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Research report

The effects of manipulations of attentional demand on cortical acetylcholine release

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Abstract

In vivo microdialysis was used to measure acetylcholine (ACh) efflux in the frontoparietal cortex while rats performed in one of two operant tasks. One task was designed and validated to generate measures of sustained attention, while the other task was designed to minimize explicit demands on sustained attentional resources (low-demand task). Transferring animals from the baseline environment into the operant chambers robustly increased cortical ACh efflux regardless of subsequent task demands. Performance in the sustained attention task further increased frontoparietal ACh efflux, and these increases were not observed when animals were simply exposed to the operant chamber without task performance. Manipulations of the task parameters within a session, to either increase or decrease explicit demands on sustained attention, were not associated with fluctuations in ACh efflux. Unexpectedly, performance in the low-demand task was also associated with significant increases in ACh efflux that were similar to those observed during the sustained attention task. However, widespread depletions of cortical cholinergic inputs produced by intra-basalis infusions of 192 IgG-saporin failed to impair performance in the low-demand task, suggesting that cholinergic transmission is not necessary for performance in this task. The present results indicate that although a wider range of instrumental processes than previously hypothesized are associated with increases in cortical ACh release, the dependence of performance on the integrity of cortical cholinergic inputs may be limited to tasks with explicit attentional demands.

Theme: Neural basis of behavior

Topic: Cognition

Keywords: Acetylcholine; Sustained attention; Microdialysis; Cortex; Basal forebrain; 192 IgG-saporin

1. Introduction

Dysregulations of cortical cholinergic afferents arising from the basal forebrain have been hypothesized to contribute to the development of attentional impairments observed in such diverse neuropsychiatric disorders as Alzheimer's disease and schizophrenia [25,40,43]. Research investigating the relationship between attentional processing and cholinergic systems in animals has provided strong evidence for a role of cortical cholinergic transmission in the mediation of attentional functions, including stimulus detection and selection [9,42]. Cortical responses to sensory inputs are facilitated by increases in cholinergic transmission, suggesting the presence of a mechanism by which the processing of relevant stimuli is enhanced [29,48]. The involvement of the population of corticopetal cholinergic neurons in attention is supported by findings that performance in tasks assessing various forms of attention is disrupted by excitotoxic or immunotoxic lesions of the basal forebrain [3,5,34,49].

In particular, the development of an operant task that generates measures of sustained attention in rats [27] has allowed for extensive investigation of the dependence of attentional processing on cortical cholinergic transmission. Widespread cortical cholinergic deafferentation produced

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by intra-basalis administration of the selective cholinergic immunotoxin 192 IgG-saporin results in severe and persistent impairments in signal detection in this task [26]. Similarly, intra-basalis infusions of the benzodiazepine receptor (BZR) agonist chlordiazepoxide (CDP) or the NMDA antagonist AP5, both of which attenuate stimulated cortical ACh release [10,31], also impair sustained attention performance by decreasing the accuracy of signal detection [17,50]. These results suggest that sustained attentional processing is dependent upon cortical cholinergic transmission.

In contrast to the above findings, cortical cholinergic deafferentation does not robustly impair performance in simple tasks such as passive avoidance or T-maze alternation [47,52]. In addition, infusions of either CDP [8] or AP5 [50] into the nucleus basalis do not impair performance on simple discrimination tasks that presumably do not require effortful processing [2]. The failure of such manipulations to affect performance in tasks that do not significantly tax controlled processing resources suggests that the effects of basal forebrain manipulations on performance interact with the specific cognitive demands of the task. In support of such an interaction, the selective depletion of cholinergic inputs to the medial prefrontal cortex does not impair baseline performance in the sustained attention task, but does exacerbate reductions in response accuracy imposed by the presentation of a visual distracter [13].

Results from several recent microdialysis experiments indicate that increases in cortical acetylcholine (ACh) release during performance of simple operant tasks are limited to early acquisition stages during which demands on attentional processing are high [33]. Cortical ACh release is enhanced during acquisition of a lever-pressing response, but not during execution of the previously learned response [22,35]. In contrast, recent experiments demonstrated increases in ACh release during tasks incorporating specific demands on attentional processes. Cortical ACh release is enhanced during performance in a five-choice serial reaction time task [38] or the sustained attention task discussed above [16]. In the latter study, increasing the attentional demand by presenting a visual distracter stimulus elicited further increases in cortical ACh release that appeared to correspond with the level of attentional effort [16], suggesting that manipulations of attentional demand may be useful in further elucidating the relationship between attentional processing and cortical cholinergic transmission.

The current series of experiments was designed to investigate further the effects of altering the level of attentional demand on cortical ACh release, by varying the parameters of the task during performance. In experiment 1, ACh efflux in the frontoparietal cortex was measured during both standard performance in the sustained attention task, and during within-session shifts in attentional demand. During selected blocks of task performance in these shift sessions, the parameters of the task were adjusted to reduce attentional requirements, as described in Section 2. As an additional control, animals received several days of exposure to the operant chambers in the absence of the task ('contextual extinction'), followed by a final extinction microdialysis session. This additional session was intended to elucidate the factors contributing to the enhancement of ACh release observed upon transfer to the operant chambers, as well as to further characterize the specificity of previously observed increases during task performance [16]. The frontoparietal cortex was chosen based on previous experiments measuring ACh efflux from this area during sustained attention task performance [16], and the hypothesis that ACh release is relatively uniform throughout the cortical mantle [42].

In experiment 2, a separate set of animals was trained exclusively in the low-demand version of the task, and cortical ACh efflux was measured during performance of this task. The effects of contextual extinction were also assessed in these animals. Finally, to investigate the necessity of cortical cholinergic inputs for performance of the low-demand task, the effects of intra-basalis administration of the immunotoxin 192 IgG-saporin on performance in this task were assessed in experiment 3.

2. Materials and methods

2.1. Subjects and apparatus

Subjects for all experiments were male Fisher/Brown-Norway rats (FBNF1, Harlan Sprague–Dawley, Indianapolis, IN) that were 3 months old at the beginning of behavioral training and 6–12 months old during microdialysis sessions. Animals were individually housed in a temperature (23°C) and humidity (45%) controlled environment on a 12:12-h light:dark schedule (lights on at 06:30 h). All animals were extensively handled prior to beginning training, and were water-deprived to ~90% of their free weight with free access to food throughout the experiment. All housing, surgery, experimentation, and euthanasia procedures were approved by the Ohio State University Animal Care and Use Committee, and were performed in accordance with the US Public Health Service Policy on the Humane Care and Use of Laboratory Animals.

Initial behavioral training took place in a set of eight operant chambers (MedAssociates, St. Albans, VT) equipped with intelligence panels and water dispensers as previously described [16]. Microdialysis experiments were performed in a set of four operant chambers that were similar to the chambers described above, with modifications for microdialysis equipment [16]. Signal presentations, lever operation, reinforcement delivery, and data collection in each system were controlled by a

Pentium PC with Med-PC for Windows software (v. 1.1, MedAssociates).

2.2. Behavioral training

All animals were initially shaped to lever-press on a modified FR-1 schedule for water reinforcement. Following 3 days of at least 100 reinforced lever presses, animals began training in either the sustained attention (experiment 1) or the low-demand task (experiments 2 and 3).

2.2.1. Sustained attention task (experiment 1)

The basic response rules of the sustained attention task are illustrated schematically in the top panel of Fig. 1. The training steps involved in acquisition of this task have been described extensively in previous publications [16,27]. Briefly, in the final version of the task, animals were required to discriminate between signal (500-, 50-, or 25-ms illumination of the central panel light) and non-signal (no illumination) events following an 18-min period of adaptation to the operant chamber. The response bin was cued by the extension of the levers into the chamber 2 s after signal or non-signal presentation. On signal trials, a response on the left lever was reinforced and termed a 'hit'; a response on the right lever was not reinforced and termed a 'miss'. On non-signal trials, a response on the right lever was reinforced and termed a 'correct rejection'; a response on the left lever was termed a 'false alarm' and was not reinforced. If no response took place within 4 s, the levers were retracted and an omission was recorded. Signal type and length were pseudo-randomly determined for each trial, with approximately equal occurrences of signal and non-signal trials throughout the session with an inter-trial interval (ITI) of 9±3 s. The houselight was illuminated throughout the 18-min pretask period and the 36-min task performance in the final version of the task.

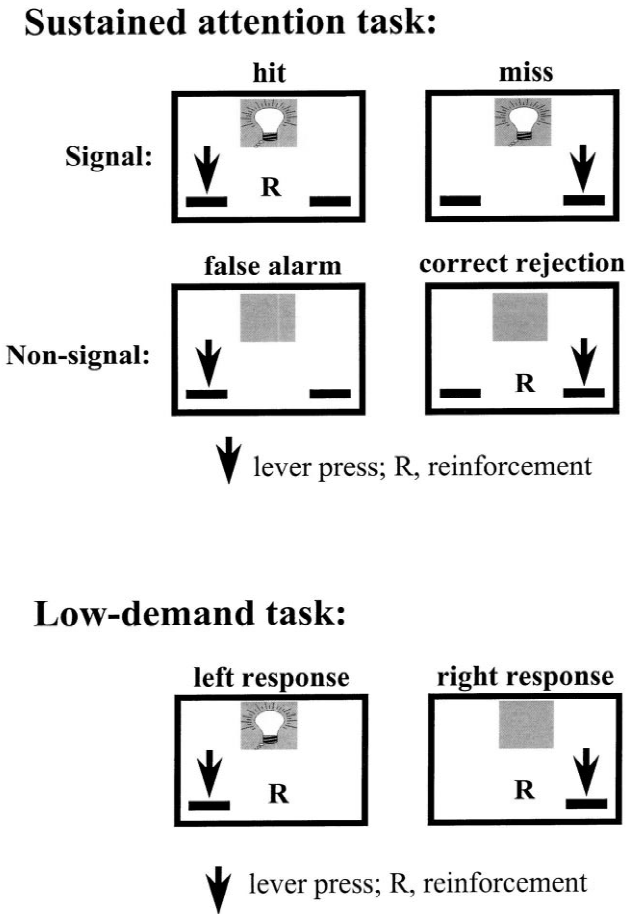

Fig. 1. (Top) Schematic illustration of the response rules of the sustained attention task (see Section 2.2.1 for full description). Signal: illumination of the central panel light; non-signal: no illumination. (Bottom) Schematic illustration of the response rules of the low-demand task. Animals were reinforced for responding on the extended lever on each trial.

The relative number of hits (hits/hits+misses) was calculated for each signal length. The relative number of correct rejections (correct rejections/correct rejections+false alarms) was also calculated. Side bias was calculated using the formula SB=(hits+false alarms)/(hits+misses+false alarms+correct rejections), such that a value of 0.5 indicates the complete absence of a side bias. Finally, the relative number of omissions was calculated (omissions/number of trials). Each behavioral measure was calculated for the entire 36-min task, and also for each of six task blocks (6 min each) to assess the effects of time on performance and to correspond with the microdialysis collection intervals. Stable performance in this task was defined as at least seven consecutive sessions with at least 70% hits to 500-ms signals and 70% correct rejections, and less than 40% omissions.

2.2.2. Low-demand task (experiments 1–3)

This task was designed to minimize explicit demands on sustained attentional processing, as described below. In this task, the basic response rules are similar to those in the sustained attention task. However, only the correct lever was extended on each trial (Fig. 1, bottom panel). This modification removes the requirement for animals to make a decision regarding stimulus presence or absence. Two additional parameters of the sustained attention task that are crucial components of testing sustained attention performance [27,37] were also manipulated in the low-demand task. Signal length remained constant (500 ms) across all signal trials, and the temporal uncertainty of the sustained attention task was removed by fixing the ITI at 9 s.

As only the correct lever was extended on each trial in the low-demand task, all animals achieved 100% accuracy with virtually no variation. Thus, behavioral measures of performance in this task were limited to the percentage of trials completed and a measure of side bias, calculated as SB=(left lever responses)/(left+right lever responses). As for the sustained attention task, these behavioral measures were calculated for the entire 36-min task, and also for

each of six 6-min task blocks to correspond with microdialysis collection intervals and to track performance across time in the task. Stable performance in this task was defined as at least seven consecutive sessions with at least 75% completed trials and a side bias between 0.4 and 0.6.

2.3. Guide cannula implantation

Upon reaching stable baseline performance in the operant chambers (as defined above) in the sustained attention task (experiment 1) or in the low-demand task (experiment 2), animals continued training in the microdialysis operant chambers. When performance was stable in these chambers, familiarization to the microdialysis procedures was initiated as outlined in the description of the individual experiments below (Sections 3.1 and 4.1). Following completion of these procedures, performance was once again allowed to become stable before surgery.

Under anesthesia (ketamine, 90 mg/kg+xylazine, 6 mg/kg), a chronic microdialysis guide cannula (10-mm plastic shaft, O.D. 720 μm, Bioanalytical Systems (BAS), West Lafayette, IN) was implanted just above the left or right frontoparietal cortex at the following stereotaxic coordinates relative to bregma: AP, − 1.5 mm; L, +2.0 mm; V, −1.0 mm from dura at a 45–50° lateral angle away from the midline. The cannula was affixed to the skull with screws and dental cement. A stainless steel stylet that ended flush with the termination of the guide cannula was inserted into the guide cannula to prevent clogging. Animals were given a post-operative injection of amoxicillin (100 mg/kg, s.c.) and were allowed to recover for 3 days with free access to water. After recovery, water deprivation levels were reinstated and animals resumed daily training in the microdialysis operant chambers.

2.4. Microdialysis sessions and acetylcholine analysis

Each animal in experiments 1 and 2 participated in multiple microdialysis sessions. We have previously established the validity of this repeated perfusion procedure for the measurement of both cortical ACh efflux [32] and striatal ACh efflux [23] by demonstrating that the effects of pharmacological manipulations do not interact significantly with session order. The specific makeup of each of these sessions for experiments 1 and 2 is detailed within the descriptions of the individual experiments in Sections 3.1 and 4.1 below.

Generally, during each microdialysis session, animals were placed into plastic test bowls (35 cm high, 38 cm diameter, CMA, Stockholm, Sweden) for 1 h to habituate, after which the stylet was removed and a concentric probe with a 2.0-mm membrane tip (O.D. 320 μm, BAS) was inserted through the guide cannula into the cortex and perfused at 2.0 μl/min with an artificial cerebrospinal fluid (CSF; pH 6.9±0.1) with the following composition (in mM): 126.5 NaCl, 27.5 $NaHCO_3$, 2.4 KCl, 0.5 Na_2SO_4, 0.5 KH_2PO_4, 1.2 $CaCl_2$, 0.8 $MgCl_2$, 5.0 glucose, and 0.1 μM of the reversible cholinesterase inhibitor neostigmine bromide (Sigma, St. Louis, MO). This moderate concentration of neostigmine was chosen in order to assure that ACh levels would be detectable with the short (6-min) collection intervals utilized. Following a 3-h discard period to allow ACh efflux to become stable and dependent upon neuronal depolarization [30], collection of dialysate samples (6 min) began. A total of five baseline collections were taken in the dialysis bowl, after which animals were transferred to the operant chamber. The timing of dialysates collected while the animal was in the operant chamber was adjusted to allow for the dead volume of the probe (0.5 μl) and outlet tubing (~50-cm FEP tubing, CMA). First, three samples were collected before the task began to assess the effects of transfer separately from task effects, and to establish a new baseline level of ACh efflux. Then, six collections were taken during performance of the task, corresponding to the six blocks of the task. Finally, three dialysate samples were collected following the completion of the task while the animal remained in the lighted operant chamber.

Dialysate samples were frozen at −80°C until analysis by high performance liquid chromatography with electrochemical detection (ESA, Chelmsford, MA) using a mobile phase containing 100 mM sodium phosphate, 0.5 mM tetramethylammonium chloride, and 2 mM 1-octanesulfonic acid. Acetylcholine and choline were separated on a 250-mm analytical column and catalyzed on a post-column solid phase reactor containing acetylcholinesterase and choline oxidase. Acetylcholine was hydrolyzed to acetate and choline, and choline oxidized to hydrogen peroxide and betaine, according to the method originally described by Potter et al. [41]. Hydrogen peroxide corresponding to ACh was then detected using a 'peroxidase-wired' glassy carbon electrode [18] with an applied potential of −200 mV. Concentration of ACh was calculated by integrating the area under the peak and fitting this value to a regression line containing standard values of ACh that were in the expected range of the in vivo dialysates. The detection limit of this system averaged 10 fmol/10-μl injection of dialysate.

2.5. Lesion surgery

Animals in experiment 3 underwent surgery to produce widespread cortical cholinergic deafferentation. Animals were anesthetized with ketamine (90 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.) and 0.5 μg/μl of the selective immunotoxin 192 IgG-saporin (Lot AT-4, Advanced Targeting Systems, San Diego, CA) was bilaterally infused into the nucleus basalis region of the basal forebrain. Bolus infusions were made in a volume of 0.5 μl per hemisphere using a 1.0-μl Hamilton syringe at the following coordinates relative to bregma: AP, −0.8 mm; L, ± 2.5 mm; DV, −7.2 mm from the dural surface. The syringe was left in

place for at least 2 min after each infusion before being slowly retracted. Animals were given a post-operative injection of amoxicillin (100 mg/kg, s.c.) and were allowed to recover for 7 days with free access to water. After recovery, water deprivation levels were reinstated and animals resumed daily training in the operant chambers.

2.6. Histological procedures

2.6.1. Verification of probe placements (experiments 1 and 2)

Within a week following the last microdialysis session, animals were deeply anesthetized and transcardially perfused with saline followed by formalin. Brains were post-fixed in formalin overnight, and transferred to a 30% sucrose phosphate buffer solution. Sections (40 μm) surrounding the probe site were mounted, stained for Nissl substance (cresyl violet), and examined for cannula and probe placements.

2.6.2. Histochemical analysis (experiment 3)

Following completion of 4 weeks of post-surgical training, animals that received cholinergic depletions were deeply anesthetized and perfused as described above. After post-fixation and cryoprotection, coronal sections (50 μm) throughout the extent of the cortex were taken and stained for acetylcholinesterase (AChE) activity according to a protocol modified from Tago et al. [45] and described extensively in Gill et al. [14]. Sections from deafferented animals were examined microscopically for AChE-positive fiber staining, and compared to sections from unoperated control animals to obtain an estimate of cholinergic depletion.

2.7. Statistical analysis

For each experiment, repeated measures ANOVAs were performed on behavioral and neurochemical data separately. Percentage data (hits, correct rejections, omissions, and completed trials) were angularly transformed ($x' = 2^*\arcsin x^{1/2}$) before analysis to correct for the skewed distribution of percentage scores [53]. To control for possible violations of the sphericity assumption of homogeneity of variances, repeated measures ANOVAs with more than two levels of any factor were evaluated using ϵ-corrected degrees of freedom [51]. Uncorrected degrees of freedom, corrected P-values, and Huynh-Feldt ϵ-values are reported here. A significance level of $\alpha = 0.05$ was used in all analyses. Significant F-values were further evaluated with multiple dependent t-tests using the modified Bonferroni correction [24]. The corrected level of significance is signified in the text as α_{comp} (per comparison), and is computed as $\alpha_{comp} = (\alpha^* df_{num})/\text{number of comparisons}$.

3. Experiment 1: cortical ACh efflux during shifts in attentional demand

This experiment investigated changes in cortical ACh efflux during both standard performance in the sustained attention task, with the intent to replicate previous findings [16], and during unpredicted shifts in the attentional demands of the task. Cortical ACh efflux was predicted to vary as a function of attentional demand in this experiment.

3.1. Experiment 1: procedures

3.1.1. Behavioral training and pre-microdialysis procedures

A total of ten animals were trained to participate in this experiment; one animal was excluded due to illness. After 124±18.5 (mean±S.E.M.) sessions, performance in the final version of the task was stable, and animals began training in the microdialysis chambers. Following 11±3.0 sessions, familiarization procedures were initiated. Animals were placed in microdialysis bowls for ~4 h daily prior to task performance. In addition, animals received two exposures to the 'shift-low' session and one exposure to the 'shift-high' session. In the shift-low session, animals performed in the standard sustained attention task for the first task block, after which the task was shifted to the low-demand task for the remaining five blocks. In the shift-high session, the first three task blocks consisted of the low-demand task, and the last three blocks of the standard sustained attention task.

Following at least 3 days of stable performance after the third and final shift exposure (mean=6±1.1 sessions), animals underwent surgery as described in Section 2.3. Of the nine animals in experiment 1, five received a cannula implanted in the left hemisphere and four in the right hemisphere. Following recovery from surgery and upon returning to stable performance in the sustained attention task (mean=10±1.0 sessions), animals were tethered inside the operant chamber during their daily training sessions. Animals then received three more exposures to the shift sessions, consisting either of two shift-low and one shift-high session ($n=3$), or one shift-low and two shift-high sessions ($n=6$). Then 2–3 days following the third shift session, the first microdialysis session took place.

3.1.2. Microdialysis sessions

Animals in experiment 1 participated in five microdialysis sessions. Two of these sessions consisted of performance in the standard sustained attention task during all six task blocks. Another two sessions consisted of one shift-low session and one shift-high session pseudo-randomly distributed across the first four sessions, with the stipulation that shift microdialysis sessions were separated by one standard microdialysis session. At least 1 but no

more than 4 days intervened between each microdialysis session (mean=3±0.3 days), during which animals continued training in the standard sustained attention task.

3.1.3. Contextual extinction

Following completion of the fourth microdialysis session, animals began exposures to contextual extinction sessions. Animals were placed in the lighted operant chamber at the accustomed time (i.e. following 4 h in the bowl), but the task was not started. Animals remained in the operant chamber for 90 min, representing the normal duration of the pretask, task, and post-task periods. During the contextual extinction protocol, animals were given free access to water in the colony room, and received no reinforcement in the operant chambers. Following 7–10 consecutive days (mean=9±0.4) of contextual extinction, animals participated in a fifth microdialysis session during which they were transferred into the lighted chamber and remained there for 90 min in the absence of the task or any other events. The one animal which did not complete the extinction session is excluded from relevant statistical analyses.

3.2. Experiment 1: results

3.2.1. Basal ACh efflux and transfer effects

For all nine animals included in statistical analyses, cannula and probe placements were located within the frontoparietal cortex, as illustrated schematically in the left panel of Fig. 2. Basal cortical ACh efflux (pmol/10 μl values) in the microdialysis bowls was analyzed for changes across both repeated microdialysis sessions and collection interval, with a two-way repeated measures ANOVA with factors of Session (four levels; sessions 1–4) and Time (five levels; baselines 1–5). Basal efflux (pmol/10 μl) decreased as a function of repeated microdialysis sessions ($F_{3, 24}=3.798$, $P=0.029$, $\epsilon=0.894$; session 1: 0.062±0.006; session 2: 0.071±0.015; session 3: 0.027±0.006; session 4: 0.045±0.010). Multiple dependent *t*-tests comparing efflux in session 1 with each subsequent session revealed that ACh efflux declined significantly from the first to the third microdialysis session ($t_8=5.684$, $P<0.001$, $\alpha_{comp}=0.05$). However, ACh values remained stable across the baseline collections within each session, as evidenced by the lack of either a main effect of Time ($F_{4, 32}=0.504$, $P=0.586$, $\epsilon=0.426$) or an interaction between Session and Time ($F_{12, 96}=0.44$, $P=0.863$, $\epsilon=0.545$). Importantly, a subsequent analysis of median bowl baselines as a function of session type, rather than chronological microdialysis session as above, indicated that basal ACh efflux (pmol/10 μl) did not differ significantly during the baseline periods of the standard, shift-low, and shift-high sessions ($F_{3, 24}=2.93$, $P=0.075$, $\epsilon=0.746$; standard 1: 0.056±0.017; standard 2: 0.026±0.004; shift-low: 0.048±0.007; shift-high: 0.067±0.012).

For subsequent analyses, ACh values were expressed as a percent change from baseline, defined either as the median of the five bowl baseline collections (for analysis of transfer and pretask effects) or the mean of pretask 2 and pretask 3 collections (for analysis of task and post-task effects, see below). The effect of transferring animals into the operant chambers was analyzed across all microdialysis sessions using a two-way repeated measures ANOVA with the factors of Session (four levels; sessions 1–4) and Time (four levels; last baseline, transfer, pretask 2, pretask 3). Fig. 3 illustrates that ACh levels increased upon transfer ($F_{3, 24}=16.974$, $P=0.001$, $\epsilon=0.51$). Multiple dependent

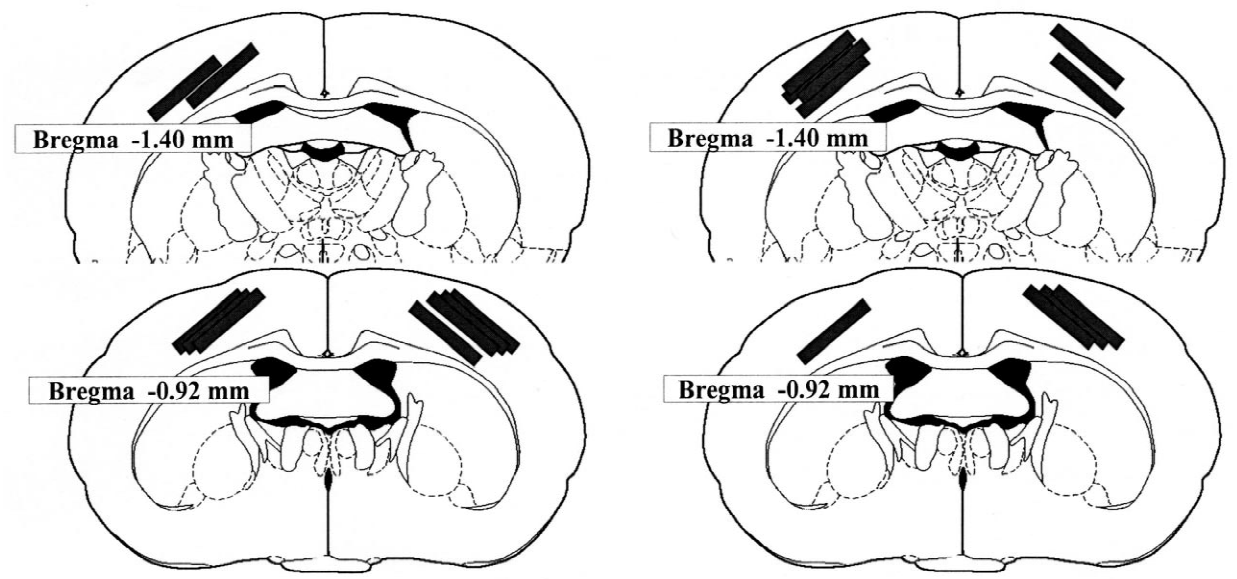

Fig. 2. Microdialysis probe placements (membrane tip, 2 mm) in the frontoparietal cortex in experiments 1 (left, $n=9$) and 2 (right, $n=10$). Drawings adapted from Paxinos and Watson [39].

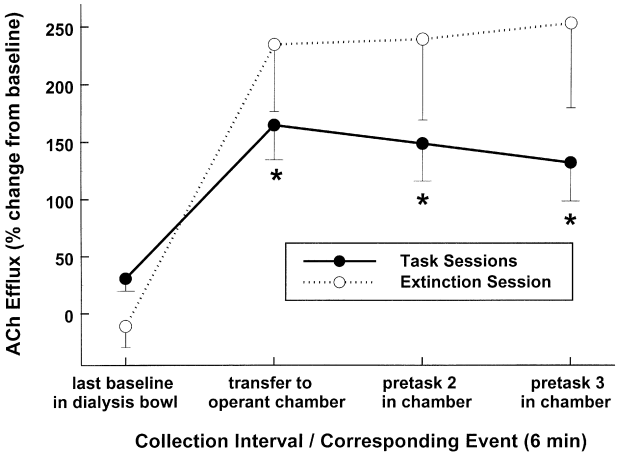

Fig. 3. Effects of transfer into the operant chamber on cortical ACh efflux (percent change from bowl baseline) during sustained attention task and extinction sessions in experiment 1. Task sessions: ACh values are collapsed across all four microdialysis sessions (baseline=0.048±0.006 pmol/10 μl); extinction session: ACh values during the fifth microdialysis session, following 7–10 days of contextual extinction (baseline=0.033±0.011 pmol/10 μl). Task sessions: *$P<0.01$ versus the last baseline collection.

t-tests revealed that ACh efflux, collapsed across all four task sessions, was significantly higher than the final bowl baseline during the transfer ($t_8=5.397$, $P=0.001$, $\alpha_{comp}=0.038$), the second pretask ($t_8=4.527$, $P=0.002$), and the third pretask collections ($t_8=3.762$, $P=0.006$). Although ACh levels remained well above baseline by the third pretask collection, there was also an attenuation of the transfer effect over time from the transfer to the third pretask collection ($t_8=2.633$, $P=0.03$). The effect of time on ACh efflux did not differ across the four sessions ($F_{9,72}=0.932$, $P=0.441$, $\epsilon=0.334$), suggesting that it was not diminished by repeated experience with the transfer protocol during microdialysis sessions.

3.2.2. Task performance during standard sessions

The relative number of hits during the two standard sessions was analyzed using a two-way repeated measures ANOVA with the factors of Session (two levels; standard 1 and 2), Task Block (six levels), and Signal Length (three levels; 500, 50, and 25 ms). Performance did not differ significantly between the two sessions in the standard sustained attention task ($F_{1,8}=3.547$, $P=0.096$). Fig. 4 (top panel) illustrates that collapsed across both standard task sessions, the relative number of hits was signal-length dependent ($F_{2,16}=26.494$, $P<0.001$, $\epsilon=1$), as previously observed [16,27]. This signal-length dependency of the relative number of hits did not interact with either Session or Block (all *P*-values>0.288).

Analyses of the relative number of correct rejections, side bias, and the relative number of omissions during the two standard sessions were carried out using repeated measures ANOVAs with the factors of Session (two) and Block (six). As illustrated in the top panel of Fig. 4, the

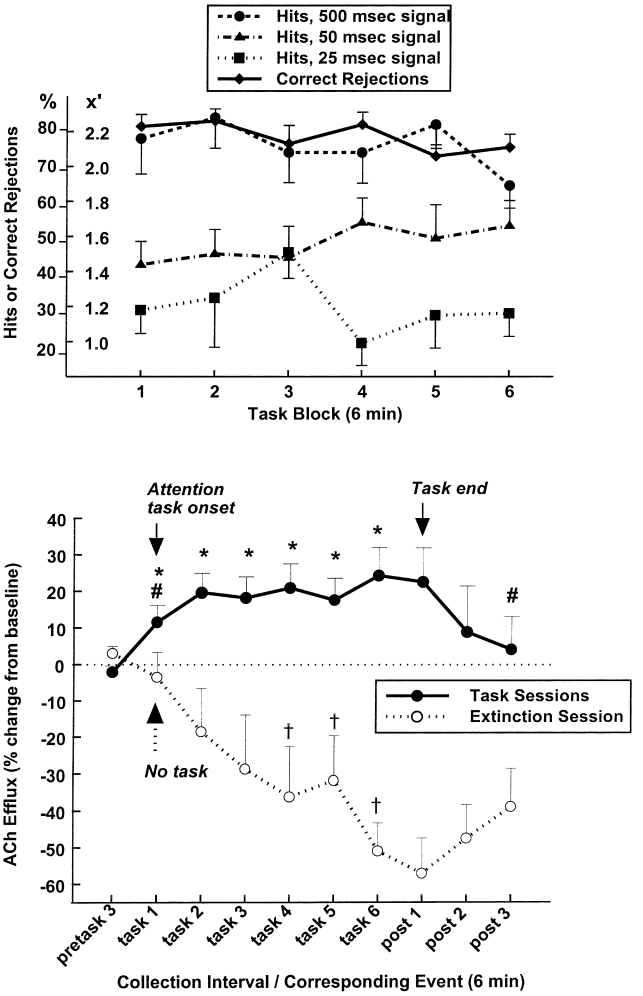

Fig. 4. (Top) Performance in the sustained attention task during the standard microdialysis sessions in experiment 1. The relative number of hits (hits/hits+misses) to each signal length and the relative number of correct rejections (correct rejections/correct rejections+false alarms) are shown across the six task blocks. In this and subsequent figures, transformed values of each measure (x') are depicted, with corresponding percentage values (%) included on the ordinate for clarity. Data are collapsed across the two standard task sessions. (Bottom) Cortical ACh efflux (percent change from operant chamber baseline) during and after task performance in the standard sustained attention task and following contextual extinction in experiment 1. For the standard task sessions, data are collapsed across the two standard task sessions (baseline=0.089±0.016 pmol/10 μl), and correspond with the behavioral data presented in the top panel. For the extinction session (baseline=0.080±0.022 pmol/10 μl), there are no corresponding behavioral data as animals were simply placed in the lighted test chamber and the task was not started. Standard task sessions: *$P<0.043$ versus pretask 3; #$P<0.032$ versus task 6. Extinction session: †$P<0.021$ versus pretask 3.

relative number of correct rejections was similar across the two standard sessions ($F_{1,8}=0.041$, $P=0.845$), and did not significantly vary across task blocks ($F_{5,40}=2.228$, $P=0.077$, $\epsilon=0.913$). There was no difference between the two standard sessions on this measure of performance over time ($F_{5,40}=1.108$, $P=0.369$, $\epsilon=0.725$). Neither side bias nor omissions exhibited significant variations across either

standard sessions or task Block (side bias, all P-values$>$ 0.12; omissions, all P-values$>$0.086; data not shown). As performance over the two sessions of the standard sustained attention task did not differ on any measure, these data were subsequently collapsed for subsequent comparisons and in relevant figures.

3.2.3. ACh efflux during standard task performance

To provide a more appropriate baseline measure of ACh efflux in the operant chambers immediately prior to task onset, a second baseline was recalculated as the mean of the second and third pretask collections (in pmol/10 μl). Analyses of task and post-task effects were conducted on percent change from these recalculated operant chamber baseline values. To analyze changes in ACh efflux during standard task performance, a two-way repeated measures ANOVA with the factors of Session (two) and Time (seven levels; pretask 3, and tasks 1–6) was conducted on percent change from (operant chamber) baseline values. As changes in ACh efflux did not differ across the standard sessions, either overall ($F_{1, 8}$=0.630, P=0.45) or across task blocks ($F_{6, 48}$=1.349, P=0.257, ϵ=0.947), data were collapsed across these two sessions for illustration and subsequent analyses. The bottom panel of Fig. 4 shows that ACh efflux significantly increased during task performance (Time: $F_{6, 48}$=5.775, P=0.002, ϵ=0.622). Planned comparisons consisting of multiple dependent t-tests indicated that ACh efflux was significantly higher during each of the six task blocks than in the third pretask collection (task 1: t_8=2.496, P=0.037, α_{comp}=0.05; task 2: t_8=3.229, P=0.012; task 3: t_8=2.98, P=0.018; task 4: t_8=2.882, P=0.02; task 5: t_8=2.825, P=0.022; task 6: t_8=3.019, P=0.017).

ACh efflux following task completion was analyzed using a two-way repeated measures ANOVA with factors of Session (two) and Time (four levels; task 6, and post-tasks 1–3). Post-task ACh values decreased in comparison to the final task block across both sessions, as indicated by a main effect of Time ($F_{3, 24}$=4.247, P= 0.032, ϵ=0.68) but no interaction between Session and Time ($F_{3, 24}$=1.013, P=0.40, ϵ=0.913). Post-hoc analyses revealed that this decline in ACh levels did not appear until the third post-task collection as compared to the final task block (t_8=2.611, P=0.031, α_{comp}=0.05), as illustrated in Fig. 4.

3.2.4. ACh efflux following contextual extinction

Although the extinction session was always the last microdialysis session, the results from this session are presented here for clarity. Cortical ACh efflux during this session was analyzed as previously, in three separate blocks of time (transfer effects, 'task' effects, and 'post-task' effects).

The effects of transfer on ACh efflux (percent change from bowl baseline) were examined with a two-way repeated measures ANOVA with the factors of Session (two levels; all four previous sessions collapsed, and extinction session) and Time (four levels; last baseline, transfer, pretask 2, pretask 3). A significant main effect of Time indicated that ACh levels were increased upon transfer during the extinction session ($F_{3, 21}$=46.135, $P<$ 0.001, ϵ=0.466). However, as depicted in Fig. 3, this transfer effect did not differ from the increase observed in the previous task sessions (Session: $F_{1, 7}$=0.572, P= 0.474; Session×Time: $F_{3, 21}$=2.713, P=0.137, ϵ=0.382).

ACh efflux (percent change from the pretask baseline) during the collection intervals in which the task would have been presented was analyzed for changes over time and in comparison to the standard task sessions, using a two-way repeated measures ANOVA with the factors of Session (two levels; collapsed standard sessions and extinction session) and Time (seven levels; time points corresponding to pretask 3 and tasks 1–6). A significant main effect of Session indicated that over all time points analyzed, ACh efflux was lower during the extinction session than during the standard sessions ($F_{1, 7}$=141.105, $P<$0.001). Furthermore, the bottom panel of Fig. 4 illustrates that the pattern of changes in ACh efflux over these time points was significantly different during the extinction session as compared to the standard sessions ($F_{6, 42}$=7.642, $P<$0.001, ϵ=0.675). A one-way ANOVA on the effects of Time during the extinction session revealed that ACh levels significantly fluctuated over the seven collection intervals corresponding to pretask 3 and task blocks 1–6 ($F_{6, 42}$=3.739, P=0.008, ϵ=0.853). Post-hoc dependent t-tests indicated that ACh efflux was significantly lower than 'pretask' levels during the blocks corresponding to task 4 (t_7=3.123, P=0.017, α_{comp}= 0.043), task 5 (t_7=2.998, P=0.02), and task 6 (t_7=6.047, P=0.001).

These results indicate that simple exposure to the test chamber during the 'task' time points is not sufficient to increase cortical ACh release. This finding supports the specificity of the task-related increases observed during the standard task sessions.

3.2.5. Task performance during the shift-low session

For the blocks of the shift sessions in which animals performed in the standard sustained attention task, performance on all measures (relative number of hits and correct rejections, side bias, and omissions) was compared to the corresponding block(s) of the standard sessions (collapsed). Analysis of behavior across all six blocks of the two shift sessions, including performance in both sustained attention and low-demand blocks, was limited to the measures of side bias and the relative number of omissions (Section 2.2.2).

Performance on all behavioral measures in block 1 of the shift-low session was compared to block 1 of the collapsed standard sessions (Fig. 4), using one-way or two-way repeated measures ANOVAs. Analysis of signal detection performance during the first block of the shift-

low session revealed that the relative number of hits was once again significantly affected by signal length ($F_{2, 16}$= 13.746, $P<0.001$, $\epsilon=1$), as depicted in the top panel of Fig. 5. Hits during the first block of the shift-low session did not significantly differ from hits in the first block of the standard sessions (Session: $F_{1, 8}=0.093$, $P=0.768$; Session×Signal Length, $F_{2, 16}=0.504$, $P=0.583$, $\epsilon=$ 0.839). The relative number of correct rejections was also similar during block 1 of these two sessions ($F_{1, 8}=0.082$, $P=0.781$). Finally, neither side bias nor the relative number of omissions differed as a function of the two different sessions (both P-values>0.74). These results indicate that overall performance during the first block of the shift-low session was similar to performance during the first block of the standard sustained attention task sessions.

Side bias and the relative number of omissions throughout the shift-low session were analyzed with separate

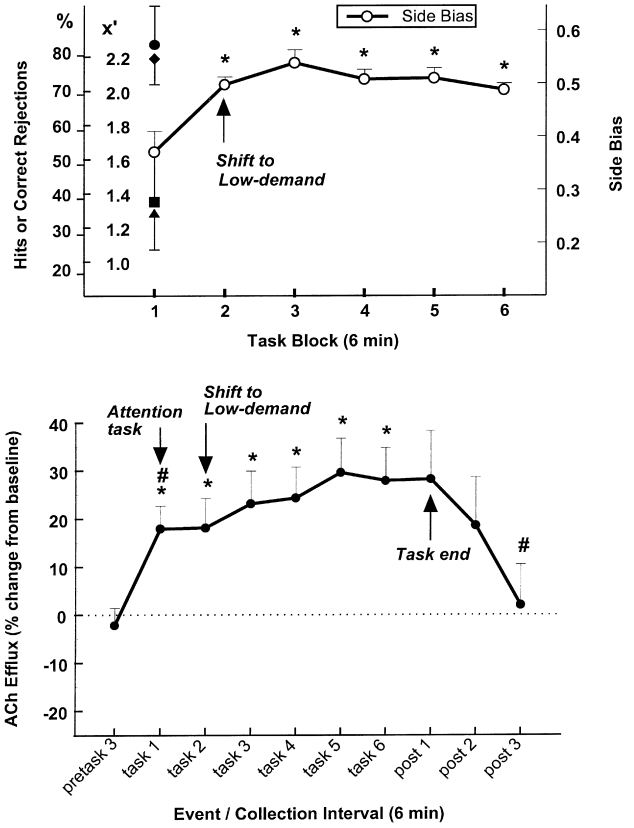

Fig. 5. (Top) Performance in the shift-low microdialysis session in experiment 1. Animals performed in the sustained attention task for block 1, and then shifted to the low-demand task (indicated by the arrow) in block 2 for the remainder of the task. The relative number of hits to each signal length, and the relative number of correct rejections (left axis) are shown for the first block only, as measures of accuracy are not meaningful for low-demand task blocks (see Materials and methods, Section 2.2.2). Symbols for block 1 are as in Fig. 4. *$P<0.05$ versus task block 1. (Bottom) Cortical ACh efflux (percent change from baseline, 0.082±0.013 pmol/10 μl) during and after task performance in the shift-low session in experiment 1. Task onset and the shift to the low-demand task are indicated by arrows. *$P<0.027$ versus pretask 3; #$P<0.023$ versus task 6.

one-way repeated measures ANOVA with the factor of Block (six levels). As predicted by the parameters of the low-demand task, animals demonstrated a significant change in side bias following the shift to this task ($F_{5, 40}=$ 6.651, $P<0.001$, $\epsilon=0.808$), as illustrated in the top panel of Fig. 5. Dependent t-tests revealed that in comparison to block 1 (sustained attention task), side bias values were significantly higher (i.e. closer to a 'neutral' side bias of 0.5) during each of the subsequent five blocks of the low-demand task (block 2: $t_8=2.589$, $P=0.032$, $\alpha_{comp}=$ 0.05; block 3: $t_8=3.986$, $P=0.004$; block 4: $t_8=3.79$, $P=0.005$; block 5: $t_8=3.456$, $P=0.009$; block 6: $t_8=$ 2.758, $P=0.025$). The shift to the low-demand task did not significantly affect the omission rate ($F_{5, 40}=2.087$, $P=$ 0.091, $\epsilon=0.950$, data not shown).

3.2.6. ACh efflux during shift-low task performance

Changes in ACh efflux during and after performance of each of the shift sessions was analyzed separately with one-way ANOVAs with the factor of Time as defined for the standard sessions above. During the shift-low session, ACh efflux was enhanced during task performance ($F_{6, 48}=8.999$, $P=0.001$, $\epsilon=0.47$). A total of 11 comparisons (dependent t-tests, evaluated using the modified Bonferroni correction as described in Section 2.7) were conducted to determine the basis of this effect. As illustrated in Fig. 5 (bottom panel), planned comparisons revealed that ACh levels were higher during each task block as compared to the final pretask collection (task 1: $t_8=3.40$, $P=0.009$, $\alpha_{comp}=0.027$; task 2: $t_8=2.728$, $P=$ 0.026; task 3: $t_8=3.768$, $P=0.005$; task 4: $t_8=3.345$, $P=0.01$; task 5: $t_8=3.53$, $P=0.008$; task 6: $t_8=3.862$, $P=0.005$).

To assess whether ACh levels varied specifically as a function of task demands in the shift-low session, ACh efflux in task 1 (standard sustained attention task) was compared to ACh efflux during each of task blocks 2–6 (low-demand task). Cortical ACh efflux did not differ between task 1 and task 2, the first low-demand task block, ($t_8=0.082$, $P=0.937$), suggesting that the shift itself did not immediately affect cortical ACh levels. Further multiple comparisons ($\alpha_{comp}=0.027$) revealed that ACh levels did not change during subsequent task blocks 3–5 relative to task 1 (all P-values>0.031), but that ACh efflux was significantly higher in task 6 ($t_8=2.801$, $P=0.023$).

Analysis of ACh values following completion of task performance in the shift-low session revealed a significant decline in ACh efflux during the post-task period ($F_{3, 24}=$ 5.196, $P=0.007$, $\epsilon=1$), as shown in Fig. 5. Post-hoc analyses indicated that, as described for the standard sessions, this effect was due to a significant decrease in ACh levels during the third post-task collection relative to the final task block ($t_8=3.733$, $P=0.006$, $\alpha_{comp}=0.05$).

3.2.7. Task performance during the shift-high session

Side bias and the relative number of omissions during

the shift-high session were analyzed as described above, using one-way repeated measures ANOVAs with the factor of Block (six). As illustrated in the top panel of Fig. 6, side bias values were significantly affected by Time in the shift-high session ($F_{5, 40}$=7.561, P<0.001, ϵ=0.935). A series of *t*-tests indicated that side bias values did not fluctuate across the first three (low-demand) blocks of this task (P-values>0.14, α_{comp}=0.042). Following the shift to the sustained attention task, animals' side bias was significantly lowered (e.g. became biased toward the right lever) during blocks 4 (t_8=5.415, P=0.001) and 5 (t_8=2.48, P=0.038), as compared to block 3. These results indicate that animals resumed their typical slight bias to the right (miss/correct rejection) lever upon shifting to the standard sustained attention task. The analysis of the relative number of omissions indicated that the shift from the low-demand to the sustained attention task did not affect response rates ($F_{5, 40}$=0.445, P=0.698, ϵ=0.524).

Performance in blocks 4–6 of the shift-high session was compared to blocks 4–6 of the standard sessions (Fig. 4) with a series of two- or three-way ANOVAs with factors of Session and Block, and Signal Length where appropriate. While the relative number of hits during the final three blocks of the shift-high session was signal-length dependent ($F_{2, 16}$=15.515, P<0.001, ϵ=0.885), this effect was different than during the standard sessions ($F_{2, 16}$=4.785, P=0.024, ϵ=1). Multiple dependent *t*-tests revealed that the relative numbers of hits to all three signal lengths were significantly different from each other during the standard sessions (all P-values<0.017, α_{comp}=0.033). However, as illustrated in the top panel of Fig. 6, in the shift-high session, there was no difference between hits to the 50-ms and hits to the 25-ms signal (t_8=0.033, P=0.974, α_{comp}=0.033). This Session by Signal Length interaction was not further affected by Block ($F_{4, 32}$=0.233, P=0.915, ϵ=0.981), nor did Session interact with Block alone ($F_{2, 16}$=0.228, P=0.799, ϵ=1).

The relative number of correct rejections was similar during the shift-high and standard sessions, both overall ($F_{1, 8}$=0.951, P=0.358) and as a function of Block ($F_{2, 16}$=2.696, P=0.098, ϵ=1, data not shown). Side bias values were statistically equivalent across the final three blocks of the standard sessions and the shift-high session (all P-values>0.14), and the relative number of omissions was similarly unchanged (all P-values>0.27).

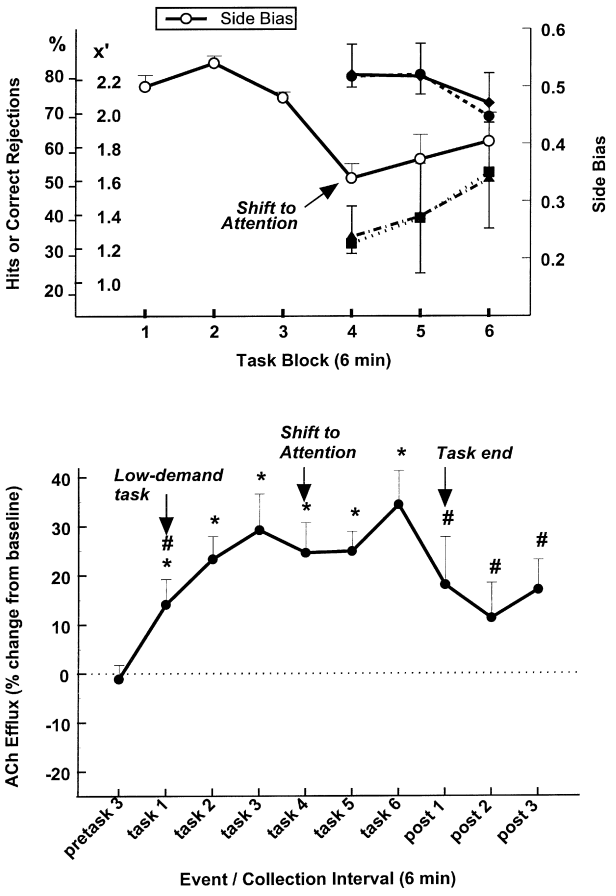

Fig. 6. (Top) Performance in the shift-high microdialysis session in experiment 1. Animals performed in the low-demand task for blocks 1–3, and then shifted to the sustained attention task (indicated by the arrow) for blocks 4–6. The relative number of hits to each signal length and the relative number of correct rejections (left axis) are shown for blocks 4–6; symbols as in Fig. 4. Side bias (right axis), calculated as in Fig. 5, is depicted across all six task blocks. (Bottom) Cortical ACh efflux (percent change from operant chamber baseline, 0.083±0.017 pmol/10 μl) during and after task performance in the shift-high session in experiment 2A. Task onset and the shift to the sustained attention task are indicated by arrows. *P≤0.03 versus pretask 3; #P<0.03 versus task 6.

3.2.8. ACh efflux during shift-high task performance

During task performance in the shift-high session, ACh efflux was once again significantly affected by Time ($F_{6, 48}$=8.405, P<0.001, ϵ=0.802), as shown in the bottom panel of Fig. 6. To explore the source of this effect, a total of nine comparisons (dependent *t*-tests) were conducted. Similar to the results reported above for both the standard sessions and the shift-low session, cortical ACh efflux was significantly enhanced from pretask values during each of the six task blocks (task 1: t_8=2.575, P=0.033, α_{comp}=0.033; task 2: t_8=3.729, P=0.006; task 3: t_8=4.595, P=0.002; task 4: t_8=3.912, P=0.004; task 5: t_8=4.637, P=0.002; task 6: t_8=5.372, P=0.001). Additional post-hoc comparisons included a comparison between ACh levels in task block 3, the final block of low-demand performance, and each of the three blocks of sustained attention performance (task blocks 4–6). As illustrated in the bottom panel of Fig. 6, ACh efflux was not different than task 3 during any of these post-shift blocks (task 4: t_8=0.743, P=0.479; task 5: t_8=0.588, P=0.573; task 6: t_8=1.659, P=0.136), suggesting that the shift in task demands did not significantly affect cortical ACh release.

Fig. 6 also illustrates that cortical ACh levels during the post-task period in the shift-high session were significantly affected by time ($F_{3, 24}$=4.328, P=0.014, ϵ=1). ACh

efflux was significantly lower during each of the three post-task collections as compared to ACh efflux in the final task block (post 1: $t_8=2.424$, $P=0.042$, $\alpha_{comp}=0.05$; post 2: $t_8=2.896$, $P=0.02$; post 3: $t_8=3.326$, $P=0.01$).

3.3. Experiment 1: discussion

The results of this experiment replicated previous findings [16] by demonstrating a significant enhancement of cortical ACh release upon transferring animals to the operant chambers, and further significant increases during sustained attention task performance. Furthermore, in the extinction session, ACh release either did not change or was significantly lower than pretask baseline levels. This finding clearly demonstrates that merely remaining in the operant chamber for an extended period of time is not sufficient to increase cortical ACh release throughout the time points corresponding to the task, further supporting the attribution of the task-associated increases in ACh release to task performance itself.

Although previous research [16] suggested that manipulating the attentional demands of the task could provide a useful method of investigating the relationship between ACh release and attention, shifts in attentional demand in the current experiment were not specifically associated with the predicted changes in ACh release. To aid in understanding the failure of shifts in attentional demand to specifically influence levels of cortical ACh efflux, ACh efflux in animals that were exclusively trained in the low-demand task were assessed in the following experiment. It was speculated that the activation of attentional processes, and potentially the stimulation of cortical ACh release, would be differentially regulated in these animals as compared to animals trained extensively in the sustained attention task but then shifted to the low-demand task.

4. Experiment 2: cortical ACh efflux during low-demand task performance

Animals in this experiment were exclusively trained in the low-demand task before participating in microdialysis sessions. It was predicted that cortical ACh efflux would not be enhanced above pretask levels during performance of this task, in accordance with previous findings [15]. Furthermore, the transfer effect on ACh efflux was predicted to be smaller in animals exclusively trained in the low-demand task.

4.1. Experiment 2: procedures

4.1.1. Behavioral training and pre-microdialysis procedures

This experiment originally consisted of 11 animals; one animal was excluded due to a misplaced probe. Animals were trained in the low-demand task in the operant chambers (mean=61±9.1 sessions) and then continued training in the modified microdialysis chambers. After 6±0.6 sessions, performance in the microdialysis chambers was stable, and familiarization procedures were initiated. Animals were placed in microdialysis bowls for ~4 h daily prior to task performance. Following at least 3 days of stable performance after exposure to the bowls (mean=5±0.6 sessions), animals underwent surgery as described in Section 2.3. Of the ten animals in experiment 2, five received a cannula implanted in the left hemisphere and five in the right hemisphere. Following recovery from surgery and upon returning to stable performance in the low-demand task (mean=5±0.4 sessions), animals were tethered inside the operant chamber during their daily training sessions. When performance was stable under these tethering conditions (mean=7±0.9 sessions), microdialysis sessions began.

4.1.2. Microdialysis sessions

Animals in experiment 2 participated in two or three microdialysis sessions. Each of the first two sessions consisted of performance in the low-demand task with no other manipulations. These two sessions were separated by at least 2 but no more than 4 days (mean=2±0.2 days) during which animals continued to perform in the low-demand task.

4.1.3. Contextual extinction

Following completion of the two low-demand sessions, a subset of animals ($n=5$) received contextual extinction sessions as described for experiment 1 above (mean=10±0.5 consecutive days). These animals then participated in a third microdialysis session during which they were transferred into the lighted chamber and remained there for 90 min in the absence of the task.

4.2. Experiment 2: results

4.2.1. Basal ACh efflux and transfer effects

Inspection of Nissl-stained sections indicated that all probe placements were located in the frontoparietal cortex, as depicted in the right panel of Fig. 2. The analysis of ACh efflux followed the same general pattern as in experiment 1. Overall basal ACh efflux (pmol/10 μl) was unaffected by repeated microdialysis sessions ($F_{1,9}=0.593$, $P=0.461$; session 1: 0.054±0.010; session 2: 0.044±0.009), and did not change significantly throughout the five baseline collections in the microdialysis bowls ($F_{4,36}=0.705$, $P=0.559$, $\epsilon=0.761$). In addition, the pattern of ACh efflux over the five baseline collections did not vary as a function of Session ($F_{4,36}=0.415$, $P=0.766$, $\epsilon=0.845$).

As in experiment 1, subsequent analyses were performed on ACh values expressed as a percent change from baseline, defined either as the median bowl baseline (for analysis of transfer and pretask effects) or the mean of

pretask 2 and pretask 3 collections (for analysis of task and post-task effects). Similar to the results of experiment 1 (depicted in Fig. 3), transferring animals into the operant chamber elicited a significant increase in cortical ACh efflux during the low-demand task sessions ($F_{3, 27}$= 28.406, $P<0.001$, $\epsilon=0.454$; data not shown). Post-hoc analyses indicated that ACh efflux was significantly higher than the last bowl baseline (4.7±13.4% above baseline) during the transfer collection (162.4±28.4%, $t_9=5.547$, $P<0.001$, $\alpha_{comp}=0.038$), as well as during both subsequent pretask collections (pretask 2: 144.6±27.2%, $t_9=5.746$, $P<0.001$; pretask 3: 140.3±25.8%, $t_9=5.44$, $P<0.001$). Although ACh efflux remained significantly above baseline levels during the third pretask collection, it declined from the original transfer-induced increase (transfer vs. pretask 3: $t_9=2.437$, $P=0.038$). The increase in ACh efflux elicited by transfer did not differ across the two low-demand sessions, as indicated by the lack of a significant effect of Session ($F_{1, 9}=3.036$, $P=0.115$) or an interaction between Session and Time ($F_{3, 27}=0.982$, $P=0.377$, $\epsilon=0.509$).

4.2.2. Low-demand task performance

Analysis of behavioral measures of low-demand task performance was limited to the percentage of completed trials (angularly transformed), to assess effects on general performance abilities; and side bias, to assess differential effects on left or right lever responses. A two-way repeated measures ANOVA with the factors of Session (two levels; low-demand one and two) and Task Block (six levels) was performed on each of these behavioral measures. As the percentage of trials completed did not differ between the two low-demand sessions (Session: $F_{1, 9}=0.666$, $P=0.435$; Session×Block: $F_{5, 45}=0.126$, $P=0.977$, $\epsilon=0.857$), data were collapsed across both sessions for illustration and subsequent analyses. As depicted in Fig. 7 (top panel), the percentage of trials completed significantly differed across task blocks during low-demand microdialysis sessions ($F_{5, 45}=15.832$, $P<0.001$, $\epsilon=0.697$). Post-hoc analyses indicated that in comparison to block 1, the percentage of completed trials significantly declined in both block 5 ($t_9=2.771$, $P=0.022$; $\alpha_{comp}=0.05$) and block 6 ($t_9=5.254$, $P=0.001$). Side bias remained unchanged across either Block ($F_{5, 45}=0.887$, $P=0.472$, $\epsilon=0.697$) or Session ($F_{1, 9}=0.039$, $P=0.848$), nor was there a significant interaction effect on side bias ($F_{5, 45}=0.952$, $P=0.437$, $\epsilon=0.683$).

4.2.3. ACh efflux during low-demand task performance

Once again, data were collapsed across the two low-demand sessions on the basis of non-significant analyses involving Session ($F_{1, 9}=0.148$, $P=0.709$; Session×Time, $F_{6, 54}=0.468$, $P=0.827$, $\epsilon=0.989$). Unexpectedly, analysis of ACh efflux during performance of the low-demand task revealed a significant effect of Time ($F_{6, 54}=8.406$, $P<0.001$, $\epsilon=0.73$), as illustrated in the bottom panel of Fig. 7.

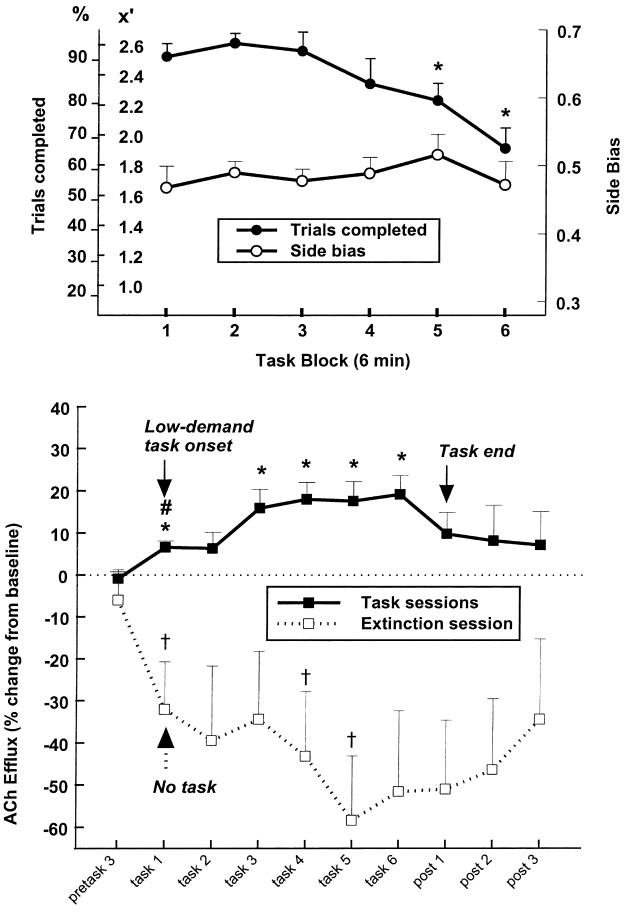

Fig. 7. (Top) Performance in the low-demand task microdialysis session in experiment 2. Data are collapsed across the two standard task sessions. *$P<0.03$ versus task block 1. (Bottom) Cortical ACh efflux (percent change from operant chamber baseline) during and after task performance in the low-demand task and following contextual extinction in experiment 2. Task sessions: ACh values are collapsed across both low-demand microdialysis sessions (baseline=0.104±0.013 pmol/10 μl); extinction session: ACh values during the third microdialysis session, following 7–10 days of contextual extinction (baseline=0.113±0.025 pmol/10 μl). Low-demand task sessions: *$P<0.01$ versus pretask 3; #$P<0.02$ versus task 6. Extinction session: †$P<0.034$ versus pretask 3.

Dependent *t*-tests further indicated that ACh efflux, during the task, was significantly enhanced above pretask baseline levels in all blocks (all *P*-values<0.01, $\alpha_{comp}=0.05$) except for task block 2 ($t_9=1.485$, $P=0.172$). No changes in ACh levels were observed during the post-task period, either as a function of session ($F_{3, 27}=1.347$, $P=0.281$, $\epsilon=0.943$) or collapsed across both sessions ($F_{3, 27}=0.775$, $P=0.502$, $\epsilon=0.862$).

As illustrated in the bottom panels of Figs. 4 and 7, the changes in ACh efflux during performance in the sustained attention task and the low-demand task, respectively, appeared to be of similar magnitude. To explore whether there were statistical differences between these changes in ACh efflux, post-hoc mixed ANOVAs were conducted on percent change from baseline ACh values over the task time points. As expected, ACh values increased signifi-

cantly over time in either task ($F_{6, 102}=12.64$, $P<0.001$). However, performance in the different tasks did not differentially increase ACh efflux over time ($F_{6, 102}=1.134$, $P=0.347$), nor were overall ACh levels different during the two tasks ($F_{1, 17}=0.555$, $P=0.467$). Thus, as is apparent in the bottom panels of Figs. 4 and 7, ACh efflux was increased to a comparable extent during performance in either the low-demand or the sustained attention task.

4.2.4. ACh efflux following contextual extinction

ACh efflux during the extinction session was compared to the two low-demand task sessions, and analyzed in a similar manner as described in Section 3.2.4 above. Once again, analysis of the transfer effect revealed that ACh efflux increased upon transfer to the operant chamber (data not shown) (Time: $F_{3, 12}=16.874$, $P=0.006$, $\epsilon=0.447$), but this effect did not vary across sessions ($F_{1, 4}=0.076$, $P=0.797$) or interact with session type ($F_{3, 12}=0.215$, $P=0.769$, $\epsilon=0.539$).

Fig. 7 (bottom panel) illustrates that overall ACh levels were lower in the extinction session than in the low-demand task sessions ($F_{1, 4}=13.674$, $P=0.021$). Furthermore, during the time points corresponding to the low-demand task, changes in ACh efflux differed across session type ($F_{6, 24}=7.82$, $P<0.001$, $\epsilon=1$). A one-way ANOVA examining ACh levels during the blocks corresponding to the task during the extinction session revealed a significant effect of Time ($F_{6, 24}=3.842$, $P=0.008$, $\epsilon=1$). Multiple dependent *t*-tests indicated that ACh efflux was significantly lower than pretask levels in three of the six 'task' blocks (task 1: $t_4=3.237$, $P=0.032$, $\alpha_{comp}=0.043$; task 4: $t_4=3.20$, $P=0.033$; task 5: $t_4=3.594$, $P=0.023$).

4.3. Experiment 2: discussion

Cortical ACh release was increased during performance in the low-demand task in a similar manner as during the sustained attention task in experiment 1, suggesting that cortical cholinergic transmission can be equally activated by operant performance regardless of explicit sustained attention demands. The similarity of the transfer-induced increase in ACh release between the two experiments, and during the extinction sessions, further suggests that this enhancement does not depend on the attentional nature of subsequent task performance. These similarities were unexpected given the results of previous experiments demonstrating no significant changes in ACh release during performance of simple operant tasks [15,22,25], as discussed further below in the General Discussion.

In light of the findings from experiment 2, it is perhaps not surprising that ACh release failed to demonstrate block-by-block fluctuations according to specific task demands in the shift sessions of experiment 1. Regardless of the theoretical demands on sustained attention imposed by each task, performance in both tasks clearly elicited increases in ACh release. Shifting between two tasks that each activate ACh release would not be predicted to produce robust changes in cortical ACh levels.

Experiments 1 and 2 suggested that cortical ACh release is enhanced by performance in either the sustained attention task or the low-demand task. However, while performance in the sustained attention task is highly dependent upon the integrity of corticopetal cholinergic neurons [26,28], the dependence of low-demand performance on these cholinergic inputs has not previously been assessed. In an attempt to identify possible dissociations between the necessity of cholinergic transmission in these two tasks, the final experiment reported below investigated the effects of cortical cholinergic depletions on low-demand task performance.

5. Experiment 3: effects of cortical cholinergic deafferentation on low-demand task performance

To assess the necessity of the cholinergic system for performance in the low-demand task, widespread cortical cholinergic depletions were administered using the selective cholinergic immunotoxin, 192 IgG-saporin. Performance in the low-demand task was predicted to be unaffected by cortical cholinergic deafferentation, in accordance with the lack of performance deficits in other tasks which place minimal demands on processing resources [47,52].

5.1. Specific methods

A total of five animals were trained exclusively in the low-demand task as described in Materials and methods. Following substantial overtraining in this task (mean=142±12.1 presurgical sessions), animals received cholinergic depletions via bilateral infusion of the immunotoxin 192 IgG-saporin into the nucleus basalis region, as detailed in Section 2.5. Following recovery, animals were returned to daily low-demand task performance for ~4 weeks.

5.2. Results

Histochemical staining procedures for acetylcholinesterase (AChE) indicated that all five subjects in this experiment sustained large depletions (~90% or greater relative to unoperated controls) of cortical cholinergic innervation. This finding was indicated both by the small amount of residual fiber staining throughout cortical areas, as illustrated in Fig. 8B, and the loss of large (presumably magnocellular) AChE-positive somata in the basal forebrain region, including areas of substantia innominata, ventral pallidum, and medial preoptic area, as illustrated in Fig. 8D.

The effects of cortical cholinergic deafferentation on percent trials completed and side bias were assessed by comparing baseline performance with post-surgical per-

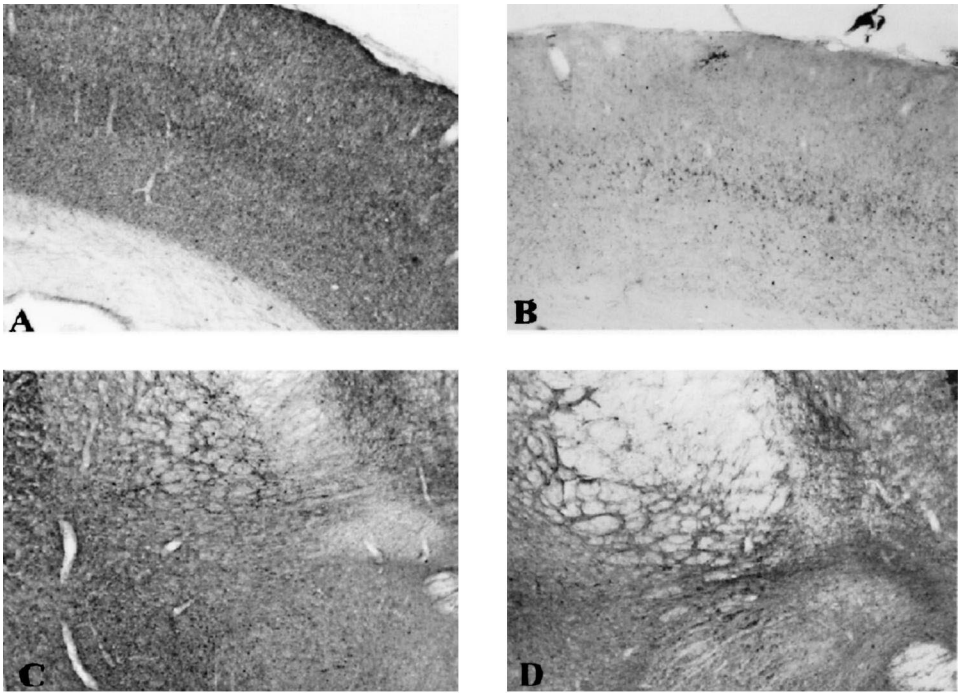

Fig. 8. Photomicrographs (3×) of AChE-stained sections demonstrating the extent of cortical cholinergic deafferentation in animals of experiment 3. A and B depict frontoparietal cortex with the midline to the left; C and D depict the basal forebrain area (nucleus basalis/substantia innominata) with the midline to the right. (A) Cortical fiber density in an unoperated control. (B) Near-total loss of AChE-positive fiber staining in the cortex of a basal forebrain lesioned animal. (C) Basal forebrain region of an unoperated control animal. Note the large darkly stained AChE-positive neurons in the nucleus basalis area near the top of the photograph. (D) Basal forebrain region of a lesioned animal. Note the presence of only a few scattered AChE-positive neurons in the nucleus basalis. Photographs were taken with a digital CC camera with a 2/3rds chip.

formance in the same animals. Baseline performance consisted of the average of the last five sessions prior to surgery. Post-surgical performance (20 sessions total) was collapsed into sets of five sessions each for a total of four sets. Thus, two-way repeated measures ANOVAs with the factors of Lesion (five levels; one pre-lesion and four post-lesion) and Task Block (six levels) were performed on transformed values of percent trials completed and on side bias.

Cholinergic deafferentation did not affect the overall percentage of trials completed at any post-surgical time point (Lesion: $F_{4,\ 16}=2.693$, $P=0.084$, $\epsilon=0.837$), nor did the lesion influence performance over blocks (Lesion× Block: $F_{20,\ 80}=0.857$, $P=0.559$, $\epsilon=0.389$). Performance during the pre-surgical and the final (fourth) post-surgical conditions is illustrated in Fig. 9. The percentage of completed trials per session declined over time in the task across all pre- and post-surgical sessions, as indicated by a main effect of Block ($F_{5,\ 20}=9.344$, $P=0.023$, $\epsilon=0.263$). Side bias was also unaffected by cortical cholinergic depletion, either overall ($F_{4,\ 16}=0.665$, $P=0.514$, $\epsilon=0.399$) or in combination with Block ($F_{20,\ 80}=1.412$, $P=0.142$), as depicted in Fig. 9.

5.3. Discussion

Near-total deafferentation of cortical cholinergic inputs failed to affect performance in the low-demand task, in stark contrast to the severe and persistent effects of cholinergic deafferentation on sustained attention performance [26,28]. The failure of cholinergic depletions to affect low-demand task performance is in agreement with previous findings regarding the inability of cholinergic lesions to disrupt performance in well-learned, relatively simple

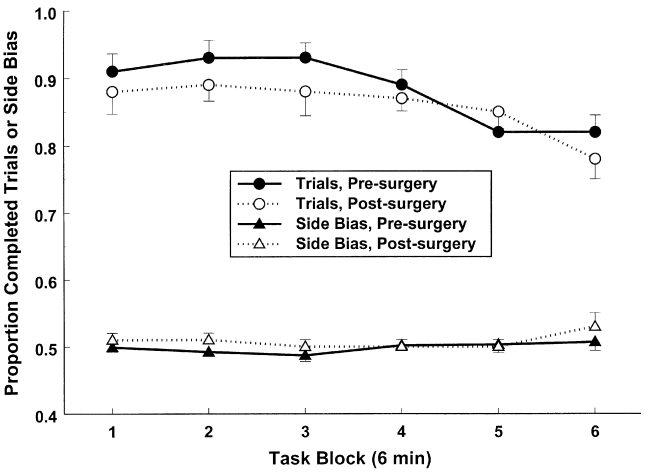

Fig. 9. Effects of cortical cholinergic depletions on percent trials completed and side bias values for task performance in the low-demand task in experiment 3. The fourth and final set of post-surgery performance (sessions 16–20) is presented to represent post-lesion performance.

tasks such as passive avoidance or T-maze alternation [47,52]. Similarly, it was recently reported that in a task of spatial cueing, performance on validly cued or non-cued trials (i.e. simple spatial discrimination performance) is not disrupted by cholinergic depletions [6].

Although the results of experiment 2 indicated that cortical ACh release is activated during performance in the low-demand task, the results of experiment 3 clearly reveal that cortical cholinergic transmission is not necessary for performance in this task. In contrast, performance of the sustained attention task is both associated with increases in cortical ACh release (current experiment 1; [16]) and is highly dependent on the integrity of cortical cholinergic inputs [26,28]. Thus, although performance in both an attentionally demanding task and a minimally demanding task were associated with similar patterns of increases in cortical ACh release, the role of cortical cholinergic transmission in the mediation of performance in these two different tasks can still be dissociated.

6. General discussion

The results of these experiments provide further information regarding the nature of the changes in cortical ACh release associated with attentional processing and operant task performance. As observed previously [16], ACh release increased during transfer to the operant chamber and during sustained attention task performance, and decreased following task completion. While the transfer-induced increase in ACh was not affected by contextual extinction, ACh release decreased during time points corresponding to the task in the extinction session. Within-session shifts in attentional demand were not associated with fluctuations in ACh release that corresponded with the particular demands of the task per se. Furthermore, exclusive performance in the low-demand version of the task was unexpectedly associated with significant increases in cortical ACh release above the pretask baseline that were similar in magnitude to the increases seen during performance of the sustained attention task. However, performance in the low-demand task remained unaffected by widespread depletions of cortical cholinergic inputs produced by intrabasalis infusions of the immunotoxin 192 IgG-saporin. Thus, although the cortical cholinergic system was activated during performance of the low-demand task, the integrity of cortical cholinergic inputs was not required for performance in this task. Below, these results are discussed in further detail.

6.1. Cortical ACh release during transfer

Transferring animals to the operant chamber elicited substantial increases in cortical ACh release that persisted throughout the pretask periods prior to either the sustained attention or the low-demand task. These increases reliably occurred despite animals' extensive experience with the transfer protocol. The similarity of the transfer-induced increase in ACh in animals trained in either task suggests that this enhancement cannot be ascribed to the sustained attention demands of subsequent task performance, as previously speculated [16]. In fact, the failure of contextual extinction to modify the transfer effect implies that it is independent of even the presence of a task. Instead, these results suggest that the transfer-induced enhancement of ACh release can be attributed to factors such as physical handling, movement to a different environment, anticipation of reinforcement, and contextual associations with the operant chamber [1,15,21,46], that do not appear to interact with subsequent task performance. This interpretation is in accordance with the conclusions reached by Acquas et al. [1], who reported identical transfer-induced increases in cortical ACh release whether animals had been aversively conditioned in the test chamber or had simply been exposed to the test chamber.

6.2. Cortical ACh release during task performance

The results of these experiments demonstrate that cortical ACh release can be stimulated during operant performance in a manner that is dissociable from the effects of mere exposure to the operant chamber, but does not appear to require explicit demands on sustained attention. Previously, the increases in cortical ACh release observed during performance of the sustained attention task were attributed to attentional processing per se [16]. However, the demonstration of similar increases in cortical ACh throughout differential shifts in attentional demand (experiment 1) and during performance in the low-demand task (experiment 2) suggests that these increases in ACh release reflect a common activational process that may encompass several components associated with operant performance.

These results did not support the prediction that sustained attentional demands per se would be selectively associated with enhanced cortical ACh release during shifts in explicit attentional task demands within the same session (experiment 1). A recent experiment by Passetti et al. [38] also failed to demonstrate differential changes in cortical ACh release during varying levels of attentional task demands in separate sessions, suggesting that the magnitude of ACh release is not directly correlated with the level of attentional processing required by the task. However, we have previously reported evidence for such a relationship with the demonstration of increased cortical ACh release under conditions of heightened attentional load produced by a distracter stimulus [16]. It could be speculated that in the present experiments, the differences in the level of attentional demand between the two tasks were not pronounced enough, and thus were not associated with differential changes in ACh release. The reduction of extrinsic attentional demand by relaxing the task requirements during the low-demand task blocks may not have

been translated into the intended cessation of attentional processing by the animals; a true disengagement of attentional processing in such overtrained animals may require shifting to a much more fundamental task such as free lever pressing.

Accordingly, one interpretation of the current results is that the low-demand task did tax attentional resources, leading to the conclusion that the increases in ACh release observed during both sustained attention and low-demand task performance can be attributed to attentional processing. Although the parameters of the low-demand task were adjusted to reduce explicit demands on sustained attention [27,37], other components of attention may have been engaged by performance in this task, such as visual search [36] and/or orienting processes [11]. In contrast, the failure of simple lever pressing to increase ACh release has been reported by three separate groups [15,22,35]. It might be speculated that while explicit sustained attentional demands do not appear to be necessary to activate cortical ACh release, some demands on monitoring of stimuli and/or response alternatives may be required. While the low-demand task is substantially less demanding than the sustained attention task, the requirement for animals to wait for each trial and to monitor the location of lever extension before responding make it more complex than previously utilized simple lever pressing tasks. However, the finding that ACh release was also not increased during discrimination performance [15], a task which can also be characterized as rule-based, does not support this speculation. Methodological differences between the current experiments and previous research, including collection interval, neostigmine level, and cortical area, limit the ability to make direct comparisons between these studies. Further research is clearly needed to reconcile the results of the current experiment with the negative findings from previous studies.

An alternate interpretation of these results is that performance in the low-demand task increased ACh release due to factors other than attentional processing. Task components such as contextual factors, sensory stimulation, motor activation, and reinforcement factors are each capable of independently eliciting increases in cortical ACh release [1,7,12,20,21]. Examination of several of these factors in the context of operant performance suggests that no single component is driving the increases in ACh release observed during task performance. The results of the extinction sessions reported here indicate that transferring animals into the context of the operant chamber elicits similar increases in ACh release regardless of the level, or even the presence, of subsequent task demands. Previous experiments demonstrated that neither substantial changes in motor activity (i.e. lever pressing rate) nor alterations in both the contingency and the delivery of reinforcements are accompanied by fluctuations in ACh release [15]. These results suggest that a combination of all of these components may be associated with increases in cortical ACh release within the context of operant task performance, regardless of the presence of explicit demands on sustained attention.

Finally, it is also possible that the magnitude of increases in ACh release during the two tasks was restricted by the large transfer-induced ACh increases prior to task onset. It may be that the lability of cortical ACh release during task performance was limited by a ‘ceiling’ effect imposed by the high pretask baseline, which may have precluded the demonstration of differential changes in ACh release during the two tasks. Future experiments may investigate the effects of lengthening the pretask period on the magnitude of ACh release elicited by transfer and task performance.

Together, the results of experiments 1 and 2 indicate that the observed increases in cortical ACh release during task performance under the current experimental conditions cannot be definitively ascribed to sustained attentional processing, but may reflect more basic attentional processes or even more general components of operant performance. The previous finding of increases in ACh release during the presentation of a distracter stimulus [16] further suggests that the demonstration of increases in cortical ACh release that can be specifically attributed to attentional processing may require a further augmentation of attentional demand.

6.3. Cortical cholinergic mediation of task performance

Collectively, these experiments demonstrate that cortical ACh release is stimulated during performance under a wider range of cognitive demand than originally predicted. However, although significant increases in ACh release were observed during performance of the low-demand task, the failure of widespread cholinergic deafferentation to produce impairments indicates that cortical cholinergic inputs are not necessary for performance in this task. Research on the cortical cholinergic modulation of learning has also revealed this type of dissociation. Cholinergic depletions do not robustly affect the acquisition of various operant tasks [47,52], but increases in cortical ACh release have been demonstrated during acquisition stages of different tasks [4,35]. The failure of selective cholinergic depletions to affect learning processes has been interpreted as a lack of significant involvement of the basal forebrain cholinergic system in learning [9]. Similarly, the current data suggest that although corticopetal cholinergic neurons are activated during low-demand task performance, cortical cholinergic transmission may not function as the crucial link mediating performance [44].

In contrast, extensive data support the cortical cholinergic mediation of sustained attentional processing. Cholinergic depletions robustly impair performance in the sustained attention task, and the severity of the impairment is related to the extent of the cortical deafferentation [26,28]. Intra-basalis administration of BZR ligands or

NMDA antagonists that attenuate stimulated ACh release [10,31] also impair task performance in a similar manner [17,50]. Finally, significant increases in cortical ACh release take place during sustained attention task performance (current experiment 1; [16,38]). This converging evidence provides convincing support for the cortical cholinergic mediation of attentional function [44]. Such a convergence of evidence from different research approaches is not found for the cholinergic mediation of low-demand performance. Together, these findings suggest that despite the demonstration of similar increases in ACh release during performance of both the low-demand and the sustained attention task, performance in these two tasks differentially depends on cortical cholinergic transmission.

It may be speculated that the differential functional significance of increased ACh release during low-demand and sustained attention task performance could be perceived at the level of ACh-dependent cortical information processing. Although similar increases in ACh release occur during performance of both tasks, these increases could have divergent effects on cortical processing. Extensive electrophysiological evidence suggests that the effects of ACh on cortical cells depend on the activation of other corticopetal inputs [48]. It seems likely that the demands of the sustained attention task activate different cortical inputs, and thus place cortical cells into a different state of responsiveness, than performance in the low-demand task. While enhanced cortical ACh release could function to increase cortical activation during performance in both tasks, this activation may only be necessary to enhance the efficacy of other cortical inputs (and thus to mediate performance) in the sustained attention task. Some support for the suggestion that the responsivity of cortical cells depends on task demands derives from the finding that prefrontal cortical cells demonstrate task-related changes in firing rate during performance of the sustained attention task, but not during performance of tasks designed to minimize explicit attentional requirements [14,19]. Further exploration of the unit firing properties of cortical neurons during performance in both types of tasks, under intact and cholinergically deafferented conditions [14], could be useful in revealing neuronal dissociations between performance in tasks with different levels of cognitive demand.

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