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Cortical cholinergic signaling controls the detection of cues

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The cortical cholinergic input system has been described as a neuromodulator system that influences broadly defined behavioral and brain states. The discovery of phasic, trial-based increases in extracellular choline (transients), resulting from the hydrolysis of newly released acetylcholine (ACh), in the cortex of animals reporting the presence of cues suggests that ACh may have a more specialized role in cognitive processes. Here we expressed channelrhodopsin or halorhodopsin in basal forebrain cholinergic neurons of mice with optic fibers directed into this region and prefrontal cortex. Cholinergic transients, evoked in accordance with photostimulation parameters determined in vivo, were generated in mice performing a task necessitating the reporting of cue and noncue events. Generating cholinergic transients in conjunction with cues enhanced cue detection rates. Moreover, generating transients in noncued trials, where cholinergic transients normally are not observed, increased the number of invalid claims for cues. Enhancing hits and generating false alarms both scaled with stimulation intensity. Suppression of endogenous cholinergic activity during cued trials reduced hit rates. Cholinergic transients may be essential for synchronizing cortical neuronal output driven by salient cues and executing cue-guided responses.

acetylcholine | cortex | attention | optogenetics

Virtually all cortical regions and layers receive inputs from cholinergic neurons originating in the nucleus basalis of Meynert, the substantia innominata, and the diagonal band of the basal forebrain (BF). Reflecting the seemingly diffuse organization of this projection system, functional conceptualizations traditionally have described acetylcholine (ACh) as a neuromodulator that influences broadly defined behavioral and cognitive processes such as wakefulness, arousal, and gating of input processing (1, 2). However, anatomical studies have revealed a topographic organization of BF cholinergic cell bodies with highly segregated cortical projection patterns (3–7). Such an anatomical organization favors hypotheses describing the cholinergic mediation of discrete cognitive-behavioral processes. Studies assessing the behavioral effects of cholinergic lesions, recording from or stimulating BF neurons in behaving animals have supported such hypotheses, proposing that cholinergic activity enhances sensory coding and mediates the ability of reward-predicting stimuli to control behavior (8–17).

In separate experiments using two different tasks, we reported the presence of phasic cholinergic release events (transients) in the medial prefrontal cortex (mPFC) of rodents trained to report the presence of cues (18, 19). These studies used choline-sensitive microelectrodes to measure changes in extracellular choline concentrations that reflect the hydrolysis of newly released ACh by endogenous acetylcholinesterase (*SI Results and Discussion*). Importantly, such cholinergic transients were not observed in trials in which cues were missed and in which the absence of a cue was correctly reported and rewarded. Cholinergic transients have thus been hypothesized to mediate the detection of cues, specifically defined as the cognitive process that generates a behavioral response by which subjects report the presence of a cue (20).

Here we used optogenetic methods to test the causal role of cortical cholinergic transients in cue detection (as defined above). We used a task that consisted of cued and noncued trials and rewarded correct responses for both trial types (hits and correct rejections). Incorrect responses (misses and false alarms, respectively) were not rewarded. We hypothesized that hit rates would be enhanced by generating transients in conjunction with cues, and that hit rates will be reduced by silencing cue-associated endogenous cholinergic signaling. We further reasoned that if cholinergic transients are a mediator of the cue detection response, generating such transients on noncued trials could force invalid detections (false alarms).

Phasic cholinergic activity was generated or silenced, in separate sessions, by photoactivation directed toward the cholinergic cell bodies of the BF or the cholinergic terminals locally in the right mPFC. The decision to target right mPFC was based on findings indicating that performance of the task used here enhances cholinergic function in the right, but not left, mPFC in mice (21) and activates right prefrontal regions in humans (19, 22). The present results support the hypothesis that the ability of cues to guide behavior is mediated by phasic cholinergic signaling. Particularly strong support for this hypothesis was obtained by the demonstration that, in the absence of cues, and thus of endogenous transients, photostimulation of either cholinergic soma in the BF or cholinergic terminals in the mPFC increased the number of invalid reports of cues (or false alarms).

Results

Optogenetic Generation of Cholinergic Transients. First we determined the optogenetic stimulation parameters required to generate choline currents with amplitudes that correspond with those observed in task-performing animals. Choline-acetyltransferase (ChAT)-Cre mice were virally transduced to express channelrhodopsin-2 (ChR2) in cholinergic neurons (Fig. 1 and Figs. S1–S3). Optical fibers were

Significance

Virtually the entire cortex is innervated by cholinergic projections from the basal forebrain. Traditionally, this neuronal system has been described as a neuromodulator system that supports global states such as cortical arousal. The presence of fast and regionally specific bursts in cholinergic neurotransmission suggests a more specialized role in cortical processing. Here we used optogenetic methods to investigate the capacity of phasic cholinergic signaling to control behavior. Our findings indicate a causal role of phasic cholinergic signaling in using external cues to guide behavioral choice. These findings indicate the significance of phasic cholinergic activity and also illustrate the potential impact of abnormal, phasic cholinergic neurotransmission on fundamental cognitive functions that involve cue-based behavioral decisions.

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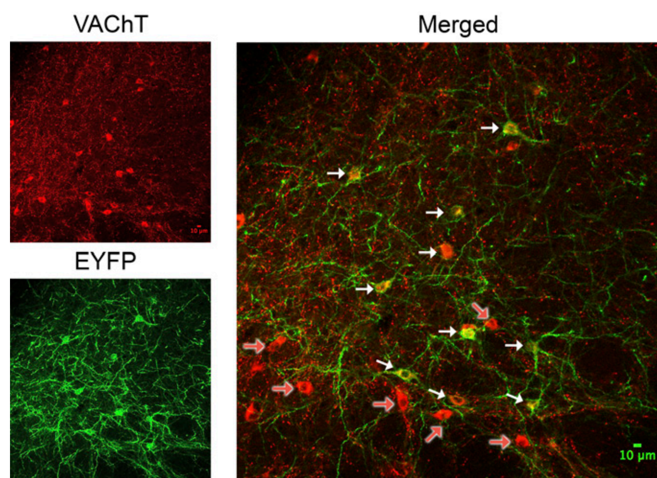

Fig. 1. Example of transfected cholinergic neurons in the basal forebrain expressing the reporter EYFP. The microphotographs show the middle slice of a confocal stack taken at the level of the ventral nucleus basalis of Meynert (coronal slice). Cholinergic neurons were visualized using an antibody against the vesicular acetylcholine transporter (VAChT; *SI Materials and Methods*; red; *Upper Left*). ChR2-H134R-EYFP–expressing neurons are in green (*Lower Left*). Merged microphotograph is on *Right*, with white arrows depicting VAChT+EYFP-immunopositive neurons and red arrows (on white contrast) depicting cholinergic neurons that were not transfected (10-µm scale inserted). Note that visualization of the colabeling of some neurons was outside this particular focal plane/slice but present in adjacent confocal slices. Neurons in the more ventral portion of this section were not transfected by the virus. The image represents the general finding that about two-thirds of cholinergic neurons in the nucleus basalis were transfected and that EYFP expression was restricted to cholinergic neurons (Figs. S1 and S3).

implanted into the right BF and the right mPFC, concurrent with a choline-sensitive microelectrode in the ipsilateral mPFC (Fig. 2 and Table S1). Because of headstage constraints, simultaneous electrochemical recording and optogenetic stimulation were performed in anesthetized mice. Cholinergic activation was induced through somatic or mPFC terminal stimulation in a series of sweeps across multiple laser intensities (5–25 mW) and durations (500 and 1,000 ms) and recorded in mPFC (Fig. 2). Control experiments confirmed that recorded currents reflected hydrogen peroxide resulting from enzymatic oxidation of choline (Fig. 2*B*).

Increasing BF stimulation power increased the amplitude of choline currents [$F(4,116) = 34.81$, $P < 0.001$; Fig. 2*D*]. Amplitudes did not vary by stimulation duration and the two factors did not interact [main effect of stimulation duration: $F(1,29) = 2.90$, $P = 0.10$, duration by power interaction: $F(4,116) = 2.07$, $P = 0.11$]. Compared with the shorter stimulation duration, 1,000-ms stimulation generated transients that peaked later [$F(1,29) = 94.40$, $P < 0.001$] and required more time to return within 50% of baseline [t_{50}; $F(1,29) = 73.32$, $P < 0.001$; Fig. 2*E*]. Stimulation power did not affect peak time [main effect of power: $F(4,116) = 2.00$, $P = 0.13$] or the rate of signal decay [main effect of power: $F(4,116) = 2.19$, $P = 0.08$]. The effects of stimulation power and duration did not interact for either measure [peak time, power by duration interaction: $F(4,116) = 1.05$, $P = 0.39$; decay rate, power by duration interaction: $F(4,116) = 0.24$, $P = 0.89$; for currents evoked by photostimulation in the mPFC see Fig. S4].

The amplitudes of endogenous cholinergic transients recorded during cue-hit trials corresponded most closely with those evoked by medium laser power (Fig. S5). However, endogenous transients rose and decayed more slowly than photostimulation-evoked transients, likely reflecting that that the dynamics of behavior-associated neurotransmitter release cannot be fully reproduced by optogenetic stimulation alone. Rather, endogenous transients likely reflect the activation of large neuronal networks that involve interactions with cholinergic neurons and are modulated by factors such as cortical state or top-down input. These conditions are not fully recreated by photostimulation of a specific neuronal population (see Fig. S6 for the impact of cortical state on transient characteristics). To assess the behavioral effects of a range of amplitudes of evoked cholinergic transients, the present behavioral experiments therefore systematically evaluated the behavioral effects of a wide range of stimulation power levels (5–25 mW).

Baseline Sustained Attention Task Performance by Chat$^{tm1(cre)}$ Mice. Chat$^{tm1(cre)}$ mice were trained to criterion on the operant sustained attention task (SAT) and then received bilateral infusions of adeno-associated virus (AAV) to induce expression of either ChR2-enhanced yellow fluorescent protein (EYFP), eNpHR3.0-EYFP [halorhodopsin (Halo)], or only EYFP in BF cholinergic neurons. Mice were next implanted with optic fibers bilaterally in the BF and unilaterally in right mPFC and then retrained to SAT criterion performance before tests of the effects of photoactivation on performance (Fig. 3*A*). As illustrated in Fig. 3*B*, the SAT consisted of cued and noncued (or blank) trials. Correct and rewarded responses were hits and correct rejections. Incorrect responses were misses and false alarms, respectively.

Baseline SAT performance, before optogenetic stimulation but after surgery, did not differ between mice previously infused with the three viral constructs [Fig. 3 *C* and *D*; main effects of group on hits and on false alarms; hits: $F(2,16) = 0.30$, $P = 0.74$; correct rejections: $F(2,16) = 0.52$, $P > 0.61$]. Hit rates were ~80% for the longest cue durations and 50% for the shortest cues, and mice correctly rejected about 75% of the noncued trials. Hits varied with cue duration [$F(2,32) = 64.593$, $P < 0.001$], but this effect did not differ between groups [group × duration: $F(4,32) = 0.287$, $P = 0.88$]. The relative number of errors of omissions did not vary between groups [$F(2,16) = 0.61$, $P = 0.56$; mean ± SEM: 14.46 ± 7.31%].

Photostimulation During Cued Trials Enhances Hits. Next we asked whether stimulation of BF cholinergic nuclei or mPFC terminals, coincident with cue presentation (Fig. 4*A*), increased the likelihood of hits. In all performance sessions, animals received trials paired with optogenetic stimulation and control trials without laser stimulation. BF ChR2 stimulation during the cue significantly increased hit rates [main effect of power; $F(5,40) = 5.20$, $P = 0.001$; Fig. 4*B*]. Moreover, the effects of power significantly interacted with cue duration [duration: $F(2,16) = 9.71$, $P = 0.002$; duration × power: $F(10,80) = 2.26$, $P = 0.03$]. Post hoc one-way ANOVAs indicated that increasing laser power resulted in increases in hits to shortest and medium-duration cues, but not to longest cues (Fig. 4*C*). Optical stimulation of mPFC cholinergic terminals alone did not significantly enhance hit rates [$F(5,40) = 1.87$, $P = 0.14$; Fig. 4*D*]. In contrast to the robust effects of laser stimulation in ChR2 mice, in control animals expressing EYFP alone, neither stimulation at the level of BF cholinergic neurons nor at cholinergic terminals in the mPFC (Fig. 4*E*) resulted in significant effects on hit rates [main effects of laser power BF: $F(5,10) = 1.40$, $P = 0.30$, mPFC: $F(5,20) = 0.75$, $P = 0.59$; for effects of photostimulation on response times for hits, see Fig. S7].

Photostimulation During Noncued Trials Enhances False Alarms. As a strong test of the hypothesis that cholinergic transients are causal in mediating cue detection, we tested whether generating transients on noncue trials, where transients do not occur (19), can force false alarms (Fig. 5). In mice expressing ChR2, the effects of photostimulation during noncued trials were profound. In the absence of stimulation, false alarm rates remained relatively low (<20%). Stimulation of either the BF or mPFC more than doubled false alarm rates [BF: $F(5,40) = 4.65$; $P = 0.002$; mPFC: $F(5,40) = 7.76$, $P < 0.001$; Fig. 5 *B* and *C*; for response times for false alarms, see Fig. S8]. In animals expressing only EYFP, neither bilateral BF nor mPFC laser activation during noncued trials affected the relative

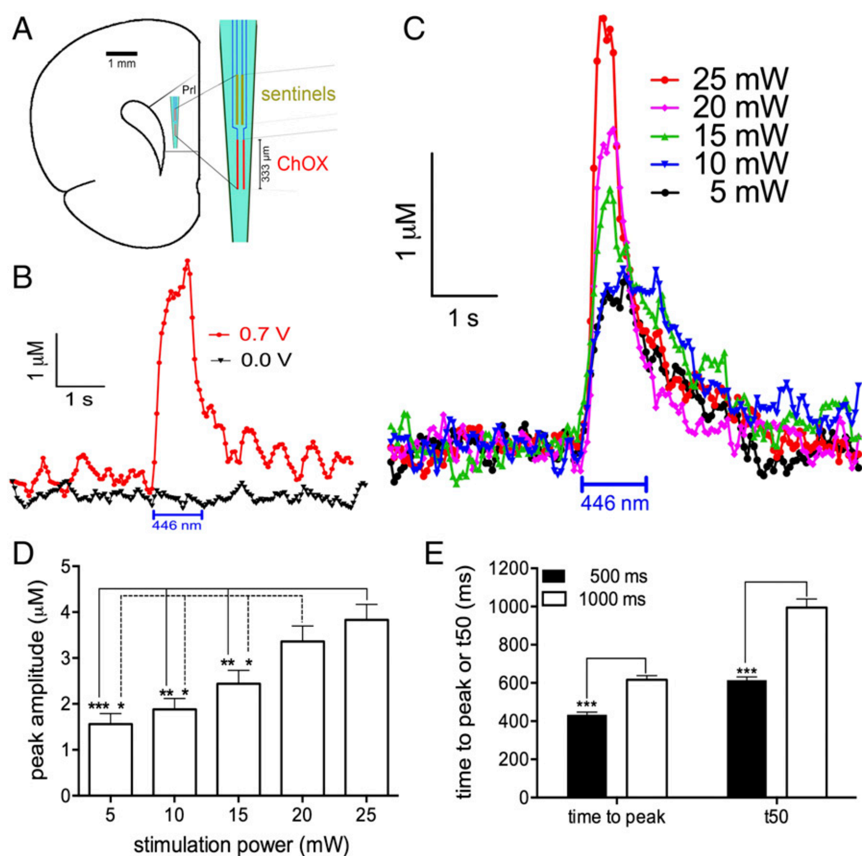

Fig. 2. Prefrontal choline currents, recorded using choline-sensitive microelectrodes, as a function of laser stimulation power and duration. (*A*) Electrode configuration and placement in the prelimbic (Prl) cortex. Choline oxidase (ChOX) was immobilized on two of four ceramic-based platinum recording sites. (*B*) Changing the applied potential of 0.7 V, the optimum oxidation potential of the reporter molecule H_2O_2 (red: vs. the reference electrode) to 0.00 V (black), eliminated optogenetically evoked currents, confirming the cholinergic basis of currents and controlling for potential confounds resulting from laser stimulation. (*C*) Mean choline currents from all trials evoked by stimulation of ChR2-expressing cholinergic neurons in the basal forebrain (BF; 5–25 mW; 1,000 ms). (*D*) Increasing stimulation power resulted in higher transient amplitudes (post hoc multiple comparisons: $*P < 0.05$; $**P < 0.01$, $***P < 0.001$). Amplitudes did not vary by stimulation duration, and the two factors did not interact. (*E*) Compared with the shorter stimulation period, 1,000-ms stimulation generated transients peaked later and required more time to return within 50% of baseline (see also Fig. S5; for cortically evoked currents, see Fig. S4; for the impact of cortical state on choline currents, see Fig. S6).

number of false alarms [BF: $F(5,10) = 0.72$, $P = 0.62$; mPFC: $F(5,20) = 1.37$, $P = 0.30$], supporting the interpretation that manipulation of BF cholinergic signaling led to the behavioral effects, as opposed to nonspecific byproducts of intracranial light delivery (Fig. 5 *D* and *E*; for response times, see Figs. S7 and S8).

We were concerned that photostimulation during noncued trials could generate an overall response bias favoring false alarms. To test this, we compared false alarm rates from nonstimulated, noncued trials, from within the laser stimulation test session, to false alarm rates from baseline performance. Nonstimulated false alarm rates were lower than at baseline [BF: $t(8) = 2.70$, $P = 0.03$; mPFC: $t(8) = 2.11$, $P = 0.07$; baseline: 20.38 ± 2.20%; mPFC: 15.59 ± 3.28%; BF: 15.02 ± 3.16%]. These false alarm rates also paralleled levels seen in EYFP control mice from their corresponding stimulation test sessions (Fig. 5 *D* and *E*). Thus, rather than developing a riskier bias toward indicating that a cue had occurred, mice adopted a more conservative criterion during sessions with laser stimulation.

Control Analyses of Potential Carryover Effects of Photostimulation. ChR2 stimulation in the presence and absence of cues enhanced the relative number of hits and false alarms, respectively. Several control analyses were conducted to determine whether these effects were associated with a more generalized shift in the animals' task-performance strategy. As already detailed above, enhancing false alarm rates with optical stimulation of ChR2 did not increase the relative number of false alarms on trials without laser stimulation.

We also compared hits and omissions on nonstimulated trials to prestimulation baseline performance levels. Hit rates did not differ between baseline and nonstimulation trials during BF stimulation test days [main effect of test session, $F(1,8) = 1.71$, $P = 0.23$] or on PFC stimulation test days [$F(1,8) = 1.41$, $P = 0.27$]. There was also no interaction with the effects of test day and cue duration [BF: $F(2,16) = 0.06$, $P = 0.92$; PFC: $F(2,16) = 0.57$, $P = 0.51$]. We next analyzed animals' performance on nonstimulated trials from each laser stimulation session, within each group (EYFP, ChR2, and Halo) using a repeated-measures ANOVA with a within-subjects factor of day. Performance on nonstimulation trials did not vary across stimulation days within any group (EYFP, ChR2, Halo; all $P > 0.10$). A follow-up analysis compared data from the nonstimulation condition across groups to further explore any potential differences in their baseline performance. This analysis was conducted using a one-way ANOVA with a between-subjects factor of group (EYFP, ChR2, Halo). Nonstimulation trial performance did not differ between groups for BF stimulation test days [main effect of group on hits: $F(2,16) = 0.34$, $P = 0.72$, false alarms: $F(2,16) = 1.10$, $P = 0.36$] or PFC stimulation test days [main effect of group on hits: $F(2,18) = 0.26$, $P = 0.77$, false alarms: $F(2,18) = 1.69$, $P = 0.22$].

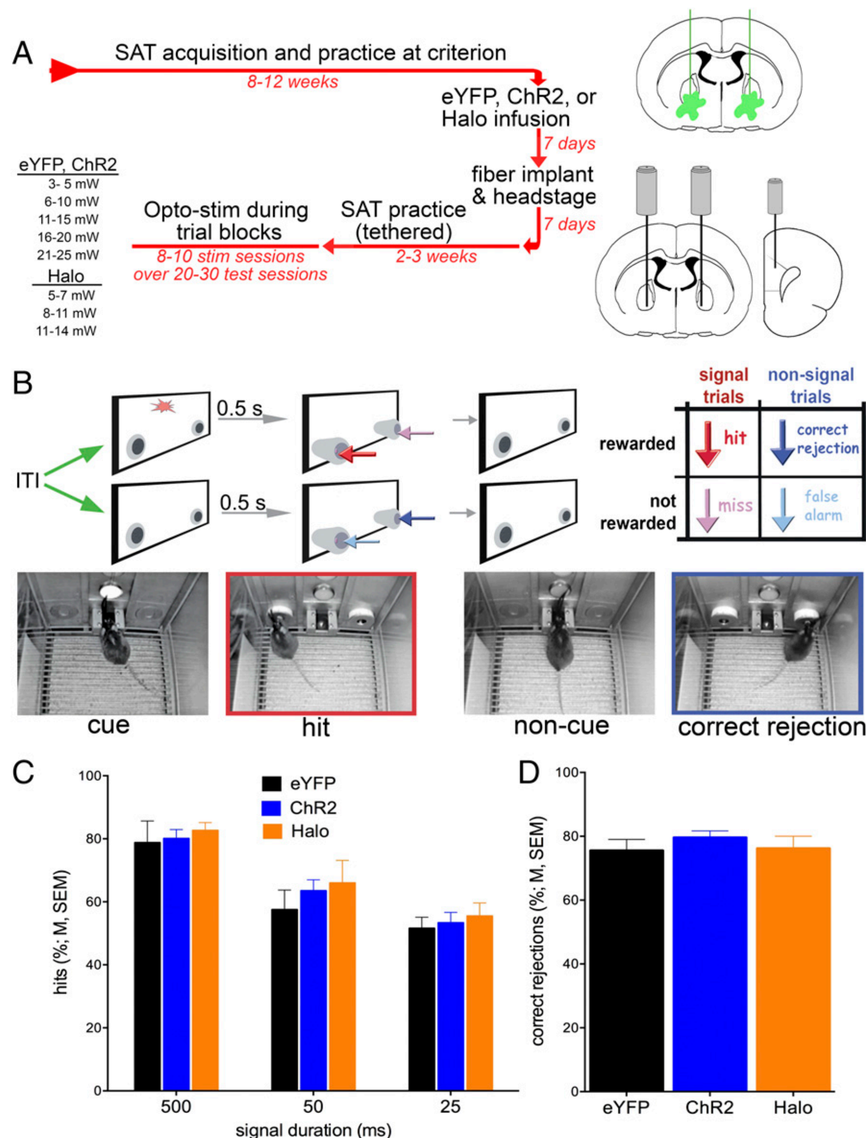

Fig. 3. Timeline of major experimental events, task trial types, and baseline performance. (*A*) ChAT-Cre mice first acquired the SAT over 8–12 wk. Thereafter, they received bilateral infusions of one of the virus constructs into the BF (*Upper Right*). Seven days later, optic fibers were implanted into the BF and mPFC. Mice resumed task practice while tethered for 2–3 wk. The effects of optical stimulation across various stimulation intensities were tested in 8–10 sessions over the next 20–30 d with tethered nonstimulation days intermixed (*Left*). (*B*) The task consisted of a random order of cued and noncued trials. Following either event, two nose-poke devices extended into the chambers and were retracted upon a nose-poke or following 4 s. Hits and correct rejections were rewarded with water, whereas misses and false alarms were not (*Right Inset*; arrows in the inset and depicting nose-poke selection are color-matched; half of the mice were trained with the nose-poke direction rules reversed). Following an intertrial interval of 12 ± 3 s, the next cue or noncue event commenced. The photographic inserts show a cue presentation with a mouse orienting toward the intelligence panel while positioned at the water port (*Left*), a subsequent hit, a noncue event, and a subsequent correct rejection (*Right*). (*C* and *D*) Baseline SAT performance during tethering by groups of mice to be infused with one of the three virus constructs (n = 9 ChR2, n = 5 Halo, n = 5 EYFP). Mice detected cues in a cue duration-dependent manner (*C*) and they correctly rejected <75% of noncue events (*D*). Performance did not differ between the three groups (see *Results* for statistical analyses).

A final control analysis used the performance data of ChR2 animals tested using the block design version of the task (*SI Materials and Methods*). This analysis allowed us to assess the possibility that photostimulation on a particular trial type within a block of trials biased performance on a subsequent block of trials without stimulation. Specifically, we compared hit and false alarm rates in the prestimulation block to hit and false alarm rates in the poststimulation block. Photoactivation of noncued trials, thus inducing false alarms, had no impact on hit rates in the poststimulation performance block [BF: $t(1) = 0.79$, $P = 0.58$; PFC: $t(1) = 0.001$, $P = 0.99$]. Similarly, photoactivation on cued trials, thus evoking increases in hit rates, had no impact on false alarm rates in the poststimulation performance block [BF: $t(1) = -3.01$, $P = 0.20$; PFC: $t(1) = 0.20$, $P = 0.87$]. Combined, the results from our control analyses suggest that carryover effects did not contribute to the behavioral impact of laser stimulation. Rather, the impact of transiently manipulating cholinergic activity was specific to the trial in which the manipulation occurred.

Photoinhibition During Cued Trials Reduces Hits. In the final series of experiments, we expressed Halo in BF cholinergic neurons to test the hypothesis that silencing endogenous ACh transients coincident with cue presentation would decrease hit rates. To ensure robust attenuation, photoinhibition began 50 ms before

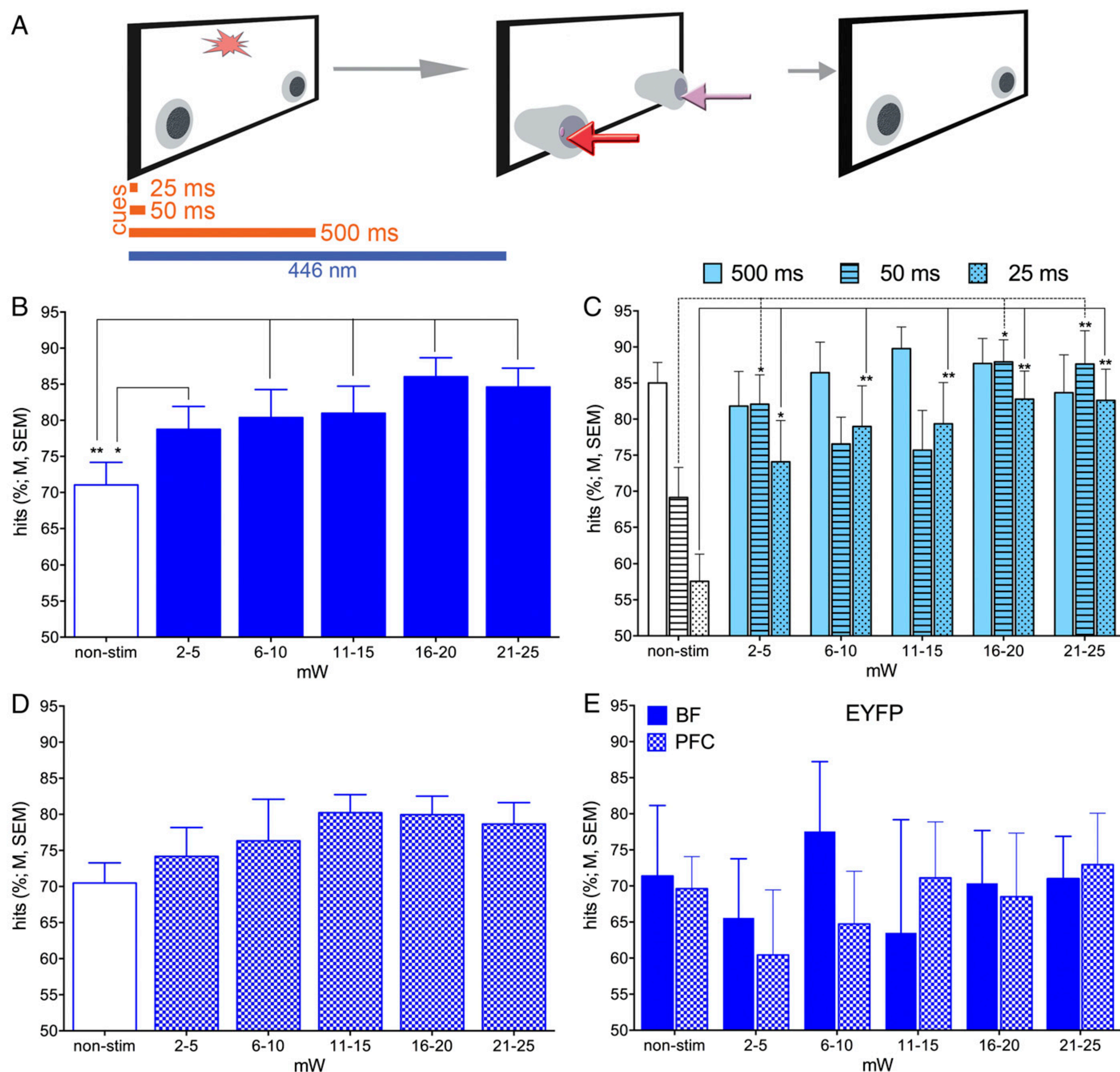

Fig. 4. Optogenetic stimulation of cholinergic neurons during cued trials (n = 9 ChR2 mice). (*A*) The onset of the blue light coincided with cue onset and light was terminated 1,000 ms later. (*B*) Hit rates, averaged over cue durations, increased in response to BF stimulation of ChR2-expressing cholinergic neurons. (*C*) The effects of power significantly interacted with cue duration, reflecting significant increases in hits to shortest and medium-duration cues. Post hoc one-way ANOVAs indicated that by increasing power, and thus the amplitude of evoked release, stimulation resulted in increases in hits to shortest and medium-duration cues, but not to longest cues (post hoc comparisons: $*P < 0.05$; $**P < 0.01$). (*D*) ChR2 stimulation in mPFC did not significantly affect hit rates. (*E*) Neither BF stimulation (n = 3) nor mPFC stimulation (n = 5) affected the hit rates in EYFP-expressing control mice.

cue onset and remained on through the entire cue period (Fig. 6*A*). BF activation in Halo-expressing mice decreased hit rates, with increasing laser power producing greater effects [$F(3,12) = 4.94$, $P = 0.02$; Fig. 6*B*; for response times, see Fig. S9]. Although the effect of Halo activation appeared most robust for hits to longest cues, the interaction between cue duration and laser power did not reach statistical significance [main effect of cue duration: $F(2,8) = 6.27$, $P = 0.03$; cue × power: $F(6,24) = 1.72$, $P = 0.19$; Fig. 6*B*]. The hit-reducing effect of Halo BF activation during cued trials did not influence noncued trial performance [false alarms: $F(3,12) = 1.66$, $P = 0.25$; Fig. 6*C*], and it did not increase the rate of omissions [$F(3,12) = 0.47$, $P = 0.71$; Fig. 6*D*]. mPFC activation of Halo was insufficient to affect hit rates [main effect of power: $F(3,16.72) = 0.12$, $P = 0.95$, and interaction with duration: $F(6,15.79) = 0.48$, $P = 0.82$; Fig. 6*E*].

Discussion

Cortical cholinergic activity is critically involved in sensory perception and attention (9, 13, 23, 24). Here we demonstrate that ACh signaling is an essential mechanism mediating the utilization of environmental stimuli to guide behavior. Optogenetically

Fig. 5. Effects of optogenetic activation of cholinergic neurons on noncued trials ($n = 9$ ChR2 mice). (*A*) On noncued trials, laser stimulation began 1,000 ms before, and ended coincident with, extension of the nose-poke devices into the operant chamber. Increasing levels of stimulation power systematically enhanced false alarms when applied bilaterally to the BF (*B*) or unilaterally just to the right mPFC (*C*; post hoc comparisons: $^{*}P < 0.05$; $^{**}P < 0.01$, $^{***}P$,0.001). In control animals expressing EYFP, neither BF (*D*; $n = 3$) nor mPFC stimulation (*E*; $n = 5$) significantly affected the false alarm rates.

generated cholinergic transients enhanced the detection of cues and, if generated during noncued trials, increased the rate of false alarms. Conversely, silencing cholinergic activity resulted in misses of long, salient cues that are normally detected (19). Generating cholinergic transients in the mPFC was sufficient to cause false alarms in the absence of a cue while enhancing or suppressing hits required bidirectional modulation of cholinergic activity in more widespread BF projection fields.

Trial-to-Trial–Based Decisions and Support for a Cholinergic Basis of Photostimulation Effects. Evoking cholinergic activity increased the probability of reporting the presence of a cue, even in noncued

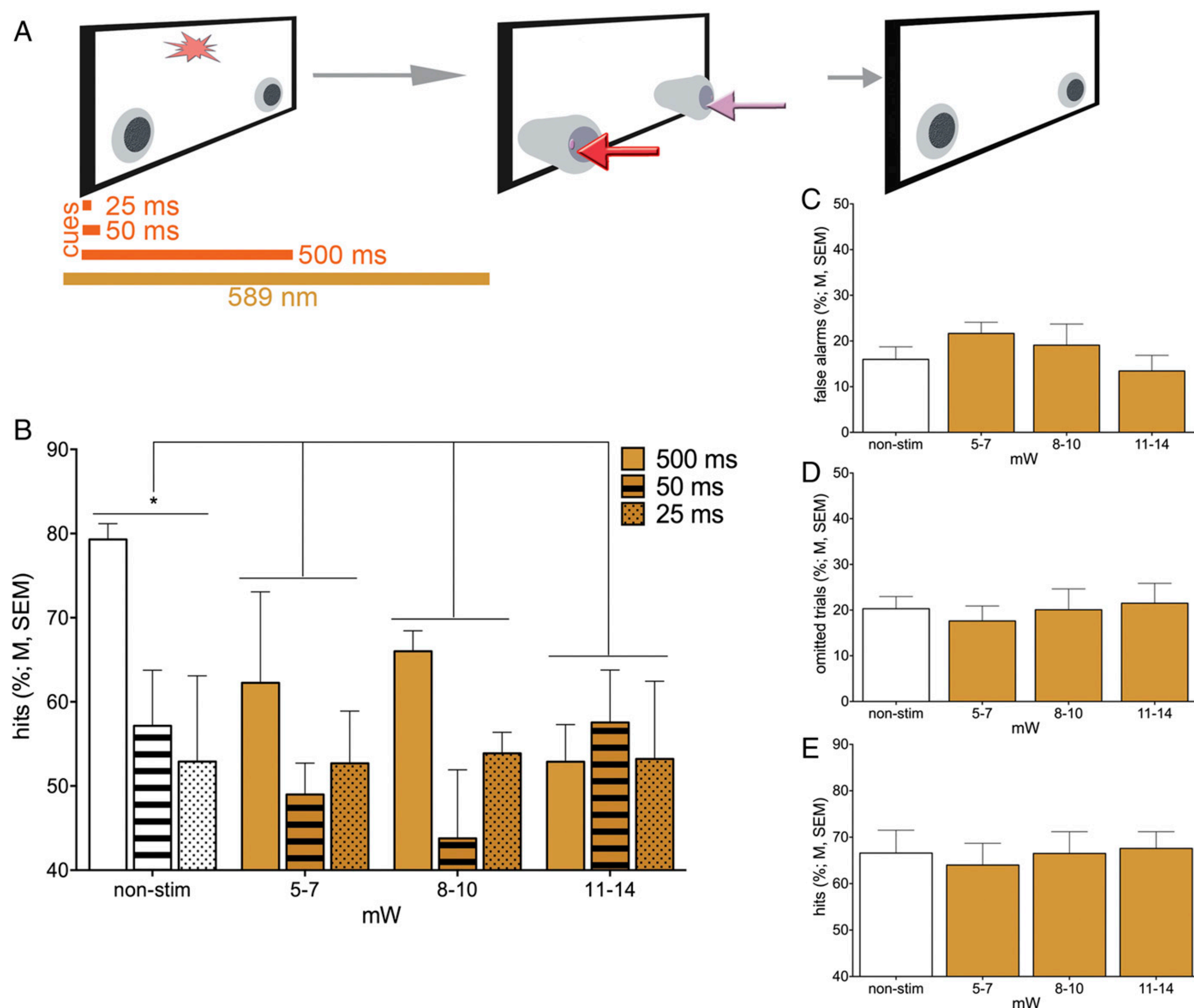

Fig. 6. Suppression of cholinergic activity on cued trials. (*A*) The 589-nm laser was turned on 50 ms before the onset of the cue to fully suppress endogenous cholinergic signaling. (*B*) BF stimulation in Halo-expressing mice ($n = 5$) decreased hit rates, with increasing laser power producing greater effects. Although the effect of Halo stimulation appeared most robust for hits to longest cues, the interaction between cue duration and laser power did not reach statistical significance (post hoc comparisons of main effect of power: $*P < 0.05$). Halo BF stimulation neither affected the relative number of false alarms (*C*) nor omissions (*D*). Halo stimulation in the mPFC did not affect hit rates (*E*).

trials. The task used in the present experiments included a random sequence of cued and noncued trials and both hits and correct rejections were rewarded. These factors allowed us to attribute the behavioral effects of photostimulation to individual trials and further to exclude potential carryover effects or cue reporting biases. Because correct responses on cued and noncued trials (hits, correct rejections) were rewarded equally, the effects of evoked cholinergic activity cannot be attributed to reward contingencies. A role of reward was also rejected in our previous electrochemical recording studies (18, 19). Furthermore, we found no evidence to suggest that photostimulation impacted the performance on nonstimulated trials. Combined, these findings reject an interpretation of stimulation effects in terms of generalized decision or response biases, the altering of value representations, or other processes that could generally alter the threshold for indicating the presence of a cue.

Current technical limitations did not allow recording cholinergic transients in conjunction with optogenetic stimulation in task-performing mice. Thus, the interpretation of the present behavioral effects, in terms of being mediated by cholinergic activity, is derived from evidence of the effects of cholinergic photostimulation in anesthetized mice (Fig. 2 and Figs. S3–S6) and the cholinergic transients previously recorded in animals performing cue detection tasks (18, 19). Despite these limitations, the selectivity of the behavioral effects suggests that our stimulation parameters evoked biologically relevant ACh release. Specifically, increasing laser power, and therefore the amplitude of cholinergic transients, produced larger behavioral effects. ChR2 stimulation during cued trials preferentially enhanced the hits to short- and medium-duration cues; such cues are missed at higher rates and thus are less likely to be associated with cholinergic transients. Increasing laser power resulted in higher hit rates specifically to these cues. Conversely, silencing cholinergic activity reduced hit rates to long cues, consistent with effects of cholinergic lesions (9). Taken together, these findings indicate systematic relationships between the salience of cues and photostimulation

power and, by extrapolation, the amplitudes of evoked and suppressed transients.

We cannot exclude the possibility that optogenetic stimulation triggered corelease of other neurotransmitters from cholinergic terminals (25), or that mechanisms secondary to cholinergic stimulation are essential for mediating the behavioral effects described here (26). However, in addition to results from our electrochemical recordings studies (18, 19), a considerable literature on the effects of pharmacological manipulations of the cholinergic system on attentional performance in animals and humans (27–29) is consistent with the present attribution of a cholinergic mechanism underlying the effects of optogenetic stimulation on cue detection processes (as defined in the introduction).

BF vs. mPFC Stimulation Effects. Bilateral BF stimulation increased hits and false alarms, while bilateral BF inhibition reduced hit rates. Right mPFC stimulation alone enhanced only false alarms. Thus, mPFC cholinergic stimulation was sufficient to modulate behavior only in the condition where endogenous transients are not normally observed (19). A single transient increase in ACh release, in a context where such release is not typically evoked, may yield a greater impact of cholinergic signaling compared with the effects of evoking transients that converge with endogenous release (cued trials). Furthermore, prior studies indicated that cholinergic activity across fronto-parietal networks contributes to cue-detection performance (11, 30); thus, enhancing and reducing hit rates may require manipulation of more widespread endogenous cholinergic activity, as resulting from BF photostimulation. An evoked, or mis-timed, cholinergic transient in the mPFC alone may be capable of sufficiently recruiting mPFC circuitry (31) and fronto-parietal networks (32) to force cue-directed behavior in select cognitive-neuronal contexts not normally associated with increased cholinergic input.

Cognitive-Neuronal Mechanisms and Relevance for Disorders. Precisely timed cholinergic transients could be essential for synchronizing cortical neuronal output driven by salient cues, thereby coordinating local network activity recruited by a cue (33, 34). Moreover, the selection of cues for guiding the behavior per se may be mediated by fronto-visual oscillations that are generated by trial-based, phasic cholinergic signaling (35). Such neuronal coordination through coherence therefore could be necessary for the engagement of a particular motor plan for executing selected cue-guided responses.

The neuronal mechanisms underlying the generation of cortical cholinergic transients are not well understood but involve glutamatergic signaling from thalamic afferents (36). Our current circuitry model suggests that separate cholinergic neuromodulatory activity, acting on a scale of minutes, can influence the generation of transients via stimulation of nicotinic ACh receptors expressed by thalamic glutamatergic terminals (37, 38). Cholinergic neuromodulation varies as a function of levels of top-down attentional control and is driven in part by mesolimbic activity (39). This hypothesis predicts that the loss of reward caused by distractors and associated error rates alters the likelihood for, and perhaps also the dynamics of, cholinergic transients. It also predicts that in subjects with reduced motivation to perform, or otherwise aberrant mesolimbic signaling, the presence and timing of transients could be sufficiently altered to yield impaired cognitive performance, including the maladaptive learning of cue-reward relationships (17).

Our demonstration of increases in false alarms, resulting from ill-timed cholinergic transients generated during noncued trials, illustrates the potential role of cholinergic dysregulation in the perceptual and cognitive impairments of neuropsychiatric and neurodegenerative disorders (40–42). Relatively subtle perturbations of the dynamics of cholinergic transients may alter large-scale network operations, such as fronto-parietal oscillatory activity (43), and thereby cause invalid perceptions and cue-oriented behavior (44, 45).

Conclusion

The cortical cholinergic input system has long been hypothesized to contribute to attentional function (46). Here we generated and silenced cholinergic transients using optogenetic methods in mice performing a task consisting of both cued and noncued trials. Our results suggest that generating cholinergic transients enhances the likelihood of reporting cues and, if generated during noncued trials, the invalid reporting of cues. Our findings expand traditional hypotheses of cholinergic functions by specifying that phasic, transient cholinergic signaling is essential for executing selected cue-guided behavior and by illustrating how dysregulated transients may cause invalid processing of cues.

Materials and Methods

Detailed materials and methods are provided in *SI Materials and Methods*, and additional results and discussion are provided in *SI Results and Discussion*. Briefly, ChAT-Cre male and female mice (3–4 mo old) were used in this study. All procedures were approved by the University of Michigan Committee on Use and Care of Animals and conducted in laboratories accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Cre-recombinase-dependent viruses encoding channelrhodopsin-2 (ChR2-H134R-EYFP), halorhodopsin (eNpHR3.0-EYFP), or EYFP alone, were made available from Karl Deisseroth (Stanford University, Stanford, CA). Transfection using AAV is detailed in *SI Materials and Methods*. Choline-selective biosensors and fixed-potential amperometry were used to measure changes in extracellular choline concentrations that reflect choline resulting from hydrolysis of newly released ACh and the oxidation of the reporter molecule H_2O_2 (47–49). Optical stimulation was achieved via a blue laser diode coupled to a fiber optic cable and modulated via a custom written software package to control laser parameters including duration and intensity. Animals underwent a total of 4–6 mo of training of the operant sustained attention task (SAT) (50, 51) with the oldest mouse being ∼9 mo old at study conclusion. Once animals achieved performance criterion they were randomly selected to undergo AAV infusions for one of three possible constructs and underwent surgery for optic fiber placement. Mice expressing EYFP or ChR2-EYFP practiced SAT sessions to determine the effects of light alone (EYFP) or photoactivation of cholinergic neurons on behavior at five intensities. In mice expressing eNpHR3.0-EYFP, effects of photosuppression on behavior were measured across three intensities ranges. Following completion of these experiments, histological analyses verified that expression of a viral reporter was limited to cells also expressing ChAT or vesicular choline transporter (VAChT). Statistical analyses were carried out to determine differences in performance associated with cholinergic activation or cholinergic suppression relative to within session performance and across groups. Statistical analyses were performed using ANOVAs and linear mixed models. Covariance structures were selected based on Akaike's information criterion (52). The α was set at 0.05, and exact P values were reported (53).

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1. Wenk GL (1997) The nucleus basalis magnocellularis cholinergic system: One hundred years of progress. *Neurobiol Learn Mem* 67(2):85–95.
2. Varela C (2013) The gating of neocortical information by modulators. *J Neurophysiol* 109(5):1229–1232.
3. Zaborszky L (2002) The modular organization of brain systems. Basal forebrain: The last frontier. *Prog Brain Res* 136:359–372.
4. Zaborszky L, et al. (2015) Neurons in the basal forebrain project to the cortex in a complex topographic organization that reflects corticocortical connectivity patterns: An experimental study based on retrograde tracing and 3D reconstruction. *Cereb Cortex* 25(1):118–137.
5. Muñoz W, Rudy B (2014) Spatiotemporal specificity in cholinergic control of neocortical function. *Curr Opin Neurobiol* 26:149–160.
6. Bloem B, et al. (2014) Topographic mapping between basal forebrain cholinergic neurons and the medial prefrontal cortex in mice. *J Neurosci* 34(49):16234–16246.
7. Zaborszky L, van den Pol A, Gyengesi E (2012) The basal forebrain cholinergic projection system in mice. *The Mouse Nervous System*, eds Watson C, Paxinos G, Puelles L (Elsevier, New York), pp 684–718.
8. Voytko ML, et al. (1994) Basal forebrain lesions in monkeys disrupt attention but not learning and memory. *J Neurosci* 14(1):167–186.

9. McGaughy J, Kaiser T, Sarter M (1996) Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: Selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. *Behav Neurosci* 110(2):247–265.
10. Dalley JW, et al. (2004) Cortical cholinergic function and deficits in visual attentional performance in rats following 192 IgG-saporin-induced lesions of the medial prefrontal cortex. *Cereb Cortex* 14(8):922–932.
11. Bucci DJ, Holland PC, Gallagher M (1998) Removal of cholinergic input to rat posterior parietal cortex disrupts incremental processing of conditioned stimuli. *J Neurosci* 18(19):8038–8046.
12. Goard M, Dan Y (2009) Basal forebrain activation enhances cortical coding of natural scenes. *Nat Neurosci* 12(11):1444–1449.
13. Pinto L, et al. (2013) Fast modulation of visual perception by basal forebrain cholinergic neurons. *Nat Neurosci* 16(12):1857–1863.
14. Picciotto MR, Higley MJ, Mineur YS (2012) Acetylcholine as a neuromodulator: Cholinergic signaling shapes nervous system function and behavior. *Neuron* 76(1):116–129.
15. Peck CJ, Salzman CD (2014) The amygdala and basal forebrain as a pathway for motivationally guided attention. *J Neurosci* 34(41):13757–13767.
16. Ljubojevic V, Luu P, De Rosa E (2014) Cholinergic contributions to supramodal attentional processes in rats. *J Neurosci* 34(6):2264–2275.
17. Hangya B, Ranade SP, Lorenc M, Kepecs A (2015) Central cholinergic neurons are rapidly recruited by reinforcement feedback. *Cell* 162(5):1155–1168.
18. Parikh V, Kozak R, Martinez V, Sarter M (2007) Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron* 56(1):141–154.
19. Howe WM, et al. (2013) Prefrontal cholinergic mechanisms instigating shifts from monitoring for cues to cue-guided performance: Converging electrochemical and fMRI evidence from rats and humans. *J Neurosci* 33(20):8742–8752.
20. Posner MI, Snyder CR, Davidson BJ (1980) Attention and the detection of signals. *J Exp Psychol* 109(2):160–174.
21. Parikh V, St Peters M, Blakely RD, Sarter M (2013) The presynaptic choline transporter imposes limits on sustained cortical acetylcholine release and attention. *J Neurosci* 33(6):2326–2337.
22. Demeter E, Hernandez-Garcia L, Sarter M, Lustig C (2011) Challenges to attention: A continuous arterial spin labeling (ASL) study of the effects of distraction on sustained attention. *Neuroimage* 54(2):1518–1529.
23. Herrero JL, et al. (2008) Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. *Nature* 454(7208):1110–1114.
24. Botly LCP, De Rosa E (2009) Cholinergic deafferentation of the neocortex using 192 IgG-saporin impairs feature binding in rats. *J Neurosci* 29(13):4120–4130.
25. Saunders A, Granger AJ, Sabatini BL (2015) Corelease of acetylcholine and GABA from cholinergic forebrain neurons. *eLife* 4, 10.7554/eLife.06412.
26. Lin S-C, Gervasoni D, Nicolelis MAL (2006) Fast modulation of prefrontal cortex activity by basal forebrain noncholinergic neuronal ensembles. *J Neurophysiol* 96(6):3209–3219.
27. Furey ML, Pietrini P, Haxby JV, Drevets WC (2008) Selective effects of cholinergic modulation on task performance during selective attention. *Neuropsychopharmacology* 33(4):913–923.
28. Vangkilde S, Bundesen C, Coull JT (2011) Prompt but inefficient: Nicotine differentially modulates discrete components of attention. *Psychopharmacology (Berl)* 218(4):667–680.
29. Bentley P, Driver J, Dolan RJ (2011) Cholinergic modulation of cognition: Insights from human pharmacological functional neuroimaging. *Prog Neurobiol* 94(4):360–388.
30. Broussard JI, Karelina K, Sarter M, Givens B (2009) Cholinergic optimization of cue-evoked parietal activity during challenged attentional performance. *Eur J Neurosci* 29(8):1711–1722.
31. Gill TM, Sarter M, Givens B (2000) Sustained visual attention performance-associated prefrontal neuronal activity: Evidence for cholinergic modulation. *J Neurosci* 20(12):4745–4757.
32. Nelson CL, Sarter M, Bruno JP (2005) Prefrontal cortical modulation of acetylcholine release in posterior parietal cortex. *Neuroscience* 132(2):347–359.
33. Gregoriou GG, Gotts SJ, Zhou H, Desimone R (2009) High-frequency, long-range coupling between prefrontal and visual cortex during attention. *Science* 324(5931):1207–1210.
34. Leventhal DK, et al. (2012) Basal ganglia beta oscillations accompany cue utilization. *Neuron* 73(3):523–536.
35. Bauer M, et al. (2012) Cholinergic enhancement of visual attention and neural oscillations in the human brain. *Curr Biol* 22(5):397–402.
36. Parikh V, Man K, Decker MW, Sarter M (2008) Glutamatergic contributions to nicotinic acetylcholine receptor agonist-evoked cholinergic transients in the prefrontal cortex. *J Neurosci* 28(14):3769–3780.
37. Parikh V, Ji J, Decker MW, Sarter M (2010) Prefrontal beta2 subunit-containing and alpha7 nicotinic acetylcholine receptors differentially control glutamatergic and cholinergic signaling. *J Neurosci* 30(9):3518–3530.
38. Hasselmo ME, Sarter M (2011) Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology* 36(1):52–73.
39. St Peters M, Demeter E, Lustig C, Bruno JP, Sarter M (2011) Enhanced control of attention by stimulating mesolimbic-corticopetal cholinergic circuitry. *J Neurosci* 31(26):9760–9771.
40. Kapur S (2003) Psychosis as a state of aberrant salience: A framework linking biology, phenomenology, and pharmacology in schizophrenia. *Am J Psychiatry* 160(1):13–23.
41. Sarter M, Lustig C, Taylor SF (2012) Cholinergic contributions to the cognitive symptoms of schizophrenia and the viability of cholinergic treatments. *Neuropharmacology* 62(3):1544–1553.
42. Higley MJ, Picciotto MR (2014) Neuromodulation by acetylcholine: Examples from schizophrenia and depression. *Curr Opin Neurobiol* 29:88–95.
43. Vossel S, et al. (2014) Cholinergic stimulation enhances Bayesian belief updating in the deployment of spatial attention. *J Neurosci* 34(47):15735–15742.
44. Spencer KM, et al. (2004) Neural synchrony indexes disordered perception and cognition in schizophrenia. *Proc Natl Acad Sci USA* 101(49):17288–17293.
45. Uhlhaas PJ, Singer W (2012) Neuronal dynamics and neuropsychiatric disorders: Toward a translational paradigm for dysfunctional large-scale networks. *Neuron* 75(6):963–980.
46. Everitt BJ, Robbins TW (1997) Central cholinergic systems and cognition. *Annu Rev Psychol* 48:649–684.
47. Parikh V, et al. (2004) Rapid assessment of in vivo cholinergic transmission by amperometric detection of changes in extracellular choline levels. *Eur J Neurosci* 20(6):1545–1554.
48. Giuliano C, Parikh V, Ward JR, Chiamulera C, Sarter M (2008) Increases in cholinergic neurotransmission measured by using choline-sensitive microelectrodes: Enhanced detection by hydrolysis of acetylcholine on recording sites? *Neurochem Int* 52(7):1343–1350.
49. Sarter M, Kim Y (2015) Interpreting chemical neurotransmission in vivo: Techniques, time scales, and theories. *ACS Chem Neurosci* 6(1):8–10.
50. Demeter E, Sarter M, Lustig C (2008) Rats and humans paying attention: Cross-species task development for translational research. *Neuropsychology* 22(6):787–799.
51. St Peters M, Cherian AK, Bradshaw M, Sarter M (2011) Sustained attention in mice: Expanding the translational utility of the SAT by incorporating the Michigan Controlled Access Response Port (MICARP). *Behav Brain Res* 225(2):574–583.
52. Verbeke G, Molenberghs G (2009) *Linear Mixed Models for Longitudinal Data* (Springer, New York).
53. Greenwald AG, Gonzalez R, Harris RJ, Guthrie D (1996) Effect sizes and p values: What should be reported and what should be replicated? *Psychophysiology* 33(2):175–183.
54. Zhang H, Lin S-C, Nicolelis MAL (2009) Acquiring local field potential information from amperometric neurochemical recordings. *J Neurosci Methods* 179(2):191–200.
55. McGaughy J, Sarter M (1995) Behavioral vigilance in rats: Task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. *Psychopharmacology (Berl)* 117(3):340–357.
56. Aravanis AM, et al. (2007) An optical neural interface: In vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. *J Neural Eng* 4(3):S143–S156.
57. Stark E, Koos T, Buzsáki G (2012) Diode probes for spatiotemporal optical control of multiple neurons in freely moving animals. *J Neurophysiol* 108(1):349–363.
58. Bass CE, et al. (2013) Optogenetic stimulation of VTA dopamine neurons reveals that tonic but not phasic patterns of dopamine transmission reduce ethanol self-administration. *Front Behav Neurosci* 7:173.
59. Clement EA, et al. (2008) Cyclic and sleep-like spontaneous alternations of brain state under urethane anaesthesia. *PLoS One* 3(4):e2004.
60. Duque A, Balatoni B, Detari L, Zaborszky L (2000) EEG correlation of the discharge properties of identified neurons in the basal forebrain. *J Neurophysiol* 84(3):1627–1635.
61. Wolansky T, Clement EA, Peters SR, Palczak MA, Dickson CT (2006) Hippocampal slow oscillation: Anovel EEG state and its coordination with ongoing neocortical activity. *J Neurosci* 26(23):6213–6229.
62. Manns ID, Alonso A, Jones BE (2000) Discharge profiles of juxtacellularly labeled and immunohistochemically identified GABAergic basal forebrain neurons recorded in association with the electroencephalogram in anesthetized rats. *J Neurosci* 20(24):9252–9263.
63. Vandecasteele M, et al. (2014) Optogenetic activation of septal cholinergic neurons suppresses sharp wave ripples and enhances theta oscillations in the hippocampus. *Proc Natl Acad Sci USA* 111(37):13535–13540.
64. Chen N, Sugihara H, Sur M (2015) An acetylcholine-activated microcircuit drives temporal dynamics of cortical activity. *Nat Neurosci* 18(6):892–902.