, the specific neural circuit is targeted by implanting electrode(s) near the specific neural circuit. Function of the neural circuit is disrupted through the application of an electrical signal to the electrode(s). In other embodiments, the specific neural circuit is targeted by implanting a device that delivers a fast-acting pharmaceutical near the specific neural circuit. Function of the neural circuit is disrupted through activation of the device to release the fast-acting pharmaceutical, which thereby inhibits function of the specific neural circuit.

[0111] In certain implementations, this disruption can be implemented during memory creation. In other implementations, this disruption can be implemented before or during memory recall. This can be particularly useful for psychiatric or neurological disorders involving memory recall, such as post-traumatic stress disorder (PTSD). Consistent with certain embodiments, the disruption can be triggered in response to a memory trigger event or other external stimulus that is presented and/or controlled for the disruption. For instance, the disruption can be provided in response to the introduction of a trigger for a memory to an animal/patient conditioned to respond to the trigger. In another instance, a patient can actively trigger the disruption. For instance, a patient may trigger the disruption when experiencing a memory associated with PTSD. Other embodiments of the present disclosure are directed towards encouraging memory acquisition, recall and/or associations between memory and emotional responses. For instance, an expressed opsin can be used to increase the susceptibility of a neural circuit to intrinsic stimulus (e.g., using stabilized step-function opsins (SSF0s) discussed herein). The encouragement can be provided to strengthen the acquisition, formation or recall of a memory. This can be used to ascertain the role of the circuit or to treat disorders associated with memory impairment. It has been discovered that (temporal) disruption of the dorsal CA1 hippocampus circuit is effective to prevent contextual fear memory acquisition. Consistent therewith, a prevailing neural network theory suggests that the process of memory consolidation starts with short term modifications in the connections between the hippocampus and the cortex, which enable the hippocampus to activate the relevant cortical sites that contribute to the complete memory, rather than store the memory itself. While these cortical traces are repeatedly co-activated, gradual long-lasting changes in the connections between them occur until eventually these connections are strong enough to support the memory without any hippocampal involvement. Surprisingly, it has been discovered that that disruption of the dorsal CA1 hippocampus circuit is effective to block fear-memory recall, even after cortical reorganization is believed to have occurred.

[0112] The following discussion, which includes a discussion of several experimental embodiments, presents a number of examples of these and other embodiments. These examples, however, are not meant to be limiting. One such embodiment concerns a production of a lentiviral vector. This lentiviral vector carries the gene encoding the light-activatable eNpHR3.1 that is fused in-frame to enhanced yellow fluorescent protein (eNpHR3.1-EYFP) under control of the calcium/calmodulin-dependent protein kinase Ha (CaMKIIa) promoter, selective for excitatory glutamatergic neurons. eNpHR3.1 is a truncated version of eNpHR3.0 with a deletion of the intrinsic N-terminal signal peptide, and is similar to eNpHR3.0 in both the photocurrent and the hyperpolarization it induces in neurons. Stereotactic delivery of CaMKIIa::eNpHR3.1 resulted in CA1 specific expression, covering its entire dorsal segment. Within the transfected area, 94% of the CaMKIIa cells expressed eNpHR3.1, and the promoter provided complete specificity, namely, all eNpHR3.1-EYFP cells were also CaMKIIa positive (FIG. **1** B). Optrode recordings in anesthetized mice confirmed that continuous green (561 nm) light illumination of excitatory CA1 neurons strongly inhibited spiking (73% decrease) in a temporally precise and reversible manner, without affecting spike amplitude. To demonstrate that optogenetic inhibition can also block the neuronal activity induced by FC in a region-specific manner, and bilateral continuous green light was delivered via two optic fibers inserted through a double cannula targeting dorsal CA1 during training, and stained for the synaptic activation marker cFos. eNpHR3.1 expressing mice demonstrated reduced cFos expression specifically in CA1 but not in two other brain regions involved in FC, the basolateral amygdala (BLA) and the anterior cingulated cortex (ACC).

[0113] Optogenetic inhibition was shown to modulate cognitive function by administering bilateral continuous green light to freely-moving mice during training in a customized FC chamber. During training, mice were introduced into context A, and then presented twice with a tone followed by a foot-shock, under continuous bilateral light delivery. Fear memory was then assessed the next day with no light. Dorsal CA1 optogenetic inhibition during training prevented contextual fear acquisition. The effect of optogenetic inhibition was shown to be reversible by re-training the mice in the same context without light administration, and testing again on the next day. eNpHR3.1 expressing mice exhibited intact contextual memory when no light was administered during training Dorsal CA1 optogenetic inhibition was also shown to interfere with memory recall. The same mice were retested, this time with light delivery during recall, and it was found that the memory that was present the day before became unavailable for recall under illumination.

[0114] It was shown that fear acquisition and fear expression mechanisms were likely not affected through testing of the same mice in a different context for their memory of the tone. eNpHr3.1 expressing mice demonstrated intact auditory-cued fear memory acquisition following light inhibition during training, as well as intact-cued fear recall with illumination during the test. Using the correlation between spatial exploration and contextual fear acquisition, the exploration time of the conditioning chamber was measured during training under light stimulation. No significant difference was found between eNpHR3.1 expressing mice and their controls. It is believed that CA1 optogenetic inhibition does not have an anxiolytic effect as mice were tested for their open field exploration with light administration. No significant differences in path length, velocity, or the percent of time spent in the center of the field (which serves as a sign of anxiety) were found between eNpHR3.1 expressing and control mice.

[0115] To test whether optogenetic inhibition can result in different behavioral phenotypes when eNpHR is expressed in different brain structures, mice were bilaterally injected with an adeno-associated virus (AAVS) carrying CaMKIIa:: eNpHR3.0-EYFP into the BLA. The acquisition of fear itself, i.e. the association between an aversive stimulus to any neutral stimulus, as well as the expression of recent and remote fear depend on the amygdala, and optogenetic activation of the BLA was sufficient to induce fear from a neutral stimulus. It was shown that optogenetic inhibition of the BLA interferes with both contextual and auditory-cued FC acquisition.

[0116] Accordingly, embodiments of the present disclosure are directed toward the introduction of third generation eNpHR in a cell-type and region specific fashion, and the use of CA1 optogenetic inhibition for interference with both acquisition and recent memory recall.

[0117] Embodiments of the present disclosure are also directed towards the use of such aspects to refine the present understanding of the role of the hippocampus in remote memory recall. Consistent with an experimental embodiment of the present disclosure, a group of mice were trained and then tested four weeks later. It was shown that CAI shutdown during recall appears to (completely) block remote fear memory. This interference with recall was also shown to be reversible, as when the mice where re-tested on the next day without illumination they appeared to express fear similarly to controls. eNpHr3.1 expressing mice demonstrated intact remote auditory-cued fear memory recall with illumination during the cued test, suggesting that their fear expression mechanism remains intact. Surprisingly, this suggests hippocampal involvement in remote fear memory.

[0118] Embodiments of the present disclosure are also directed towards the ability of CA1 inhibition (optogenetic or otherwise) to reversibly affect remote fear recall by preventing recall of long-term memories in real time, after repeated recall and reconsolidation. Experimental results were obtained by training another group of mice and then testing them five weeks later to verify the persistence of a memory trace (without light in both training and testing) Similar performance was found in both groups. On the next day, the same mice were tested under illumination, and the eNpHR3.1 group appeared to fail to recall the aversive memory. This effect was shown to be reversible, as on the next day, when tested without light delivery, eNpHR3.1 expressing mice demonstrated intact contextual memory. Moreover, after the mice had already recalled the aversive context and expressed fear, the fear response quickly ceased as soon as the light was delivered again, from the middle of the testing trial and onward.

[0119] Embodiments of the present disclosure are thereby directed toward reversible interference of remote fearful memory in real-time, even after the memory may have already been retrieved. This can be particularly useful for therapeutic treatments, e.g., in which a disturbing memory may be stopped as it appears, for example in PTSD patients, without permanently affecting other memories that are stored in the same brain structure.

[0120] The apparent direct involvement of the hippocampus in accessing remote memories, suggests a surprising finding that the intact hippocampus is still the default activator of the memory trace. Experimental tests were conducted to determine the effects of the temporal nature and/or resolution of the inhibition. The remote memory experiment was repeated with either precise illumination during the duration of the test only (as before), or prolonged light exposure, in which light was administered for 30 minutes before testing and then continuously throughout the test. Precise optogenetic inhibition significantly inhibited remote memory retrieval, whereas prolonged inhibition had no significant effect on remote memory retrieval. When the prolonged group mice were re-tested on the next day with precise light administration (during the test only), they showed inhibited fear recall. The lack of effect of prolonged light administration is not believed to be attributable to a decreased inhibition by eNpHR3.1 over time or reduction in cell health due to prolonged light exposure, as whole cell patch recordings on eNpHR3.1 positive cells in slices prepared from the same mice showed that the ability of eNpHR to suppress spiking remained the same throughout a 30 minutes period and was reversible. This data suggest that whereas the intact hippocampus is the default activator of the remote memory trace, the memory trace is not stored in the hippocampus, as when given enough time to compensate for its inactivation, the memory trace can still be retrieved by other brain structures.

[0121] Embodiments of the present disclosure are directed toward the inhibition of remote memory through inhibition of the anterior cingulated cortex (ACC). Experiments were conducted by targeting the ACC with a CaMKIIa::eNpHR3.0-EYFP virus, and testing the effect of optogenetic inhibition both one day and one month following training ACC optogenetic inhibition had no apparent effect on recent memory, but significantly impaired remote memory. Together, these findings suggest that even following cortical reorganization the most efficient way to activate the memory trace still involves the hippocampus.

[0122] The hippocampus is believed to provide continuous input to the cortex. Accordingly, an experiment was performed to determine whether the disrupted remote recall is a byproduct of the sudden drop in input from the hippocampus to the cortex, even if this input is unrelated to the recall task. Another major cortical input source, the olfactory bulbs (OB), was targeted with a CaMKIIa::eNpHR3.0-EYFP virus, and the effect of optogenetic inhibition was tested during both recent and remote fear recall. OB optogenetic inhibition had no significant effect on memory recall at either time point, suggesting that a sudden drop of otherwise unrelated excitatory input into the cortex is not sufficient to interfere with recall. When remote memories are retrieved they become available for reconsolidation, which renders them susceptible to disruption but this may also strengthen the trace. Aspects of the present disclosure relate to therapy for PTSD patients, in which a recurring disturbing memory may be stopped as it appears by reversibly shutting down a remote fearful memory in real-time, before and after reconsolidation, or in real-time after it has already been retrieved.

[0123] Consistent with another embodiment of the present disclosure, memories related to drugs of abuse can be inhibited to reduce drug seeking behavior. Other embodiments are directed toward the ability to instantaneously affect cognition by optogenetic modulation of different brain areas in order to study the role of specific neuronal populations in memory processes and enable a finer temporal, genetic and spatial dissection of the neuronal circuits that underlie them.

[0124] Specific aspects of the present invention relate to memory switching using microbial opsin genes adapted for neuroscience, allowing transduction of light pulse trains into millisecond-timescale membrane potential changes in specific cell types within the intact mammalian brain (e.g., channelrhodopsin (ChR2), Volvox channelrhodopsin (VChR1) and halorhodopsin (NpHR)). ChR2 is a rhodopsin derived from the unicellular green alga *Chlamydomonas reinhardtii*. The 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, affording generation of precisely timed action potential trains.

[0125] 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 1 mW/mm2 light for virtually the entire time in which depolarization is desired, which can be less than desirable. Much of the discussion herein is directed to ChR2. Unless otherwise stated, the 30 invention includes a number of similar variants. Examples include, but are not limited to, Chop2, ChR2-310, Chop2-310, and Volvox channelrhodopsin (VChR1. For further details onVChR1 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, which is fully incorporated herein by reference. 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 variants can be used in combination with light-activated ion pumps.

[0126] Embodiments of the present invention include relatively minor amino acid variants of the naturally occurring sequences. In one instance, the variants are greater 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 techniques known in the field of sequence analysis. 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 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.

[0127] As used herein, stimulation of a target cell is generally used to describe 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.

[0128] For further details on light-responsive opsins, reference can be made to PCT publication No. WO 2010/056970, entitled "Optically-Based Stimulation of Target Cells and Modifications Thereto," to Deisseroth et al., which is fully incorporated herein by reference.

[0129] Embodiments of the present disclosure are directed towards implementation of bistable changes in excitability of targeted populations. This includes, but is not necessarily limited to, the double-mutant ChR2-C128S/D156A. This double-mutant ChR2-C128S/D156A has been found to be well-tolerated in cultured hippocampal neurons and preserved the essential SFO properties of rapid step-like activation with single brief pulses of blue light, and deactivation with green or yellow light. In particular, the activation spectrum of ChR2-C128S/D156A peaks at 445 nm. A second deactivation peak was found at 390-400 nm, with faster but less complete deactivation by comparison with the 590 nm deactivation peak. Peak photocurrents in cells expressing ChR2-C128S/D156A were found to be robust, and comparable to those of ChR2-D156A (231.08±31.19 s.e.m; n=9 cells and 320.96±78.26 s.e.m; n=7 cells, respectively).

[0130] Individual transfected and patch-clamped neurons were next activated with 100 ms pulses of 470 nm light, and to ensure over very long recordings that current decay would not be attributable to cell rundown, each cell was deactivated with prolonged 590 nm light pulses at distinct intervals to determine the magnitude of remaining SFO current at each time point. Surprisingly, neurons expressing ChR2-C128S/D156A gave rise to sustained photocurrents that were more stable than those from cells expressing either single mutant alone. Fitting a mono-exponential decay curve to the ratio of Ideactivation/Iactivation over time revealed a spontaneous decay time constant of 29.3 min for ChR2-C128S/D156A, indicating that the C128 and D156 mutations act synergistically to delay the decay of the open state of ChR2.

[0131] Consistent with the required improvement for the anticipated application to complex mammalian behaviors, significant portions of the double-mutant SFO current were still present up to 20 minutes after the single photoactivation pulse. Based on these surprisingly slow decay kinetics, the double-mutant gene is referred to as SSFO (for stabilized step-function opsin) gene. SSFO is also used as shorthand for the active protein. Both residues likely are involved in ChR2 channel closure (gating), and both mutations likely stabilize the open state configuration of the channel. Without being limited by theory, aspects of the present disclosure relate to the discovery that SSFO may be completely blocked in photocycle progression, and may therefore represent the maximal stability possible with photocycle engineering. For instance, in contrast to ChR2-C128X and ChR2-D156A, the SSFO photocycle does not appear to access additional inactive deprotonated side products which likely split off the photocycle at later photocycle stages not reached in this mutant, in turn making the SSFO even more reliable for repeated use in vivo than the parental single mutations.

[0132] Embodiments of the present disclosure are directed toward the sensitivity of the SSFO to light. For instance, channelrhodopsins with slow decay constants effectively act as photon integrators. This can be particularly useful for more-sensitive, less-invasive approaches to optogenetic circuit modulation, still with readily titratable action on the target neuronal population via modulation of light pulse length. It has been discovered that, even at extraordinarily low light intensities (as low as 8 pW/mm^2), hundreds of picoamps of whole-cell photocurrents could be obtained from neurons expressing SSFO, which increased with monoexponential kinetics in response to 470 nm light during the entire time of illumination. Other aspects relate to the use of activation time constants that are linearly correlated with the activation light power on a log-log scale, which is indicative of a power-law relationship and suggesting that the SSFO is a pure integrator, with total photon exposure over time as the only determinant of photocurrent. For instance, it is believed that the number of photons per membrane area required for photocurrents to reach a given sub-maximal activation (time to T) is constant regardless of activation light power.

[0133] Example embodiments of the present disclosure relate to the use of a hybrid ChR1/VChR1 chimera contains no ChR2 sequence at all, is derived from two opsins genes that do not express well individually, and is herein referred to as C1V1. Embodiments of the present disclosure also relate to improvements of the membrane targeting of VChR1 through the addition of a membrane trafficking signal derived from the $K_{ir}2.1$ channel Confocal images from cultured neurons expressing VChR1-EYFP revealed a large proportion of intracellular protein compared with ChR2; therefore, to improve the membrane targeting of VChR1, we added a membrane trafficking signal derived from the $K_{ir}2.1$ channel. Membrane targeting of this VChR1-is-EYFP was slightly enhanced compared with VChR1-EYFP; however, mean photocurrents recorded from cultured hippocampal neurons expressing VChR1ts-EYFP were only slightly larger than those of VChR1-EYFP. Accordingly, embodiments of the present disclosure relate VChR1 modified by exchanging helices with corresponding helices from other ChRs. For example, robust improvement has been discovered in two chimeras where helices 1 and 2 were replaced with the homologous segments from ChR1. It was discovered that whether splice sites were in the intracellular loop between helices 2 and 3 (at ChR1 residue A1a145) or within helix 3 (at ChR1 residue Trp163), the resulting chimeras were both robustly expressed and showed similarly enhanced photocurrent and spectral properties. This result was unexpected as ChR1 is only weakly expressed and poorly integrated into membranes of most mammalian host cells. The resulting hybrid ChR1/VChR1 chimera is herein referred to as C1V1.

[0134] Aspects of the present disclosure relate to the expression of C1V1 in cultured hippocampal neurons. Experimental tests have shown a number of surprising and useful results, which are discussed in more detail hereafter. C1V1-EYFP exhibits surprisingly improved average fluorescence compared with VChR1-EYFP. Whole cell photocurrents in neurons expressing C1V1 were much larger than those of VChR1-EYFP and VChR1-ts-EYFP, and ionic

selectivity was similar to that of ChR2 and VChR1. The addition of the Kir2.1 trafficking signal between C1V1 and YFP further enhanced photocurrents by an additional 41% (C1V1-tsEYFP mean photocurrents were extremely large, nearly tenfold greater than wild type (WT) VChR1). Mean fluorescence levels closely matched the measured photocurrents (mean fluorescence 9.3±1, 19.6±3.4, 19.8±2.8 and 36.3±3.8 for VChR1-EYFP, VChR1-ts-EYFP, C1V1-EYFP and C1V1-ts-EYFP, respectively), suggesting that the increase in photocurrent sizes resulted mainly from the improved expression of these channels in mammalian neurons. Total somatic fluorescence (measured as integrated pixel density) was linearly correlated with photocurrent size in individual recorded/imaged cells across the different constructs (VChR1, VChR1-ts-EYFP, C1V1, C1V1-ts-EYFP). This suggests (without being limited by theory) that the increased photocurrent of C1V1 results from functional expression changes in neurons.

[0135] Various embodiments of the present disclosure relate to opsins with fast decay constants. This property can be particularly useful for providing precise control over spiking, e.g., in order to interfere minimally with intrinsic conductances, trigger single spikes per light pulse and/or minimize plateau potentials during light pulse trains. Experimental results suggest that the light-evoked photocurrents recorded in C1V1-ts-EYFP decayed with a time constant similar to that of VChR1. Aspects of the present disclosure are therefore directed toward modifications in the chromophore region to improve photocycle kinetics, reduced inactivation and/or possible further red-shifted absorption.

[0136] One embodiment is directed toward a corresponding ChETA mutation E162T, which experiments suggest provides an accelerated photocycle (e.g., almost 3-fold), (reference can be made to Gunaydin, et al., Ultrafast optogenetic control, *Nat Neurosci,* 2010, and which is fully incorporated herein by reference). Surprisingly, this mutation was shown to shift the action spectrum hypsochromic to 530 nm, whereas analogous mutations in ChR2 or other microbial rhodopsins have caused a red-shift.

[0137] Another embodiment is directed toward a mutation of glutamate-122 to threonine (C1V1-E122T). Experimental tests showed that C1V1-E122T is inactivated only by 26% compared to 46% inactivation of ChR2; in addition, the spectrum was further red-shifted to 546 nm.

[0138] Another embodiment of the present disclosure is directed toward a double mutant of C1V1 including both E122T and E162T mutations. Experimental tests have shown that the inactivation of the current was even lower than in the E122T mutant and the photocycle was faster compared to E162T. This suggests that multiple useful properties of the individual mutations were conserved together in the double mutant.

[0139] Embodiments of the present disclosure include the expression of various light-responsive opsins in neurons. Experimental tests of C1V1 opsin genes in neurons were carried out by generating lentiviral vectors encoding C1V1-ts-EYFP and various point mutation combinations discussed herein. The opsins were then expressed in cultured hippocampal neurons and recorded whole-cell photocurrents under identical stimulation conditions (2 ms pulses, 542 nm light, 5.5 mW/mm^2) Photocurrents in cells expressing C1V1, C1V1-E162T and C1V1-E122T/E162T were all robust and trended larger than photocurrents of ChR2-H134R. The experiments also included a comparison of integrated somatic YFP fluorescence and photocurrents from cells expressing C1V1-E122T/E162T and from cells expressing ChR2H134R. Surprisingly, C1V1-E122T/E162T cells showed stronger photocurrents than ChR2H134R cells at equivalent fluorescence levels. This suggests that C1V1 could possess a higher unitary conductance compared with ChR2-H134R. The test results suggest that the kinetics of C1V1-E122T were slower than those of C1V1-E122T/E162T and that cells expressing C1V1-E122T responded more strongly to red light (630 nm) than cells expressing the double mutant. This can be particularly useful for generating optogenetic spiking in response to red-light.

[0140] Consistent with various embodiments of the present disclosure, inhibitory and/or excitatory neurons residing within the same microcircuit are be targeted with the introduction of various opsins. Experimental tests were performed by separately expressed C1V1-E122T/E162T and ChR2-H134R under the CaMKIIa promoter in cultured hippocampal neurons. Cells expressing C1V1-E122T/E162T spiked in response to 2 ms green light pulses (560 nm) but not violet light pulses (405 nm). In contrast, cells expressing ChR2-H134R spiked in response to 2 ms 405 nm light pulses, but not 2 ms 561 nm light pulses.

[0141] Various embodiments of the present disclosure relate to independent activation of two neuronal populations within living brain slices. Experimental tests were performed by CaMKIIa-C1V1-E122T/E162Tts-eYFP and EFla-DIO-ChR2-H134R-EYFP in mPFC of PV::Cre mice. In non-expressing PYR cells, 405 nm light pulses triggered robust and fast inhibitory postsynaptic currents (IPSCS) due to direct activation of PV cells, while 561 nm light pulses triggered only the expected long-latency polysynaptic IPSCs arising from C1V1-expressing pyramidal cell drive of local inhibitory neurons.

[0142] Various embodiments described above or shown in the figures may be implemented together and/or in other manners. One or more of the items depicted in the drawings/figures can also be implemented in a more separated or integrated manner, or removed and/or rendered as inoperable in certain cases, as is useful in accordance with particular applications. In view of the description herein, those skilled in the art will recognize that many changes may be made thereto without departing from the spirit and scope of the present invention.

EXAMPLES

[0143] Neurons employing the neurotransmitter acetylcholine are widespread but relatively rare, with cell bodies and projections sparsely distributed throughout much of the mammalian brain. Pharmacological modulation of the various acetylcholine receptors is known to affect numerous brain processes, but often with side effects due to limitations in drug specificity for receptor type and target cell population, such that the ultimate causal role of cholinergic neurons in circuit function and behavior has been unclear. In a paradigmatic case, the giant cholinergic interneurons of the nucleus accumbens (NAc) are a structurally distinctive and sparsely distributed group of neurons whose function has remained elusive due to experimental inability to precisely activate or inactivate these cells in living tissue or in behaving animals. Here we integrate several optical control technologies, in the setting of freely moving mammalian behavior, in vivo electrophysiology, and slice physiology, to causally probe the function of these neurons by direct

excitation or inhibition with high cellular and temporal precision. Remarkably, despite representing a tiny (<1%) fraction of the local neuronal population, we found that the cholinergic neurons in the NAc have a dominant control role, exerting powerful, bidirectional modulation of activity in the surrounding circuit. Furthermore, we found that these neurons were directly activated by cocaine, and that silencing this drug-induced activity during cocaine exposure in freely behaving mammals disrupted cocaine reward. Importantly, manipulation of the cholinergic interneurons was not aversive by itself, suggesting that these unique cells play a role in specifically implementing hedonic behaviors relevant to drugs of abuse via their potent influence on the NAc circuitry.

[0144] Acetylcholine is an important and widely-studied neurotransmitter, which acts upon an enormous diversity of receptors and target cells (1-8). Pioneering pharmacological and genetic studies have elucidated the complex and often opposing influences of the individual subtypes of muscarinic and nicotinic acetylcholine receptors on numerous biological processes (9-15), but no study has yet resolved the fundamentally distinct question of the causal role of cholinergic neurons themselves within a CNS tissue, despite the hypothesized importance of these neurons in learning, memory, attention, and reward (16-22). Addressing such a question would require a novel paradigm for selective and temporally precise control (activation and inhibition) of cholinergic neurons within living mammalian tissues, since previous investigations have resulted in contradictory findings likely due to challenges with specificity and temporal resolution. For example, elegant in vivo pharmacological approaches have reported (23-26) that cholinergic transmission in the NAc (a structure involved in natural reward-related behaviors and responses to drugs such as cocaine (27-33)) is required for reward learning, but novel studies of molecular ablation of cholinergic interneurons within the NAc instead have reported enhanced reward learning (34). These cholinergic interneurons within the NAc are particularly intriguing as they constitute less than 1% of the local neural population (35), yet project throughout the NAc and provide its only known cholinergic input (36, 37). Relevant cholinergic receptors are expressed locally, and nicotinic and muscarinic pharmacological agonists can exert complex influences on medium spiny neurons (MSNs, which represent >95% of the local neuronal population and constitute the output of the NAc) (38-41), but the net effect (if any) of the extremely rare cholinergic interneurons on any aspect of NAc physiology or behavior is, as with other brain regions, unknown.

Example 1: Expression of Light-Responsive Opsin Proteins in Cholinergic Interneurons of the Nucleus Accumbens

[0145] We undertook an optogenetic approach (42-44) to resolve this question by selectively driving or blocking action potential firing in these cells, with both high temporal resolution and high cell-type specificity. To express microbial opsin genes specifically in cholinergic interneurons, we employed a transgenic mouse line expressing Cre recombinase under the choline acetyltransferase (ChAT) promoter (45). We stereotaxically injected into the NAc (FIG. **1**A) a Cre-inducible adeno-associated virus (AAV) vector carrying the opsin gene fused in-frame with coding sequence for enhanced yellow fluorescent protein (eYFP) (46, 47); the opsin gene encoded either the blue-light gated cation channel channelrhodopsin-2 (ChR2) (48, 49) or the yellow-light gated third-generation chloride pump halorhodopsin (eNpHR3.0) (50).

[0146] Materials and Methods

[0147] Subjects

[0148] BAC transgenic Choline Acetyltransferase (ChAT)::Cre mice were obtained from GENSAT (stock name: Tg(Chat-cre) 24Gsat/Mmcd) (Gong et al., *J. Neurosci* 27, 9817-9823 (2007)) and mated with C57BL6 mice from Charles River. Experimental mice were either heterozygous for Cre (+/−) or else control littermates (−/−). Mice were group housed in a colony maintained on a reversed 12 hr light/dark cycle and given food and water ad libitum. Experimental protocols were approved by Stanford University IACUC to meet guidelines of the National Institutes of Health guide for the Care and Use of Laboratory Animals.

[0149] Virus Production

[0150] As described previously (Tsai et al., *Science* 324, 1080-1084 (2009); Sohal et al., *Nature* 459, 698-702 (2009)), Cre-inducible recombinant AAV vectors were based on a DNA cassette carrying two pairs of incompatible lox sites (loxP and lox2722) with the opsin (either ChR2 (H134R) or eNpHR3.0) inserted between the lox sites in the reverse orientation. This double-floxed reverse opsin cassette was cloned into a version of the pAAV2-MCS vector carrying the EF-1a promoter and the Woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) to enhance expression. Full maps of the Cre-inducible ChR2 AAV construct, as well as the eNpHR3.0 transgene, are available at www.stanford.edu/group/dlab/optogenetics/sequence_info.html. The recombinant AAV vectors were serotyped with AAVS coat proteins and packaged by the viral vector core at the University of North Carolina. The final viral concentration was 3×10^{12} particles/mL for the ChR2 virus and 1.5×10^{12} particles/mL for the eNpHR3.0 virus.

[0151] Stereotactic Virus Injection, Cannula/Patchcord Implantation, and Light Delivery

[0152] Mice were anesthetized with ketamine/xylazine (60 μl/mouse of a mixture of 80 mg/ml ketamine and 12 mg/ml xylazine), and then placed in a stereotactic head apparatus. Surgeries were performed on 4-6 week old mice for physiology experiments and 8-12 week old mice for behavior experiments. Ophthalmic ointment was applied to prevent the eyes from drying. A midline scalp incision was made followed by a craniotomy, and then virus was injected with a 10 μl syringe and a 34 gauge metal needle. The injection volume and flow rate (1p1 at 0.15 μl/min) were controlled by an injection pump. Each NAc received two injections (injection 1: AP 1.15 mm, ML 0.8 mm, DV −4.8 mm; injection 2: AP 1.15 mm, ML 0.8 mm, DV −4.2 mm). The virus injection and fiber position were chosen so that virtually the entire shell was stimulated. Given the small size of the shell in mice, it is not possible to limit the virus spread and the light entirely to the medial shell, and the medial part of the core was included (medial to the anterior commissure). After injection the needle was left in place for 5 additional minutes and then very slowly withdrawn. For behavioral experiments mice were injected bilaterally, and then bilateral cannulas with a center-to-center distance of 1.5 mm were placed above the injection sites (AP 1.15 mm, DV 3.8 mm). To manipulate neuronal activity during behavior, light was bilaterally delivered through two 300 μm diameter

optic fibers (0.37 N.A.) that were inserted through the cannulae to allow the fiber to project 200-300 μm past the end of the cannulae.

[0153] Immunohistochemistry

[0154] To determine the specificity of opsin expression in ChAT neurons, mice were anesthetized with beuthanasia and perfused transcardially, first with PBS and then with 4% paraformaldehyde (PFA) dissolved in phosphate-buffered saline (PBS, pH 7.4). The brains were removed and post-fixed in 4% PFA overnight at 4° C., and then equilibrated in 30% sucrose in PBS. 40 μm-thick coronal sections were prepared on a freezing microtome (Leica) and stored in cryoprotectant (25% glycerol and 30% ethylene glycol in PBS) at 4° C. Free-floating sections were washed in PBS and then incubated for 30 min in 0.3% Triton X-100 (Tx100) and 3% normal donkey serum (NDS). Slices were incubated at 4° C. overnight with primary antibody in 3% NDS (Goat anti-ChAT 1:200, Millipore). Sections were then washed with PBS and incubated for 2 hr at room temperature with secondary antibodies (Donkey anti-goat conjugated to Cy3 or Cy5, Jackson Laboratories). Slices were then washed, incubated with DAPI (1:50,000) for 20 min, washed again, and mounted on slides with PVA-DABCO. Confocal fluorescence images were acquired on a scanning laser microscope using 5× or 10× air objectives, or a 40× oil immersion objective

[0155] Results

[0156] We validated the specificity and functionality of this targeting strategy in vivo, and found that eYFP expression was highly specific to neurons that expressed ChAT; moreover, the vast majority of neurons that expressed ChAT also expressed eYFP (FIG. **1** B,C). The observed specificity was particularly impressive for such a sparse neural population, as there was a very low pre-test probability that any given cell would be a ChAT interneuron and therefore even rare targeting leak would dramatically decrease effective targeting specificity. Both opsins were expressed on the surface membranes of ChAT neurons (FIG. **1** D), and the targeted neurons responded to current injection in a manner corresponding to previously established responses of cholinergic interneurons in dorsal striatum (FIG. **1** E) (51). In further agreement with dorsal striatum physiology, both the resting potential and input resistance were higher for ChAT neurons (YFP+ neurons) than MSNs (identified as YFP− neurons; Table S1; $p<10^{-4}$ for V_M and p=0.004 for Rinput, two-tailed t-test). Finally, both opsins were potently functional in ChAT cells, as eNpHR3.0 drove massive hyperpolarizations (FIG. **1** F; mean±s.e.m.: −83.8±11.9 mV, n=4) and ChR2 reliably drove spiking up to 20-30 Hz (FIG. **1**G, H).

TABLE 3

Membrane voltage (VM) and input resistance (RINPUT) in brain slices of ChAT neurons expressing ChR2-eYFP and of MSNs that did not express a fluorophore. Both VM and RINPUT are higher for ChAT neurons than MSNs. (p = 0.00003 for VM; p = 0.002 for RINPUT; two-tailed t-test; mean ± S.E.M.)

ChAT (ChR2-eYFP) (n = 19)		MSN (n = 13)	
V_M (mV)	R_{INPUT} (MΩ)	V_M (mV)	R_{INPUT} (MΩ)
−49.47 ± 1.07	382.02 ± 47.30	−65.43 ± 2.88	223.64 ± 31.92

Example 2: Effects of Depolarization of Choline Acetyltransferase (ChAT) Interneurons

[0157] ChAT interneurons are thought to be tonically active in vivo (3-10 Hz (52, 53)), but it has remained mysterious how (or even if) this kind of slow activity in the sparse ChAT cells could be causally involved in affecting local circuit activity or behavior. We capitalized on optogenetic control to address this question with a combination of slice electrophysiology, in vivo electrophysiology, and freely-moving behavior.

[0158] Materials and Methods

[0159] Acute Brain Slice Physiology

[0160] Coronal cerebral brain slices were prepared from adult mice with virus previously injected (>2 weeks prior to slicing), using standard techniques in strict accordance with a protocol approved by the Animal Care and Use Committee at Stanford University. Coronal slices 250 μm thick were cut with a vibratome using a sapphire blade in ice cold N-methyl-D-glucamine (NMDG)-based cutting solution containing 135 mM NMDG, 1 mM KCl, 1.2 mM KH_2PO_4, 20 mM choline bicarbonate, 10 mM glucose, 1.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$. Slices were maintained thereafter in artificial cerebral spinal fluid (ACSF) containing 119 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgCl_2$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$ and 11 mM glucose. Slices were maintained in ACSF at 37° C. for 30 minutes, and thereafter at room temperature. ACSF was bubbled constantly with 95% O_2/5% CO_2 and heated to 34° C. for all experiments. Neurons were visualized on an upright microscope (Leica DM-LFSA) equipped with both DIC optics and a filter set for visualizing eYFP using a ×40 water-immersion objective and a charge-coupled device (CCD) camera. Whole-cell recordings were made from neurons using the electrode solution containing 120 mM potassium gluconate, 20 mM HEPES, 10 mM EGTA, 1 mM $MgCl_2$, 2 mM Na-ATP, and 0.2 mM Na-GTP (pH 7.3, 290 mOsm/L); in experiments recording IPSCs, KCl was used to replace potassium gluconate. Pipette resistances were 3-5 MΩ, and recordings were made without series resistance compensation. Membrane potentials have been corrected for the error resulting from the liquid junction potentials. The holding potential (VM) for voltage-clamp experiments was −70 mV. The following agents were added as indicated: 5 μM SR-95531; 5 μM 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(F) quinoxaline (NBQX); 5 μM (R,S)-CPP; mecamylamine (10 μM); 5 μM cocaine hydrochloride. The cocaine concentration of 5 μM was carefully chosen by several criteria. First, it was consistent with the choices in previous slice work (Thompson et al., *Neuropharmacology* 49, 185-194 (2005)). Second, significantly higher concentrations would result in local anesthetic effects (Thompson et al., *Neuropharmacology* 49, 185-194 (2005)). Finally, according to studies of cocaine pharmacokinetics in mice, an i.p. injection of 10 mg/kg will yield 4.7 μM of cocaine in the brain after 15 minutes, and 20 mg/kg will yield 9.4 μM, comparable to the levels used in behavioral experiments (Shah et al., *Toxicology and Applied Pharmacology* 53, 279-284 (1980)). Photocurrents were evoked using an optical switch with a 300 W xenon lamp and either a 470±20 nm or a 580±20 nm bandpass filters; light power at the specimen was 11.52 mW mm^{-2} (470 nm) or 10.64 mW mm^{-2} (580 nm). Currents filtered at 2 kHz, digitized at 50 kHz, and recorded to disk using pClamp10 software (Axon Instruments). Data are expressed as mean±standard error of the

mean, and statistical significance was determined using the paired or unpaired t-test, as appropriate. For IPSC measurements in MSNs (FIG. **2**B-E and FIG. **5**A-B), 10 repetitions without light preceded 10 repetitions with light. Each repetition was 5 seconds in length and separated by a 5 second rest period. For testing the cocaine response of ChAT cells in slice (FIG. **4**A-C), whole-cell recordings were obtained from the ventral portion of the medial shell, where elevations in spiking were variable as summarized in FIG. **4**, but contrasted with typical rundown of spiking in control conditions; exploratory work suggested that ChAT cells in the core and elsewhere in the shell were less responsive to cocaine.

[0161] In Vitro Optrode Recordings

[0162] Simultaneous optical stimulation and extracellular electrical recording were performed as described previously (Gradinaru et al., *J. Neurosci* 27, 14231-14238 (2007)). Optrodes consisted of a tungsten electrode (1 MΩ; 0.005 in; parylene insulation) glued to an optical fiber (300 μm core diameter, 0.37 N.A.), with the tip of the electrode projecting beyond the fiber by 300-500 μm. The electrode was lowered through the NAc in approximately 100 μm increments, and optical responses were recorded at each increment. The optical fiber was coupled to a 473 nm or 560 nm laser. The power density was ~140 mW/mm^2 at the fiber tip for both wavelengths, which corresponds to a density at the tip of the electrode of about ~7-17 mW/mm^2 for 470 nm light and ~10-22 mW/mm^2 for 560 nm light. Signals were amplified and band-pass filtered (300 Hz low cut-off, 10 kHz high cut-off) before digitizing and recording to disk. At each site, 5 stimulation repetitions were presented and saved. Each stimulation epoch lasted 10-15 seconds with a recovery period of 80-90 seconds between the onset time of each repetition, and 50 seconds of data were recorded to disk for each repetition.

[0163] Results

[0164] First, postsynaptic currents in MSNs were monitored (ChR2-eYFP non-expressing cells) in acute NAc slices during optogenetic photostimulation of ChAT cells expressing ChR2-eYFP (FIG. **2**A), targeted as in FIG. **1**. Stimulating ChAT neurons in this setting robustly increased the frequency of γ-aminobutyric acid type A ($GABA_A$) receptor-mediated inhibitory postsynaptic currents (IPSCs) recorded in MSNs (FIGS. **2**, B and C). Evoked inhibitory currents were generally synchronized to the light pulse, with a modal latency of 6 ms (FIG. **2**D), coupled with a smaller enhancement of asynchronous IPSCs (FIG. **5**A-C). Across all recorded cells, the mean frequency of IPSCs observed in the MSNs increased by 525.8±154.3% during light stimulation of the ChAT neurons (n=7; mean±SEM, P=0.01, paired t test), whereas the mean IPSC amplitude was unaffected (P>0.05, paired t test; n=7, FIG. **2**E). This effect was attenuated by the nicotinic antagonist mecamylamine (FIG. **7**, n=5, P<0.05, paired t test).

[0165] Next it was asked if and how these dramatic changes in inhibitory current frequency would translate into changes in MSN spiking in vivo. We recorded neural activity extracellularly with an optrode in the NAc during optogenetic activation of the ChAT interneurons in vivo (FIG. **2**F). At sites where single units were not isolated, we observed neural population firing that tracked the light stimulation at 10 Hz but not 100 Hz (FIG. **5**D), likely representing population spiking across the sparse but synchronously activated ChAT cells in the neighborhood of the electrode. In contrast to these population spikes, the isolated units in the NAc displayed a markedly different response to the optogenetic photostimulation. In agreement with the observed increase in IPSC frequency in MSNs in slices, we observed in vivo a proud inhibition of background firing during stimulation of the ChAT cells in vivo (representative cell, FIG. **2**G). Across the population, most significantly modulated sites showed a suppression of background firing, although a few responded with an increase in firing (FIGS. **2**, H and I), consistent with known recurrent inhibition among MSNs and corresponding release from inhibition, during ChAT neuron drive, that had been previously exerted by the broader MSN population.

Example 3: Effects of Hyperpolarization of Choline Acetyltransferase (ChAT) Interneurons

[0166] Next, the consequences of specifically inhibiting ChAT interneurons were explored, employing Cre-dependent eNpHR3.0 expression in vivo.

[0167] Materials and Methods

[0168] Mice, optrode recordings, and brain slices were prepared as above.

[0169] Results

[0170] In contrast to what was observed with ChAT neuron excitation, firing of NAc neurons was typically increased in likely MSNs by optogenetic inhibition of the ChAT cells; a representative cell is shown in FIG. **3**A). Power spectral analysis revealed a striking frequency peak in the firing pattern at ~4 Hz in these in vivo recordings (FIG. **3**B). Summary data are presented in FIG. **3**C; across the population of significantly modulated sites, most neurons were excited by the optogenetic inhibition of ChAT neurons (n=17). We were able to obtain a single-unit recording from a rare putative ChAT interneuron, which was completely shut down by eNpHR3.0 (FIG. **3**D) and displayed the long action potential duration characteristic of ChAT interneurons (37) (2.0 ms for this cell, while spike durations for MSNs in our recordings ranged from 1.1-1.7 ms) Summary data (FIG. **3**E) show the dynamics of excitation and inhibition for all recorded sites, illustrating the dominant pattern of excitation (firing increased by 130.5+/−17.5% in sites that were excited by light). Taken together, the results from in vivo optogenetic excitation and inhibition of ChAT neurons are consistent with our findings from slice physiology, pointing to a surprisingly powerful role for these rare cells in controlling local circuit activity in the NAc.

Example 4: Effects of Cholinergic Interneuron Manipulation on Reward Behavior in Freely Moving Mice

[0171] It was decided to test if this potent NAc control mechanism was relevant to accumbens-dependent reward behavior in freely moving mice. First the effect of acutely administered cocaine on activity of these identified ChAT neurons in acute NAc slices was tested. Next, eNpHR3.0 was used to test for causal roles in either this cocaine-induced activity or baseline activity of ChAT cells in the reward-related behavior of cocaine conditioned place preference (CPP), in which animals learn to associate an environment with cocaine.

[0172] Materials and Methods

[0173] Conditioned Place Preference

[0174] All behavioral experiments were performed 4-6 weeks after virus injections during the animals' dark (active)

cycle. The conditioned place preference (CPP) protocol was similar to those from previous reports of unbiased, balanced place-preference (Bardo et al., *Neurosci Biobehav Rev* 19, 39-51 (1995)). The CPP apparatus consisted of a rectangular chamber with one side compartment measuring 23 cm×26 cm with black walls and a grating on the floor, a central compartment measuring 23 cm×11 cm with clear plexiglass walls and a plexiglass floor, and another side compartment measuring 23 cm×26 cm with white walls and a punched metal floor. Mouse position during each day of testing was monitored using a video-tracking system. Floors were selected such that mice did not display average baseline bias for a particular chamber, and any mouse with a strong initial preference for a chamber was excluded (more than five minute difference spent in the side chambers on day 1). The CPP test consisted of the following. On day 1, each mouse was placed in the central chamber and allowed to freely explore the entire apparatus for 20 minutes (pre-test). Day 2 consisted of conditioning. In the morning, each mouse was confined to one of the side chambers for 20 minutes, and in the afternoon was confined to the other side chamber for the same period of time. For the cocaine CPP experiments, subjects received i.p. cocaine injections (20 mg/kg unless otherwise specified) before placement in one chamber, while subjects received i.p. saline injections of an equivalent volume before placement in the other chamber. (This concentration of cocaine allowed for robust conditioning with a single day of training in control animals, facilitating the optogenetic intervention). Mice received either yellow or bluelight during the 20 minutes in which they explored the compartment that was paired with the cocaine injection, whereas they were connected to a "dummy" fiber that was not emitting light when exploring the other chamber. The intensity of the blue light (470 nm) was chosen to generate power density of 140-200 mW/mm^2 at the fiber tip, which should correspond to a power density of ~4-7 mW/mm2 in the middle of the NAc. The intensity of the yellow light (590 nm) was chosen so that there was a power density of 70-140 mW/mm^2 at the fiber tip, which should correspond to a power density of ~3.5-7 mW/mm^2 in the middle of the NAc. On day 3, exactly as in day 1, mice were placed in the center chamber and allowed to freely explore the entire apparatus for 20 min (posttest). CPP experiments that did not involve cocaine were performed identically, except that the i.p. injections of cocaine or saline were omitted.

[0175] Open Field

[0176] The open field test was conducted in an open plastic arena (50 cm long x 50 cm wide x 40 cm deep). Mice were individually placed in the center of the chamber and allowed to freely explore for 3 min. Activity in both the center and periphery of the field was measured using an automated video-tracking system (Viewer II, BiObserve). Time in center refers to time the mouse spent in the central 35×35 cm area of the open field.

[0177] Fear Conditioning

[0178] The fear conditioning apparatus consisted of a square conditioning cage (18×18×30 cm) with a grid floor wired to a shock generator and a scrambler, surrounded by an acoustic chamber. The top of the chamber was modified to enable light delivery during training by introducing an opening for the fiber. All mice received continuous yellow light during training but not during testing on the following day (590 nm; same power density as for the CPP experiments). To induce fear conditioning, mice were placed in the cage for 120 seconds; a pure tone (2.9 KHz) was then played for 20 seconds, followed immediately by a 2 second foot-shock (0.5 mA). This procedure was repeated, and 30 seconds after the delivery of the second shock mice were returned to their home cage. Freezing (complete immobility) was quantified for the 30 seconds before the first tone on the conditioning day to assess baseline freezing, as well as the 30 seconds immediately after the final shock on the conditioning day to assess immediate freezing. Contextual and auditory-cued fear conditioning were assessed the day after conditioning. To test contextual fear conditioning, mice were placed in the original conditioning cage, and freezing was measured for 5 min. To test auditory-cued fear conditioning, mice were placed in a different context: a pyramid shaped cage with a plexiglass floor. As a control for the influence of the novel environment, freezing was measured for 2.5 minutes in this new cage, and then the 2.9 KHz tone was played for 2.5 minutes, during which conditioned freezing was measured.

[0179] Results

[0180] In ventro-medial NAc ChAT cells, cocaine was found to markedly increase spontaneous firing (representative ChAT neuron shown in FIGS. **4**, A and B) Summary data revealed that cocaine increased firing rates from 0.60±0.41 Hz to 1.74±0.56 Hz at 10 min in ChAT neurons (n=7; $P<0.005$, paired t test), while in the control group of cells receiving only vehicle, firing rates decreased from 0.69±0.24 Hz to 0.09±0.09 Hz over the same time period (n=6; $P<0.05$ comparing the two groups, two-tailed t test) (FIG. **4**C).

[0181] We next used eNpHR3.0 to test for causal roles of either this cocaine-induced activity, or baseline activity, of ChAT cells in the reward-related behavior of conditioned place preference (CPP), in which animals learn to associate an environment with cocaine. After injecting virus and implanting cannulae bilaterally (FIG. **4**D) to silence ChAT neurons during cocaine exposure (FIG. **4**E), we found that mice that expressed eNpHR3.0 in the ChAT cells exhibited significantly less cocaine-induced conditioned place preference (CPP) than did their control (Cre recombinase-negative) littermates which had received the same virus, surgery, and light-delivery protocol [20 mg/kg intraperitoneally(ip), FIGS. **4**, F and G; n=10 ChAT::Cre^+, n=12 ChAT::Cre^- (left panel); $P<0.01$ for two-tailed t test; three cohorts; see also FIG. **6**A. We observed no behavioral effect of inhibiting the ChAT cells in the absence of cocaine, and ChAT neuron inhibition by itself was not aversive, as conditioning with eNpHR3.0 alone did not affect place preference (FIG. **4**G, right panel; n=9 ChAT::Cre^+, n=7 ChAT::Cre^-; $P>0.05$ for two-tailed t test; three cohorts; FIG. **6**B; see also FIG. **8**A for cocaine dose-response curve). Activation of the cells with ChR2 at 10 Hz was not sufficient to drive place preference by itself or enhance cocaine place preference (10 and 20 mg/kg ip, FIG. **8**B to D; Table 4), with our data from ChAT cell inhibition instead demonstrating necessity of these cells. Finally, in control experiments, we found that ChAT neuron inhibition by itself had no effect on mobility or anxiety in the open field (FIGS. **4**, H and J), and contextual- and auditory-cued fear conditioning were not disrupted by inhibition of the ChAT cells (FIG. **9**).

TABLE 4

Total time spent on the cocaine-paired side on the testing day of the cocaine place preference paradigm (for various cocaine concentrations) when inhibition of the ChAT neurons (with eNphR3.0) was paired with cocaine exposure.

		mg/kg		
	0	10	20	40
ChAT::Cre+	N	11	10	4
	Conditioned side (min)	10.3	10.7	10.5
ChAT::Cre−	N	12	12	3
	Conditioned side (min)	8.5	14.2	9.2

DISCUSSION

[0182] Together, these results from specific and acute optogenetic control of the defined ChAT neuron population point to a powerful role for these rare and sparsely distributed neurons in controlling local circuit activity and implementing reward-related behavior in freely moving mammals. The fact that acute silencing of ChAT interneurons disrupts drug-related learning without affecting place preference in the absence of drug suggests the tantalizing possibility that control over this microcircuit could be used to selectively disrupt the addictive properties of drugs of abuse without affecting appetitive or aversive responses in general, a possibility that would be of immense clinical benefit. Interestingly, the behavioral results do not support conclusions arising from chronic ablation of the cholinergic interneurons (34), but instead are more consistent with interpretations arising from faster but less cellularly-targeted pharmacological modulation in the NAc (23-26). Ablation of the cholinergic interneurons could lead to indirect effects, such as a compensatory increase in dopamine in the NAc, which could in turn enhance cocaine reward. In fact, a fundamental difference between acute and chronic manipulations could explain clinically-relevant apparent contradictions in our understanding of acetylcholine/dopamine balance in the brain, such as the finding that an acute increase in nicotine (presumably acting upon cholinergic receptors) causes a corresponding acute increase in dopamine (54, 55), whereas chronic changes in dopamine or acetylcholine levels can cause opposing changes in the levels of the other neuromodulator (56), for example as seen in the dopamine depletion of Parkinson's disease (57).

[0183] The speed and specificity of this approach may enable elucidation of the causal role of acetylcholine neurons, in both healthy and diseased neural circuitry, in other regions of the nervous system as well. For example, much like the dopamine neurons that project to striatum (58, 59), the ChAT interneurons in dorsal striatum are thought to carry information about rewards and stimuli that predict rewards (60). Such stimuli generate a brief pause in the activity of striatal tonically-active neurons in primates, often preceded and followed by an excitatory burst of activity (16, 17). Structural homology between dorsal and ventral striatum suggests that similar principles to those outlined here for NAc could extend to ChAT cell activity in dorsal striatum, which indeed appears to be the case (English et al., accompanying manuscript). These results, taken together with our findings that the activity of these cells in the NAc drives powerful time-locked inhibition and controls reward-learning behavior, suggest that ChAT neurons serve as a potent control node suitable for versatile neuromodulatory regulation of circuit activity and behavior in the mammalian brain.

[0184] The examples, which are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way, also describe and detail aspects and embodiments of the invention discussed above. The foregoing examples and detailed description are offered by way of illustration and not by way of limitation. All publications, patent applications, and patents cited in this specification are herein incorporated by reference as if each individual publication, patent application, or patent were specifically and individually indicated to be incorporated by reference. In particular, all publications cited herein are expressly incorporated herein by reference for the purpose of describing and disclosing compositions and methodologies which might be used in connection with the invention. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

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Thr Pro Leu Leu Leu Ile Asp Leu Gly Leu Val Ala Gly Val Ser Arg
                85                  90                  95

Trp Asp Ile Met Ala Leu Cys Leu Ser Asp Val Leu Met Ile Ala Thr
            100                 105                 110

Gly Ala Phe Gly Ser Leu Thr Val Gly Asn Val Lys Trp Val Trp Trp
        115                 120                 125

Phe Phe Gly Met Cys Trp Phe Leu His Ile Ile Phe Ala Leu Gly Lys
    130                 135                 140

Ser Trp Ala Glu Ala Ala Lys Ala Lys Gly Gly Asp Ser Ala Ser Val
145                 150                 155                 160

Tyr Ser Lys Ile Ala Gly Ile Thr Val Ile Thr Trp Phe Cys Tyr Pro
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                165                 170                 175

Val Val Trp Val Phe Ala Glu Gly Phe Gly Asn Phe Ser Val Thr Phe
            180                 185                 190

Glu Val Leu Ile Tyr Gly Val Leu Asp Val Ile Ser Lys Ala Val Phe
        195                 200                 205

Gly Leu Ile Leu Met Ser Gly Ala Ala Thr Gly Tyr Glu Ser Ile
    210                 215                 220


<210> SEQ ID NO 5
<211> LENGTH: 310
<212> TYPE: PRT
<213> ORGANISM: Chlamydomonas reinhardtii

<400> SEQUENCE: 5

Met Asp Tyr Gly Gly Ala Leu Ser Ala Val Gly Arg Glu Leu Leu Phe
1               5                   10                  15

Val Thr Asn Pro Val Val Val Asn Gly Ser Val Leu Val Pro Glu Asp
            20                  25                  30

Gln Cys Tyr Cys Ala Gly Trp Ile Glu Ser Arg Gly Thr Asn Gly Ala
        35                  40                  45

Gln Thr Ala Ser Asn Val Leu Gln Trp Leu Ala Ala Gly Phe Ser Ile
    50                  55                  60

Leu Leu Leu Met Phe Tyr Ala Tyr Gln Thr Trp Lys Ser Thr Cys Gly
65                  70                  75                  80

Trp Glu Glu Ile Tyr Val Cys Ala Ile Glu Met Val Lys Val Ile Leu
                85                  90                  95

Glu Phe Phe Phe Glu Phe Lys Asn Pro Ser Met Leu Tyr Leu Ala Thr
            100                 105                 110

Gly His Arg Val Gln Trp Leu Arg Tyr Ala Glu Trp Leu Leu Thr Cys
        115                 120                 125

Pro Val Ile Leu Ile His Leu Ser Asn Leu Thr Gly Leu Ser Asn Asp
    130                 135                 140

Tyr Ser Arg Arg Thr Met Gly Leu Leu Val Ser Asp Ile Gly Thr Ile
145                 150                 155                 160

Val Trp Gly Ala Thr Ser Ala Met Ala Thr Gly Tyr Val Lys Val Ile
                165                 170                 175

Phe Phe Cys Leu Gly Leu Cys Tyr Gly Ala Asn Thr Phe Phe His Ala
            180                 185                 190

Ala Lys Ala Tyr Ile Glu Gly Tyr His Thr Val Pro Lys Gly Arg Cys
        195                 200                 205

Arg Gln Val Val Thr Gly Met Ala Trp Leu Phe Phe Val Ser Trp Gly
    210                 215                 220

Met Phe Pro Ile Leu Phe Ile Leu Gly Pro Glu Gly Phe Gly Val Leu
225                 230                 235                 240

Ser Val Tyr Gly Ser Thr Val Gly His Thr Ile Ile Asp Leu Met Ser
                245                 250                 255

Lys Asn Cys Trp Gly Leu Leu Gly His Tyr Leu Arg Val Leu Ile His
            260                 265                 270

Glu His Ile Leu Ile His Gly Asp Ile Arg Lys Thr Thr Lys Leu Asn
        275                 280                 285

Ile Gly Gly Thr Glu Ile Glu Val Glu Thr Leu Val Glu Asp Glu Ala
    290                 295                 300
```

-continued

Glu Ala Gly Ala Val Pro
305 310

<210> SEQ ID NO 6
<211> LENGTH: 310
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 6

Met Asp Tyr Gly Gly Ala Leu Ser Ala Val Gly Arg Glu Leu Leu Phe
1 5 10 15

Val Thr Asn Pro Val Val Val Asn Gly Ser Val Leu Val Pro Glu Asp
20 25 30

Gln Cys Tyr Cys Ala Gly Trp Ile Glu Ser Arg Gly Thr Asn Gly Ala
35 40 45

Gln Thr Ala Ser Asn Val Leu Gln Trp Leu Ala Ala Gly Phe Ser Ile
50 55 60

Leu Leu Leu Met Phe Tyr Ala Tyr Gln Thr Trp Lys Ser Thr Cys Gly
65 70 75 80

Trp Glu Glu Ile Tyr Val Cys Ala Ile Glu Met Val Lys Val Ile Leu
85 90 95

Glu Phe Phe Phe Glu Phe Lys Asn Pro Ser Met Leu Tyr Leu Ala Thr
100 105 110

Gly His Arg Val Gln Trp Leu Arg Tyr Ala Glu Trp Leu Leu Thr Ser
115 120 125

Pro Val Ile Leu Ile His Leu Ser Asn Leu Thr Gly Leu Ser Asn Asp
130 135 140

Tyr Ser Arg Arg Thr Met Gly Leu Leu Val Ser Asp Ile Gly Thr Ile
145 150 155 160

Val Trp Gly Ala Thr Ser Ala Met Ala Thr Gly Tyr Val Lys Val Ile
165 170 175

Phe Phe Cys Leu Gly Leu Cys Tyr Gly Ala Asn Thr Phe Phe His Ala
180 185 190

Ala Lys Ala Tyr Ile Glu Gly Tyr His Thr Val Pro Lys Gly Arg Cys
195 200 205

Arg Gln Val Val Thr Gly Met Ala Trp Leu Phe Phe Val Ser Trp Gly
210 215 220

Met Phe Pro Ile Leu Phe Ile Leu Gly Pro Glu Gly Phe Gly Val Leu
225 230 235 240

Ser Val Tyr Gly Ser Thr Val Gly His Thr Ile Ile Asp Leu Met Ser
245 250 255

Lys Asn Cys Trp Gly Leu Leu Gly His Tyr Leu Arg Val Leu Ile His
260 265 270

Glu His Ile Leu Ile His Gly Asp Ile Arg Lys Thr Thr Lys Leu Asn
275 280 285

Ile Gly Gly Thr Glu Ile Glu Val Glu Thr Leu Val Glu Asp Glu Ala
290 295 300

Glu Ala Gly Ala Val Pro
305 310

<210> SEQ ID NO 7
<211> LENGTH: 310

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 7

Met Asp Tyr Gly Gly Ala Leu Ser Ala Val Gly Arg Glu Leu Leu Phe
1 5 10 15

Val Thr Asn Pro Val Val Val Asn Gly Ser Val Leu Val Pro Glu Asp
20 25 30

Gln Cys Tyr Cys Ala Gly Trp Ile Glu Ser Arg Gly Thr Asn Gly Ala
35 40 45

Gln Thr Ala Ser Asn Val Leu Gln Trp Leu Ala Ala Gly Phe Ser Ile
50 55 60

Leu Leu Leu Met Phe Tyr Ala Tyr Gln Thr Trp Lys Ser Thr Cys Gly
65 70 75 80

Trp Glu Glu Ile Tyr Val Cys Ala Ile Glu Met Val Lys Val Ile Leu
85 90 95

Glu Phe Phe Phe Glu Phe Lys Asn Pro Ser Met Leu Tyr Leu Ala Thr
100 105 110

Gly His Arg Val Gln Trp Leu Arg Tyr Ala Glu Trp Leu Leu Thr Ser
115 120 125

Pro Val Ile Leu Ile His Leu Ser Asn Leu Thr Gly Leu Ser Asn Asp
130 135 140

Tyr Ser Arg Arg Thr Met Gly Leu Leu Val Ser Ala Ile Gly Thr Ile
145 150 155 160

Val Trp Gly Ala Thr Ser Ala Met Ala Thr Gly Tyr Val Lys Val Ile
165 170 175

Phe Phe Cys Leu Gly Leu Cys Tyr Gly Ala Asn Thr Phe Phe His Ala
180 185 190

Ala Lys Ala Tyr Ile Glu Gly Tyr His Thr Val Pro Lys Gly Arg Cys
195 200 205

Arg Gln Val Val Thr Gly Met Ala Trp Leu Phe Phe Val Ser Trp Gly
210 215 220

Met Phe Pro Ile Leu Phe Ile Leu Gly Pro Glu Gly Phe Gly Val Leu
225 230 235 240

Ser Val Tyr Gly Ser Thr Val Gly His Thr Ile Ile Asp Leu Met Ser
245 250 255

Lys Asn Cys Trp Gly Leu Leu Gly His Tyr Leu Arg Val Leu Ile His
260 265 270

Glu His Ile Leu Ile His Gly Asp Ile Arg Lys Thr Thr Lys Leu Asn
275 280 285

Ile Gly Gly Thr Glu Ile Glu Val Glu Thr Leu Val Glu Asp Glu Ala
290 295 300

Glu Ala Gly Ala Val Pro
305 310

<210> SEQ ID NO 8
<211> LENGTH: 344
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 8

-continued

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Met Ser Arg Arg Pro Trp Leu Leu Ala Leu Ala Leu Ala Val Ala Leu
1               5                  10                  15

Ala Ala Gly Ser Ala Gly Ala Ser Thr Gly Ser Asp Ala Thr Val Pro
            20                  25                  30

Val Ala Thr Gln Asp Gly Pro Asp Tyr Val Phe His Arg Ala His Glu
        35                  40                  45

Arg Met Leu Phe Gln Thr Ser Tyr Thr Leu Glu Asn Asn Gly Ser Val
    50                  55                  60

Ile Cys Ile Pro Asn Asn Gly Gln Cys Phe Cys Leu Ala Trp Leu Lys
65                  70                  75                  80

Ser Asn Gly Thr Asn Ala Glu Lys Leu Ala Ala Asn Ile Leu Gln Trp
                85                  90                  95

Ile Thr Phe Ala Leu Ser Ala Leu Cys Leu Met Phe Tyr Gly Tyr Gln
            100                 105                 110

Thr Trp Lys Ser Thr Cys Gly Trp Glu Glu Ile Tyr Val Ala Thr Ile
        115                 120                 125

Glu Met Ile Lys Phe Ile Ile Glu Tyr Phe His Glu Phe Asp Glu Pro
    130                 135                 140

Ala Val Ile Tyr Ser Ser Asn Gly Asn Lys Thr Val Trp Leu Arg Tyr
145                 150                 155                 160

Ala Glu Trp Leu Leu Thr Cys Pro Val Leu Leu Ile His Leu Ser Asn
                165                 170                 175

Leu Thr Gly Leu Lys Asp Asp Tyr Ser Lys Arg Thr Met Gly Leu Leu
            180                 185                 190

Val Ser Asp Val Gly Cys Ile Val Trp Gly Ala Thr Ser Ala Met Cys
        195                 200                 205

Thr Gly Trp Thr Lys Ile Leu Phe Phe Leu Ile Ser Leu Ser Tyr Gly
    210                 215                 220

Met Tyr Thr Tyr Phe His Ala Ala Lys Val Tyr Ile Glu Ala Phe His
225                 230                 235                 240

Thr Val Pro Lys Gly Ile Cys Arg Glu Leu Val Arg Val Met Ala Trp
                245                 250                 255

Thr Phe Phe Val Ala Trp Gly Met Phe Pro Val Leu Phe Leu Leu Gly
            260                 265                 270

Thr Glu Gly Phe Gly His Ile Ser Pro Tyr Gly Ser Ala Ile Gly His
        275                 280                 285

Ser Ile Leu Asp Leu Ile Ala Lys Asn Met Trp Gly Val Leu Gly Asn
    290                 295                 300

Tyr Leu Arg Val Lys Ile His Glu His Ile Leu Leu Tyr Gly Asp Ile
305                 310                 315                 320

Arg Lys Lys Gln Lys Ile Thr Ile Ala Gly Gln Glu Met Glu Val Glu
                325                 330                 335

Thr Leu Val Ala Glu Glu Glu Asp
            340
```

<210> SEQ ID NO 9
<211> LENGTH: 344
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 9

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```
Met Ser Arg Arg Pro Trp Leu Leu Ala Leu Ala Leu Ala Val Ala Leu
1               5                  10                  15

Ala Ala Gly Ser Ala Gly Ala Ser Thr Gly Ser Asp Ala Thr Val Pro
            20                  25                  30

Val Ala Thr Gln Asp Gly Pro Asp Tyr Val Phe His Arg Ala His Glu
        35                  40                  45

Arg Met Leu Phe Gln Thr Ser Tyr Thr Leu Glu Asn Asn Gly Ser Val
    50                  55                  60

Ile Cys Ile Pro Asn Asn Gly Gln Cys Phe Cys Leu Ala Trp Leu Lys
65                  70                  75                  80

Ser Asn Gly Thr Asn Ala Glu Lys Leu Ala Ala Asn Ile Leu Gln Trp
                85                  90                  95

Ile Thr Phe Ala Leu Ser Ala Leu Cys Leu Met Phe Tyr Gly Tyr Gln
            100                 105                 110

Thr Trp Lys Ser Thr Cys Gly Trp Glu Thr Ile Tyr Val Ala Thr Ile
        115                 120                 125

Glu Met Ile Lys Phe Ile Ile Glu Tyr Phe His Glu Phe Asp Glu Pro
    130                 135                 140

Ala Val Ile Tyr Ser Ser Asn Gly Asn Lys Thr Val Trp Leu Arg Tyr
145                 150                 155                 160

Ala Glu Trp Leu Leu Thr Cys Pro Val Leu Leu Ile His Leu Ser Asn
                165                 170                 175

Leu Thr Gly Leu Lys Asp Asp Tyr Ser Lys Arg Thr Met Gly Leu Leu
            180                 185                 190

Val Ser Asp Val Gly Cys Ile Val Trp Gly Ala Thr Ser Ala Met Cys
        195                 200                 205

Thr Gly Trp Thr Lys Ile Leu Phe Phe Leu Ile Ser Leu Ser Tyr Gly
    210                 215                 220

Met Tyr Thr Tyr Phe His Ala Ala Lys Val Tyr Ile Glu Ala Phe His
225                 230                 235                 240

Thr Val Pro Lys Gly Ile Cys Arg Glu Leu Val Arg Val Met Ala Trp
                245                 250                 255

Thr Phe Phe Val Ala Trp Gly Met Phe Pro Val Leu Phe Leu Leu Gly
            260                 265                 270

Thr Glu Gly Phe Gly His Ile Ser Pro Tyr Gly Ser Ala Ile Gly His
        275                 280                 285

Ser Ile Leu Asp Leu Ile Ala Lys Asn Met Trp Gly Val Leu Gly Asn
    290                 295                 300

Tyr Leu Arg Val Lys Ile His Glu His Ile Leu Leu Tyr Gly Asp Ile
305                 310                 315                 320

Arg Lys Lys Gln Lys Ile Thr Ile Ala Gly Gln Glu Met Glu Val Glu
                325                 330                 335

Thr Leu Val Ala Glu Glu Glu Asp
            340
```

<210> SEQ ID NO 10
<211> LENGTH: 344
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 10

-continued

```
Met Ser Arg Arg Pro Trp Leu Leu Ala Leu Ala Leu Ala Val Ala Leu
1               5                  10                  15

Ala Ala Gly Ser Ala Gly Ala Ser Thr Gly Ser Asp Ala Thr Val Pro
            20                  25                  30

Val Ala Thr Gln Asp Gly Pro Asp Tyr Val Phe His Arg Ala His Glu
        35                  40                  45

Arg Met Leu Phe Gln Thr Ser Tyr Thr Leu Glu Asn Asn Gly Ser Val
    50                  55                  60

Ile Cys Ile Pro Asn Asn Gly Gln Cys Phe Cys Leu Ala Trp Leu Lys
65                  70                  75                  80

Ser Asn Gly Thr Asn Ala Glu Lys Leu Ala Ala Asn Ile Leu Gln Trp
                85                  90                  95

Ile Thr Phe Ala Leu Ser Ala Leu Cys Leu Met Phe Tyr Gly Tyr Gln
            100                 105                 110

Thr Trp Lys Ser Thr Cys Gly Trp Glu Glu Ile Tyr Val Ala Thr Ile
        115                 120                 125

Glu Met Ile Lys Phe Ile Ile Glu Tyr Phe His Glu Phe Asp Glu Pro
    130                 135                 140

Ala Val Ile Tyr Ser Ser Asn Gly Asn Lys Thr Val Trp Leu Arg Tyr
145                 150                 155                 160

Ala Thr Trp Leu Leu Thr Cys Pro Val Leu Leu Ile His Leu Ser Asn
                165                 170                 175

Leu Thr Gly Leu Lys Asp Asp Tyr Ser Lys Arg Thr Met Gly Leu Leu
            180                 185                 190

Val Ser Asp Val Gly Cys Ile Val Trp Gly Ala Thr Ser Ala Met Cys
        195                 200                 205

Thr Gly Trp Thr Lys Ile Leu Phe Phe Leu Ile Ser Leu Ser Tyr Gly
    210                 215                 220

Met Tyr Thr Tyr Phe His Ala Ala Lys Val Tyr Ile Glu Ala Phe His
225                 230                 235                 240

Thr Val Pro Lys Gly Ile Cys Arg Glu Leu Val Arg Val Met Ala Trp
                245                 250                 255

Thr Phe Phe Val Ala Trp Gly Met Phe Pro Val Leu Phe Leu Leu Gly
            260                 265                 270

Thr Glu Gly Phe Gly His Ile Ser Pro Tyr Gly Ser Ala Ile Gly His
        275                 280                 285

Ser Ile Leu Asp Leu Ile Ala Lys Asn Met Trp Gly Val Leu Gly Asn
    290                 295                 300

Tyr Leu Arg Val Lys Ile His Glu His Ile Leu Leu Tyr Gly Asp Ile
305                 310                 315                 320

Arg Lys Lys Gln Lys Ile Thr Ile Ala Gly Gln Glu Met Glu Val Glu
                325                 330                 335

Thr Leu Val Ala Glu Glu Glu Asp
            340
```

<210> SEQ ID NO 11
<211> LENGTH: 344
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 11

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Met Ser Arg Arg Pro Trp Leu Leu Ala Leu Ala Leu Ala Val Ala Leu
1               5                  10                  15

Ala Ala Gly Ser Ala Gly Ala Ser Thr Gly Ser Asp Ala Thr Val Pro
            20                  25                  30

Val Ala Thr Gln Asp Gly Pro Asp Tyr Val Phe His Arg Ala His Glu
        35                  40                  45

Arg Met Leu Phe Gln Thr Ser Tyr Thr Leu Glu Asn Asn Gly Ser Val
    50                  55                  60

Ile Cys Ile Pro Asn Asn Gly Gln Cys Phe Cys Leu Ala Trp Leu Lys
65                  70                  75                  80

Ser Asn Gly Thr Asn Ala Glu Lys Leu Ala Ala Asn Ile Leu Gln Trp
                85                  90                  95

Ile Thr Phe Ala Leu Ser Ala Leu Cys Leu Met Phe Tyr Gly Tyr Gln
            100                 105                 110

Thr Trp Lys Ser Thr Cys Gly Trp Glu Thr Ile Tyr Val Ala Thr Ile
        115                 120                 125

Glu Met Ile Lys Phe Ile Ile Glu Tyr Phe His Glu Phe Asp Glu Pro
    130                 135                 140

Ala Val Ile Tyr Ser Ser Asn Gly Asn Lys Thr Val Trp Leu Arg Tyr
145                 150                 155                 160

Ala Thr Trp Leu Leu Thr Cys Pro Val Leu Leu Ile His Leu Ser Asn
                165                 170                 175

Leu Thr Gly Leu Lys Asp Asp Tyr Ser Lys Arg Thr Met Gly Leu Leu
            180                 185                 190

Val Ser Asp Val Gly Cys Ile Val Trp Gly Ala Thr Ser Ala Met Cys
        195                 200                 205

Thr Gly Trp Thr Lys Ile Leu Phe Phe Leu Ile Ser Leu Ser Tyr Gly
    210                 215                 220

Met Tyr Thr Tyr Phe His Ala Ala Lys Val Tyr Ile Glu Ala Phe His
225                 230                 235                 240

Thr Val Pro Lys Gly Ile Cys Arg Glu Leu Val Arg Val Met Ala Trp
                245                 250                 255

Thr Phe Phe Val Ala Trp Gly Met Phe Pro Val Leu Phe Leu Leu Gly
            260                 265                 270

Thr Glu Gly Phe Gly His Ile Ser Pro Tyr Gly Ser Ala Ile Gly His
        275                 280                 285

Ser Ile Leu Asp Leu Ile Ala Lys Asn Met Trp Gly Val Leu Gly Asn
    290                 295                 300

Tyr Leu Arg Val Lys Ile His Glu His Ile Leu Leu Tyr Gly Asp Ile
305                 310                 315                 320

Arg Lys Lys Gln Lys Ile Thr Ile Ala Gly Gln Glu Met Glu Val Glu
                325                 330                 335

Thr Leu Val Ala Glu Glu Glu Asp
            340
```

<210> SEQ ID NO 12
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 12

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Lys Ser Arg Ile Thr Ser Glu Gly Glu Tyr Ile Pro Leu Asp Gln Ile
1 5 10 15

Asp Ile Asn

<210> SEQ ID NO 13
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)...(2)
<223> OTHER INFORMATION: x = any amino acid

<400> SEQUENCE: 13

Phe Xaa Tyr Glu Asn Glu
1 5

<210> SEQ ID NO 14
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 14

Phe Cys Tyr Glu Asn Glu Val
1 5

<210> SEQ ID NO 15
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 15

Met Thr Glu Thr Leu Pro Pro Val Thr Glu Ser Ala Val Ala Leu Gln
1 5 10 15

Ala Glu

1.-**8**. (canceled)

9. A method for disrupting reward-related behavior in an individual comprising: administering a polynucleotide encoding a light-responsive opsin protein to the individual, wherein the light-responsive opsin protein is expressed on the cell membrane of cholinergic interneurons in the nucleus accumbens or the striatum of the individual, and the protein is responsive to light and is capable of inducing membrane hyperpolarization of the interneurons when the interneurons are illuminated with the light, whereby activating the protein by the light disrupts at least one reward-related behavior in the individual.

10.-**33**. (canceled)

34. A system comprising:

a) a set of cholinergic neurons of a nucleus accumbens, wherein the cholinergic neurons express a light-responsive opsin polypeptide;

b) an optical delivery system for exciting the cholinergic neurons by activating the light-responsive opsin;

c) a drug delivery device for providing a drug to the set of cholinergic neurons; and

d) a monitoring device for assessing the activity of the set of cholinergic neurons in response to the drug.

35. (canceled)

36. The system of claim **34**, wherein the monitoring device is further configured to assess the activity of the set of cholinergic neurons by monitoring the release of acetylcholine.

37. The system of claim **34**, wherein the light-responsive opsin polypeptide is a hyperpolarizing opsin.

38. The system of claim **37**, wherein the light-responsive opsin polypeptide is a light-responsive chloride ion pump and comprises an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence set forth in NpHR (SEQ ID NO:1).

39. The system of claim **38**, wherein the light-responsive opsin polypeptide comprises an endoplasmic reticulum (ER) export signal.

40. The system of claim **39**, wherein the ER export signal comprises the amino acid sequence FXYENE, wherein X is any amino acid.

41. The system of claim **38**, wherein the light-responsive opsin polypeptide comprises a trafficking signal that enhances transport of the opsin polypeptide to the plasma membrane.

42. The system of claim **37**, wherein the light-responsive opsin polypeptide comprises an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence set forth in GtR3 (SEQ ID NO:4).

43. The system of claim **42**, wherein the light-responsive opsin polypeptide comprises an endoplasmic reticulum export signal.

44. The system of claim **34**, wherein the optical delivery system comprises a light emitting diode.

45. The system of claim **34**, wherein the optical delivery system comprises a solid state laser diode.

46. A method for assessing the addictive properties of a substance, the method comprising:

a) exposing cholinergic neurons of a nucleus accumbens present in the system of claim **34** to the substance;

b) activating the light-responsive opsin polypeptide with light, thereby modulating the activity of the cholinergic neurons; and

c) monitoring the activity of the modulated cholinergic neurons after exposure to the substance.

47. The method according to claim **46**, wherein the activity of modulated cholinergic neurons after exposure to the substance is monitored through electrical activity.

48. The method according to claim **47**, wherein the electrical activity comprises action potentials/firing of modulated cholinergic neurons after exposure to the substance.

49. The method according to claim **46**, wherein the activity of modulated cholinergic neurons after exposure to the substance is monitored through the release of acetylcholine.

50. The method according to claim **46**, wherein activating the light-responsive opsin polypeptide further comprises applying an electrical pulse through one or more electrodes positioned near the cholinergic neurons.

51. The method according to claim **46**, wherein activating the light-responsive opsin polypeptide further comprises releasing a drug at a location proximate to the cholinergic neurons.

52. The method according to claim **46**, wherein activating the light-responsive opsin polypeptide further comprises applying a magnetic field to a location proximate to the cholinergic neurons.

* * * * *