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Nucleus Accumbens Cholinergic Interneurons Oppose Cue-Motivated Behavior

Anne L. Collins, Tara J. Aitken, I-Wen Huang, Christine Shieh, Venuz Y. Greenfield, Harold G. Monbouquette, Sean B. Ostlund, and Kate M. Wassum

ABSTRACT

BACKGROUND: Environmental reward–predictive stimuli provide a major source of motivation for adaptive reward pursuit behavior. This cue-motivated behavior is known to be mediated by the nucleus accumbens (NAc) core. The cholinergic interneurons in the NAc are tonically active and densely arborized and thus well suited to modulate NAc function. However, their causal contribution to adaptive behavior remains unknown. Here we investigated the function of NAc cholinergic interneurons in cue-motivated behavior.

METHODS: We used chemogenetics, optogenetics, pharmacology, and a translationally analogous Pavlovian-to-instrumental transfer behavioral task designed to assess the motivating influence of a reward-predictive cue over reward-seeking actions in male and female rats.

RESULTS: The data show that NAc cholinergic interneuron activity critically opposes the motivating influence of appetitive cues. Chemogenetic inhibition of NAc cholinergic interneurons augmented cue-motivated behavior. Optical stimulation of acetylcholine release from NAc cholinergic interneurons prevented cues from invigorating reward-seeking behavior, an effect that was mediated by activation of β_2-containing nicotinic acetylcholine receptors.

CONCLUSIONS: NAc cholinergic interneurons provide a critical regulatory influence over adaptive cue-motivated behavior and therefore are a potential therapeutic target for the maladaptive cue-motivated behavior that marks many psychiatric conditions, including addiction and depression.

Keywords: Acetylcholine, Biosensors, Chemogenetics, Dopamine, Motivation, Optogenetics, Pavlovian-to-instrumental transfer, Tonically active neurons

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Environmental reward–predictive stimuli provide a major source of motivation for adaptive reward pursuit behaviors (1). This incentive motivational value can become dysfunctional in many psychiatric disease states (2). Indeed, it can become amplified, allowing cues to become potent triggers for maladaptive compulsive overeating (3), alcohol abuse (4–7), or drug seeking (8–12). Stress, anxiety, and depression (13–16) can also disrupt the motivating influence of appetitive cues, resulting in dampened or inappropriate motivation. The nucleus accumbens (NAc) core has been implicated in cue-motivated behavior (17–19). However, little is known about the function of the major NAc neuromodulator acetylcholine. Such information is crucial given the purported importance of cholinergic signaling in many mental illnesses (20,21).

Cholinergic interneurons provide the primary, though not exclusive (22), source of acetylcholine in the NAc (23). Despite accounting for only 1% to 2% of the population, these large-bodied, tonically active neurons are densely arborized (24–29), making them ideally suited to modulate NAc function and associated behaviors. Cholinergic interneurons have also been shown to locally regulate striatal dopamine release (30–32). NAc cholinergic signaling is elevated under conditions that discourage vigorous reward seeking, such as satiety (33,34), and has been implicated in anxiety-like and depression-like states (35,36) marked by blunted motivation. Cholinergic interneurons are also transiently activated by informative environmental stimuli. Cues that discourage motivated behavior activate the cholinergic interneurons (37,38), whereas reward-predictive cues that encourage motivated behavior cause a characteristic pause in cholinergic interneuron activity (29,37,39–46). Still, very little is known of the causal contribution of NAc cholinergic interneurons to motivation.

We sought to fill this gap in knowledge by assessing the function of NAc cholinergic interneurons in cue-motivated behavior. Working from the evidence that cholinergic interneurons increase their activity when vigorous motivated behavior is disadvantageous and pause when active reward pursuit is encouraged, we tested the hypothesis that NAc cholinergic interneuron activity functions to oppose the motivating influence of appetitive cues. Chemogenetic and optogenetic methods were used to selectively manipulate NAc cholinergic interneuron activity. We used the Pavlovian-to-instrumental transfer (PIT) test to measure cue-motivated behavior. This test is translationally analogous to that used in humans in health and disease (5,11,17,47–55) and assesses

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the invigorating influence of an environmental reward-predictive stimulus over instrumental reward-seeking activity. Because the Pavlovian and instrumental components are trained separately, PIT isolates the incentive motivational value of the cue from other processes through which cues trigger action, such as via discriminative control or a stimulus-response relationship.

METHODS AND MATERIALS

Subjects

Adult (3–5 months) male and female ChAT::Cre$^+$ bacterial artificial chromosome transgenic rats (Long-Evans background) (56) were used for all experiments. Although bacterial artificial chromosome transgenic ChAT::Cre$^+$ mice have been shown to overexpress the vesicular acetylcholine transporter, which can lead to behavioral and electrophysiological changes (57), we found normal expression of the behaviors of interest here, similar to our prior reports in wild-type rats (58–60). Pups were weaned at postnatal day 21 and housed in groups until experiment onset. Handling occurred daily, beginning at postnatal day 60. Training and testing were performed during the dark phase of a 12-hour reverse dark/light cycle. Rats were food restricted to approximately 85% free-feeding body weight, and water was provided ad libitum in the home cage. All procedures were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and were approved by the University of California, Los Angeles, Institutional Animal Care and Use Committee.

Surgery

Standard surgical procedures, described previously (58,61,62), were used for infusion of adeno-associated viruses (AAVs) and implantation of optical fiber or microinfusion injector/optical fiber guide cannula into the NAc core. Rats were anesthetized with isoflurane, and a nonsteroidal anti-inflammatory agent was administered preoperatively and postoperatively to minimize pain and discomfort. Surgical details for each experiment are provided in Supplemental Methods. Expression and placement were verified with standard histological procedures (see Supplemental Methods).

Behavioral Procedures

General Training and Testing. Rats received Pavlovian and instrumental training in conditioning chambers (Med Associates, Inc., Fairfax, VT), as described previously (58–60). Rats first received 8 days of Pavlovian training, in which one of two auditory stimuli (75-dB tone or white noise; counterbalanced across rats) was paired with noncontingent delivery of 45-mg chocolate-flavored, grain-based pellets (Bio-Serv, Flemington, NJ). During each 2-minute presentation of the conditional stimulus (CS$^+$), pellets were presented on a random-interval 30-second schedule. The CS$^+$ was presented 6×/session with a random 2- to 4-minute intertrial interval (mean 3 minutes). The lever was never present during these sessions.

All rats then received 8 days of instrumental conditioning in which lever pressing earned delivery of a single chocolate pellet. Each session lasted until 20 outcomes had been earned or 30 minutes had elapsed. Rats received 1 day each of continuous, random-interval 15-second and random-interval 30-second schedules of reinforcement, followed by 5 days on the final random-interval 60-second schedule. The CS$^+$ was never present during this training.

Rats received one session of habituation to the neutral control stimulus (CS$^\varnothing$), which consisted of six 2-minute presentations of the CS$^\varnothing$ (opposite stimulus as the CS$^+$), with a 2- to 4-minute intertrial interval. No rewards were delivered during this session.

On the day before each PIT test, rats were given a single 30-minute instrumental extinction session in which no cues were present and the lever was available, but presses were unrewarded. During each PIT test, the lever was continuously available, but pressing was not reinforced. Responding was extinguished for 5 minutes to establish a low rate of baseline performance, after which each CS was presented 4 times in pseudorandom order, also without accompanying reward. Each CS lasted 2 minutes with a 4-minute fixed intertrial interval. Rats received one Pavlovian and two instrumental retraining sessions identical to the above sessions in between subsequent PIT tests. In all cases, testing began at least 4 weeks after viral infusion to allow construct expression.

Chemogenetic Inactivation of NAc Cholinergic Interneurons. Before training, ChAT::Cre$^+$ rats were bilaterally infused with a Cre-inducible AAV vector to express the inhibitory designer receptor human M4 muscarinic receptor [hM4D(Gi)] or control fluorophore mCherry selectively in cholinergic interneurons of the NAc. Following training, rats received PIT tests, counterbalanced for order, one following vehicle and one following intraperitoneal injection of the hM4D(Gi) ligand clozapine-*N*-oxide (CNO) (5 mg/kg) (see Supplemental Methods). These experiments were run in two separate cohorts, and data were collapsed across cohorts following analyses indicating no interaction between cohort and any of the variables of primary interest [hM4D(Gi): highest $F_{1,17} = 3.23$, $p = .09$; mCherry: highest $F_{1,14} = 3.88$, $p = .07$]. Final hM4D(Gi) cohort was $n = 19$ (8 female; 2 rats were excluded owing to off-target viral spread) and mCherry cohort was $n = 16$ (8 female). Following PIT testing, a subset of subjects were tested for the influence of NAc cholinergic interneuron inactivation on food consumption and lever pressing on a progressive-ratio response requirement (see Supplemental Methods).

Optical Stimulation of NAc Cholinergic Interneurons. Before training, ChAT::Cre$^+$ rats were bilaterally infused with a Cre-inducible AAV vector to express the excitatory opsin channelrhodopsin-2 (ChR2) or control fluorophore enhanced yellow fluorescent protein (eYFP) selectively in NAc cholinergic interneurons. Optical fibers were implanted bilaterally in the NAc. From the last 2 days of instrumental training and for a single additional Pavlovian retraining session, rats were tethered to the patch cord, but no light was delivered to allow habituation to the optical tether. Following training, rats received four PIT tests, counterbalanced for order, with intervening retraining. During each test, optical fibers were connected via ceramic

sleeves to patch cords attached to a commutator. Blue light (473 nm, 10 Hz, 10 mW, 5-ms pulse width, 120-second duration) (see also Supplemental Methods) was delivered for optical activation of ChR2-expressing NAc cholinergic interneurons. For the main experimental condition, light was delivered concurrent with each of the four CS^+ presentations, with light and CS^+ onset and offset synced. There were three separate control conditions: light delivered concurrent with each $CS^\varnothing$ presentation, light delivered during the CS-free 2-minute baseline periods immediately before each CS^+ presentation, or light delivered during the CS-free 2-minute baseline periods immediately before each $CS^\varnothing$ presentation. There were no significant differences in performance between the pre-CS^+ and pre-$CS^\varnothing$ stimulation tests, and thus data were collapsed across these tests into a single baseline stimulation control condition (see Supplemental Figure S4). Final ChR2 cohort was $n = 9$ (5 female; 5 subjects excluded for lack of expression and/or optical fiber misplacement), and final eYFP cohort was $n = 8$ (5 female).

Optical Stimulation of NAc Cholinergic Interneurons and Inactivation of NAc β_2-Containing Nicotinic Acetylcholine Receptors. Before training, ChAT::Cre^+ rats were bilaterally infused with a Cre-inducible AAV vector to express ChR2 selectively in NAc cholinergic interneurons. Microinfusion injector/optical fiber guide cannulas were implanted bilaterally above the NAc. Following training, rats received 4 PIT tests, counterbalanced for order with intervening retraining. Before each test, rats were bilaterally infused with either the selective $\alpha_4\beta_2$-containing nicotinic receptor competitive antagonist dihydro-β-erythroidine (DhβE) (15 μg/0.5 μL/side) (see Supplemental Methods) or artificial cerebrospinal fluid vehicle via an injector inserted through the guide cannula designed to protrude 2.5 mm to just above the NAc (−6.5 mm). Following infusion, injectors were removed, and optical fibers, also designed to protrude 2.5 mm and thus target the NAc, were placed through guide cannulas and secured via ceramic sleeves. During two of the tests, one each following vehicle or DhβE, blue light (473 nm, 10 Hz, 10 mW, 5-ms pulse width, 120-second duration) was delivered for optical activation of ChR2-expressing NAc cholinergic interneurons concurrent with each CS^+ presentation. During the other two tests, an optical fiber was attached, but no light was delivered. Thus, each rat received four tests: vehicle/no stimulation, vehicle/stimulation during CS^+, DhβE/no stimulation, DhβE/stimulation during CS^+. Following the PIT tests, optical fibers were removed, and dummies were placed in the guide cannulas. Final cohort was $n = 11$ (all male, 1 rat was excluded owing to a clogged cannula).

Data Analysis

Behavioral Analysis. Lever pressing and entries into the food-delivery port were the primary behavioral output measures for the PIT test. These measures were counted for each 2-minute CS period, with behavioral output during the 2-minute periods before each CS serving as the baseline. For both the chemogenetic inhibition and the optical stimulation experiments, there was no interaction between trial and any of the other variables on lever pressing during the test (highest $F_{6,108} = 1.84$, $p = .13$). Thus, in all cases, data were collapsed across trials.

Sex Differences. Approximately half the subjects in the chemogenetic and optical manipulation experiments were female. In neither experiment was there a main effect of sex [hM4D(Gi): $F_{1,7} = 2.72$, $p = .12$; ChR2: $F_{1,7} = 0.71$, $p = .43$], and sex did not significantly interact with the effect of CS and/or drug or stimulation period on lever pressing (highest $F_{1,17} = 3.41$, $p = .08$). Thus, all data were collapsed across sexes. Because sex did not influence results of the initial optogenetic experiment, the follow-up experiment assessing the influence of intra-NAc DhβE on the behavioral effect of optical stimulation included only male subjects.

Statistical Analysis. Data were processed with Microsoft Excel (Microsoft Corp., Redmond, WA). Statistical analyses were conducted with GraphPad Prism, version 7 (GraphPad Software, San Diego, CA) and SPSS (IBM Corp., Armonk, NY) software. Data were analyzed with Student's t tests, one-way, two-way, and three-way repeated-measures analysis of variance (Geisser-Greenhouse correction). Corrected post hoc comparisons were used to clarify main effects and interactions. All datasets met equal covariance assumptions, justifying analysis of variance interpretation (63). α levels were set at $p < .05$.

Approach Validation

Optical stimulation and chemogenetic inhibition of NAc cholinergic interneurons was validated in vivo with electroenzymatic choline biosensors and constant-potential amperometry as detailed in Supplemental Methods. Briefly, to confirm chemogenetic inhibition of NAc cholinergic interneurons, silicon wafer–based platinum microelectrode array choline biosensors packaged with an optical fiber affixed to the back surface of the probe (to reduce the photovoltaic artifact) were lowered into the NAc of anesthetized rats expressing ChR2 and hM4D(Gi) in cholinergic interneurons. The ability of blue light (473 nm, 20 Hz, 5–30 mW, 10-ms pulse width, 5-second duration) to evoke acetylcholine release continuously monitored by the sensor was assessed following injection of vehicle or CNO (5 mg/kg, intraperitoneal). Final recording locations were $n = 4$ in 2 subjects. To confirm stimulation of NAc cholinergic interneurons with the exact light parameters used in the behavioral experiments, choline biosensors/optical fibers were lowered into the NAc of anesthetized rats expressing ChR2 or eYFP in cholinergic interneurons. Choline fluctuations were monitored, and blue light (473 nm, 10 Hz, 10 mW, 5-ms pulse width, 120-second duration) was delivered to evaluate its ability to evoke acetylcholine release in ChR2-expressing subjects. Final ChR2 recording locations were $n = 5$ in 4 subjects, and final eYFP recording locations were $n = 5$ in 3 subjects.

RESULTS

Chemogenetic Inhibition of NAc Cholinergic Interneurons Augments Cue-Motivated Behavior

To evaluate the contribution of NAc cholinergic interneurons to cue-motivated behavior, we first chemogenetically inactivated these cells during a PIT test. Inactivation was achieved by using ChAT::Cre^+ rats and a Cre-inducible AAV vector to

express the inhibitory designer receptor hM4D(Gi) selectively in cholinergic interneurons of the NAc (Figure 1A–C). In separate subjects expressing both hM4D(Gi) and ChR2 in cholinergic interneurons, CNO (5 mg/kg, intraperitoneal) activation of hM4D(Gi) in cholinergic interneurons was found to effectively attenuate optically evoked NAc acetylcholine release in vivo (Figure 1D).

Rats received Pavlovian training to pair a 2-minute auditory stimulus (CS^+) with a food pellet reward (Figure 1E). An alternative 2-minute auditory stimulus was presented unpaired with reward and served as a control ($CS^{\varnothing}$). Rats were then instrumentally conditioned, in the absence of the stimuli, to lever press to earn food rewards (see Supplemental Table S1 for training data). At the PIT test, the lever was available and each CS was presented in pseudorandom order to assess the motivating influence of the CS^+ over lever-pressing activity. No rewards were delivered during this test. Increased lever-press rate during the CS^+ provided the measure of cue-motivated behavior (i.e., expression of PIT). Each rat was tested twice, once following injection of vehicle and once following CNO, counterbalanced for order (Figure 1E).

Inactivation of NAc cholinergic interneurons augmented the expression of PIT (CS period: $F_{2,36} = 8.15$, $p = .001$; drug: $F_{1,18} = 0.78$, $p = .39$; CS × drug: $F_{2,36} = 5.2$, $p = .01$) (Figure 1F). Demonstrating PIT, the CS^+ elevated lever pressing relative to both the baseline and the $CS^{\varnothing}$ periods under vehicle control conditions ($p < .05$). Inactivation of NAc cholinergic interneurons enhanced the invigorating influence of the CS^+ relative to the vehicle control condition ($p < .01$). NAc cholinergic interneuron inactivation predominantly influenced CS^+-invigorated responding; neither baseline nor $CS^{\varnothing}$ lever-press rate was significantly altered in the CNO condition ($p > .05$). There was no effect of CNO on the expression of PIT in subjects lacking the hM4D(Gi) transgene (CS period: $F_{2,30} = 4.47$, $p = .02$; drug: $F_{1,15} = 0.31$, $p = .58$; CS × drug: $F_{2,30} = 0.45$, $p = .64$) (Figure 1G). Inactivation of NAc cholinergic interneurons did not alter the expression of Pavlovian conditional food-port approach responses during the PIT test. It also did not alter lever pressing during a progressive ratio test or basic food consumption (Supplemental Figure S2). Thus, inactivation of NAc cholinergic interneurons selectively enhanced the motivating influence of a reward-predictive cue over instrumental behavior.

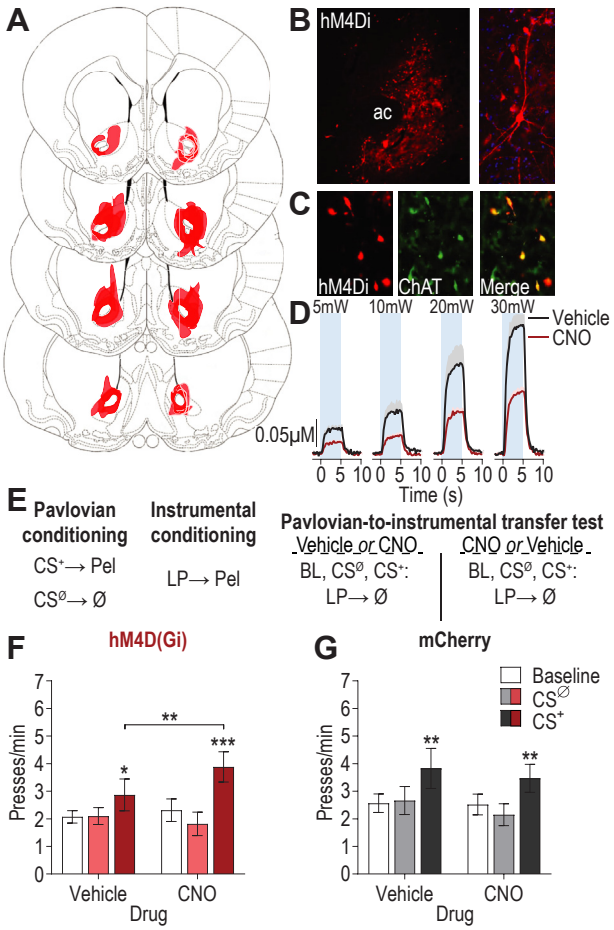

Figure 1. Chemogenetic inhibition of nucleus accumbens (NAc) cholinergic interneurons augments cue-motivated behavior. **(A)** Schematic representation of human M4 muscarinic receptor [hM4D(Gi)]–mCherry expression in the NAc for all subjects. Slides represent 0.7–1.7 mm anterior to bregma. **(B)** Representative immunofluorescent images of hM4D(Gi)-mCherry expressing cholinergic interneurons in the NAc. **(C)** Colocalization of choline acetyltransferase (ChAT) staining and hM4D(Gi)-mCherry expression in the NAc. **(D)** CNO:hM4D(Gi) attenuation of optically evoked (473 nm, 20 Hz, 5–30 mW, 10-ms pulse width, 5-second duration) acetylcholine release in the NAc in vivo [see Supplemental Figure S1 for histology demonstrating hM4D(Gi) and channelrhodopsin-2 expression in cholinergic interneurons; $n = 4$]. Mean ±1 SEM. **(E)** Procedure schematic. **(F, G)** Lever press rate during each 2-minute period of the Pavlovian-to-instrumental transfer test, averaged across trials compared between the conditional stimulus (CS)–free (baseline) (BL), neutral control stimulus ($CS^{\varnothing}$), and reward-predictive cue (CS^+) periods for the vehicle-treated and CNO-treated conditions in hM4D(Gi) ($n = 19$) **(F)** or mCherry control ($n = 16$) **(G)** subjects. Mean ±1 SEM. $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$. ac, anterior commissure; CNO, clozapine *N*-oxide; LP, lever press; Ø, no reward; Pel, pellet reward; Veh, vehicle. [Images in panel **(A)** reproduced with permission from Paxinos and Watson (100).]

Optical Stimulation of NAc Cholinergic Interneurons Concurrent With Reward Cue Presentation Blunts Cue-Motivated Behavior

The chemogenetic inactivation results suggest that NAc cholinergic interneurons function to oppose cue-motivated behavior. To further test this, we next evaluated the influence of activation of NAc cholinergic interneurons on expression of PIT. We used optical stimulation to provide temporal specificity. The excitatory opsin ChR2 was selectively expressed in NAc cholinergic interneurons (Figure 2A–C) of ChAT::Cre^+ rats. Optical stimulation (473 nm, 10 Hz, 10 mW, 2 minutes) of these cells at a frequency in the upper range of their normal firing rate (64,65) was found to increase acetylcholine release in vivo. This increase was restricted to the light-on period ($F_{2,8} = 15.15$, $p = .01$) and did not occur in subjects lacking the ChR2 transgene (Figure 2D). Following Pavlovian and instrumental training, during the PIT test, we used a within-subject design to stimulate NAc cholinergic interneurons concurrent with either each 2-minute CS^+ presentation or, in separate control tests, each $CS^{\varnothing}$ presentation or an equivalent number and duration of CS-free baseline periods (Figure 2E).

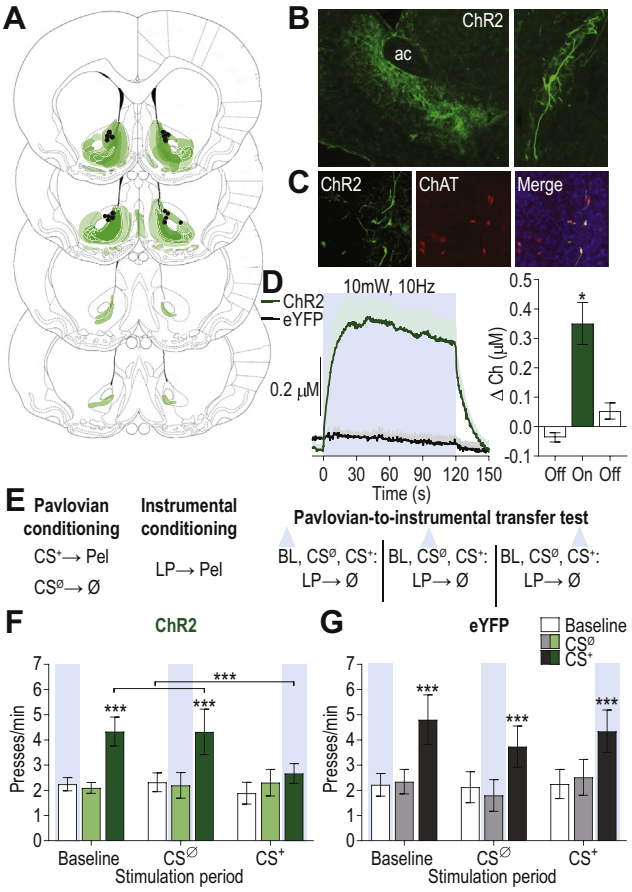

Figure 2. Optical stimulation of nucleus accumbens (NAc) cholinergic interneurons concurrent with reward-predictive cue blunts cue-motivated behavior. **(A)** Schematic representation of channelrhodopsin-2 (ChR2)–enhanced yellow fluorescent protein (eYFP) expression and fiber tips in the NAc for all subjects. Slides represent 0.7–1.7 mm anterior to bregma. **(B)** Representative immunofluorescent images of ChR2-eYFP–expressing cholinergic interneurons in the NAc. **(C)** Colocalization of choline acetyltransferase (ChAT) staining and ChR2-eYFP expression in the NAc. **(D)** Optically evoked acetylcholine release in vivo by blue light delivery (473 nm, 10 Hz, 10 mW, 5-ms pulse width, 120-second duration) to ChR2-expressing cholinergic interneurons in the NAc (see Supplemental Figure S3 for histology; n = 5/group). Mean ±1 SEM. **(E)** Procedure schematic. The blue triangle indicates light delivery. **(F, G)** Lever press rate during each 2-minute period of the Pavlovian-to-instrumental transfer test, averaged across trials compared between the conditional stimulus (CS)–free (baseline) (BL), neutral control stimulus ($CS^{\varnothing}$), and reward-predictive cue (CS^{+}) periods for tests in which optical stimulation occurred during the baseline stimulation, $CS^{\varnothing}$, and CS^{+} periods in ChR2 (n = 9) **(F)** or eYFP control (n = 8) **(G)** subjects. Mean ±1 SEM. ***$p < .001$. ac, anterior commissure; LP, lever press; Ø, no reward; Pel, pellet reward.

Optical stimulation of NAc cholinergic interneurons during CS^{+} presentation blunted the expression of PIT (CS period: $F_{2,16}$ = 8.07, p = .004; stimulation period: $F_{2,16}$ = 0.71, p = .50; CS × stimulation period: $F_{4,32}$ = 3.79, p = .01) (Figure 2F). Neither baseline nor $CS^{\varnothing}$ period stimulation altered lever pressing during those periods ($p > .05$) or the significant enhancement in such pressing induced by the CS^{+} ($p < .001$). However, stimulation of NAc cholinergic interneurons concurrent with CS^{+} presentation prevented that cue from increasing lever pressing ($p > .05$). Light delivery had no effect on the expression of PIT in subjects lacking the ChR2 transgene (CS period: $F_{2,14}$ = 8.66, p = .004; stimulation period: $F_{2,14}$ = 0.27, p = .77; CS × stimulation period: $F_{4,28}$ = 1.04, p = .41) (Figure 2G). Optical stimulation of NAc cholinergic interneurons did not prevent the CS^{+} from eliciting Pavlovian conditional food-port approach responses (Supplemental Figure S5), suggesting no deficit in CS^{+} recognition. Thus, optical stimulation of NAc cholinergic interneurons blunted the expression of cue-motivated behavior.

Acetylcholine Release From NAc Cholinergic Interneurons Works via β_2-Containing Nicotinic Receptors to Blunt Cue-Motivated Behavior

These data suggest that cholinergic interneuron activity tempers the motivating influence of reward-predictive cues over reward-seeking actions. Acetylcholine receptors are broadly distributed in the NAc and consist of two major subtypes: metabotropic muscarinic acetylcholine receptors and ionotropic nicotinic acetylcholine receptors (nAChRs). We previously found that activity of the NAc nAChRs, in particular, works to restrain the expression of cue-motivated behavior (58). Moreover, nAChRs containing the β_2 subunit have been shown to be located exclusively on dopamine axons and terminals (66), where they regulate phasic dopamine release (67–72), which has itself, in the NAc, been shown to track and mediate cue-motivated behavior (9,59,60,73–76). Thus, we next asked whether the attenuating effect of optical stimulation of NAc cholinergic interneurons over cue-motivated behavior is mediated via these β_2-containing nAChRs. To achieve this, we again selectively expressed ChR2 in NAc cholinergic interneurons (Figure 3A–C) and evaluated the influence of intra-NAc infusion of DhβE (15 μg/side), a selective $\alpha_4\beta_2$-containing nAChR antagonist, on the suppressive influence of NAc cholinergic interneuron stimulation over PIT expression (Figure 3D).

Blockade of β_2-containing nAChRs recovered the impairment of PIT induced by optical stimulation of NAc cholinergic interneurons during the CS^{+} (CS period: $F_{2,22}$ = 22.69, $p < .0001$; optical stimulation: $F_{1,11}$ = 0.08, p = .78; drug: $F_{1,11}$ = 0.003, p = .96; CS × stimulation: $F_{2,22}$ = 5.19, p = .02; CS × drug × stimulation: $F_{2,22}$ = 5.10, p = .02) (Figure 3E). We replicated the suppressive effect of optical stimulation of NAc cholinergic interneurons during CS^{+} presentation on the expression of PIT relative to a nonstimulated control condition ($p > .001$). Whereas intra-NAc infusion of DhβE alone at this dose did not influence PIT expression relative to the vehicle-infused control condition ($p > .05$), it did alleviate the suppressive effect of cholinergic interneuron stimulation ($p < .01$), allowing subjects to show a significant PIT effect ($p < .001$). These data demonstrate that acetylcholine release from NAc cholinergic interneurons acts via β_2-containing nAChRs to blunt the motivating influence of cues. Secondarily, the data indicate that the effect of optical stimulation of cholinergic interneurons was not due to nAChR desensitization.

DISCUSSION

Using a combination of chemogenetic, optogenetic, and pharmacological approaches, we investigated the function of

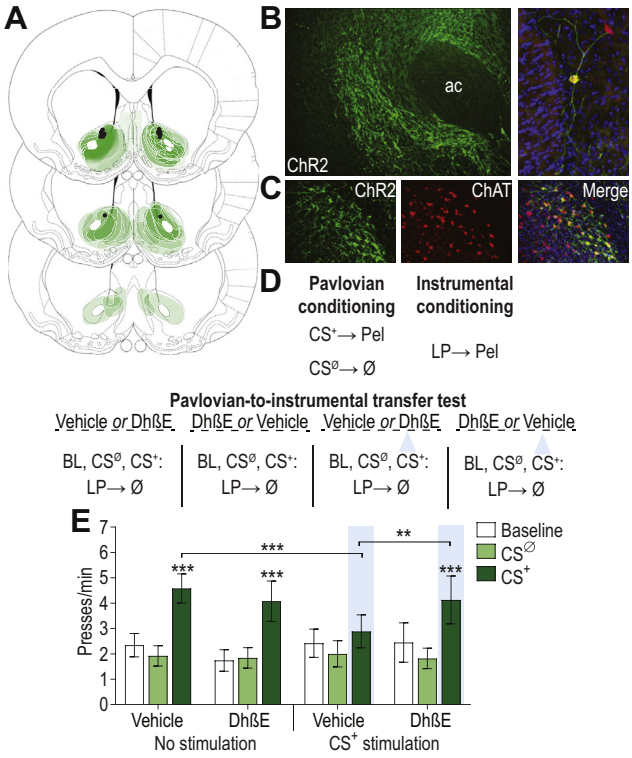

Figure 3. Acetylcholine release from nucleus accumbens (NAc) cholinergic interneurons works via β_2-containing nicotinic receptors to blunt cue-motivated behavior. **(A)** Schematic representation of channelrhodopsin-2 (ChR2)–enhanced yellow fluorescent protein expression and fiber/injector tips in the NAc for all subjects. Slides represent 0.7–1.7 mm anterior to bregma. **(B)** Representative immunofluorescent images of ChR2–enhanced yellow fluorescent protein–expressing cholinergic interneurons in the NAc. **(C)** Colocalization of choline acetyltransferase (ChAT) staining and ChR2–enhanced yellow fluorescent protein expression in the NAc. **(D)** Procedure schematic. The blue triangle indicates light delivery. **(E)** Lever press rate during each 2-minute period of the Pavlovian-to-instrumental transfer test, averaged across trials compared between the conditional stimulus (CS)–free (baseline) (BL), neutral control stimulus ($CS^{Ø}$), and reward-predictive cue (CS^{+}) periods for the tests with either intra-NAc vehicle or DhβE with or without optical stimulation during CS^{+} (n = 11). Mean ±1 SEM. $^{**}p < .01$, $^{***}p < .001$. ac, anterior commissure; DhβE, dihydro-β-erythroidine; LP, lever press; Ø, no reward; Pel, pellet reward.

NAc cholinergic interneurons in cue-motivated behavior. The data revealed that cholinergic interneuron activity in the NAc functions to limit the motivational influence of reward-predictive cues over reward-seeking actions. Chemogenetic inactivation of NAc cholinergic interneurons augmented cue-motivated behavior, whereas optical stimulation of these cells temporally restricted to cue presentation prevented cues from motivating action. This mitigating function is achieved via acetylcholine activation of β_2-containing nAChRs.

These data accord well with evidence of the activity patterns of striatal cholinergic interneurons collected in nonhuman primates and rodents. Striatal cholinergic interneurons both tonically and phasically increase their activity when vigorous motivated behavior is discouraged, for example, in states of satiety (33,34), or when cues signal unfavorable (e.g., high effort, low reward) conditions (37). Cholinergic interneurons also transiently increase their activity when cues signal that reward is available contingent on a no-go response (38), i.e., when motivated movement must be withheld. Striatal cholinergic interneurons transiently pause their activity in response to cues signaling that vigorous reward seeking is advantageous. For example, cholinergic interneurons will pause in response to reward-predictive cues (29,37,39–46) and when cues signal favorable low-effort/high-reward conditions (37). The current data provide an important causal addition to this literature and reveal that increases in NAc cholinergic interneuron activity function to oppose cue-motivated behavior and that decreases or pauses in such activity are permissive to cue-motivated action. These results also indicate that the NAc inputs that regulate cholinergic interneuron excitability, activity, or synchrony, such as thalamostriatal projections (69), are well positioned to influence cue-motivated behavior. Indeed, recent evidence from the dorsal striatum indicates that stimulation of rostral intralaminar thalamic inputs can regulate motivated behavior by triggering a rapid burst and then pause in cholinergic interneuron activity (77).

We found the suppressive effect of optical stimulation over cue-motivated behavior to depend on the activity of β_2-containing nAChRs. Acetylcholine release from NAc cholinergic interneurons acts at β_2-containing nAChRs to curtail the motivating influence of appetitive cues. This is consistent with our previous evidence that general nAChR, but not muscarinic acetylcholine receptor, blockade augments cue-motivated behavior (58). Moreover, that inactivation of β_2-containing nAChRs completely recovered the suppressive influence of optical stimulation of NAc cholinergic interneurons over cue-motivated behavior suggests that, although other acetylcholine receptor subtypes may contribute, β_2-containing nAChRs are a critical locus of action for cholinergic regulation of cue-motivated behavior.

NAc core dopamine release is a major substrate of cue-motivated behavior. Its activity correlates with (58,60,74,78) and is necessary (59,76,79) and sufficient (75,80,81) for the motivational influence of reward-predictive cues. β_2-containing nAChRs are located exclusively on NAc dopamine axons and terminals (66), where they have been found to modulate dopamine release (67–72). The present data may be considered surprising in light of evidence that optical stimulation of striatal cholinergic interneurons can evoke dopamine release from terminals via action at β_2-containing nAChRs (69,70). However, a growing body of literature indicates that cholinergic regulation of dopamine release depends on the activity state of the dopamine cells (82,83). β_2-containing nAChR activity facilitates low probability (32,67,84) and tonic dopamine release (85) but will actually suppress dopamine release that results from high-frequency stimulation, which mimics dopamine neuron burst firing (32,67,84). Indeed, inactivation of β_2-containing nAChRs in the NAc will augment dopamine release induced by high-frequency stimulation ex vivo (68,71), and general nAChR inactivation in the NAc will potentiate the phasic dopamine release response to reward-predictive cues in awake-behaving animals (58). Thus, we speculate that NAc cholinergic interneuron activity may restrain the motivating influence of reward-predictive cues via attenuating their ability to elicit dopamine release, with pausing in their signaling being permissive to such release and associated motivation.

The suppressive function of NAc cholinergic interneurons over cue-motivated behavior is interesting in light of how these cells are regulated. NAc cholinergic interneurons are controlled by several factors that mediate food-related motivation and responsivity to food cues. For example, they express receptors for the adiposity and satiety signal insulin, activation of which increases their activity and modulates NAc dopamine signaling through an nAChR-dependent mechanism (86). They also express receptors for corticotropin-releasing factor, which mediates the positive and negative effects of stress (87–90). NAc corticotropin-releasing factor receptor activation increases cholinergic interneuron activity (91) and acetylcholine release (92) and regulates dopamine release (91). Moreover, serotonin, a neuromodulator long linked to motivation and mood and recently in the NAc linked to adaptive social behavior (93), attenuates the excitability of NAc cholinergic interneurons via presynaptic 5-hydroxytryptamine 1A and postsynaptic 5-hydroxytryptamine 1B receptors (94).

Thus, NAc cholinergic interneurons are well positioned to mitigate cue-motivated behavior when vigorous motivated action would not be beneficial and to promote cue-motivated behavior when it is adaptive. Dysfunction in this mechanism could therefore lead to the dysregulated motivation underlying some mental illnesses. Indeed, cues can become unnaturally strong motivators of drug-seeking behavior in addiction (4,8,95,96), and NAc cholinergic interneurons have been linked to addiction-like behaviors (97,98). Depression can be characterized by avolitional symptoms (95,99), and NAc cholinergic interneurons have been linked to depression-like behavior (35). Therefore, these results have implications for the understanding and treatment of these and other diseases marked by maladaptive motivation.

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ALC, KMW, and SBO conceptualized and designed the experiments and interpreted the data. ALC and KMW analyzed the data. ALC conducted the optogenetic experiments and optogenetic validation, with assistance from VYG. TJA and ALC conducted the chemogenetic experiments, with assistance from VYG. CS and KMW conducted the chemogenetic validation experiments. I-WH, HGM, and KMW designed the choline biosensors, and I-WH prepared and tested all sensors. KMW and ALC wrote the manuscript with assistance from TJA and SBO.

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ARTICLE INFORMATION

From the Departments of Psychology (ALC, TJA, CS, VYG, KMW) and Chemical Engineering (I-WH, HGM) and Brain Research Institute (KMW), University of California, Los Angeles, Los Angeles; and Department of Anesthesiology and Perioperative Care (SBO), University of California, Irvine, Irvine, California.

Address correspondence to Kate M. Wassum, Ph.D., Department of Psychology, University of California, Los Angeles, 1285 Franz Hall, Box 951563, Los Angeles, CA 90095-1563; E-mail: kwassum@ucla.edu.

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