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Prefrontal Acetylcholine Release Controls Cue Detection on Multiple Timescales

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SUMMARY

Cholinergic neurons originating from the basal forebrain innervate the entire cortical mantle. Choline-sensitive microelectrodes were used to measure the synaptic release of cortical acetylcholine (ACh) at a subsecond resolution in rats performing a task involving the detection of cues. Cues that were detected, defined behaviorally, evoked transient increases in cholinergic activity (at the scale of seconds) in the medial prefrontal cortex (mPFC), but not in a nonassociational control region (motor cortex). In trials involving missed cues, cholinergic transients were not observed. Cholinergic deafferentation of the mPFC, but not motor cortex, impaired cue detection. Furthermore, decreases and increases in precue cholinergic activity predicted subsequent cue detection or misses, respectively. Finally, cue-evoked cholinergic transients were superimposed over slower (at the timescale of minutes) changes in cholinergic activity. Cortical cholinergic neurotransmission is regulated on multiple timescales to mediate the detection of behaviorally significant cues and to support cognitive performance.

INTRODUCTION

Attentional capacities and mechanisms, such as the sustained readiness for input processing, the ability to monitor and discriminate between multiple stimulus sources and modalities, and associated executive processes (such as response selection, error detection, and effortful control) collectively determine the efficacy with which stimuli control behavior. Ascending neuronal projection systems, particularly the cholinergic and noradrenergic projections arising from basal forebrain and brainstem areas, respectively, have been proposed to contribute to attentional performance by modulating the processing of information in the fronto-parietal attentional network (Aston-Jones and Cohen, 2005; Everitt and Robbins, 1997; Hasselmo and McGaughy, 2004; Mesulam, 1990; Posner and Dehaene, 1994; Sarter et al., 2005a, 2006). The persistent attentional impairments that result from lesions of the basal forebrain or the selective removal of the cortical cholinergic input system indicated the necessary role of this neuromodulator for attentional performance (Chiba et al., 1995; Dalley et al., 2004; McGaughy et al., 1996, 2000, 2002; Muir et al., 1992, 1994; Turchi and Sarter, 1997; Voytko et al., 1994). Furthermore, studies measuring acetylcholine (ACh) release using microdialysis revealed increases in cortical ACh release, specifically in association with demands on attentional processes but not with the basic behavioral operations associated with cognitive task performance (Arnold et al., 2002; Dalley et al., 2001; Himmelheber et al., 2000; McGaughy et al., 2002; Passetti et al., 2000).

However, the precise cognitive operations supported by changes in cortical cholinergic activity have remained unknown. The low temporal resolution of measures of ACh release using microdialysis (minutes) limits the attribution of changes in cholinergic neurotransmission to specific behavioral or cognitive operations. Moreover, such measures of ACh release supported the traditional notion that this neuromodulator system acts at a timescale of minutes to influence cortical "arousal" states. However, the presence of a highly potent metabolizing enzyme for the neurotransmitter, acetylcholinesterase (AChE), and fast ionotropic nicotinic acetylcholine receptors (nAChRs) suggest that the functions of the forebrain cholinergic system are not sufficiently described by such notions.

Cortical cholinergic inputs, particularly to prefrontal regions, have been hypothesized to mediate the detection of cues (Sarter et al., 2005a). The term "detection" refers to multiple cognitive processes involving "…the entry of information concerning the presence of a signal into a system that allows the subject to report the existence of the signal by an arbitrary response indicated by the experimenter" (Posner et al., 1980). This definition further implies that detection involves response preparation and timing, response outcome expectation, and the timing of such an outcome. The hypothesis that the cortical cholinergic input system mediates cue detection is consistent with neurophysiological evidence indicating that ACh augments the processing of thalamic inputs (Ashe et al., 1989; Kilgard and Merzenich, 1998; Tremblay et al.,

1990; Weinberger, 2003) and that the effects of lesions of the cortical cholinergic input system on attention performance selectively manifest in trials in which cues are presented, while sparing the animals' ability to reject noncue events (McGaughy et al., 1996). However, direct evidence indicating that the cholinergic system is selectively active during cue detection has not been available, due largely to the absence of methods for the monitoring of cholinergic activity at a sufficiently high temporal resolution.

To test the hypothesis that cholinergic activity in the mPFC mediates cue detection, we employed, in task-performing animals, ceramic-based multisite microelectrode arrays for the electrochemical measurement of synaptic ACh release at a subsecond resolution (Burmeister and Gerhardt, 2003; Burmeister et al., 2003; Parikh et al., 2004). The measurement scheme underlying this technique is illustrated in Figure S1 (in the Supplemental Data available with this article online). Our previous experiments indicated the validity of this technique in terms of measuring choline resulting from AChE-induced hydrolysis of newly released ACh (Parikh et al., 2004, 2006; Parikh and Sarter, 2006). Cholinergic activity was recorded in the medial prefrontal cortex (mPFC) and a nonassociational control region (motor cortex) of animals performing a cued appetitive response task (Figure 1). This task involves the presentation of a cue predicting subsequent reward delivery and therefore evoking attentional shifts from ongoing behavior to the monitoring of the two reward ports (detection). Although this task involves less well-defined demands on attentional processes than operant procedures involving computerized control of levers and reward delivery devices, it allows for manual operation of task events and thus is devoid of sources of static energy that were found to interfere with the recording of small currents (picoamperes), despite extensive shielding. The collective results from these experiments indicate that the cortical cholinergic input system acts on multiple timescales (at the scales of seconds, tens of seconds, and minutes) to support cue detection and attentional performance.

RESULTS

Task Acquisition and Performance during Recording Sessions

Animals reached criterion performance for each stage of learning of the cued appetitive response task within about 2 weeks of training. The latencies between cue presentation and reward retrieval decreased continuously during the two stages of task acquisition (Figure 1C), as indicated by a significant effect of day (stage 1, 10 s cue followed by immediate reward: $F_{(4,20)} = 27.25$, $p < 0.001$; stage 2: 1 s cue followed by reward 6 ± 2 s later: $F_{(4,20)} = 8.98$, $p < 0.001$).

In sessions during which cholinergic activity was recorded, animals detected significantly more cues than they missed (58.7% ± 2.3% of the cues were detected; 25 trials/session; $t_{(10)} = 5.03$, $p < 0.001$; Figure 2A). As would be expected, the latencies between reward delivery and reward retrieval were longer in trials in which cues were missed ($t_{(10)} = 2.26$, $p = 0.048$; Figure 2B).

Cue-Evoked Transient Increases in Cholinergic Activity in the mPFC

Details concerning electrode preparation, in vitro calibration, and electrode properties in vivo following completion of the recording experiments are described in the Supplemental Materials. Amperometric recordings of cholinergic activity in the mPFC, but not motor cortex (Supplemental Materials), revealed transient increases that were evoked by cues that were detected (Figures 2C–2G). Cue-evoked cholinergic signal amplitudes were significantly higher for detected cues when compared with missed cues (highest choline signal levels observed during the 6 ± 2 s cue-reward interval; $t_{(10)} = 4.21$, $p = 0.002$; Figure 2G). The time required for cholinergic signal amplitudes to decrease by 50% from peak (t_{50}) was 3.17 ± 0.27 s. As will be further substantiated below, during trials involving missed cues, cholinergic activity remained unchanged from precue levels (Figures 2D and 2F).

Additional analysis indicated that reward delivery and retrieval did not evoke cholinergic activity. First, choline signal levels recorded for 2 s prior to and 5 s following reward delivery did not differ by trial type (detected/missed; $t_{(10)} = 1.18$, $p = 0.27$). Second, in trials involving missed cues, choline signal levels recorded for 5 s following the (missed) cue and following reward delivery did not differ ($t_{(10)} = 2.17$, $p = 0.10$; Figure 2F). The conclusion that reward-related processes did not confound cholinergic activity is further supported by the demonstration of regular cue-evoked cholinergic transients in catch trials not involving reward delivery, and by the absence of such transients early into the acquisition of the task (for these results see Supplemental Materials).

As the definition of detection involves the initiation of a behavioral response that indicates the entrance of a behaviorally significant cue into the processing stream (Introduction), the onset of the cue-evoked behavioral response was expected to correlate with the onset of the increase in cholinergic activity. Such increase in cholinergic activity was defined as the time point, relative to cue presentation, when cholinergic activity increased by 25% over precue levels. As illustrated in Figure 2H, the time of onset of the choline spike correlated significantly with the onset of the behavioral shift (Pearson's $r = 0.79$, $p < 0.001$).

In this task, the efficacy of the cue detection process is indicated by response latencies. Choline signal amplitudes correlated significantly with the latencies between cue presentation and reward retrieval (Pearson's $r = -0.37$, $p = 0.045$). Analysis of the regression between these two variables indicated that an increase in choline signal amplitude by 1 μM was associated with a decrease of 1.75 s in response latency.

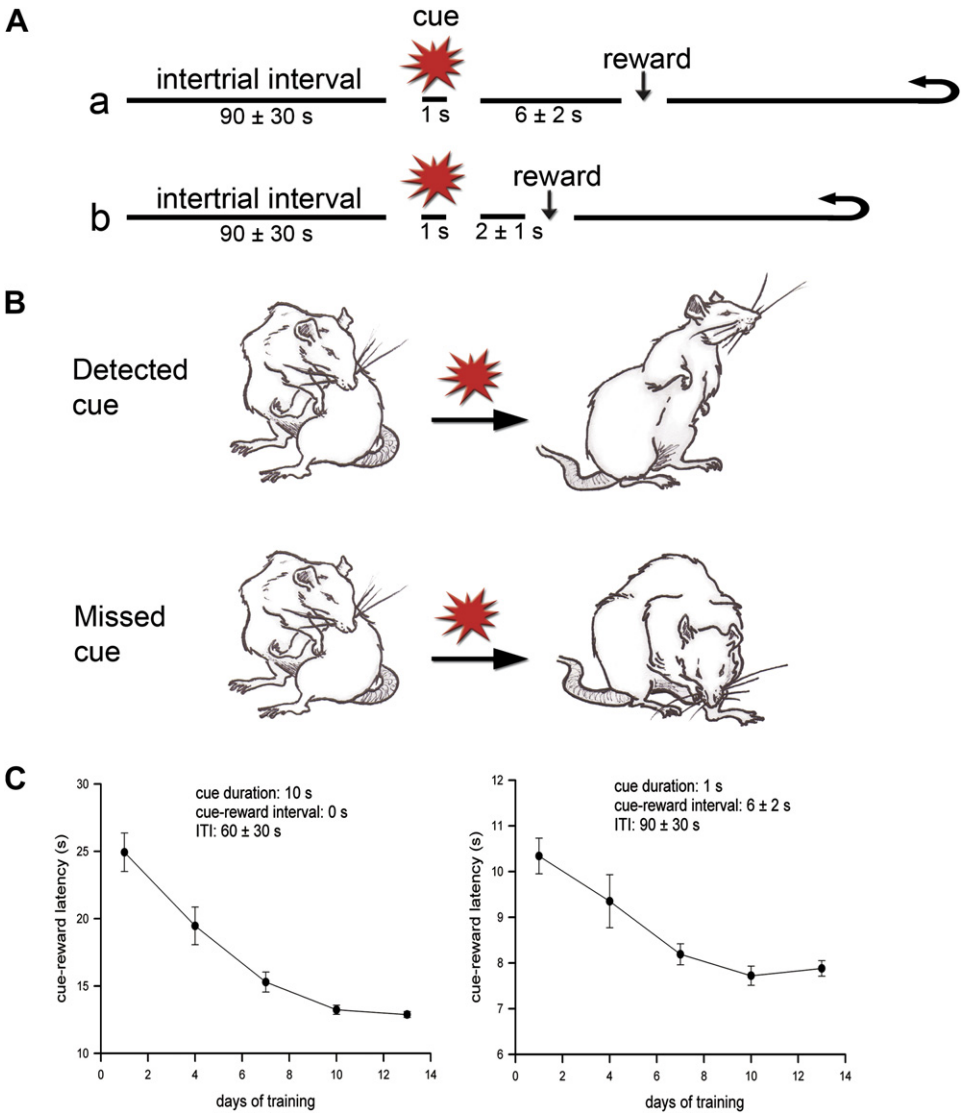

Figure 1. Task Description, Trial Classification, and Task Acquisition

(A) Illustration of the main events constituting the cued appetitive response task and the two task versions, differing only by the interval between cue presentation and reward delivery (Aa and Ab). Following the intertrial interval (ITI), a cue was presented and always followed by reward delivery at one of two reward ports (random selection). Separate groups of rats were trained to perform versions of the task involving a 6 ± 2 s or a 2 ± 1 s interval between cue and reward delivery. Reward was consistently delivered, irrespective of cue-evoked behavior. The long ITI served to foster disengagement from the task and endogenously generated behavior (mostly grooming).

(B) Trials involving cue detection were classified as such based on cue-elicited disengagement from ongoing behavior and monitoring of the food ports. A missed cue was defined as such based on the absence of a cue-evoked shift in behavior. Note that in trials involving missed cues, proximal stimuli associated with reward delivery ensured port approach and reward retrieval, albeit involving longer latencies when compared with trials involving cue detection.

(C) Latencies between cue presentation and reward delivery during the two stages of acquisition of the cued appetitive response task. In stage 1 (left graph), a 10 s cue was followed immediately by reward delivery. Latencies decreased significantly during 2 weeks of training in this stage. In the second stage (right graph), a 1 s cue was presented and followed by reward 6 ± 2 s later. Furthermore, the ITI was increased to 90 ± 30 s. Latencies decreased further during this stage of task acquisition (data based on n = 6). Data are mean with SEM.

Left-Shift of Cue-Evoked Cholinergic Signals

The evidence described above was based on recordings in the mPFC of rats performing the cued appetitive response task involving a 6 ± 2 s interval between cue and reward delivery (Figure 1A). Cholinergic activity was recorded in a separate group of animals trained to perform the cued appetitive response task involving a shorter (2 ± 1 s; Figure 1A) interval, in order to test the following hypothesis: if cue-evoked cholinergic transients merely reflect the sensory encoding of the cue, the timing of

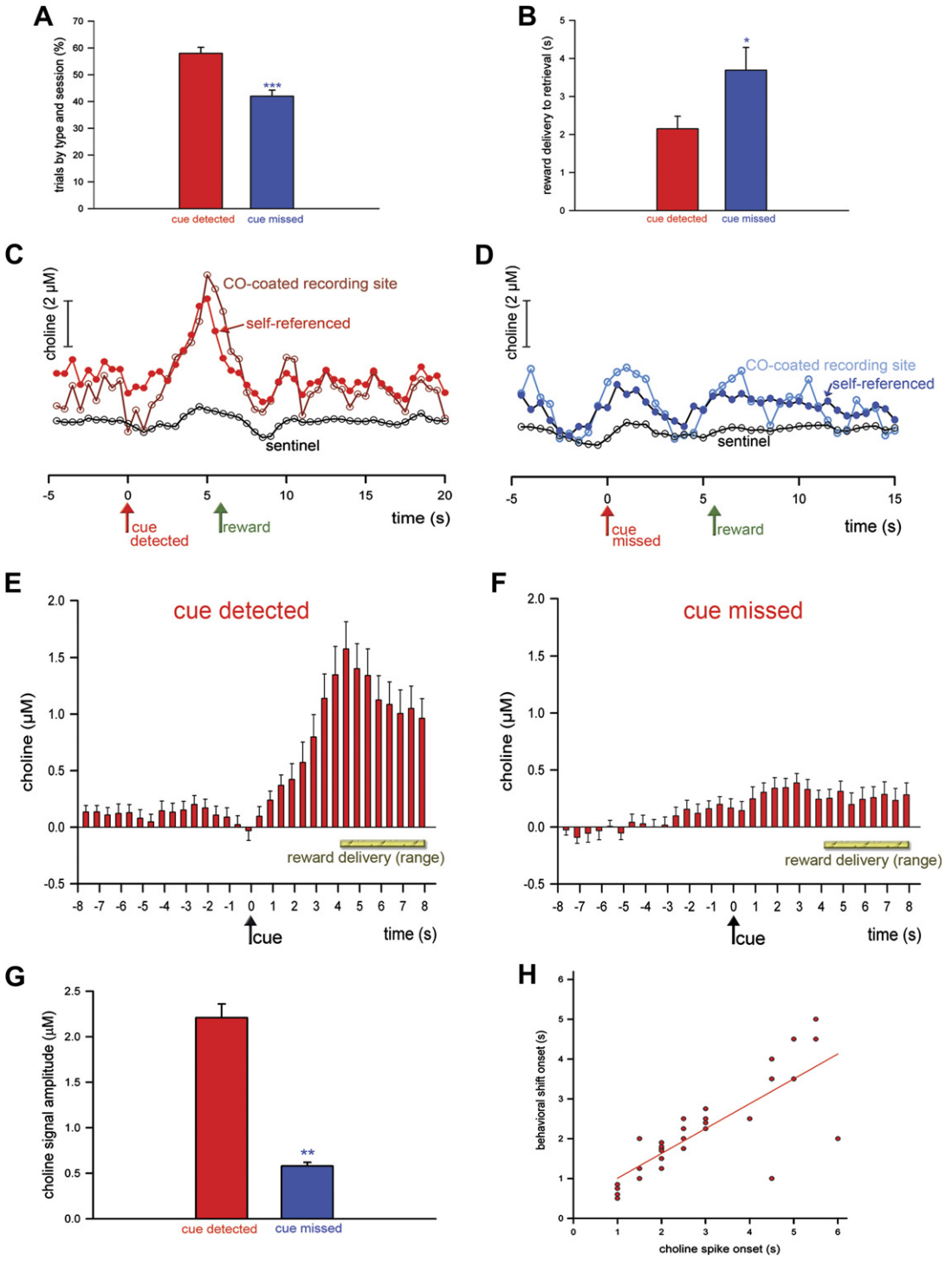

Figure 2. Detected Cue-Evoked Transient Increases in Cholinergic Activity

(A) Bar chart depicting the proportion of cues that were detected or missed during recording sessions (25 trials/session). The greater proportion of cues was detected (***$p < 0.001$).

(B) Latencies between reward delivery and reward retrieval were longer in trials in which animals missed the cue (*$p < 0.05$).

(C) Raw traces obtained from a ChOase-coated (dark red) and a non-ChOase-coated ("sentinel;" black) recording site, recorded at 2 Hz during a trial involving cue detection. Furthermore, the self-referenced and boxcar-filtered trace (averaged over two points; red; filled circles) is shown (arrows on the abscissa depict the time of cue and reward delivery).

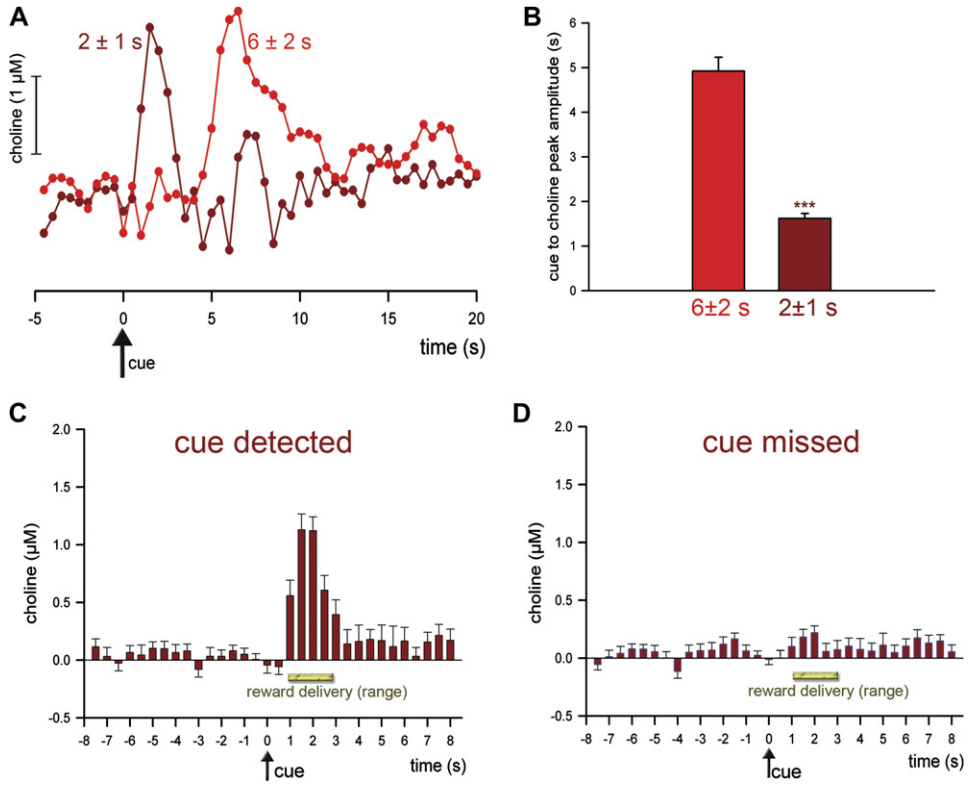

Figure 3. Effects of a Shorter Cue-Reward Interval on the Timing of Cholinergic Transients

(A) Self-referenced, detected cue-evoked cholinergic activity recorded in the mPFC of separate groups of animals performing the task with a 6 ± 2 s (red trace) or 2 ± 1 s (dark red trace) interval between cue presentation and reward delivery. Note the leftward shift of the cue-evoked cholinergic signal in animals performing the task involving the shorter interval.

(B) The latency from cue presentation to (detected) cue-evoked choline signal peak amplitude differed significantly between the two task versions (***$p < 0.001$). However, the amplitudes of the increases in cholinergic activity did not differ between the two task versions ($p > 0.05$).

(C and D) Population data (n = 5) depicting choline signal levels (using mean and SEM) in trials involving detected and missed cues (short cue-reward interval; because the cue-reward interval was variable, the range in time during which reward was delivered is depicted by bars superimposed over the abscissa).

Error bars = SEM.

cue-evoked cholinergic activity should be insensitive to variation of the interval between cue and reward delivery. In contrast, if variation of this interval causes variation of the timing of the cue-evoked cholinergic transients, such a finding would indicate that cholinergic transients reflect a shift in the timing of cue-evoked cognitive operations that collectively define detection (Introduction). As illustrated in Figure 3, the latency from cue presentation to the (detected) cue-evoked choline signal peak amplitude was significantly shorter in animals performing the task involving the shorter cue-reward interval ($t_{(53)} = 9.26$, $p < 0.001$; Figure 3B). The amplitudes of the cholinergic transients did not differ between the two task versions ($t_{(9)} = 1.72$, $p > 0.12$). As was the case for recordings from

(D) Raw traces (ChOase-coated: blue; sentinel: black) and a self-referenced trace (dark blue) from a trial during which the cue was missed. Transient increases in cholinergic activity were not observed in such trials.

(E and F) Histograms depicting choline signal levels (using mean and SEM) in trials involving detected and missed cues (based on n = 6 animals; from each animal, 10 trials per trial type were selected as described in Supplemental Methods). Data were recorded at 2 Hz over a total of 16 s per trial (8 s precue and 8 s postcue period) and expressed relative to the average choline signal levels measured during a 2 s pretrial period. Data indicate averages over 30 trials each with detected (E) and missed (F) cues (see arrows to indicate the cue presentation at time zero; because the cue-reward interval was variable, the range in time during which reward was delivered is depicted by bars superimposed over the abscissa). Because the population averages describing cue-evoked cholinergic activity were time-locked to cue presentation ([E]; cue time: zero), the relatively slow postpeak decline in cholinergic activity suggested in (E) in fact reflects the observation that the timing of the choline peak amplitude varied across trials and animals (see also the left bar in Figure 5B).

(G) Cue-evoked choline signal amplitudes were significantly higher for detected cues when compared with missed cues (**$p < 0.01$).

(H) Correlation between the time of onset, relative to cue presentation, of the detected cue-evoked shift in behavior and a 25% increase in cholinergic activity (Pearson's $r = 0.79$, $p < 0.001$).

Error bars = SEM.

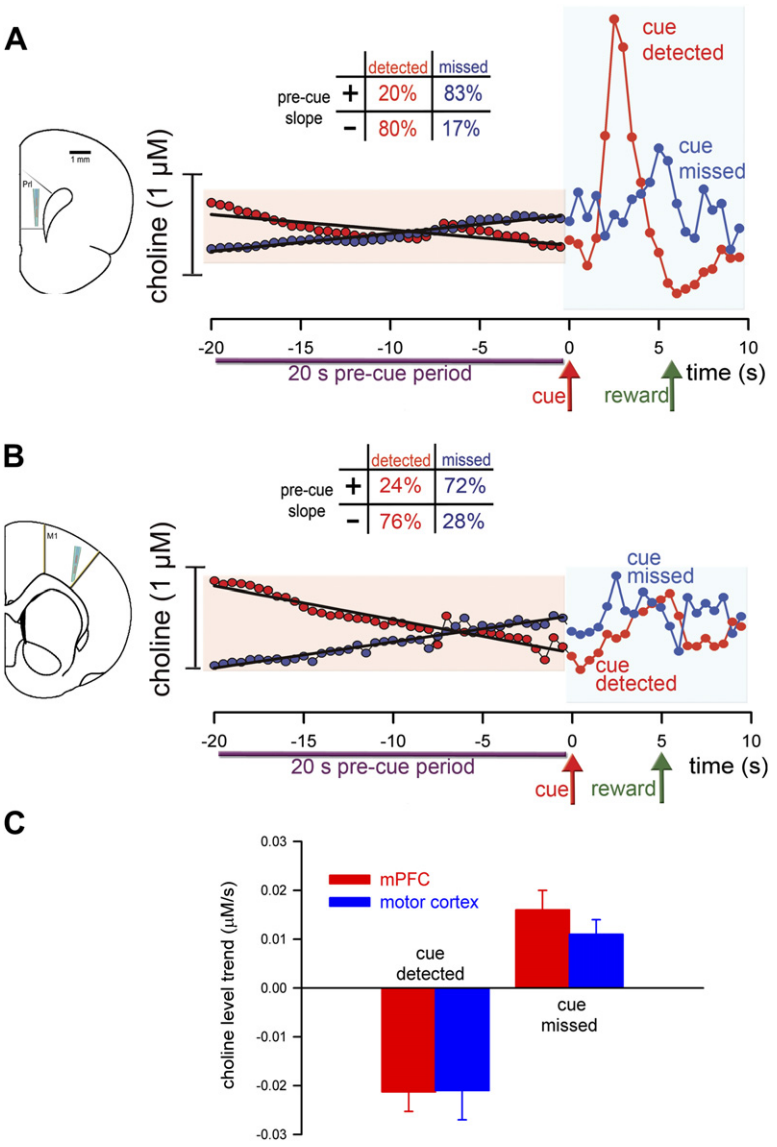

Figure 4. Precue Trends in Cholinergic Activity Predict Trial Outcome

(A and B) Relatively small decreases and increases in cholinergic activity, which occur in the mPFC and motor cortex over tens of seconds prior to cue presentation, predict subsequent cue detection and misses, respectively. The traces placed over the yellowish background in (A) and (B) are self-referenced traces that were boxcar-filtered over 20 points in order to calculate the slope of cholinergic activity during this period (red traces, trial with cue detection; blue traces, trial with missed cue; linear regressions indicated by black solid lines). The traces placed over blue background are self-referenced traces that were boxcar-filtered over two points. Note that cue-evoked cholinergic transients were not observed in motor cortex.

(C) Precue changes in cholinergic activity, expressed as µM/s, preceding the two outcomes (detection/miss), did not differ between mPFC and motor cortex (data based on the analysis of a total of 55 trials per trial type, obtained from a total of n = 11 animals, 6 with electrodes in mPFC, 5 with electrodes in motor cortex). Data are mean with SEM.

the mPFC of animals performing the task involving the longer cue-reward interval, cholinergic activity evoked by detected cues was significantly higher when compared with missed cues ($t_{(8)} = 6.97$, $p < 0.001$). Cholinergic activity in trials involving missed cues and reward-delivery-evoked port approach remained at pretrial levels (Figure 3D; see below for statistical results).

Based on the choline signal population data for detected trials from both task versions, over the entire 16 s period (see Figure 2E and Figure 3C), the effects of the variation of the cue-reward interval were indicated by a significant interaction between the effects of time (data across 16 s) and cue-reward interval (long, short) on choline signal levels (main effect of time: $F_{(1,31)} = 13.28$, $p < 0.001$; main effect of interval: $F_{(1,53)} = 21.38$, $p < 0.001$; time × interval: $F_{(31,1643)} = 10.72$, $p < 0.001$). In the analysis of choline signal levels recorded during trials in which the cue was missed, neither an effect of time or interval nor an interaction between these two factors was found (both $p > 0.05$), reflecting the absence of changes in cholinergic activity (Figure 2F and Figure 3D). Cue-evoked cholinergic transients were not observed in separate experiments in which cholinergic activity was recorded in the motor cortex (Supplemental Materials).

Precue Trends on Cholinergic Activity

In the analysis of cholinergic signal levels across trials involving cue detection and misses, respectively, systematic relationships between precue trends in cholinergic signal levels in the mPFC and trial outcome (detection or miss) were discovered. For a systematic analysis of this relationship, data from a 20 s period prior to the cue were boxcar-filtered, and the slope of the linear regression was determined (see Supplemental Methods). As

illustrated in Figure 4A, in 80% of trials involving cue detection, mPFC precue cholinergic activity showed a negative trend; conversely, 83% of misses were preceded by increases in cholinergic activity ($\chi^2 = 24.15$, $p < 0.001$). Moreover, for trials with detected cues, steeper decreases in precue cholinergic activity correlated with greater amplitudes of cue-evoked cholinergic activity (Pearson's $r = -0.32$, $p = 0.01$).

A similar result was found in the analysis of cholinergic activity recorded in the motor cortex (76% and 72%, respectively; $\chi^2 = 9.70$, $p = 0.002$; Figure 4B). The magnitude of these trends did not differ between mPFC and motor cortex (Figure 4C; decreases preceding cue detection: $t_{(41)} = 0.038$, $p = 0.97$; increases preceding misses: $t_{(41)} = 0.93$, $p = 0.36$).

Cholinergic Deafferentation of the Recording Area Abolishes Cue-Evoked Cholinergic Transients

In order to confirm that the demonstration of evoked cholinergic activity, measured by choline-sensitive microelectrodes, requires the presence of cholinergic terminals, cholinergic activity was recorded following the unilateral removal of cholinergic inputs to the recording region (see Experimental Procedures). In contrast to bilateral cholinergic deafferentation of the mPFC (below), such restricted deafferentation is insufficient to impair attentional performance (Gill et al., 2000) and, likewise, did not affect the proportion of cues that was detected ($t_{(9)} = 1.75$, $p = 0.22$). Detected cue-evoked cholinergic activity was not observed in the deafferented recording region, confirming the validity of the measure in terms of reflecting ACh released from cholinergic neurons (Figures 5A and 5B).

Bilateral Cholinergic Deafferentation-Induced Disruption of Cue Detection

Bilateral removal of mPFC cholinergic inputs decreased the proportion of detected cues ($F_{(3,16)} = 8.68$, $p = 0.001$; Figure 5C). Multiple comparisons indicated that this impairment was present during all 3 weeks of postsurgery training and testing (all $p < 0.025$). The number of port approaches was recorded across test sessions (see Experimental Procedures), regardless of whether such approaches were evoked by cue or reward delivery. The effects of the lesions on this measure were analyzed in order to reveal potential confounds based on general exploratory or activity changes. Although the lesion produced a significant effect on this measure ($F_{(3,16)} = 3.46$, $p = 0.041$), multiple comparisons indicated that this was due to an increased frequency of port approaches observed during the second week after the infusions of the immunotoxin (Figure 5D). Immunotoxin-induced deafferentation typically reaches asymptotic levels 2 weeks postinjection (Waite et al., 1994).

In contrast to the effects of bilateral cholinergic deafferentation of the mPFC, a similar deafferentation of the motor cortex did not affect cue detection rate ($F_{(3,16)} = 0.55$, $p = 0.67$; see Supplemental Materials).

Minute-Based, Performance-Session-Associated Changes in Cholinergic Activity in mPFC and Motor Cortex

The transient increases in cholinergic activity that were recorded in the mPFC during trials involving detected cues were superimposed over more slowly changing (on the scale of minutes), or tonic, changes in cholinergic activity. Such tonic cholinergic activity was also observed in the motor cortex (Figure 6). ANOVA confirmed that session-related changes in cholinergic activity occurred in both cortical regions (main effect of time: $F_{(39,351)} = 2.13$, $p < 0.001$) and did not differ in magnitude (main effect of region: $F_{(1,9)} = 0.32$, $p = 0.59$).

Performance-associated increases in mPFC tonic cholinergic signal levels were positively correlated with the amplitudes of cue-evoked cholinergic transients (Pearson's $r = 7.21$, $p < 0.001$; Figure 6B) and with a greater proportion of detected cues (analyzed over blocks of five trials each; $r = 0.46$, $p = 0.01$). Tonic signal levels recorded in the motor cortex were not correlated with performance ($r = 0.04$, $p = 0.86$). Furthermore, the total number of port approaches, a measure of task-related locomotor and exploratory activity, did not correlate with tonic levels of cholinergic activity recorded in mPFC or motor cortex (both $p > 0.05$). Session-related tonic cholinergic activity corresponded with levels of ACh release measured by using microdialysis in both cortical regions (Supplemental Materials).

In animals trained to perform the task that were placed into the test chamber without activating the task, no such tonic changes in cholinergic activity were observed, indicating that performance of the task is necessary to evoke such tonic changes, and that context alone and expectation of performance were not sufficient to evoke tonic increases in cholinergic activity (mPFC: $F_{(5,17)} = 0.49$, $p = 0.78$; motor cortex: $F_{(5,17)} = 0.83$, $p = 0.55$; Figures 6A and 6C). Finally, session-related tonic changes in mPFC cholinergic activity were not observed following unilateral removal of cholinergic inputs to the recording region ($F_{(5,29)} = 0.77$, $p = 0.58$).

DISCUSSION

The results from these experiments support the following main conclusions. Transient or "phasic" increases in mPFC cholinergic activity are evoked by attended cues. In trials involving missed cues, the delivery of reward triggered port approach and reward retrieval; as these events did not evoke cholinergic transients, cholinergic transients mediate cue-evoked cognitive operations, but not port approach and reward retrieval. This conclusion is further supported by the evidence from catch trials not involving reward delivery, and from trials early into the acquisition of the task when rewards were delivered and retrieved, but cues did not yet evoke a behavioral response. The demonstration of the shift in the timing of cholinergic transients in response to shorter cue-reward intervals is consistent with the hypothesis that these

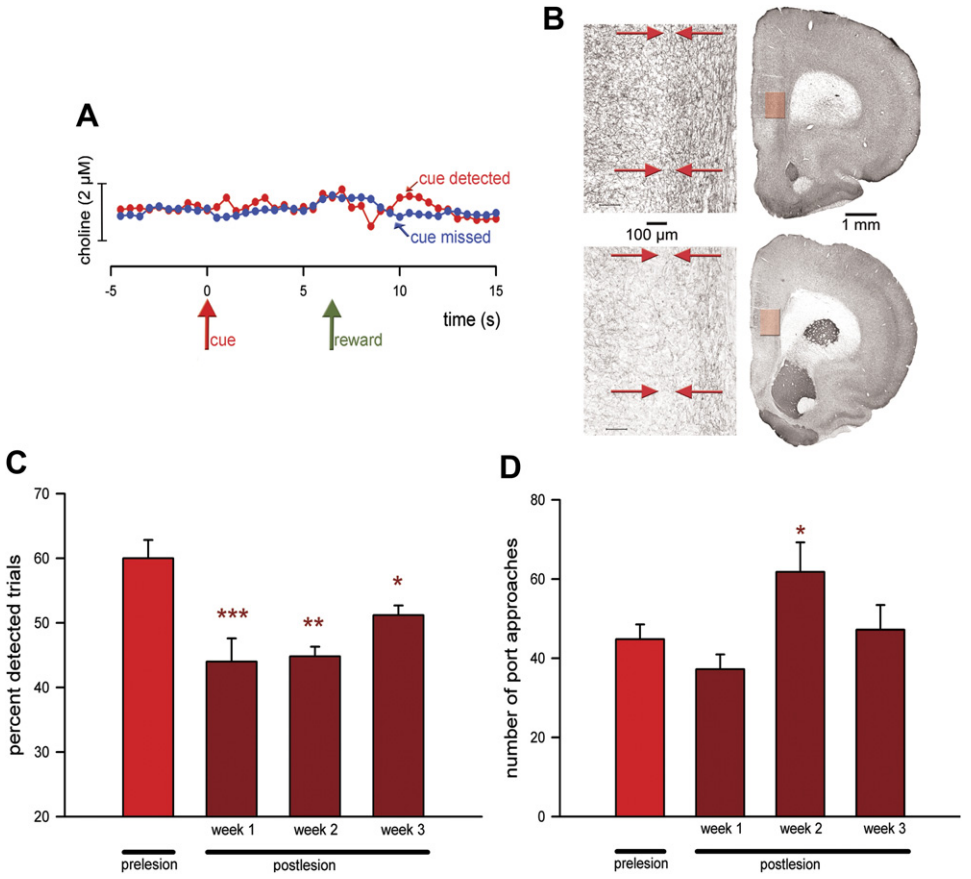

Figure 5. Cholinergic Deafferentation-Induced Attenuation of Cue-Evoked Cholinergic Signals and Cue Detection

(A) Unilateral, restricted removal of cholinergic inputs to the recording region attenuated detected cue-evoked increases in cholinergic activity (self-referenced boxcar-filtered trace: detected cue: red; missed cue: blue), confirming the neuronal (cholinergic) origin of such increases in cholinergic signals.

(B) Coronal sections illustrating the loss of cholinergic innervation following infusion of the immunotoxin 192 IgG-saporin into the mPFC (lower microphotographs) compared with a section from an intact brain (upper microphotographs). Sections were stained for the visualization of AChE-positive fibers. The inserts on the coronal sections depict the areas shown by the photomicrographs (left). The arrows indicate the approximate position and dimension of the four recording sites when placed into this region. On average, infusions of the immunotoxin resulted in the removal of over 80% of the cholinergic innervation (AChE-positive fiber counts: intact: 70.22 ± 2.78 [n = 6]; lesioned: 11.87 ± 1.35 [n = 5]; $t_{(9)} = 17.62$, $p < 0.001$).

(C) Bilateral removal of cholinergic inputs to the mPFC reduced the proportion of cues that were detected (see Results for ANOVA; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus prelesion).

(D) Number of port approaches per session. During week 2, lesioned animals exhibited a transient increase in the number of port approaches; thus, the lesion-induced impairment in the number of cue detections was not associated with a reduction of task-related exploratory or locomotor activity. Data are mean with SEM.

transients mediate a cognitive operation, as opposed to merely indicating the sensory processing of the cue. As removal of cholinergic inputs to the mPFC, but not motor cortex, impaired cue detection, cue-evoked cholinergic activity in the mPFC is necessary for cue detection. Performance-session-related, tonic changes in cholinergic activity occur over minutes, with higher tonic levels predicting greater amplitudes of phasic signals and enhanced cue detection (as indicated by shorter cue-reward retrieval latencies). Finally, precue increases or decreases in cholinergic activity, observed over tens of seconds prior to cue presentation, predict subsequent misses or cue detection, respectively.

Is Cue Detection Necessarily Mediated via Cholinergic Transients, and Exactly What Mechanisms Trigger Cholinergic Transients and Detection Processes?

Removal of cholinergic inputs to the mPFC, but not motor cortex, impaired cue detection. Since precue cholinergic trends and task-session-related tonic changes in cholinergic activity were also recorded in motor cortex, and were also abolished as a result of deafferentation, mPFC cholinergic activity is necessary for cue detection (below). The significant correlation between the amplitudes of these transients and response latencies, and the temporal left-shift of these transients in response to

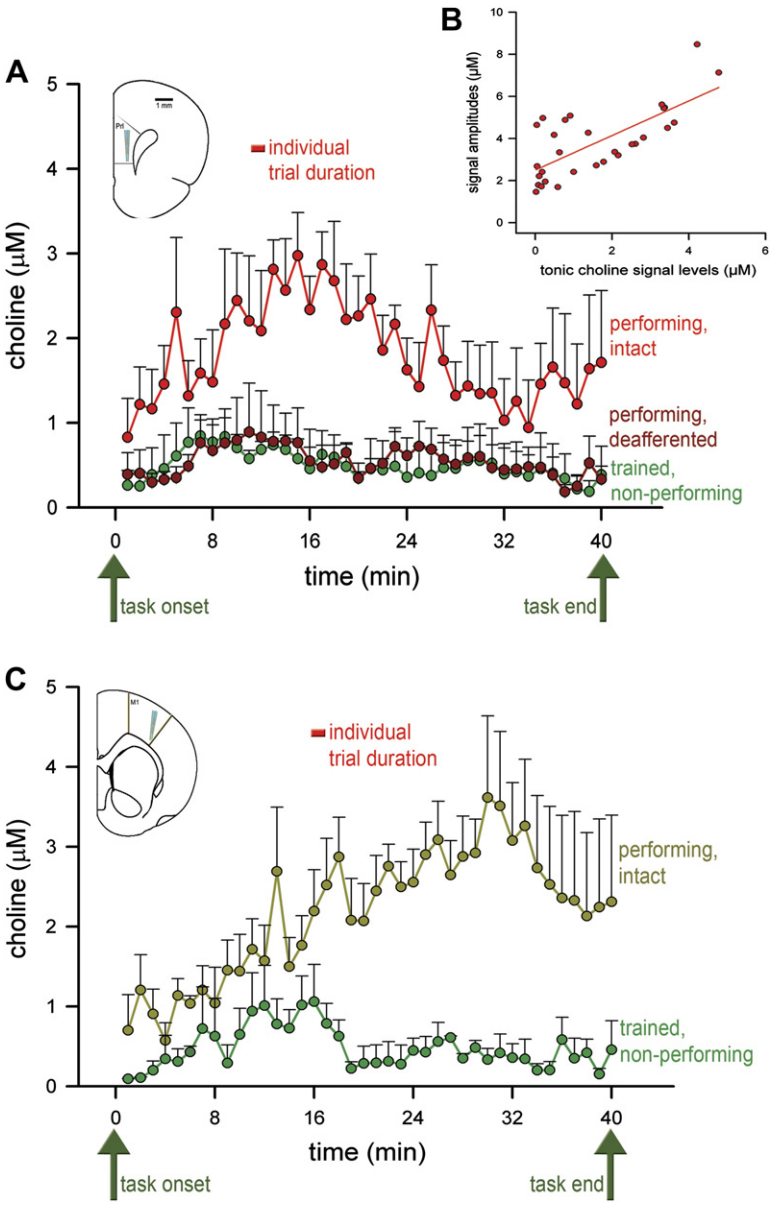

Figure 6. Tonic Changes in Cholinergic Activity

(A and C) Performance-session-related tonic changes (using mean and SEM) in cholinergic activity in the mPFC and motor cortex. These data were extracted from amperometric recordings at 2 Hz by boxcar-filtering self-referenced traces over 20 data points and expressed minute-based data points as a change from average signal levels recorded during a 3 min presession baseline (based on recordings in a total of n = 22 animals). (A) Session-related changes in cholinergic activity in the mPFC of intact (red trace), trained but not performing (animals placed into chambers but task not turned on; green trace), and task-performing animals after unilateral removal of cholinergic inputs to the recording region (dark red trace). Note the bar indicating the duration of a single trial relative to the abscissa depicting the entire 40 min session. (B) Significant correlation between session-related tonic choline levels recorded in the mPFC, taken from 2 s precue periods, and the amplitudes of cue-evoked transient increases in cholinergic activity (for this correlation, amplitudes were calculated relative to the average of 3 min presession baseline levels). (C) Session-related changes in cholinergic activity in the motor cortex of intact (light green trace) and trained but nonperforming (dark green trace) animals. In intact rats, session-related changes in cholinergic activity occurred in both regions, but they did not differ in magnitude. The different time course of minute-based changes in cholinergic activity in the two regions was reflected by a significant interaction between time and region. Increases in cholinergic activity were not observed in trained animals placed in the test chamber that were not allowed to perform. Likewise, following unilateral cholinergic deafferentation of the recording region, session-related increases in cholinergic activity were not observed.

shorter cue-reward intervals, further substantiate this conclusion.

As discussed in the Introduction, cue detection involves a range of cognitive processes, including attentional shifts away from ongoing, task-irrelevant activities to task-related behavioral and cognitive processes, including reward port monitoring, response rule processing and preparation, reward anticipation, and the timing of responses and reward delivery. The present evidence is consistent with the hypothesis that cue-evoked cholinergic transients mediate cue detection.

The present evidence collectively rejects the possibility that reward delivery and reward retrieval evoked transient increases in cholinergic activity. First, reward port approach and reward retrieval also occurred in trials involving missed cues; yet cholinergic transients were not evoked by these events. Second, in trials involving detected cues, reward delivery occurred during the decay of the cholinergic transient; therefore, potential reward-delivery-associated cholinergic spikes would have been readily observed. Third, in catch trials not involving reward delivery, cue-evoked cholinergic transients were identical to those observed in regular trials, indicating that reward delivery and retrieval did not confound cue-evoked cholinergic transients. Fourth, early into training, while cues did not yet control behavior but while rewards were delivered and effectively retrieved, cholinergic transients were not observed. Therefore, the presence or absence of transient cholinergic activity indicates the differences between the cognitive and behavioral operations elicited by the distal

(cue) versus proximal (reward delivery-associated) conditioned stimuli. For spatially and temporally distal stimuli to guide behavior, they need to trigger cognitive operations such as attentional shifts away from task-irrelevant activities toward anticipation and timing of the reward, port monitoring, response rule processing, and the timing of the response (Holland, 1993; Holland and Gallagher, 1999). In contrast, stimuli that are spatially and temporally bound with reward delivery can elicit port approach and reward retrieval without requiring such cognitive operations.

As discussed earlier (Sarter et al., 2005a, 2006), detection represents a top-down process that requires representation of the presence of the cue and information about the associative significance of the cue. Consistently predictive cues evoke attentional shifts toward outcome-related behaviors and events and, as indicated by the present results, such shifts are necessarily mediated by transient increases in cholinergic activity in the mPFC. Increases in mPFC cholinergic neurotransmission are hypothesized to be necessary for recruitment of prefrontal neuronal assembles that orchestrate, top-down, the components of the detection process. Therefore, in the absence of cholinergic inputs to the mPFC, cues are missed at a higher frequency and, in animals performing more demanding attention tasks, performance is persistently disrupted (references above).

Results from neurophysiological recordings of basal forebrain neuronal activity correspond with the present conclusions. First, evidence for both phasic and tonic firing characteristics of basal forebrain neurons was described (Detari et al., 1999). Second, neurophysiological studies conducted in task-performing primates indicated that basal forebrain neuronal activity reflects decision-making processes and cue-evoked reward expectation and timing (Richardson and DeLong, 1990; Wilson and Rolls, 1990).

Why Are Cues Missed and What Do Precue Trends in Cholinergic Activity Signify?

The processes underlying missed cues remain necessarily speculative. Given the parameters of cue presentation (1 s duration, ceiling-mounted), it is unlikely that the cue failed to stimulate the retina; rather, misses demand an explanation in terms of postsensory, cognitive processes. This view is supported by the observation, based on videotape analyses, that missed cues triggered brief (<1 s) disturbances in the sequencing of grooming behavior but failed, by definition, to trigger termination of such behavior. Effective cue detection involves a state of readiness for input processing, meaning the allocation of attentional resources for input processing and the disengagement from ongoing behavior and task-irrelevant cognitive activity. A miss could be attributed to a low readiness for input processing and may be similar to phenomena described as inattentional blindness or attentional lapses (Simons and Chabris, 1999; Weissman et al., 2006).

The present evidence suggests that precue decreases in cholinergic activity in the mPFC and motor cortex, and therefore perhaps cortex-wide, foster subsequent cue detection, while increases in precue cholinergic activity were followed by misses (Figure 4). Moreover, for recordings in the mPFC, steeper precue decreases predicted greater cue-evoked cholinergic signal amplitudes and therefore faster response latencies. Therefore, precue negative slopes in cholinergic activity are hypothesized to indicate, or even contribute to, a more effective manifestation of the brain resting default state, while positive slopes reflect a less effective suspension of task-related activity. This interpretation is consistent with findings from human studies indicating that attentional lapses are more likely if task-irrelevant cognitive activity prevents the return to the resting default state (Weissman et al., 2006). The hypothesis that trends in precue cholinergic activity determine trial outcome requires research in which these trends are controlled experimentally by, for example, varying the duration of the intertrial interval (ITI) and thereby controlling the suspension of task-related processes.

Do Session-Related Tonic Changes in Cholinergic Activity Contribute to the Mediation of Attentional Performance?

Session-related, tonic increases in cholinergic activity recorded in the mPFC correlated with higher cue detection rates and with greater amplitudes of cue-evoked cholinergic transients. Furthermore, greater amplitudes predicted shorter response latencies. These findings suggest functionally significant interactions between the multiple components of cholinergic neurotransmission. Minute-based changes in mPFC cholinergic activity contribute to the general readiness for cortical input processing and therefore also influence the efficacy of the detection process.

Because lesions of the cholinergic input to the motor cortex did not affect the animals' performance, the role of tonic cholinergic activity elsewhere in the cortex remains unclear. The performance of cognitive tasks involving multimodal stimuli and complex instrumental behaviors may generally be optimized by tonic cholinergic activity, including that in the motor cortex to support skilled motor responses (Conner et al., 2003, 2005). As the present task did not tax such motor functions, the removal of cholinergic inputs to motor cortex was inconsequential.

Which Neuronal Mechanisms May Be Responsible for the Manifestation of Cholinergic Transients?

The present evidence is consistent with a model that assumes multiple cholinergic modules and a regulation of cholinergic activity in a modality-specific and cortical-area-specific manner (Zaborszky, 2002). Moreover, our results suggest that performance-related cholinergic activity manifests on multiple timescales. The anatomical characteristics of the basal forebrain cholinergic system do not suggest a topographic organization that would

readily explain the presence of such functional modules and multiple modes of action (Mesulam, 1990; Sarter and Bruno, 1997; Zaborszky et al., 1999). However, there is evidence that the cholinergic inputs to the mPFC represent a critical component of neuronal circuits that consist of prefrontal projections to the basal forebrain and the nucleus accumbens (NAc) and projections from the NAc to the basal forebrain, suggesting that in addition to local, intra-PFC mechanisms contributing to the orchestration of cholinergic transients, larger loops involving mesolimbic circuitry influence mPFC cholinergic activity and therefore cue detection (Neigh et al., 2004; Zmarowski et al., 2005, 2007). It is intriguing to speculate that phasic dopamine signals recorded in the NAc in response to cues predicting reward (Day et al., 2007) contribute, via NAc projections to the basal forebrain, to the manifestation of mPFC cue-evoked cholinergic transients. Reward prediction may be thereby integrated with prefrontally controlled attentional shifts and response processing, collectively giving rise to the cholinergically mediated detection of cues.

Relevance for Cognitive Disorders

The findings that transient increases in cholinergic activity mediate cue detection and that the cholinergic system acts on multiple timescales to support cognitive performance form the basis for a significant expansion of hypotheses concerning the role of cholinergic dysregulation in the manifestation of the cognitive symptoms of neuropsychiatric disorders and the dementias (Mesulam, 2004; Sarter et al., 2005b). Specifically, abnormalities in the orchestration of cue-evoked cholinergic transients may precede more global and structural decline in cholinergic function. Dysregulated transients would be expected to disrupt the ability to utilize external stimuli in order to shift attentional resources toward goals. Indeed, such deficits have been extensively documented in patients with Alzheimer's disease and have been attributed to dysregulation and loss of cholinergic neurons (Mesulam, 2004). Likewise, deficits in target detection represent a core cognitive symptom of schizophrenia (Braff and Light, 2004) and have been attributed to dysregulation in forebrain cholinergic systems (Sarter et al., 2005b). Future efforts designed to understand the role of cholinergic dysfunction in the manifestation of cognitive impairments and the usefulness of cholinergic treatments will need to dissociate between the regulation and functions of the multiple phasic and tonic components of forebrain cholinergic neurotransmission.

EXPERIMENTAL PROCEDURES

Subjects

Adult male Fisher/Brown Norway hybrid rats (FBNF1; Harlan, Indianapolis, IN), weighing 250–300 g at the beginning of the experiments, were used. Animals were individually housed in a temperature- (23°C) and humidity- (45%) controlled environment on a 12 hr light/dark cycle (lights on at 06:30 a.m.). Food and water was available ad libitum until the commencement of behavioral training. Rats were mildly food-deprived by providing them with 30 g of lab chow in their home cages following each daily test session, thereby maintaining them at 85% of their free-feeding body weights at least. Water was always available ad libitum. All procedures were conducted in adherence with protocols approved by the University Committee on Use and Care of Animals (UCUCA) of the University of Michigan.

Behavioral Apparatus and Behavioral Training

The test environment is described in Supplemental Materials. For 2 weeks, food-deprived animals were handled daily for 5 min and then placed into the test chamber for an additional 30 min. Four pieces (12 mg each) of Kellogg's Fruit Loops were placed in the chamber to allow familiarization with the food used subsequently as reinforcement. Once animals rapidly consumed the pellets, they were then trained to accept the pellets presented by an experimenter using plastic tweezers inserted through one of the two food ports (random selection).

Training of the cued appetitive response task consisted of two stages. In the first stage, the light cue was illuminated for 10 s and a pellet was delivered immediately after cue offset (25 trials/day). The ITI was 60 ± 30 s. Animals were trained in this version until latencies between cue-onset and pellet retrieval were <13 s for at least 75% of the trials/session. During the second stage of training, cue duration was shortened to 1 s and the latency between cue presentation and pellet delivery was increased to 6 ± 2 s or, in a separate group of animals, 2 ± 1 s (Figure 1A). In addition, the ITI was increased to 90 ± 30 s. Individual training and test sessions lasted for approximately 40 min and included an 8 min waiting period between placing the animal into the chamber and the onset of the first trial (25 trials total). Training continued until the latencies between cue presentation and reward delivery were ≤9 s in at least 80% of the trials. Figure 1C depicts the learning of this response in terms of decreasing response latencies during the two stages of training.

Animals' performance was videotaped for the off-line classification of trials by experimenters blind to the choline recording data. Trials involving cue detection were classified as such based on cue-evoked behavior, characterized by disengagement from ongoing behavior (typically grooming), and orientation to and monitoring of the two reward ports. Trials involving a failure to detect the cue (missed cue) were characterized by the absence of cue-elicited changes in behavior (Figure 1B). It is important to note that in trials involving missed cues, the salient auditory and visual stimuli associated with food delivery reliably evoked the animals' approach to the baited port and food retrieval, albeit with longer latencies between cue presentation and food retrieval when compared with trials involving cue detection (see Results). Thus, trials involving missed cues served as an additional control for the test of the hypothesis that port approach and reward consumption and associated locomotor activity did not evoke transient cholinergic activity (see Results). On average, animals detected ~65% of the cues. After reaching stable criterion performance in the task, animals were habituated, for 1 additional week, to performing the task in a shielded test chamber used for subsequent electrochemical recordings. Animals were then prepared for either microelectrode implantation or lesion surgery (below).

During postsurgery retraining, which lasted 4–6 days/sessions, animals were placed into the chambers 90 min prior to task onset to foster habituation to tethering (described in Supplemental Materials). Postsurgery training sessions, including sessions during which cholinergic activity was recorded, were videotaped. Trials were classified off-line as having involved detected or missed cues by experimenters blind to the recording data.

The following measures of behavioral performance were obtained or calculated from each test session: (1) the number and proportion of cues that were detected; (2) for trials involving detected cues, the latency between cue presentation and disengagement of ongoing behavior (to generate this measure, experimenters blind to the recording data rated the time of onset of cue-evoked change in behavioral activity, typically indicated by termination of grooming behavior); (3) the latency between food delivery and food retrieval; and (4) general

food port approach behavior, independent of trial-related activity, which was determined off-line by dividing the floor into nine squares and counting the number of entries into the two squares underneath the ports throughout the session.

Preparation and Calibration of Choline-Sensitive Microelectrodes, Surgery, and In Vivo Recording of Cholinergic Activity

Ceramic-based, multisite microelectrodes featuring four 15 × 333 μm Platinum recording sites arranged in side-by-side pairs (Quanteon LLC, Nicholasville, KY; see Figure S1A) were prepared for enzyme coatings and calibrated in vitro. These methods, as well as surgical methods and procedures used for in vivo recording of cholinergic activity, are described in detail in the Supplemental Methods.

Microelectrode Sensitivity In Vivo

After completion of recording sessions, choline was infused through the guide cannula to determine the sensitivity of the microelectrode to choline. Additionally, and in order to confirm that the responses of the implanted microelectrode reflects choline resulting from the hydrolysis of endogenously generated ACh, the effect of neostigmine, an AChE inhibitor, on potassium-evoked choline signals was determined (see the Supplemental Materials for methods and results).

Choline Signal Analysis and Group Sizes

Methods used for self-referencing of choline signal recordings, the analysis of event-evoked cholinergic signals and session-related tonic changes in cholinergic activity, methods used for the microdialysis experiments and comparison of session-related tonic changes in cholinergic activity with microdialysis release data, and the number of animals per group, are described in the Supplemental Materials.

Amperometric Recordings Following the Removal of Cholinergic Inputs

In order to confirm that changes in choline levels recorded in the mPFC of task-performing animals reflect choline resulting from hydrolysis of newly released ACh from cholinergic terminals, electrodes were implanted in the mPFC following cholinergic deafferentation of the recording area by infusion of the immunotoxin 192 IgG-saporin (192-SAP; ATS, San Diego, CA). Animals (n = 5) received unilateral infusions of 192-SAP (100 ng/0.5 μl) into the right mPFC using the following coordinates: AP: +3.2 mm, ML: −0.7 mm; DV: −3.5 mm. Infusions were made at a rate of 0.25 μl/min using a 1 μl Hamilton microsyringe; the needle remained in place for an additional 4 min following the infusion. Animals were returned to daily test sessions and microelectrodes were implanted 3 weeks later. Importantly, such unilateral, restricted deafferentation of the recording region does not affect the rats' performance on attention-demanding tasks (Gill et al., 2000).

Effects of Bilateral Removal of Cholinergic Inputs on Task Performance

To determine whether cholinergic innervation of the mPFC is necessary for the performance of the cued appetitive response task, the hypothesis that bilateral removal of cholinergic inputs into the mPFC reduces cue detection rate was tested in a separate group of animals (n = 5). These animals were trained to task criterion. In order to remove cholinergic inputs to the mPFC (including prelimbic and infralimbic region and anterior cingulate cortex), 192-SAP (100 ng/0.5 μl) was infused bilaterally at two sites per hemisphere (AP: +3.7 and +2.6; ML: ± 0.7 mm; DV: −3.5 mm). Following 2 days of postsurgery recovery with food and water ad libitum, the animals were returned to the deprivation regimen and daily test sessions. Animals were tested for 3 more weeks. Sessions were videotaped once a week for analysis (see Supplemental Materials for histological methods).

Statistical Analyses

Statistical analyses were performed using SPSS/PC+ (V13.0; SPSS, Chicago, IL). Repeated-measure mixed factor ANOVAs were used to analyze the effects of group (intact and unilateral lesion, two levels; prelesion [bilateral] and postlesion; four levels), task (standard and shorter cue-reward interval; two levels), and trial blocks (five levels) on behavioral performance. Post hoc multiple comparisons for analysis of significant main effects were performed using Least Significance Difference (LSD) test or independent t tests. One-way ANOVAs or planned multiple two-tailed unpaired t tests were employed to test group differences with respect to the proportion of detected cues, reward retrieval latencies, and port approach frequencies. The effect of trial blocks on the proportion of cues that were detected was examined using one-way ANOVA (for more details see Supplemental Materials).

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/56/1/141/DC1/.

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