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Research report

Increases in cortical acetylcholine release during sustained attention performance in rats

Anne Marie Himmelheber, Martin Sarter, John P. Bruno *

Departments of Psychology and Neuroscience, Ohio State University, 31 Townshend Hall, 1885 Neil Avenue, Columbus, OH 43210, USA

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Abstract

Acetylcholine (ACh) efflux in the frontoparietal cortex was studied with in vivo microdialysis while rats performed in an operant task designed to assess sustained attention. Transferring animals from the baseline environment into the operant chambers elicited a robust increase in cortical ACh efflux that persisted throughout the 18-min pre-task period. Subsequent performance in the 36-min sustained attention task was associated with further significant increases in frontoparietal ACh efflux, while the termination of the task resulted in a delayed decline in ACh levels. Upon the 12-min presentation of a visual distracter (flashing houselight, 0.5 Hz) during task performance, animals initially developed a significant response bias to the left lever in the first 6-min distracter block, reflecting a reduction of attentional effort. Under continued conditions of increased attentional demand, performance recovered during the second 6-min distracter block. This return to attentional processing was accompanied by an increase in cortical ACh efflux, suggesting that the augmentation of attentional demand produced by the distracter elicited *further* increases in ACh release. The enhancement of cortical ACh efflux observed prior to task performance implies the presence of complex relationships between cortical ACh release and anticipatory and/or contextual factors related to operant performance and attentional processing. This finding, along with the further increases in cortical ACh efflux associated with task performance, extends hypotheses regarding the crucial role of cortical cholinergic transmission for attentional functions. Furthermore, the effects of the distracter stimulus provide evidence for a direct relationship between attentional effort and cortical ACh release.

Keywords: Acetylcholine; Sustained attention; Microdialysis; Cortex; Basal forebrain; Operant performance

1. Introduction

The functions of the cholinergic corticopetal projections have been of particular interest since the discovery that degeneration of basal forebrain neurons is associated with the development of Alzheimer's disease [2,29]. Cortical acetylcholine (ACh) has been hypothesized to augment the processing of sensory information by enhancing the responsiveness of cortical neurons to other afferent inputs [30], sharpening the processing of behaviorally relevant stimuli [19,20]. Accordingly, increases in cortical ACh efflux are observed upon presentation of behaviorally relevant unconditioned or conditioned stimuli that presumably increase animals' levels of arousal and/or attention [1,8,11,22]. This amplification of stimulus-related processing may provide a basis for an involvement of cortical cholinergic transmission in attentional processes [7,31].

Recent experiments have revealed that increases in cortical ACh efflux occur during early stages of learning, when attentional processes are engaged for the identification and selection of relevant information in the environment and the formation of new stimulus associations. ACh efflux in somatosensory cortical areas is enhanced in parallel with early improvements in performance in a tactile discrimination task [5], and increases in pre-frontal cortical ACh efflux correspond with increases in the number of reinforced lever presses during initial acquisition of a simple operant task [27]. Conversely, cortical ACh efflux did not change during performance in well-learned simple operant tasks, despite large experimental variations in reinforcement and lever pressing rate [9].

Experiments that investigate the effects of cholinergic manipulations on performance in tasks designed to assess specific aspects of attention have established the existence of a critical relationship between cholinergic transmission and attentional processing. For example, performance in a task incorporating components of selective and sustained

* Corresponding author. Fax: +1-614-688-4733; e-mail: bruno.1@osu.edu

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attention is disrupted by blockade of high-affinity choline uptake or excitotoxic lesions of the basal forebrain [25,26]. Previous experiments in our laboratory have demonstrated that the loss of cortical cholinergic inputs produced by infusions of the selective cholinergic immunotoxin 192 IgG-saporin decreases animals' ability to process signal events in a sustained attention task, and that the severity of these deficits depends on the extent of the lesion [16,18]. This selective impairment of signal detection can be reproduced by intra-basalis infusions of benzodiazepine receptor agonists, which decrease the excitability of basal forebrain neurons and attenuate stimulated cortical ACh efflux [10,22].

The studies described above clearly demonstrate the *necessity* of basal forebrain activation and cortical cholinergic transmission for sustained attention performance. However, these experiments are limited in their ability to describe the direct relationships between attention and cholinergic transmission in *intact* animals. Furthermore, the effects of lesions or drugs on attention and cortical ACh efflux described above were assessed in separate, parallel experiments, precluding the investigation of the covariance of attentional performance and cholinergic transmission in the same animal. Thus, the current experiment was designed to address these issues and extend previous findings by *simultaneously* assessing sustained attention performance and cortical ACh efflux, with the intent of directly characterizing the magnitude of changes in cholinergic transmission that are presumed to be necessary to support sustained attention performance. In vivo microdialysis was used to directly measure ACh efflux in the frontoparietal cortex during sustained attention performance under both baseline conditions, and during the manipulation of attentional demand by the presentation of a visual distracter that has previously been shown to increase the level of ''background noise'' and disrupt task performance. As the release of ACh throughout all cortical areas is hypothesized to be relatively uniform [31], the assessment of ACh efflux in the frontoparietal cortex was predicted to reveal task-related changes in cortical ACh release. The results of this experiment, in conjunction with previous findings on the lack of correlates between *non-attentional* operant performance and cortical ACh efflux [9], reveal the dynamic changes in cortical cholinergic transmission that mediate attentional processing.

2. Materials and methods

2.1. Subjects

The subjects were 11 male Brown–Norway/Fisher rats that were 3 months old at the beginning of the behavioral training and between 8 and 14 months old during the microdialysis sessions. Two animals were excluded due to undetectable ACh levels in one or more dialysis sessions, and one animal was excluded due to illness, resulting in eight animals in the final analysis. Animals were individually housed in a temperature- (23°C) and humidity-controlled (45%) environment on a 12 h:12 h light:dark schedule (lights on at 0630 h). All animals were extensively handled prior to beginning training, and were water-deprived to approximately 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 U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals.

2.2. Behavioral training

2.2.1. Apparatus

The initial behavioral training took place in a set of eight operant chambers (MedAssociates, St. Albans, VT) located inside larger sound-attenuating chambers. Each operant chamber was equipped with an intelligence panel consisting of three panel lights (2.8 W), two retractable levers, and a water dispenser (40–45 μl water per delivery) on the front wall. A houselight (2.8 W) was located on the rear wall. Microdialysis experiments were performed in a set of four operant chambers that were similar to the chambers described above, with the following modifications: (1) increased height of the recessed water delivery area to allow room for cannulated animals to drink, and (2) an opening in the top of the operant chamber to allow for placement of a liquid swivel and microdialysis tubing. 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).

2.2.2. Training

Animals were initially shaped to lever-press on a modified FR-1 schedule for water reinforcement. Following at least 3 days of 100 or more reinforced lever presses, animals began training in the sustained attention task. This task has previously been described and validated as generating a measure of sustained attention [17]. The basic response rules of the task are illustrated schematically in Fig. 1. In the first step of training, the animals were required to discriminate between signal (1-s illumination of the central panel light) and non-signal events following a 5-min period of adaptation to the operant chamber. Each trial 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

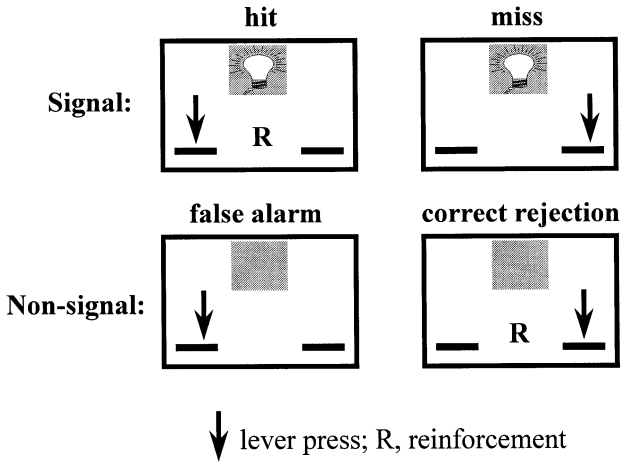

Fig. 1. Schematic illustration of the response rules and contingencies of the sustained attention task. Signal and non-signal trials were presented in a pseudo-random sequence, such that approximately half of the total trials were signal events and half were non-signal events. The occurrence of a signal trial (illumination of the central panel light for 500, 50, or 25 ms) required the animals to respond on the left lever for water reinforcement (R); this response was termed a hit. An incorrect response on the right lever was not reinforced and termed a miss. Following a non-signal trial (no illumination of the central panel light), a right lever response was reinforced and termed a correct rejection, while a left lever response was not reinforced and termed a false alarm. The beginning of each trial was cued by the extension of both levers into the operant chamber 2 s after signal or non-signal presentation. Animals experienced between 170 and 180 trials over the course of each 36-min daily test session.

response took place within 4 s, the levers were retracted and an omission was recorded. The signal and non-signal trials were presented in pseudo-random order, with 81 signal and 81 non-signal trials throughout the session; the inter-trial interval (ITI) was 12 ± 3 s. During this training step, incorrect responses were followed by up to three correction trials, in which the previous trial (signal or non-signal) was repeated. In the event of an incorrect response on the third correction trial, a forced trial was initiated, in which only the correct lever was extended for 90 s or until the animal made a response. When the forced trial was a signal trial, the central panel light was also illuminated while the left lever was extended. The houselight was not illuminated during this training step to facilitate the detection of signals.

Following at least 5 days of stable performance at 70% hits and 70% correct rejections in this task, the animals began a second training step. In this task, variable signal lengths (500, 50, 25 ms) were introduced, with signal type and length pseudo-randomly determined for each trial. The length of the adaptation period was increased to 18 min and the session length was set at 36 min to correspond with the timing of subsequent microdialysis experiments, resulting in a variable number of trials per session. The pseudo-random selection of signal type and length insured that approximately half of these trials were signal trials and the other half were non-signal trials, and that approximately one-third of the signal trials were of each signal length. In this training step, correction trials and forced trials were discontinued, and the event rate was increased by reducing the ITI to 9 ± 3 s. The houselight remained off during this step. Following at least 7 days of stable performance (at least 70% hits to 500 ms signals and 70% correct rejections, and less than 50% omissions to 25 ms signals), animals began training in the final version of the task.

In the final version of the sustained attention task, in which the animals were trained and tested for the remainder of the experiment including microdialysis sessions, the parameters remained identical to those described for the second training step except that the houselight was illuminated throughout the task including the 18-min adaptation period. Stable performance in this task was defined as at least 7 days with at least 70% hits to 500-ms signals and 70% correct rejections, and less than 40% omissions.

2.2.3. Behavioral measures

Measures of accuracy in the sustained attention task included hits, misses, correct rejections, and false alarms. 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, a measure of animals' tendency to respond preferentially on one lever, was calculated using the formula SB = (hits + false alarms)/(hits + misses + false alarms + correct rejections). A value of 0.5 indicates the complete absence of a side bias; values less than 0.5 indicate a bias to the right (miss/correct rejection) lever, while values greater than 0.5 indicate a bias to the left (hit/false alarm) lever. However, as animals typically attain a stable performance level of about 75% hits to longest signals (500 ms) and 75% correct rejections, but only about 50% hits to the shortest signals (25 ms), they are consequently making more right lever responses than left lever responses, resulting in a ''neutral'' side bias score of approximately 0.3–0.4. Finally, errors of omission were recorded, and 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.

2.3. Habituation training and guide cannula implantation

Upon reaching stable baseline performance in the operant chambers (as defined above) in the final version of the sustained attention task (mean $\pm$ S.E.M., 99 ± 35.0 sessions), the animals continued training in the microdialysis operant chambers. When performance was once again stable (11 ± 1.3 sessions), habituation to the microdialysis procedures were initiated. Animals were placed in microdialysis bowls for approximately 4 h daily prior to task

performance. In addition, animals received two presentations of the flashing houselight distracter to minimize the potential effects of novelty associated with presentation of this stimulus. In these two sessions, separated by at least three regular training sessions (mean = 6 ± 1.4 sessions) during which performance was monitored, the houselight was flashed at 0.5 Hz throughout the 36 min of the task. This distracter stimulus provides a means of manipulating attentional demands placed on animals by producing an increase in visual ''background noise'', and has previously been demonstrated to robustly impair performance in the sustained attention task [16,17]. Following at least 3 days of stable performance after the second distracter presentation (mean = 10 ± 3.8 sessions), the animals underwent 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 frontoparietal cortex at the following stereotaxic coordinates relative to bregma: AP: −1.0 mm, L: +2.0 mm, V: −1.0 mm from dura at an angle of 45° away from the midline. Of the eight animals included in statistical analyses, six received cannula implanted in the left hemisphere and two in the right hemisphere. The cannula was affixed to the skull with screws and dental cement, and the wound was sutured closed if necessary. A stainless steel stylet that ended flush with the termination of the guide cannula was inserted into the guide cannula to prevent clogging of the cannula. The animals were given a post-operative injection of amoxycillin (100 mg/kg, s.c.) and were allowed to recover for 3 days with free access to water. After recovery, the water deprivation levels were reinstated and the animals resumed daily habituation procedures and training in the microdialysis operant chambers.

Once the animals were retrained to stable performance levels in the sustained attention task (mean = 8 ± 1.9 sessions), they began habituation to the tethering apparatus used in microdialysis experiments. Additionally, animals received three more sessions (again separated by at least three regular sessions; mean = 5 ± 1.0 sessions) in which the flashing houselight distracter was presented throughout the task. Two days following the third and final distracter presentation, the first microdialysis session took place.

2.4. Microdialysis sessions and ACh analysis

Each animal participated in four microdialysis sessions. We have previously established the validity of this repeated perfusion procedure for the measurement of both cortical ACh efflux [23] and striatal ACh efflux [14] by demonstrating that the effects of pharmacological manipulations do not interact significantly with session order. Three of these four sessions consisted of performance in the standard sustained attention task, while the fourth session consisted of presentation of the visual distracter in the third and fourth (of six) blocks of the task. Occurrence of the distracter session was randomly distributed across the four sessions. At least 2 days, but no more than 5 days, intervened between each microdialysis session, during which animals continued training in the standard sustained attention task and performance was monitored.

During each microdialysis session, animals were placed into plastic test bowls (35 cm high, 38 cm diameter) for 1 h to habituate, after which the stylet was removed and a removable 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 $MgCl_2$, and 5.0 glucose, and 0.1 μM of the reversible cholinesterase inhibitor neostigmine bromide. 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 [21], collection of dialysate samples (every 6 min) began. 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 and outlet tubing. 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. 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. Following probe removal, an in vitro estimate of probe efficiency was obtained by placing the probe in a standard (1.0 pmol) ACh solution and taking an additional 6-min collection. The ACh values (pmol/min) for each collection interval were subsequently corrected according to individual probe recoveries.

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. ACh and choline were separated on a 250-mm analytical column and catalyzed on a post-column solid phase reactor containing acetylcholinesterase and choline oxidase. ACh was hydrolyzed to acetate and choline, and choline oxidized to hydrogen peroxide and betaine. Hydrogen peroxide corresponding to ACh was then detected using a ''peroxidase-wired'' glassy carbon electrode 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 contain-

ing 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.

2.5. Histological verification of probe placement

Within a week following the last microdialysis session, the animals were deeply anesthetized and transcardially perfused with saline followed by formalin. The 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, and examined for cannula and probe placements.

2.6. Statistical analysis

Statistical analyses (repeated measures ANOVAs) were performed on behavioral and neurochemical data separately to assess changes in each variable. Percentage data (relative numbers of hits, correct rejections, and omissions) were angularly transformed ($x' = 2 \arcsin x^{1/2}$; [35]) before analysis. 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 (df) [33]. Uncorrected df, corrected p values, and Huynh–Feldt ϵ values less than 1 are presented 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 [15]; the corrected level of significance is given in the text as α_{comp} (per comparison).

2.6.1. Attentional performance

Attentional performance during the standard sustained attention task microdialysis session was analyzed with a three-way repeated measures ANOVA on the relative number of hits, with the factors of Session (three levels; standard sessions 1, 2, and 3), Task Block (six levels), and Signal Length (three levels; 500, 50, and 25 ms). A similar repeated measures ANOVA was performed on the relative number of correct rejections, but with the factors of Session (3) and Block (6) only, as this measure represents an index of accuracy to non-signals. Side bias and omissions were each analyzed using a two-way repeated measures ANOVA with factors of Session (3) and Block (6). Each of these analyses was then repeated for performance measures in the distracter session, minus the factor of Session as there was only one distracter session. The critical post-hoc analysis in evaluating the effects of the distracter was the comparison between the blocks of the distracter session in which the houselight was flashing (Blocks 3 and 4) and the blocks before it began (Blocks 1 and 2), during which the task was identical to the standard sustained attention task.

2.6.2. ACh efflux

Baseline ACh values in the microdialysis bowls were defined as the median of the first five collections (in pmol/min). The potential effect of repeated microdialysis sessions on basal ACh efflux was assessed with a dependent t-test comparing median bowl baselines during Sessions 1 and 4 (regardless of session type). Further analyses were conducted on % change from baseline measures calculated for each animal. The effects of transferring animals into the operant chamber from microdialysis bowls were assessed using a two-way repeated measures ANOVA with the factors of Session (four levels; three standard sessions and one distracter session) and Time (four levels) on % change from bowl baseline values. This analysis included the last baseline collection, the transfer collection, and the second and third pre-task collections (Pre-tasks 2 and 3). Significant results were further analyzed using multiple dependent t-tests with the modified Bonferroni correction.

For the analysis of ACh efflux during task performance, it was considered important to compare ACh efflux during attentional performance with ACh efflux immediately prior to task onset while the animals were waiting in the operant chamber, rather than with efflux while animals were in the microdialysis bowls. The transfer from the bowl to the operant chamber was itself expected to significantly increase ACh efflux, an increase which may or may not be associated with the attentional aspects of the task which starts 18 min later. Thus, to provide a more appropriate measure of baseline ACh efflux immediately prior to task onset, the baseline was recalculated as the mean of the second and third pre-task collections (in pmol/min). To assess the potential effects of repeated sessions on this new baseline measure, a dependent t-test was conducted to compare the mean operant chamber values from Sessions 1 and 4. All subsequent analyses were conducted on % change from the recalculated operant chamber baseline values. This redefinition of the baseline also prevented potential misinterpretation of task-related changes in ACh efflux due to increases potentially associated with the change in context.

Changes in ACh efflux during standard task performance were analyzed using a two-way repeated measures ANOVA with the factors of Session (three levels; standard sessions 1, 2, and 3) and Task Collection (seven levels), including the last pre-task interval (Pre-task 3) and the six collections taken during the task. Multiple dependent t-tests were performed to compare ACh efflux during Pre-task 3 with efflux in each of the six task blocks. Changes in ACh efflux following task completion were analyzed with a two-way repeated measures ANOVA with the factors of Session (three levels) and Post Collection (four levels), which included % change from baseline values during the last task collection (Task 6) and the three dialysates collected after the termination of the task. Post-hoc analyses included multiple dependent t-tests between the last task

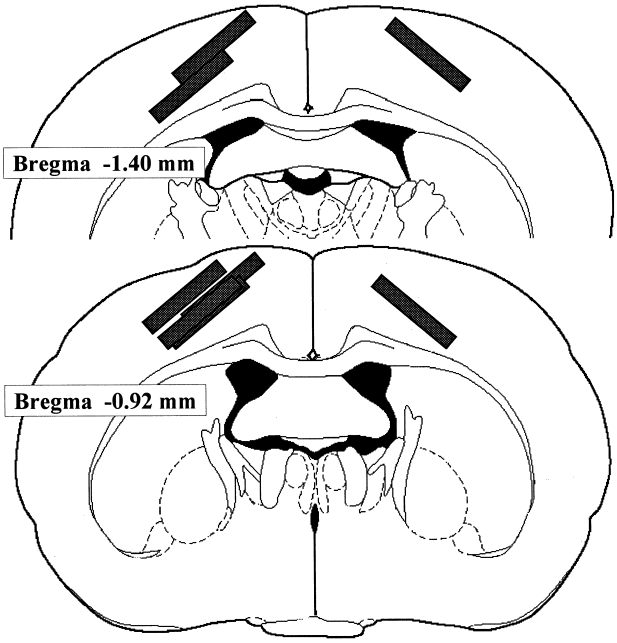

Fig. 2. Microdialysis probe placements in the frontoparietal cortex ($n = 8$). Drawings are adapted from the atlas of Paxinos and Watson [28]. The length of the 2-mm membrane tip is indicated on each section. All placements fell within the bounds of the frontoparietal cortex.

collection and each of the three post-task collections in turn. These analyses were then repeated for the distracter session, without the repeated factor of Session. However, post-hoc *t*-tests in the distracter sessions were limited to the following comparisons relevant to the two distracter blocks: Blocks 3 and 4 were each compared to Pre-task 1, Block 1, and Block 2, and were then compared to each other for a total of seven comparisons. In addition, examination of the data revealed consistent directional changes in ACh efflux between Blocks 3 and 4 that were not present between other task blocks. Thus, a Wilcoxon Signed Rank Test was performed comparing Blocks 3 and 4 of the distracter session to assess the statistical reliability of the direction of changes in ACh efflux. Blocks 1 and 2 of the distracter session were also analyzed with a Wilcoxon test as a control comparison.

3. Results

3.1. Basal ACh efflux and transfer effects

All cannula and probe insertions were located within the boundaries of the frontoparietal cortex, as illustrated in Fig. 2. The repeated insertions of microdialysis probes did not compromise the amount of recoverable cortical ACh efflux, as bowl baseline ACh values did not change significantly across the four microdialysis sessions [$t(7) = 0.753$, $p = 0.476$; means $\pm$ S.E.M.: Session 1, 0.097 ± 0.028 pmol/min, Session 4, 0.111 ± 0.030 pmol/min]. Thus, the subsequent effects of transfer into the operant chamber on ACh efflux were calculated as % change from these baselines.

Fig. 3 illustrates that cortical ACh efflux increased significantly when animals were transferred to the operant chamber [$F(3,21) = 9.819$, $p = 0.008$, $\epsilon = 0.452$]. The ACh values were significantly higher than the last micro-

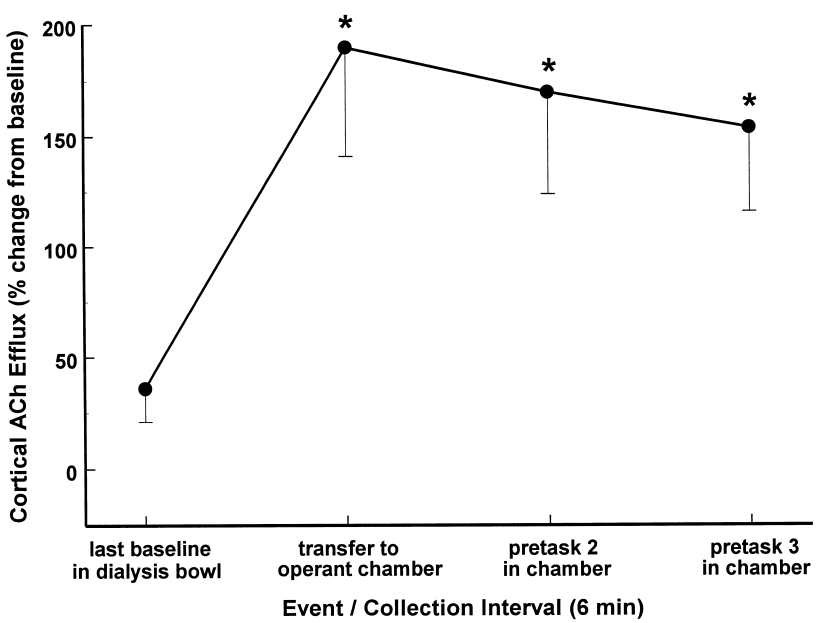

Fig. 3. Increase in cortical ACh efflux resulting from the transfer of animals into the operant chamber. Data are expressed as % change from baseline, where baseline refers to the median of the five dialysates collected while the animals are in the microdialysis bowls (mean $\pm$ S.E.M. = 0.088 ± 0.020 pmol/min). ACh values are collapsed across all four sessions (three standard and one distracter) as the time points depicted all occur prior to task onset, and thus do not differ across the two types of sessions. *: $p < 0.03$ vs. the last baseline collection.

dialysis bowl baseline value during the transfer collection [$t(7) = 3.805$, $p = 0.007$, $\alpha_{comp} = 0.038$], and during both the second pre-task collection [$t(7) = 2.799$, $p = 0.027$] and the third pre-task collection [$t(7) = 3.834$, $p = 0.006$]. Furthermore, this elevation of cortical ACh efflux did not attenuate over the pre-task period, as ACh levels during the third pre-task collection were not significantly different than levels during the transfer collection [$t(7) = 1.571$, $p = 0.160$]. As would be expected, these changes were not different either across the three standard sessions or the distracter session, as revealed by a nonsignificant main effect of Session [$F(3,21) = 1.593$, $p = 0.233$, $\epsilon = 0.766$] and lack of interaction between Session and Time [$F(9, 63) = 0.894$, $p = 0.482$, $\epsilon = 0.454$]. Across session types, the repeated microdialysis sessions did not affect baseline values of ACh in the operant chamber [$t(7) = 0.221$, $p = 0.832$; Session 1, 0.183 ± 0.028 pmol/min, Session 4, 0.175 ± 0.032 pmol/min]. The similarity of efflux across sessions permitted the expression of subsequent ACh efflux as % change from operant chamber baseline.

3.2. Standard sessions

3.2.1. Attentional performance

The accurate detection of signals was highly dependent on signal duration, as revealed by a main effect of Signal Length on the relative number of hits [$F(2,14) = 75.270$, $p < 0.001$]. Fig. 4 illustrates that the animals were more accurate in detecting the 500-ms signal than either the 50-ms signal [$t(7) = 10.006$, $p < 0.001$, $\alpha_{comp} = 0.033$] or the 25-ms signal [$t(7) = 10.253$, $p < 0.001$], and more accurate to the 50-ms signal than to the 25-ms signal [$t(7) = 3.482$, $p = 0.010$]. This signal-length dependency did not change over blocks [$F(10,70) = 0.154$, $p = 0.353$]. A main effect of Session indicated that signal detection improved significantly over the three standard sessions [$F(2,14) = 2.857$, $p = 0.041$, $\epsilon = 0.923$]. The relative number of hits, collapsed over all three signal lengths, was greater during the third than the second standard session [$t(7) = 3.320$, $p = 0.013$, $\alpha_{comp} = 0.033$; Session 2:50 $\pm$ 2.9%; Session 3:58 $\pm$ 3.8%]. Although the relative number of hits did not change significantly over blocks of time on task [$F(5,35) = 0.554$, $p = 0.734$], the data are presented for each of the six task blocks in Fig. 4 to correspond with neurochemical data presented below. Additionally, as the effect of Session did not interact with any other factor (all $p > 0.31$), the pictured data are collapsed across the three standard sessions. Fig. 4 also illustrates that the relative number of correct rejections also remained stable across blocks [$F(5,35) = 0.306$, $p = 0.870$, $\epsilon = 0.790$], and did not change across standard sessions [$F(2,14) = 0.652$, $p = 0.536$]. While there was a trend for an interaction between Session and Block on the relative number of correct rejections, this effect did not reach significance [$F(10,70) = 1.734$, $p = 0.09$].

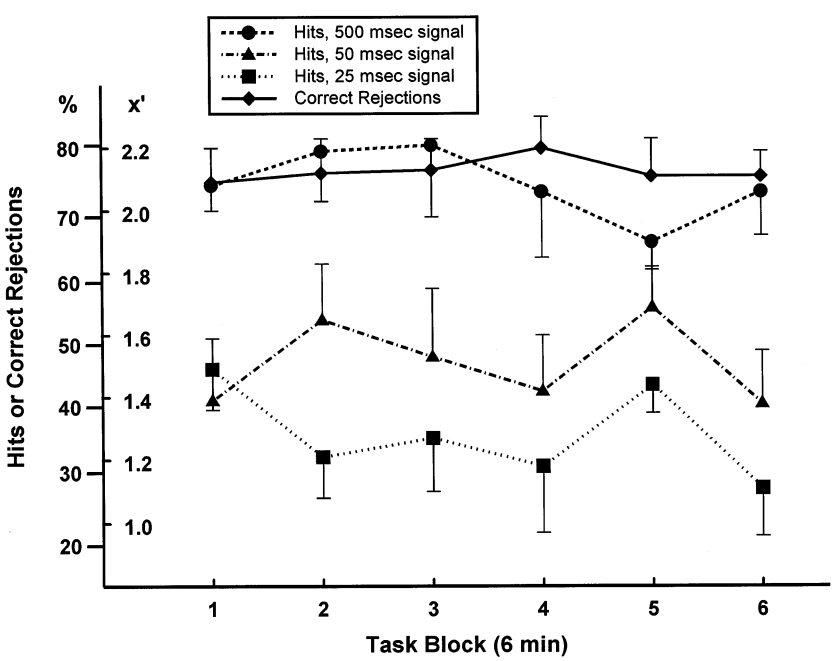

Fig. 4. Performance in the sustained attention task during the standard microdialysis session. Signal detection was dependent on signal length, but did not change across the six blocks of the task. The relative number of hits (hits/hits + misses) for each signal length are shown as well as the relative number of correct rejections (correct rejections/correct rejections + false alarms) for each of the six task blocks. Each 6-min block consisted of approximately 27–30 trials consisting of both signal and non-signal trials presented in pseudo-random order (see Section 2.2.2). The ordinate depicts both transformed values of each measure (x′), used in statistical analyses, and corresponding percentage values (%) for descriptive purposes. The data are collapsed across the three standard task sessions (see Section 3.2).

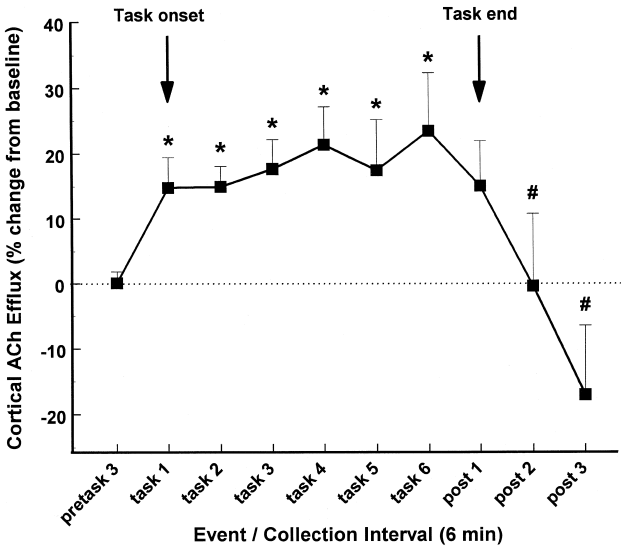

Fig. 5. Cortical ACh efflux during and after task performance in the standard sustained attention task. The cortical ACh values increased relative to baseline during the performance of the task, and decreased following task completion. The baseline in this figure (mean ± S.E.M. = 0.176 ± 0.021 pmol/min) has been recalculated as the mean of the second and third pre-task collections to represent a true operant chamber baseline; thus, although the ACh value at the time point Pre-task 3 is approximately zero in this figure, it is functionally equivalent to the same time point in Fig. 3, where the value for standard task sessions is 154% above the bowl baseline. Thus, while the increases in ACh efflux associated with task performance are moderate in comparison to the pre-task operant chamber baseline, these changes represent much larger increases above the original bowl baseline values. The data are collapsed across the three standard task sessions (see Section 3.2). *: $p < 0.05$ vs. Pre-task 3; #: $p < 0.01$ vs. Task 6.

Side bias was unaffected by either Session or Block, and these two factors did not interact (all $p > 0.10$). The response rate, indexed by the relative number of omissions, significantly changed over time in the standard task [$F(5,35) = 11.405$, $p < 0.001$, $\epsilon = 0.595$). As omissions were expected to increase over time in the task [17], comparisons were made between the relative number of omissions in Block 1 vs. each other block. Results indicated that the relative number of omissions was significantly lower in Block 2 than in Block 1 ($t(7) = 7.807$, $p < 0.001$, $\alpha_{comp} = 0.05$; Block 1:6.7 ± 0.93%; Block 2:2.3 ± 0.54%] and significantly greater in Block 6 than in Block 1 ($t(7) = 3.3$, $p = 0.013$; Block 6: 27.6 ± 7.0%].

3.2.2. ACh efflux

Performance in the standard vigilance task was associated with significant increases in cortical ACh efflux, as illustrated in Fig. 5 [$F(6,42) = 3.543$, $p = 0.036$, $\epsilon = 0.464$]. Specifically, the cortical ACh efflux in each of the six task blocks was greater than in Pre-task 3, the block immediately prior to task onset [Task 1: $t(7) = 2.906$, $p = 0.023$, $\alpha_{comp} = 0.05$; Task 2: $t(7) = 3.740$, $p = 0.007$; Task 3: $t(7) = 3.916$, $p = 0.006$; Task 4: $t(7) = 3.498$, $p = 0.010$; Task 5: $t(7) = 2.395$, $p = 0.048$; Task 6: $t(7) = 2.822$, $p = 0.026$]. Fig. 5 also shows that cortical ACh efflux decreased during the post-task period in the standard sessions [$F(3,21) = 7.25$, $p = 0.005$, $\epsilon = 0.755$]. While efflux in the first post-task block did not differ from efflux in Task Block 6 [$t(7) = 1.313$, $p = 0.231$, $\alpha_{comp} = 0.05$], the ACh levels during the second and third post-task blocks declined in comparison to Task Block 6 [Post 2: $t(7) = 4.458$, $p = 0.003$; Post 3: $t(7) = 4.196$, $p = 0.004$].

3.3. Distracter session

3.3.1. Attentional performance

As the flashing houselight was presented in Blocks 3 and 4 of the distracter session, the analysis of Block was considered most important in determining the effects of the distracter on task performance. Analysis of the relative number of hits during the distracter session suggested that accuracy of signal detection was significantly affected by the distracter presentation [$F(5,35) = 2.623$, $p = 0.046$, $\epsilon = 0.929$], as presented in Table 1. Post-hoc comparisons between Block 1 and each of the distracter blocks (3 and 4) revealed that the relative number of hits was greater in Block 3, the first distracter block, than in Block 1 [$t(7) = 2.588$, $p = 0.036$, $\alpha_{comp} = 0.05$]; however, the relative number of hits in Block 4 (the second distracter block) and Block 1 were not significantly different [$t(7) = 0.333$, $p = 0.749$]. Furthermore, the relative number of hits was significantly greater in Block 3 than in Block 4 [$t(7) = 3.592$, $p = 0.009$], suggesting that the flashing houselight had differential effects on the detection of signal events over the two blocks of its presentation. These effects of the

Table 1
Hits and correct rejections during the six blocks of the distracter test session
Relative numbers (%) of hits and correct rejections [means (S.E.M.)] for each of the six task blocks of the distracter session. % Hits = hits/(hits + misses); % correct rejections = correct rejections/(correct rejections + false alarms). The distracter stimulus (flashing houselight, 0.5 Hz) was presented during Blocks 3 and 4. The relative number of hits is collapsed across all three signal lengths.
Statistical analyses were performed on transformed data; percentage data are presented for descriptive purposes.

Measure	Task Block					
	1	2	3 (Distracter 1)	4 (Distracter 2)	5	6
% Hits	42 (7.6)	50 (6.6)	60 (4.2)*	42 (6.4)	34 (6.3)	32 (6.1)
% Correct rejections	78 (3.8)	75 (5.9)	37 (5.8)†	65 (8.1)	78 (7.1)	83 (3.4)

*: $p < 0.037$ vs. Block 1 or Block 4; †: $p < 0.004$ vs. Block 1 or Block 4.

distracter did not depend on signal duration [$F(10,70) = 1.144$, $p = 0.343$], and the overall relative number of hits during the distracter session remained signal-length dependent [$F(2,14) = 29.823$, $p < 0.001$]. The detection rate of 500 ms signals was higher than that of 50-ms signals [$t(7) = 6.107$, $p < 0.001$, $\alpha_{comp} = 0.033$] or 25-ms signals [$t(7) = 6.556$, $p < 0.001$], but the relative number of hits to the 50- and 25-ms signal did not significantly differ [$t(7) = 1.559$, $p = 0.163$].

Table 1 suggests that the relative number of correct rejections was also affected by the distracter presentation in Blocks 3 and 4 [$F(5,35) = 13.629$, $p < 0.001$]. In contrast to the pattern of hits, the relative number of correct rejections was *lower* in Block 3 than in Block 1 [$t(7) = 6.194$, $p < 0.001$, $\alpha_{comp} = 0.05$], but was not different in Block 4 in comparison to Block 1 [$t(7) = 1.117$, $p = 0.301$]. Furthermore, the relative number of correct rejections was significantly lower in Block 3 than in Block 4 [$t(7) = 4.373$, $p = 0.003$]. Thus, while significant departures were observed in Block 3 relative to Block 1 for measures of performance to both signals (relative number of hits) and non-signals (relative number of correct rejections), the direction of these effects was opposite in nature: the relative number of hits increased, while the relative number of correct rejections decreased, upon the introduction of the flashing houselight in Block 3.

The basis of the distracter's effects on the relative numbers of hits and correct rejections becomes clearer upon examination of the analysis of side bias during the distracter session. Fig. 6 (top panel) clearly illustrates that side bias was robustly affected by the distracter [$F(5,35) = 9.353$, $p < 0.001$, $\epsilon = 0.990$]. Post-hoc analyses revealed that side bias initially *increased* during Block 3 in comparison to Block 1 [$t(7) = 4.840$, $p = 0.002$, $\alpha_{comp} = 0.05$], and subsequently *decreased* from Block 3 to Block 4 [$t(7) = 5.879$, $p = 0.001$]. In other words, during the initial presentation of the flashing houselight, baseline side bias values (0.33 ± 0.04 in Block 1), which reflect a slight bias to the right (miss/correct rejection) lever, increased to values above 0.5 (0.64 ± 0.05 in Block 3), reflecting a shift in bias to the left (hit/false alarm) lever. The resulting overall increase in responses to the left lever thus accounts for both the increased relative number of hits and the decreased relative number of correct rejections (due to increased false alarms) observed in Block 3. The side bias values then returned to near baseline levels in Block 4 (0.39 ± 0.7), presumably reflecting animals' ability to regain performance during the second distracter block.

Time in the distracter session significantly affected response rates, as indexed by the relative number of omissions [$F(5,35) = 4.337$, $p = 0.009$, $\epsilon = 0.741$]. However, this effect did not appear to be related to the presentation of the distracter, as comparisons between Block 1 and all subsequent blocks revealed only that the relative number of omissions was lower in Block 2 than in Block 1 [data not shown; $t(7) = 2.942$, $p = 0.022$, $\alpha_{comp} = 0.04$; Block 1, $8.3 \pm 3.4\%$; Block 2, $1.3 \pm 6.3\%$]. To assess potential differences between the 2 distracter blocks, an additional comparison was made between Block 3 and Block 4; the relative number of omissions was not different between these two blocks [$t(7) = 1.384$, $p = 0.209$].

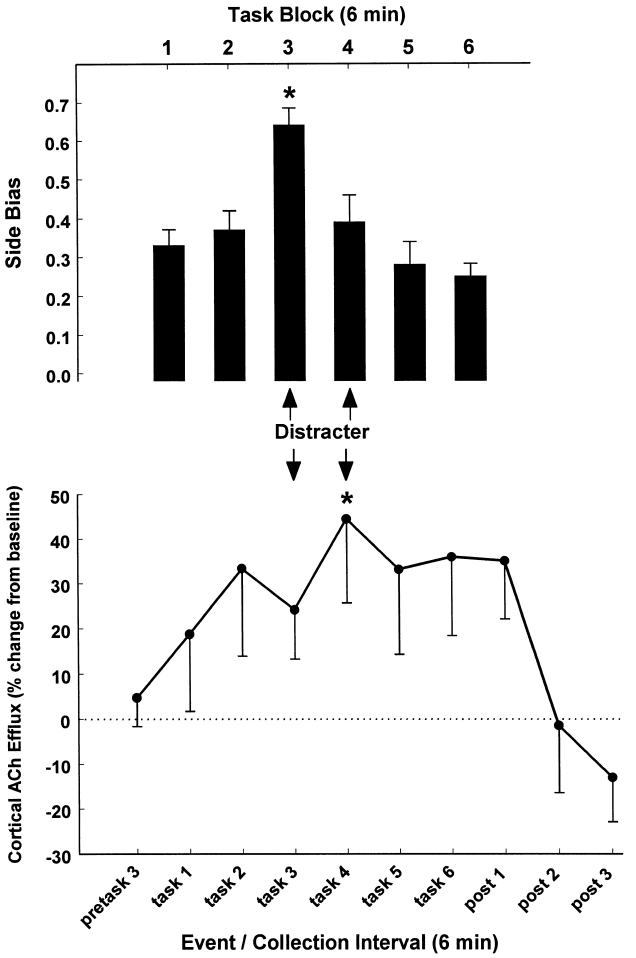

Fig. 6. Top: Side bias during the six blocks of the distracter session. Side bias is a measure of the animals' tendency to favor one lever over the other, and is computed using the formula (left lever responses)/(left lever responses + right lever responses). The initial presentation of the distracter in Block 3 shifted the side bias toward the left lever (hit/false alarm lever; see Fig. 1). Side bias values returned to baseline during the second block of distracter presentation (Block 4). These changes in side bias fully account for the changes observed in both hits and correct rejections during Blocks 3 and 4 (see Table 1 and Section 3.3). *: $p = 0.002$ vs. Block 1; $p = 0.001$ vs. Block 4. Bottom: Cortical ACh efflux during and after task performance in the distracter session. The baseline ACh value (0.172 ± 0.020 pmol/min) was recalculated as described for Fig. 5. Note the significant increase in ACh efflux during Block 4, the second distracter block, that corresponds with a return to normal task performance, as reflected by side bias values shown in the top panel. *: $p = 0.042$ vs. Pre-task 3; $p = 0.030$ vs. Task 1; $p = 0.036$ vs. Task 3 (Wilcoxon's Signed Rank Test).

3.3.2. ACh efflux

Cortical ACh efflux during task performance in the distracter session appeared to be increased, as illustrated in the bottom panel of Fig. 6 [$F(6,42) = 3.055$, $p = 0.052$,

$\epsilon = 0.494$]. Although this effect of Block did not quite reach the a priori level of statistical significance ($\alpha = 0.05$), post-hoc analyses were conducted on these data because examination of the changes in ACh efflux during task performance revealed that the average change during task performance in the distracter session (32%) was actually greater in magnitude than the average change in ACh efflux during standard task performance (18%), suggesting that meaningful increases in ACh release were occurring during the distracter task performance but that the statistical significance of these changes may have been obscured by the high variability associated with these values.

ACh efflux was elevated relative to pre-task baseline levels in Block 4 [$t(7) = 2.487$, $p = 0.042$, $\alpha_{comp} = 0.043$], but not in Block 3 [$t(7) = 1.798$, $p = 0.115$]. The cortical ACh levels during Block 4 were also significantly higher than in the first (non-distracter) task block [$t(7) = 2.721$, $p = 0.030$]. Additionally, there were trends for ACh efflux to be higher in Block 4 than in both Block 2 [$t(7) = 2.242$, $p = 0.060$] and Block 3 [$t(7) = 2.148$, $p = 0.069$]. Although an inspection of Fig. 6 suggests that ACh levels may have decreased from Block 2 to Block 3, the beginning of the distracter, this was not supported statistically [$t(7) = 0.886$, $p = 0.405$]. There was a trend for cortical ACh efflux to decrease following task completion during the distracter session [$F(3,21) = 3.61$, $p = 0.069$, $\epsilon = 0.533$]; as can be seen in Fig. 6, this effect likely did not reach significance due to the high variability associated with these data points.

Further inspection of the data revealed that while the magnitude of individual ACh efflux values per block was rather variable, there appeared to be a consistent increase in ACh efflux between the two distracter blocks across animals. Specifically, efflux increased from Block 3 to Block 4 in seven out of eight animals, while such systematic changes did not appear to be present between other non-distracter task blocks. A Wilcoxon Signed Rank Test indicated that there was a significant difference between efflux in Blocks 3 and 4 [$T = 33$, $z = 2.1$, $p = 0.036$]. In contrast, there was no such directional change between Blocks 1 and 2 in the distracter session [$T = 27$, $z = 1.26$, $p = 0.208$]. These results suggest that during the initial block of distracter presentation, animals' side bias was significantly shifted to the left (hits/false alarms) lever, and cortical ACh efflux was not significantly elevated above pre-task levels. However, during the second block of distracter presentation, baseline side bias values were reinstated, and corresponding ACh efflux increased relative to the first distracter block, as well as in comparison to both pre-task and pre-distracter levels.

4. Discussion

The results of this experiment can be summarized as follows. First, upon the transfer of animals into operant chambers, a robust (190% above baseline) and sustained (18 min) increase in frontoparietal ACh efflux was observed. Cortical ACh efflux was further increased above pre-task levels at the onset of the standard sustained attention task, remained elevated throughout the 36 min of task performance, and declined to below pre-task levels following termination of the task. Overall, performance in this task was dependent on stimulus length, remained relatively stable across multiple sessions, and was sensitive to the presence of a distracter (see also Ref. [17]). During the manipulation of attentional demand by the presentation of a visual distracter stimulus during two 6-min blocks (Blocks 3 and 4) in one session, the animals' side bias significantly shifted to the left lever in Block 3 and then recovered in Block 4; these changes were accompanied by an increase in cortical ACh efflux from Block 3 to Block 4.

The transfer of animals into the operant chamber was accompanied by a large increase in cortical ACh efflux that did not significantly decline over the three collection intervals (18 min) of the pre-task period. This increase is likely to reflect the combined effects of factors such as movement into a different (but not novel) environment, anticipation of reinforcement, and physical handling that have been demonstrated to enhance cortical ACh efflux [1,9,12,32]. However, the magnitude and persistence of this effect lead to the speculation that anticipatory and contextual factors specifically associated with *explicit* attentional performance may be significant additional factors contributing to the transfer-induced increase in ACh efflux. Thus, when animals enter the operant chamber, a context which presumably has strong associations with subsequent attentional task performance due to the extensive experience animals have received in this environment, the large and sustained increase in ACh efflux may be interpreted to partially reflect an enhancement in attentional processing, in anticipation of upcoming task demands. The current data do not permit the dissociation of the relative contributions of attentional and non-attentional factors to the transfer-related increase in ACh efflux; future experiments will explicitly address this issue. Furthermore, the presence of a moderate concentration (0.1 μM) of neostigmine in the perfusion fluid may have partially contributed to the persistence of the transfer-related increase in ACh efflux. However, it seems unlikely that the duration of the transfer effect was *entirely* due to the effects of neostigmine, as previous experiments utilizing a higher concentration of neostigmine (0.5 μM) demonstrated that upon transfer to operant chambers prior to performance in simple, well-learned tasks, the significant increase in cortical ACh efflux does not persist after the first post-transfer collection [9]. Additional experiments have illustrated that neither the magnitude nor the duration of increases in cortical ACh efflux following tactile stimulation are dependent on the concentration of neostigmine (0.05 vs. 0.5 μM; see Ref. [8]).

Changes in ACh efflux during performance in the sustained attention task were expressed as the % change from a new baseline calculated from pre-task ACh levels in the operant chamber, rather than from the original microdialysis bowl baseline values. This method provides a more appropriate measure of the magnitude of changes that occur during task performance, as neurotransmitter levels are directly compared to levels in the test environment immediately prior to performance [3]. Using this method, average changes in ACh efflux during standard task performance ranged from 15% to 24% above the pre-task baseline; in contrast, these changes expressed as changes from the original bowl baseline range from 194% to 216% above baseline. Clearly, the latter numbers are confounded by transfer-related increases, and do not represent true performance-related changes in cortical ACh efflux.

Relative to pre-task levels, ACh efflux in the frontoparietal cortex was significantly enhanced upon task onset and remained elevated throughout performance. These increases were associated with task performance itself, as ACh efflux exhibited further increases above the already elevated pre-task baseline upon onset of the task, and significantly declined within 6 min of task completion. The temporal separation of task onset from the transfer of animals into the operant chamber, by the inclusion of a pre-task period, supports the argument that the enhanced ACh efflux observed during task performance reflected more than contextual or other transfer-related factors. Additionally, the specificity of these increases for the duration of the task itself makes it unlikely that the inclusion of neostigmine in the perfusion fluid represents the *main* determinant of increases in ACh efflux. Finally, previous experiments have failed to demonstrate significant increases in cortical ACh efflux relative to pre-task levels during performance of simple operant tasks in which explicit attentional demands are minimized, even though measures of performance such as lever presses and number of reinforcements underwent marked changes over the course of these tasks [9]. The significant decline of cortical ACh efflux observed following completion of the sustained attention task was also not consistently demonstrated following completion of these simpler tasks [9].

Together, these results suggest that the increases in ACh efflux observed during sustained attention task performance can be attributed primarily to attentional processes, and not to secondary components of operant performance such as changes in motor activity or reinforcement delivery. Thus, these data provide a direct link between changes in cortical ACh release and attentional processing in intact animals, and extend our previous findings that cholinergic transmission is necessary to support sustained attention task performance [16,18]. The current findings are also in agreement with other previous research demonstrating the necessity of the basal forebrain cholinergic system for attentional processing (e.g., Refs. [6,25]); as well as experiments showing enhancements of cortical ACh efflux during early acquisition stages of learning [5,27] when attentional load is presumably high [24].

In the current experiment, sustained attention performance was stable across all six blocks of the standard task. Previous experiments utilizing this task have provided evidence for a vigilance decrement, defined as a decline in signal detection accuracy over time in the task [10,16–18]. The variability of the demonstration of a vigilance decrement here may be due to inter-related factors such as the substantial amount of training that animals receive in the task prior to microdialysis sessions, the high motivation levels of the animals, and/or the relatively short duration of the task itself (36 min). It has been suggested that vigilance decrements are most likely to be manifested as early, sharp decreases in performance in tasks where feedback is not given and signal probability is low [4]. Aside from the conceptual issues regarding the presence or absence of a vigilance decrement, the stability of sustained attention performance observed here may limit the ability to make conclusive statements about the covariance of attentional processing and cholinergic transmission. The presence of a direct relationship between attentional processing and increases in ACh efflux was indirectly supported by the observation that both performance and ACh efflux were stable across all six task blocks. However, the results of the distracter session, where attentional demand was explicitly manipulated in order to assess corresponding changes in ACh efflux, provides even more convincing evidence for this relationship.

The extreme shift in side bias observed during the initial 6-min block of distracter presentation (Block 3, Fig. 6) can be interpreted as a disengagement from active attentional processing, reflecting a lessening of animals' effort in the sustained attention task. Cortical ACh efflux was not significantly higher than pre-task baseline during this period of reduced effort. During the second 6-min block of distracter presentation (Block 4), side bias values recovered to baseline levels as animals became re-engaged in the attentional demands of the task. This return to active attentional processing under continued conditions of an augmentation in attentional load appeared to be accompanied by an increase in frontoparietal ACh efflux during the second 6-min block of distracter presentation, relative to both pre-task and Block 3 ACh levels. The distracter stimulus was not a novel stimulus during the microdialysis session, and did not appear to have general effects on motivation, as the number of omissions did not change during the distracter blocks. In addition, this pattern of changes in cortical ACh efflux was not observed when the same stimulus (flashing houselight) was presented during performance of simple operant tasks, nor did this stimulus significantly affect any measure of performance in these tasks [9].

Together, these results suggest that the effects of the distracter on performance and cortical ACh efflux in the current experiment were primarily due to its ability to

increase background noise and disrupt active attentional processing. These results demonstrate that changes in cortical ACh efflux can be elicited by manipulations of attentional demand, and imply that increases in attentional effort may be directly related to increases in cortical cholinergic transmission. Future studies are designed to more explicitly and thoroughly investigate the effects of bidirectional shifts in attentional demand on both performance and cortical ACh efflux.

This experiment provides the first direct investigation of the dynamics of cortical ACh efflux in rats during simultaneous performance in a complex operant task that explicitly assesses attention. Significant increases in frontoparietal ACh efflux were observed upon transfer into the test chamber and during the pre-task period, and additional increases in ACh above and beyond pre-task levels were elicited during sustained attention task performance. Additionally, cortical ACh efflux increased with recovery of active attentional processing during presentation of a distracter stimulus. While the current experiment assessed ACh efflux only in the frontoparietal cortex, it has been hypothesized that excitatory input to the basal forebrain increases ACh release throughout the entire cortical mantle [31,34], and evidence for the similarity of ACh efflux across different cortical areas has recently been provided [8,13]. Thus, the current results support and extend previous research demonstrating a crucial role for the basal forebrain cholinergic system in attentional processing [6,10,16,18,25,26]. More broadly, these findings demonstrate the feasibility of using microdialysis techniques to measure neurotransmitter release during cognitive processing in rats, thus providing a powerful means to investigate the neurochemistry of cognition.

Acknowledgements

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