

Research report

Operant performance and cortical acetylcholine release: role of response rate, reward density, and non-contingent stimuli

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

Department of Psychology and Neuroscience Program, 31 Townshend Hall, Ohio State University, Columbus, OH 43210, USA

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Abstract

The relationship between acetylcholine (ACh) efflux in medial prefrontal cortex (mPFC) and performance in a visual discrimination task and a variable interval (VI) schedule of reinforcement was studied in rats. Animals were pretrained in one of the two tasks and then unilaterally implanted with microdialysis guide cannula into the mPFC. Animals were then dialyzed, during 12 min collection intervals, in the operant chambers prior to task onset and during and after task performance. Each animal was dialyzed for a total of four sessions: two standard task sessions, one session in which a houselight was flashed at 0.5 Hz during the third 12 min block, and an extinction session (always the last session) in which reinforcement was withheld during the final three blocks. Response accuracy in the discrimination task was very high (> 95% correct) and stable across the four blocks with a progressive increase in omissions. The flashing houselight did not affect performance whereas the loss of reinforcement led to an increase in omissions. VI performance was associated with a high number of lever presses and a high reward rate that declined over the four blocks. Again, the flashing houselight did not affect VI performance whereas lever pressing declined markedly during the extinction session. ACh efflux did not change, relative to baseline, during performance in either task, or with the presentation of the flashing houselight or the loss of reinforcement. These data contrast with the changes in cortical ACh efflux observed in situations characterized by the presentation of novel stimuli or changing demands on attentional processing and, therefore, assist in the specification of hypotheses on the cognitive functions of cortical ACh. © 1997 Elsevier Science B.V.

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1. Introduction

The basal forebrain cholinergic system and its projections to the telencephalic structures have traditionally been implicated in learning and memory processes [4,5,16,31,39]. For example, neurons in the basal forebrain have been shown to respond selectively to reinforcing stimuli in learning and memory tasks [35,47]. However, further attempts to investigate the effects of cortical cholinergic manipulations on mnemonic processes have been inconclusive [9,38,45,46], prompting a refinement of the hypothesized involvement of cholinergic transmission in cognition [11]. It has been proposed that the importance of cortical cholinergic activity in learning and memory may be reflected in earlier stages of information processing, such as attentional mechanisms [8,18,28,29,37,41,42,45].

Much of the research supporting the role of cortical

acetylcholine (ACh) in attention, including studies conducted in this laboratory, has focused on *sustained attention*, a psychological construct that describes the variables which determine a subject's readiness to detect unpredictably and rarely occurring stimuli over relatively long periods of time [33,34]. We have developed and validated a measure of sustained attention, or vigilance, in rats using operant procedures that require the animals to discriminate between signals of variable length and non-signal trials [12,18,19,44]. Measures of performance in this task include hits, misses, correct rejections, and false alarms. Importantly, performance in this task has been demonstrated to depend on signal length, time-on-task, event rate, and signal probability [19]. Furthermore, increases in the demands on attentional processing by presenting a visual distractor have repeatedly been demonstrated to impair sustained attention performance (e.g., [19]). Using this task to measure sustained attention, we have shown that both systemic and intra-basalis administration of benzodiazepine receptor (BZR) agonists (which decrease cortical

* Corresponding author. Fax: +1 (614) 292-4537; e-mail: jpbruno+@osu.edu

ACh efflux through their ability to positively modulate GABAergic transmission [24,25]) impair vigilance task performance [12,19]. Additionally, partial deafferentation of cortical cholinergic afferents via intracortical injections of the selective cholinotoxin 192 IgG-saporin both decreases basal cortical ACh levels [10] and results in a decline in vigilance performance [20].

The research described above has provided strong evidence for the involvement of cortical cholinergic activity in attention; however, to understand fully the putative attentional functions of the intact cortical cholinergic system, experiments must be conducted which manipulate attentional demand and subsequently *directly* assess changes in ACh release. In vivo microdialysis provides a potentially powerful means of elucidating this relationship by providing a direct measurement of cortical ACh efflux in situ as an animal is simultaneously performing in a task designed to assess sustained attention.

A few recent studies utilizing microdialysis techniques coupled with behavioral performance have presented evidence for increased cortical ACh efflux during acquisition of simple operant behaviors, such as tactile discrimination [2] and lever pressing for food reinforcement [32]. In addition, we have recently completed a preliminary study pairing microdialysis of cortical ACh with vigilance task performance, the results of which suggest a correlation between cortical ACh efflux and attentional demand [44]. In this experiment, when attentional demand was increased by presenting a visual distractor during sustained attention performance (the validity of which has been previously demonstrated; see above and [19]), cortical ACh efflux showed a corresponding increase. However, the observed changes in cortical ACh efflux in these studies may not have been driven exclusively by the cognitive demands of the different tasks, as increases in cortical ACh efflux have repeatedly been shown to correlate with other factors, such as locomotor activity [3], novelty [1,13], sensory stimulation [13], and anticipation of reward [14,24,25]. In particular, the visual distractor stimulus used in our preliminary study not only increases attentional demand, but also has sensory properties and is correlated with a decline in reward density due to the decrease in correct responses [44]. In order to attribute more accurately changes in cortical ACh to ongoing cognitive processes, the specific effects of such non-attentional variables on cortical ACh efflux need to be systematically investigated within the framework of operant performance [44]. It should be noted that the term “non-attentional variables” is defined here as the absence of *explicit* demands on attentional functions as described conceptually by the construct [33,34], and as defined operationally by the sustained attention task described above [19].

The present experiment was designed to determine the effects on cortical ACh efflux of non-attentional components of basic operant performance, such as sensory stimuli (e.g., visual discriminanda), motor activity (e.g., lever

pressing), and reward density. To accomplish this, cortical ACh efflux was assessed during performance in two operant tasks specifically designed to incorporate the sensory and motor elements of the sustained attention task described above, but with factors such as novelty and stress excluded and attentional demands minimized. *Explicit* demands on attention were not assessed by the two tasks employed in this study, as neither involved task parameters or processing demands that represent valid measures of the construct of attention (see Section 2 for a detailed description of each task). *Implicit* demands on attention were minimized by an extensive overtraining of the animals in either task, and by extensive habituation training of the animals to all aspects of a daily training session and to the environment and procedures associated with microdialysis. In this way, the variables listed above were largely isolated and examined for their ability to influence cortical ACh efflux in the absence of *explicit* attentional demands, thus laying the groundwork for clearer interpretations of changes in cortical ACh efflux during attentional performance shown in previous and subsequent experiments.

2. Materials and methods

2.1. Subjects

Ten male Brown-Norway/Fisher-344 rats (National Institute of Aging colony, Charles Rivers, MA) served as subjects. Animals were 4 months old at the beginning of behavioral training and were 9–12 months old during microdialysis sessions. Animals were individually housed in an environment controlled for temperature (23°C) and humidity (45%), on a 12 h/12 h light/dark schedule (lights on at 06.30 h). All animals were handled extensively prior to beginning training. Animals were water-deprived to approximately 90% of their free-feeding weight throughout the course of the experiment; food was available ad libitum. The care and use of animals in these experiments were approved by the University's Laboratory Animal Care and Use Committee.

2.2. Behavioral training

Animals were trained in one of two operant tasks, either a *visual discrimination* ($n = 5$) or a *variable interval (VI) schedule of reinforcement* ($n = 5$), described below. The operant chambers (MedAssociates, St. Albans, VT) used in these behavioral tasks were equipped with two retractable levers, three panel lights (2.8 W), a houselight (2.8 W), and a water dispenser which delivered 40–45 μ l of water per reinforcement. The intelligence panel (lights and levers) and the water dispenser were located on the front wall of the chamber, with the houselight on the rear wall. Each operant chamber was located inside a large sound attenuating chamber. Signal presentation, lever operation, and reinforcement delivery, as well as data collection, were

controlled using an IBM PC clone and Med-PC software (MedAssociates).

All animals were initially shaped to press a lever for water reinforcement on a modified FR1 schedule. Following 3 consecutive days of at least 50 presses during a 40 min session, animals were pseudorandomly assigned to either the visual discrimination or the variable interval group and began further training. After animals reached stable baseline performance as defined below for each task, they were trained in two additional sessions while a flashing houselight (2.8 W, 0.5 Hz) was presented in the third 12 min block (minutes 25–36) of the task. At least a week of regular training separated the first and second presentation of the flashing houselight. The effects of this stimulus were tested because it has been used as a distractor in a vigilance task [19] and its presentation during vigilance performance was previously shown to be associated with increases in cortical ACh release [44]. As the flashing houselight was not expected to affect performance in the VI schedule of reinforcement or the visual discrimination task, it did *not* serve as a distractor in the present experiments. Instead, the flashing houselight was presented to assess the potential role of the sensory properties of this stimulus on cortical ACh release in animals trained in elementary operant tasks. It is also important to note that the flashing houselight was repeatedly presented to the animals (see below) in order to minimize the implicit attentional effects of novel stimuli. Following the two flashing houselight sessions, animals resumed regular training and were allowed to return to baseline performance before undergoing surgery for implantation of chronic guide cannula.

2.2.1. Visual discrimination task

While the visual discrimination task requires animals to press a cued lever, it does not *explicitly* tax attentional processing [22,30]. Performance in this task has frequently been demonstrated to fulfill the criteria of a habit in well-trained animals (e.g., resistance to reversal learning [40]). Moreover, the parameters of the visual discrimination task (e.g., lasting presentation time of the discriminanda and relatively long response period) further minimized demands on attention. At the beginning of each session, the houselight was illuminated and remained on throughout the session. Following a 24 min adaptation period, one reinforcement was delivered to signal the beginning of the task. On each trial, the left or right panel light was illuminated for 3 s, with an equal number of presentations to each side within each session (pseudorandomized sequence of side). Animals were reinforced for pressing the cued lever (i.e., the lever located directly below the illuminated light). Following a response to the correct lever, a reinforcement was delivered, the light was extinguished, and a new trial was initiated after a 12 ± 3 s inter-trial interval (ITI). A response to the incorrect lever or expiration of the 3 s response interval caused the light

to be extinguished and a new trial to be initiated. Each task session lasted 48 min and was analyzed in four blocks of 12 min each. Following completion of the task, animals remained in the operant chambers for an additional 36 min with the houselight illuminated.

2.2.2. Variable interval (VI) schedule of reinforcement

The particular VI schedule of reinforcement used for this experiment results in high rates of lever pressing, thus providing a fruitful basis for an assessment of the role of motor activity in the regulation of cortical ACh release. Performance in a VI schedule of reinforcement is based on an internal motor program and thus, the explicit demands on attentional processes are minimized. At the beginning of each session, the houselight was illuminated and remained on for the entire session. Following a 24 min adaptation period, one reinforcement was delivered to signal the beginning of the task. Animals were trained to press either lever for reinforcement on a VI schedule of 12 ± 3 s. In addition, either the left or the right panel light was illuminated for 1.5 s at a randomly selected time once during each inter-response interval (IRI). In contrast to the discrimination task in which the panel lights served as a discriminative stimulus, illumination of the panel lights was not explicitly related to any component of the VI schedule of reinforcement. Thus, any potential relationships between performance and ACh efflux in either task were not confounded by the presence or absence of the sensory effects of the panel lights. Each task session lasted 48 min, consisting of four blocks of 12 min each. Following completion of the task, animals remained in the operant chambers for 36 min with the houselight illuminated.

2.2.3. Behavioral measures

For the *visual discrimination* task, measures of performance were percent correct responses and percent omissions. The percent correct measure was calculated by dividing the number of correct responses by the total number of responses on trials (correct + incorrect). The criterion for stable performance in this task, prior to the implantation of dialysis guide cannula, was defined as 3 consecutive days with at least 95% correct responses and less than 40% omissions across the four blocks of the task. The mean (\pm S.E.M.) number of sessions to criterion was 110 ± 25 . The performance in the *variable interval* task was described by the number of reinforcements received and number of total lever presses (reinforced plus non-reinforced responses). Stable performance in this task was defined as 3 consecutive days with at least 120 reinforcements. The mean (\pm S.E.M.) number of sessions to criterion was 113 ± 37 .

2.3. Guide cannula implantation and post-surgical training

Following training to stable baseline levels, animals were implanted with microdialysis guide cannulae. Ani-

mals were anesthetized with sodium pentobarbital (50.0 mg/kg, i.p.), and a stainless steel guide cannula (0.65 mm o.d., Carnegie Medicin, Stockholm) was implanted unilaterally just above the medial prefrontal, prelimbic cortex, at a 10° angle towards the midline at the following coordinates relative to bregma: 3.2 mm anterior, 1.0 mm lateral, and 1.2 mm ventral (from dura mater). The microdialysis probe membrane (see below) extended 2 mm beyond the tip of the guide cannula into the medial prefrontal cortex. The cannulae were permanently fixed to the skull with stainless steel screws and dental acrylic.

Following guide cannula implantation, animals were allowed to recover for 3 days, during which they were allowed ad libitum access to food and water. Upon recovery, they were returned to water deprivation and were retrained in the respective tasks. Following their return to pre-surgery levels of performance, which typically required 3–5 days, animals continued daily training and were habituated to dialysis testing procedures. These procedures, which were identical for animals in both the visual discrimination and variable interval tasks, included placing the animal in the dialysis bowl for 3 h prior to operant training, and tethering the animal to the swivel arm inside the operant chamber with a short length of elastic. Finally, animals were again exposed to the flashing house-light during task performance for 3 consecutive days, followed by an additional day of training in the standard task. Flashing house-light was presented before the animals entered the dialysis phase of the experiment in order to minimize potential novelty effects. On the next day, the first dialysis session was conducted.

2.4. Microdialysis sessions

Each animal received four microdialysis sessions, with 1–3 days in between each session during which the animal was trained in the operant chamber without probes inserted. A typical microdialysis session was organized as follows. The animal was placed in a clear parabolic dialysis bowl (35.0 ± 1.0 cm high, 38.0 ± 1.0 cm in diameter) and allowed to habituate for approximately 30 min, after which a concentric dialysis probe (0.5 mm o.d., 2.0 mm membrane tip; CMA 10, Carnegie Medicin, Stockholm) was inserted into the guide cannula. The animal was perfused at a rate of 2.0 µl/min with an artificial CSF (pH = 6.9) containing (in mM): NaCl 126.5; NaHCO₃ 27.5; KCl 2.4; Na₂SO₄ 0.5; KH₂PO₄ 0.5; CaCl₂ 1.2; MgCl₂ 0.8; glucose 5.0; and neostigmine bromide 0.5 µM (Sigma, St. Louis, MO). Collection of dialysates began 3 h after probe insertion to allow basal ACh efflux to become stable and dependent on axonal depolarization [23]. Dialysate samples were collected every 12 min to correspond with the blocks in the operant tasks. While the animal was resting in the bowl, three baseline samples were collected, after which the animal was transferred to the operant chamber. Two more baseline samples (B1 and

B2) were collected during the initial adaptation period in the operant chamber. During task performance, four samples corresponding to the four blocks of the task (T1–T4) were collected; finally, three post-task samples (P1–P3) were collected following completion of the task while the animal remained in the lighted test chamber. Thus, each dialysis session lasted approximately 6 h from initial habituation to the final post-task collection.

The first three microdialysis sessions consisted of two standard sessions and one session with flashing house-light presentation, with the order of the three counterbalanced across sessions for each animal. In standard sessions, animals performed in the task as trained, with no additional manipulations during any of the four 12 min task blocks. In the flashing house-light session, the house-light (2.8 W) was flashed at a frequency of 0.5 Hz during the third 12 min block of the task. The fourth and final session for each animal consisted of an extinction session during which reinforcement was withheld during the second, third and fourth 12 min blocks of the task. In addition, three animals were perfused during the final post-task period with artificial CSF containing tetrodotoxin (TTX; Sigma), which blocks voltage-dependent sodium channels. The administration of TTX (10 µM) was used to confirm that ACh release under these operant test conditions was dependent upon membrane depolarization and neuronal activity [23].

2.5. Analysis of ACh

Dialysate samples and standard solutions were placed on dry ice immediately after being collected and stored at –80°C until analysis. Analysis of ACh was performed on a high performance liquid chromatography with electrochemical detection system (Bioanalytical Systems (BAS), West Lafayette, IN) using a sodium phosphate mobile phase (pH = 8.5). ACh and choline were separated on a polymer-packed 530 mm column, then catalyzed on a post-column enzyme reactor containing acetylcholinesterase and choline oxidase. ACh was hydrolyzed to acetate and choline, and choline was then oxidized to hydrogen peroxide and betaine. Hydrogen peroxide corresponding to ACh or choline was detected with an electrochemical detector (LC-4C, BAS) using a glassy carbon working electrode coated with peroxidase (BAS), and ChromGraph software (BAS). ACh was quantified by integrating the area under the peak and comparing the area to a standard curve generated from the areas corresponding to four standard values of ACh. The detection limit for ACh under these conditions was 20 fmol/20 µl injection.

2.6. Verification of probe placement

Within 1 week after the final operant microdialysis session, animals were given an overdose of sodium pentobarbital and transcardially perfused with saline and forma-

lin. The brains were removed and soaked in 30% sucrose phosphate buffer prior to being sectioned at 40 μm . Mounted sections were processed with a Nissl stain (Cresyl violet) to verify probe placement.

2.7. Statistical analysis

All statistical analyses were carried out separately for the visual discrimination task and the variable interval task. Where analyses were performed across dialysis session type (standard, flashing, and extinction), an analysis of variance (ANOVA) was first carried out comparing the two standard sessions. A non-significant result of session type resulted in the two standard sessions being collapsed in subsequent ANOVAs. Unless stated differently, the use of “standard” as a level of session type refers to the mean of the two standard sessions in all analyses described below. To control for possible violations of the sphericity assumption of homogeneity of variances, all repeated measures analyses with more than two levels of any factor were evaluated using μ -corrected degrees of freedom. Original degrees of freedom, corrected P values, and Huynh–Feldt ϵ values are presented in the text. A significance level of $\alpha = 0.05$ was used throughout all analyses.

2.7.1. Behavioral data

Within each task, two-way repeated measures ANOVAs consisting of Session Type (3 levels) by Task Block (4 levels) were performed on each of two dependent measures. For the variable interval task, these measures were number of rewards and total number of presses. The measures analyzed in the visual discrimination task were percent correct and percent omissions. To normalize these percentage data, an angular transformation was performed ($2 \times \arcsin \sqrt{x}$ [48]) and ANOVAs were carried out using these transformed values. The analysis of transformed percent correct in the visual discrimination session did not include the extinction session due to the fact that all animals omitted most or all trials in the final blocks of that session. Significant comparisons were further analyzed with multiple paired t -tests using the Bonferroni correction to control for family-wise error.

2.7.2. ACh efflux data

To assess the effect of repeated dialysis sessions on basal cortical ACh efflux, median bowl baselines in the first and last sessions were compared using a paired samples t -test. The effect of transferring animals from the dialysis bowl into the operant chamber was assessed with a two-way completely within-subjects ANOVA, using Session Type (4 levels) by Collection (3 levels), conducted on percent change from baseline efflux. Baseline in this analysis referred to the median of the first three dialysates collected while the animal was in the dialysis bowl. The standard sessions were *not* collapsed in this analysis. Multiple paired t -tests, with Bonferroni corrected alpha

levels, were performed to further investigate significant comparisons.

Changes in ACh efflux during the task were assessed using a two-way repeated measures ANOVA with factors of Session Type (3 levels; Standard, Flashing Houselight, Extinction) and Task Collection (5 levels) on percent change from baseline (as defined above). The levels of the factor Task Collection included the final collection taken in the operant chamber prior to task onset, and the four collections during performance of the task. Baseline for this analysis was defined as the mean of the collections taken while the animal was in the operant chamber prior to the beginning of the task. To investigate differences in ACh efflux during the post-task period, a separate two-way repeated measures ANOVA was conducted with the factors of Session Type (3 levels) and Post Collection (4 levels) on percent change from baseline. The four levels of Post Collection consisted of the final baseline collection in the operant chamber, as described above, plus the three collections taken after the task had ended. Baseline was again defined as the mean of the pre-task collections taken in the operant chamber. Significant main effects and interactions for Task and Post-task effects were again analyzed by multiple paired t -tests using the Bonferroni correction.

3. Results

3.1. Visual discrimination task

3.1.1. Behavioral analysis

The animals' performance did not differ across the two standard dialysis sessions (percent correct: $F_{1,4} = 7.64$; $P = 0.051$; percent omissions: $F_{1,4} = 3.20$; $P = 0.148$); thus, in all subsequent analyses, data from these two sessions were collapsed at each time point. The top panels of Figs. 1 and 2 show that response accuracy did not change either across Session Type (Standard (Fig. 1) vs. Flashing Houselight (Fig. 2): $F_{1,4} = 1.38$; $P = 0.305$) or over Blocks (i.e., T1–T4) of the task ($F_{3,12} = 0.78$; $P = 0.527$; $\epsilon = 1$). The data from the Extinction session were excluded from the analysis of Session Type due to extremely high omission rates that resulted in an extremely low number of responses. The addition of the flashing houselight in block 3 (see Fig. 2, top panel) had no effect on accuracy, as indicated by the lack of an interaction between Session Type and Block ($F_{3,12} = 0.54$; $P = 0.664$; $\epsilon = 1$). Although percent correct data from the Extinction session were not analyzed (due to the 100% omission rate during T4), inspection of the top panel of Fig. 3 suggests that accuracy in blocks 2 and 3 was also not affected by the loss of reinforcement during the Extinction session.

Animals decreased their response rate over the course of the visual discrimination task in all sessions, as evidenced by a significant main effect of Block on percent omissions ($F_{3,12} = 104.67$; $P < 0.001$; $\epsilon = 0.492$). While

the effect of Session Type on percent omissions was not statistically significant ($F_{2,8} = 5.07$; $P = 0.056$; $\epsilon = 0.761$), inspection of the data suggested a higher overall omission rate during the Extinction session (see top panel of Fig. 3). Also, the omission rate increased more sharply over time in the Extinction session, as indicated by a significant interaction between Session Type and Block ($F_{6,24} = 3.62$; $P = 0.011$; $\epsilon = 1$). Post-hoc tests revealed that, as expected, percent omissions in block 3 of the Extinction session ($96 \pm 2\%$) was significantly higher than

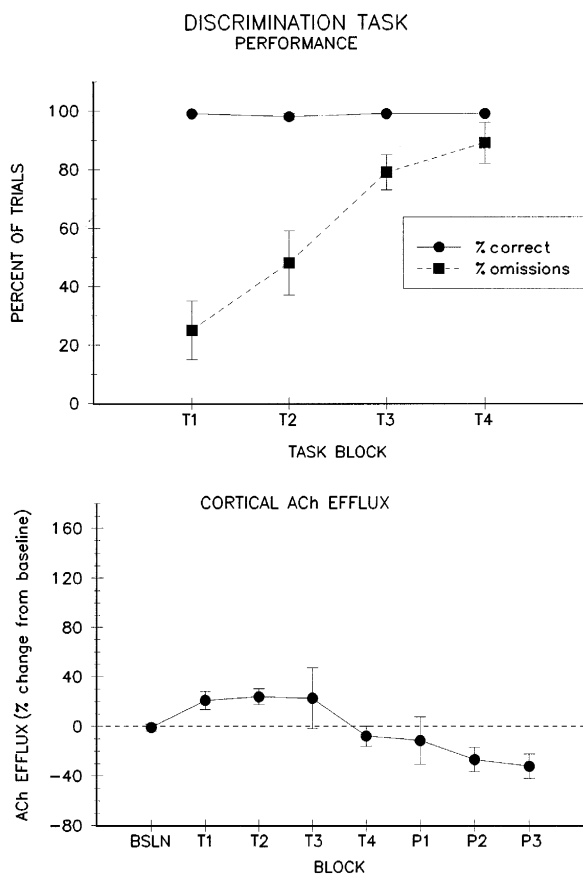


Fig. 1. Behavior (top panel) and cortical ACh efflux (bottom panel) during performance in the Standard session of the visual discrimination task (data from the two Standard sessions are collapsed). For ease of presentation, behavioral data in the top panels of Fig. 1Fig. 2Fig. 3 are presented as non-transformed values (means \pm S.E.M.), although statistics were conducted on transformed values (see text). Performance measures are presented at each of the four 12 min blocks of the task, indicated by T1 through T4. Accuracy, as measured by percent correct, remained high across the four blocks of the task, while the omission rate increased over time on task. Cortical ACh efflux values in the bottom panels of Fig. 1Fig. 2Fig. 3Fig. 4Fig. 5Fig. 6 are expressed as percent change from baseline (means \pm S.E.M.), where baseline is defined as the mean of the two baseline collections in the operant chamber prior to task onset. Dialysate collection intervals (12 min each) are represented on the abscissa, with BSLN representing the final pre-task collection in the operant chamber; T1 through T4 corresponding to the four blocks of the task; and P1 through P3 representing the three post-task collection periods. Medial prefrontal ACh efflux did not change significantly across the course of the entire Standard visual discrimination session (baseline efflux = 0.33 ± 0.05 pmol/min).

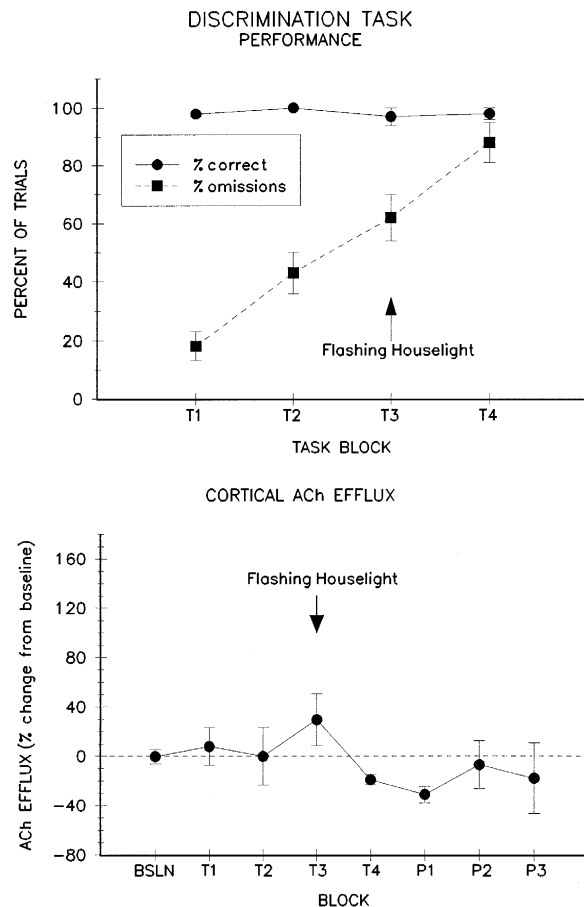


Fig. 2. Behavior (top panel) and cortical ACh efflux (bottom panel) during performance in the Flashing Houselight session of the visual discrimination task. Neither percent correct nor percent omissions were affected by the addition of the flashing houselight in the third block (T3). Cortical ACh efflux did not change significantly across the course of the distractor session (baseline efflux = 0.30 ± 0.05 pmol/min).

in block 3 of the Standard session ($79 \pm 6\%$) ($t_4 = 2.92$; $P = 0.043$).

3.1.2. ACh efflux

Examination of Nissl-stained sections indicated that all microdialysis guide cannulae were located within the bounds of the medial prefrontal cortex. In all animals in both the visual discrimination and VI groups, the tip of the guide cannula was located between 2.7 and 3.7 mm anterior to bregma. The tip of the dialysis probe, which extended 2 mm past the end of the guide cannula, was located between 3 and 4.5 mm ventral from the dura, and between 0.5 and 1.2 mm lateral from the midline.

Basal cortical ACh efflux (mean \pm S.E.M.) in the dialysis bowls remained stable across dialysis sessions ($t_4 = 0.41$; $P = 0.700$; *Session 1*, 0.28 ± 0.10 pmol/min; *Session 4*, 0.23 ± 0.10). Cortical ACh efflux, indicated by percent change from median bowl baseline (collapsed across all sessions: 0.21 ± 0.06 pmol/min), increased upon transfer of the animal from the dialysis bowl into the

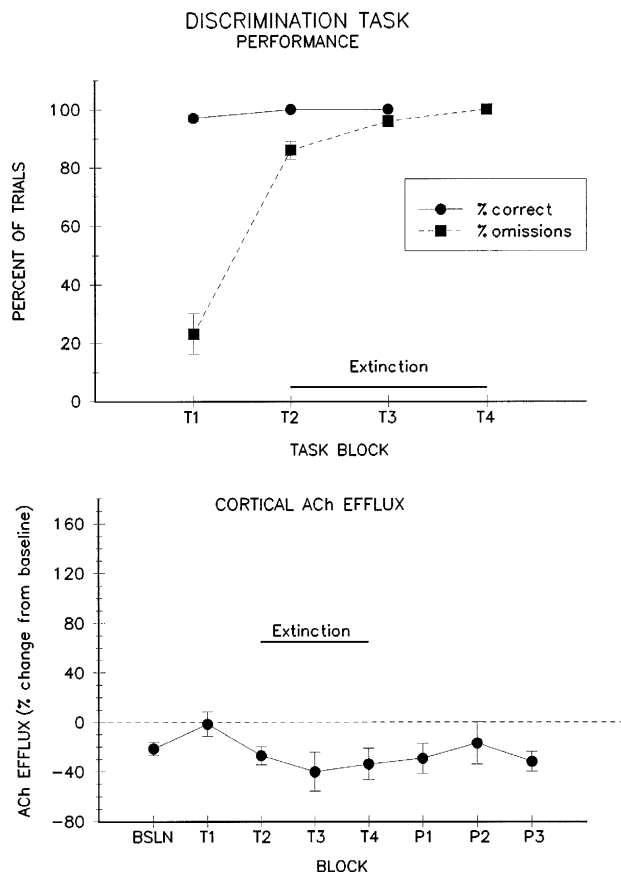


Fig. 3. Behavior (top panel) and cortical ACh efflux (bottom panel) during performance in the Extinction session of the visual discrimination task. Percent correct remained high throughout the first three blocks of the task, while percent omissions increased sharply with the onset of the extinction trials in block 2 (T2) and remained high throughout the remainder of the session. Cortical ACh efflux remained stable across the course of the Extinction session (baseline efflux = 0.28 ± 0.09 pmol/min).

operant chamber (Collection: $F_{2,8} = 7.21$; $P = 0.032$; $\epsilon = 0.722$). Post-hoc analyses revealed that this effect was due to a significant increase in efflux during the first collection in the operant chamber as compared to the last collection in the dialysis bowl ($t_4 = 3.11$, $P = 0.036$; last collection in the bowl, $-5 \pm 5\%$; first collection in operant chamber; $99 \pm 30\%$ change from baseline).

Cortical ACh efflux, as measured by percent change from operant chamber baseline, did not significantly differ during the two Standard dialysis sessions, either while animals were engaged in the visual discrimination task ($F_{1,4} = 4.57$; $P = 0.099$) or after the task had ended (post-task; $F_{1,4} = 2.68$; $P = 0.177$). Thus, for subsequent analyses of task and post-task effects, data from the two Standard sessions were collapsed at each time point. ACh efflux during the task (i.e., collections T1 through T4) was significantly affected by Session Type, as revealed by a significant main effect ($F_{2,8} = 9.2$; $P = 0.008$; $\epsilon = 1$). Paired t -tests revealed that the basis for this effect was a significant decrease from baseline efflux during the Extinction session as compared to the Standard session ($t_4 =$

-4.46 ; $P = 0.011$; Standard, $12 \pm 5\%$; Extinction, $-25 \pm 6\%$ change from baseline). However, as illustrated in the bottom panels of Figs. 1–3, there were no significant differences among cortical ACh efflux during the baseline and T1–T4 collection intervals ($F_{4,16} = 2.16$; $P = 0.138$; $\epsilon = 0.818$). Thus, cortical ACh efflux remained stable throughout performance of the visual discrimination task, despite evidence for significant changes in concurrent behavior such as increasing omission rates (see above). Additionally, neither the flashing houselight (Fig. 2) nor the loss of reinforcement in the Extinction session (Fig. 3) significantly affected cortical ACh efflux, as indicated by the lack of an interaction between Session Type and Task Collection ($F_{8,32} = 1.33$; $P = 0.294$; $\epsilon = 0.610$).

Overall, cortical ACh efflux following the conclusion of the visual discrimination task (i.e., baseline collections and P1–P3) was not significantly affected by Session Type ($F_{2,8} = 1.2$; $P = 0.349$; $\epsilon = 1$). Similar to the pattern of efflux observed during performance of the task, ACh efflux did not change across the three Collection periods after the task ($F_{3,12} = 1.32$; $P = 0.314$; $\epsilon = 1$). Finally, post-task ACh efflux was not affected by either the flashing houselight (Fig. 2) or by loss of reinforcement (Fig. 3), as evidenced by a non-significant interaction between Session Type and Collection ($F_{6,24} = 0.66$; $P = 0.680$; $\epsilon = 0.966$).

Basal cortical ACh efflux from animals in the operant chambers was dependent on sodium-gated membrane depolarization, as demonstrated by the effects of administering TTX ($10 \mu\text{M}$) through the dialysis probe. The perfusion of TTX in three animals following the conclusion of the discrimination task and three post-task collections resulted in an average decrease of 71% from baseline efflux. As ACh values declined to below detectable limits (less than 20 fmol) in two of the three animals a statistical analysis of this effect was not conducted. However, the reported decrease of 71% is likely to underestimate the actual decrease in ACh efflux resulting from TTX perfusion.

3.2. Variable interval task

3.2.1. Behavioral analysis

Comparison of animals' performance during the two Standard sessions showed that behavior during these sessions was not significantly different either for number of rewards ($F_{1,4} = 2.03$; $P = 0.227$) or for total number of lever presses ($F_{1,4} = 0.84$; $P = 0.412$). Thus, data from the Standard sessions were collapsed at each time point for all subsequent analyses. As illustrated in the top panels of Fig. 4, Fig. 5 and Fig. 6, the number of rewards (i.e., reinforced lever presses) declined over Blocks of the task in all sessions ($F_{3,12} = 145.4$; $P < 0.001$; $\epsilon = 1$) and was significantly lower overall in the Extinction session ($F_{2,8} = 9.29$; $P = 0.008$; $\epsilon = 1$). (Note that all reinforcement was withheld in the final three blocks of this session; thus,

in these blocks, the measure “number of rewards” refers to the number of presses made during reinforcement opportunities, i.e., those responses that would ordinarily have been rewarded.) The decrease in the number of rewards across blocks was different across sessions, as revealed by a significant interaction of Session Type and Block ($F_{6,24} = 3.33$; $P = 0.016$; $\epsilon = 1$). Post-hoc analyses revealed that the number of rewards declined more sharply across time in the Extinction session; this measure was significantly lower during block 2 (T2) of the Extinction session than during block 2 (T2) of the Standard session ($t_4 = -4.59$; $P = 0.010$). Similar differences between number of rewards during the Standard and Extinction sessions was observed for Block 3 ($t_4 = -4.29$; $P = 0.013$) and Block 4 ($t_4 = -4.4$; $P = 0.012$).

The top panels of Fig. 4, Fig. 5 and Fig. 6 also indicate that animals’ overall response rates in the variable interval task (as indexed by number of total presses) paralleled the

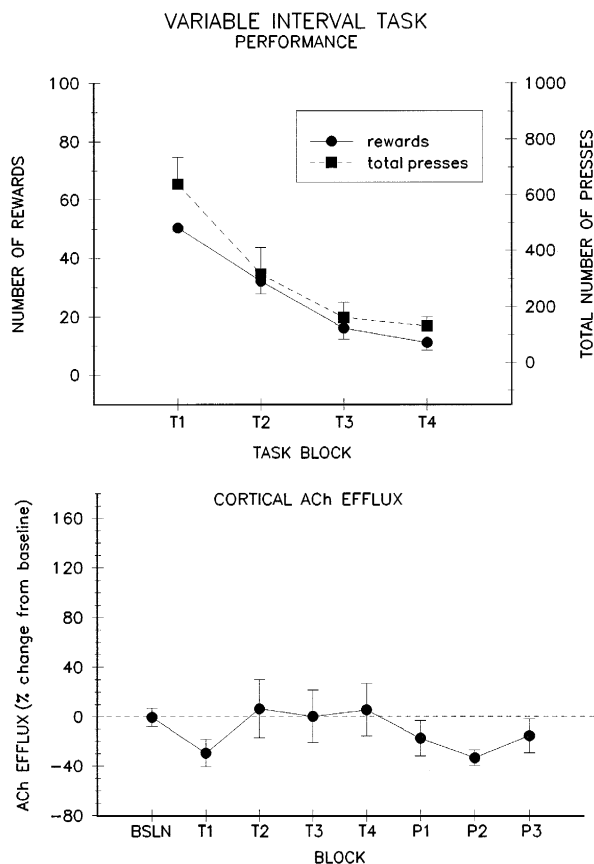


Fig. 4. Behavior (top panel) and cortical ACh efflux (bottom panel) during performance in the Standard session of the variable interval task (data from the two Standard sessions are collapsed). The behavioral measures of number of rewards and total number of lever presses during each block of the task, T1 through T4, are expressed as means \pm S.E.M. in the top panels of Figs. 4–6. In the Standard variable interval session, both number of rewards and total presses decreased across blocks of the task. Despite these changes in activity level, cortical ACh efflux did not vary across the course of the Standard session (baseline efflux = 0.42 ± 0.16 pmol/min).

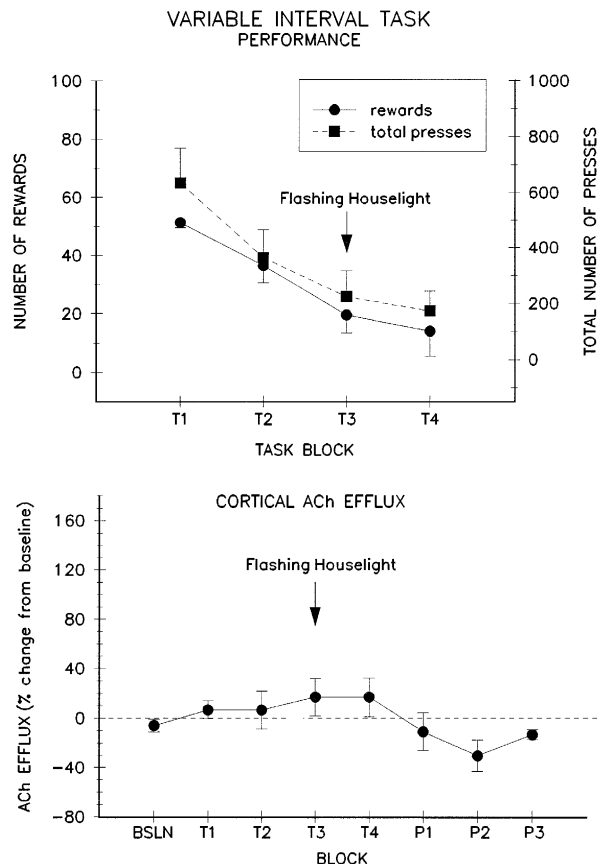


Fig. 5. Behavior (top panel) and cortical ACh efflux (bottom panel) during performance in the Flashing Houselight session of the variable interval task. The number of rewards and total presses again declined over blocks, but were not affected by the flashing houselight in the third block (T3). Medial prefrontal ACh efflux did not change over the course of the session and was not affected by the addition of the distractor (baseline efflux = 0.42 ± 0.12 pmol/min).

decrease in number of rewards over time, as indicated by a significant main effect of Block ($F_{3,12} = 49.43$; $P < 0.001$; $\epsilon = 0.468$). However, total presses did not differ across Session Types ($F_{2,8} = 1.43$; $P = 0.296$; $\epsilon = 0.868$). The decline in overall response rate over time was similar in each session, as evidenced by the lack of an interaction between Session Type and Block ($F_{6,24} = 2.04$; $P = 0.167$; $\epsilon = 0.472$).

3.2.2. ACh efflux

Again, repeated dialysis sessions did not significantly affect basal cortical ACh efflux (mean \pm S.E.M.) in the dialysis bowls ($t_4 = 0.62$; $P = 0.567$; *Session 1*, 0.31 ± 0.11 pmol/min; *Session 4*, 0.23 ± 0.10). Cortical ACh efflux did not change significantly when these animals were transferred into the operant chamber to begin the variable interval task (Collection: $F_{2,8} = 1.43$; $P = 0.300$; $\epsilon = 0.634$).

Task and post-task effects were analyzed as for the visual discrimination animals, using percent change from

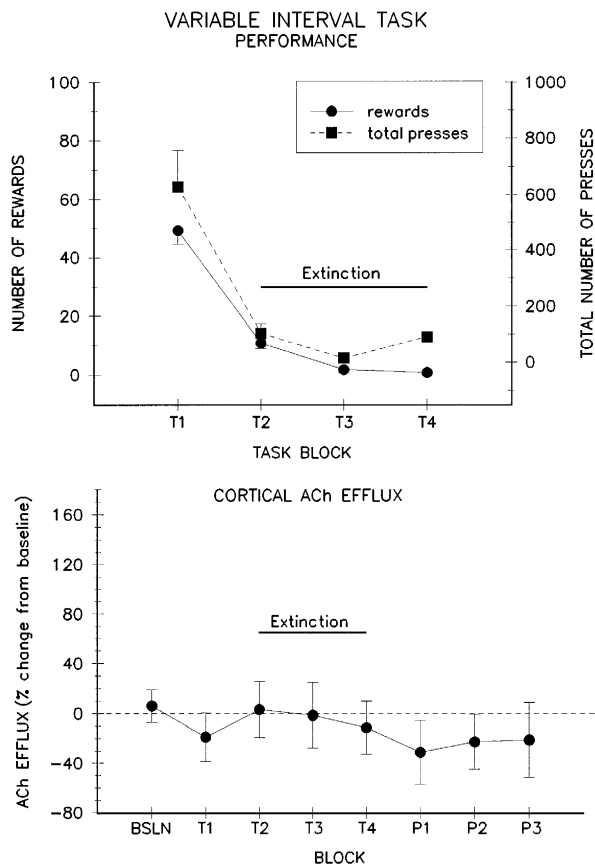


Fig. 6. Behavior (top panel) and cortical ACh efflux (bottom panel) during performance in the Extinction session of the variable interval task. The decreases in both number of rewards and total presses across blocks were more drastic in this session. In contrast, cortical ACh efflux did not vary significantly across the blocks of the task or throughout the session (baseline efflux = 0.32 ± 0.14 pmol/min).

baseline efflux as the dependent measure. Efflux data from the two standard sessions were collapsed at each time point, as ACh efflux during these two sessions was not significantly different either during the task collections (T1–T4: $F_{1,4} = 0.22$; $P = 0.666$) or during post-task collections (P1–P3: $F_{1,4} = 0.71$; $P = 0.447$). The bottom panels of Figs. 4–6 illustrate that cortical ACh efflux did not change significantly over the course of the variable interval task (T1–T4) during any of the dialysis sessions, as evidenced by the lack of a main effect of either Session Type ($F_{2,8} = 0.24$; $P = 0.794$; $\epsilon = 1$) or Task Collection ($F_{4,16} = 1.83$; $P = 0.210$; $\epsilon = 0.612$) and a non-significant interaction ($F_{8,32} = 0.56$; $P = 0.798$; $\epsilon = 1$).

Analysis of post-task collections (P1–P3) also revealed no changes in cortical ACh efflux as a function of Session Type ($F_{2,8} = 0.01$; $P = 0.990$; $\epsilon = 1$), but indicated that collapsed across sessions, ACh efflux decreased relative to baseline during Post-task Collections ($F_{3,12} = 3.64$; $P = 0.045$; $\epsilon = 1$). Post-hoc analyses showed that relative efflux during the second post-task collection (P2) was significantly lower than efflux during the baseline collection ($t_4 = 3.99$; $P = 0.016$; Baseline, $0 \pm 3\%$; Post 2, $-29 \pm$

6% change from baseline). This effect remained constant across all sessions, as revealed by a non-significant interaction of Session Type by Post-task Collection ($F_{6,24} = 0.5$; $P = 0.806$; $\epsilon = 1$).

4. Discussion

The results of the present study suggest that ACh release within the medial prefrontal cortex (mPFC) is not significantly correlated with the performance of a simple visual discrimination task or a variable interval (VI) schedule of reinforcement. ACh efflux, expressed as a percent change from baseline, did not change with performance in either task, suggesting that the demands of these tasks were not sufficient to activate the mPFC cholinergic system. Moreover, mPFC ACh efflux was not systematically influenced by variations of sensory, motivational, or motor aspects of operant performance. No changes in ACh efflux were observed in response to presentation of a flashing houselight. The high rates of lever presses and reinforcements in the VI task, and their steep decline over blocks, were also not correlated with changes in ACh efflux. While there was an overall reduction in ACh efflux during the baseline and task period in the Extinction session relative to the Standard session, this effect cannot be attributed simply to a response to the removal of expected reinforcement (see discussion below). Below, the discussion will focus on: (1) interpretational issues critical to the demonstrated lack of relationship between mPFC ACh and performance, and on the absence of significant changes in ACh efflux in general; (2) the relationship between these results and recent studies on task-related changes in cortical ACh efflux; and (3) the implications of these results for subsequent analyses of the role of cortical cholinergic transmission in attention.

4.1. Interpretational issues

There are several issues regarding the interpretation of the negative effects observed in this study. First, it could be argued that the lack of task-induced increases in mPFC ACh efflux was due to a “ceiling effect”, i.e., that baseline cortical ACh levels were maximal prior to the introduction of any behavioral manipulations, thus precluding further stimulation of ACh efflux. Several findings suggest that this interpretation is unlikely. Using identical strains and methods of baseline collection in dialysis bowls, we have repeatedly demonstrated increases from baseline cortical (medial prefrontal or frontoparietal) ACh efflux resulting from environmental/behavioral (i.e., exposure to darkness associated with food reward) and pharmacological (i.e., atropine, high K^+ concentrations, benzodiazepine receptor inverse agonists) manipulations [10,23–27]. In addition, animals in the current study were extensively

habituated to all of the testing procedures, rendering it unlikely that baseline cortical ACh levels would be maximal under these conditions. Finally, the absolute levels of baseline cortical ACh efflux in this experiment corresponded well with the absolute baseline release measured in previous experiments in which increases in ACh were produced, thus excluding the possibility that unusually high absolute baseline levels of cortical ACh efflux may have confounded the results from the present experiment.

Increases in ACh efflux were observed upon transfer from the dialysis bowl to the operant chamber in animals performing in the visual discrimination task, but not the VI schedule of reinforcement, thus raising the possibility that ACh levels had reached a ceiling level in the operant chambers prior to task onset. However, the observed increases following transfer to the operant chambers were not prohibitively large (93–98% change from baseline). As our previous studies have reported changes of up to 200% from baseline efflux measured in dialysis bowls [10,23], it is doubtful that these transfer effects represent maximal increases from baseline. Finally, in a preliminary study we have demonstrated that cortical ACh efflux can be increased (120%) from operant chamber baseline in animals performing in an operant task designed to measure sustained attention [44]. For these reasons, it is reasonable to assume that in this experiment, baseline ACh efflux in the operant chambers was not at a ceiling level.

A second issue raised by this type of research concerns the temporal resolution of microdialysis procedures. For example, in the current study, the validity of correlating cortical ACh efflux, measured on the order of minutes, with task performance, where events are changing on the order of seconds, may be questioned. It is conceivable that each 12 min collection may actually reflect an average of rapidly occurring trial-to-trial increases and decreases in cortical ACh efflux, the exact nature of which would thus be masked by the length of the collection interval. However, our initial experiments on cortical ACh efflux in animals performing a sustained attention task suggest that both task onset and the presence of a flashing houselight (as a distractor) are associated with increases in cortical ACh efflux that persisted throughout the 12 min collection intervals [44]. In a series of studies on the role of cortical and hippocampal ACh on arousal and attentional processes, Fibiger and co-workers have demonstrated persistent increases in ACh efflux, over comparably large collection intervals, associated with the anticipation of a palatable meal [14], the transfer from home cage to test chamber [1], and to unconditioned or conditioned sensory stimuli [1,13]. Finally, given the putative arousal and stimulus amplification functions of cortical ACh [21,36,41,44], it is unlikely that rapid, discrete changes in efflux would be manifested following individual trials of the task. Instead, cortical ACh efflux would be expected to reflect a rather slow, general increase during task performance [42].

It has previously been reported that transfer of an

animal from one environment to another, such as from a home cage to a testing apparatus, results in reliable increases in cortical ACh efflux that persist even when animals are handled extensively prior to testing [1,14]. In our previous experiment, we also observed a significant increase upon transferring animals from dialysis bowls to the operant chambers [44]. Given these observations, the finding that transfer effects in this study were not more consistently demonstrated was unexpected. Increases in relative ACh efflux were observed in both groups of animals, but the effect was statistically significant only in the visual discrimination group. It is unlikely that this transfer effect reflects an anticipation of reward, as this was presumably present in both tasks. It may be speculated that although the visual discrimination task did not increase cortical ACh efflux during performance, the response rules of the task were sufficiently more demanding (compared to the variable interval task, which had no externally driven response rules) to produce increases in ACh efflux prior to task onset. Generally, however, the animals of the current experiments were extremely well-handled and adapted to the experimental settings and procedures, which may have diminished the potential transfer-associated increases in cortical ACh release.

Another methodological issue surrounding the interpretation of these data centers around the extent to which dialysis studies in one cortical region (i.e., mPFC) can be generalized to the entire cortical cholinergic system. Our selection of the mPFC in the current experiment was based on our previous selection of this region in a study on the relationship between ACh efflux and attentional processing [44]. The relationship between the mPFC and attention is supported by a wealth of experimental data from human and animal experiments. We have argued elsewhere that stimulation of the basal forebrain cortical cholinergic system increases cortical ACh efflux similarly throughout the cortical mantle [42]. In fact, our own microdialysis data suggest similar responsivity to pharmacological and environmental stimuli in mPFC vs. frontoparietal cortex [23–27]. Moreover, a recent microdialysis study has suggested relatively global changes in cortical ACh release as rats transition through behavioral states during day/night phases [15].

A final methodological issue worthy of discussion is the relationship between the use of a cholinesterase inhibitor such as neostigmine and the ability to detect changes in cortical ACh efflux. As we have discussed previously [24,41,42], the addition of neostigmine to the aCSF is necessary to detect extracellular ACh using concentric dialysis probes and relatively short collection intervals in the cerebral cortex. It is conceivable that pharmacologically elevated levels of extracellular ACh dampen the reactivity of cholinergic neurons through over-stimulation of autoreceptor mechanisms. Thus, small changes in ACh efflux, that might not have reached statistical significance in the present experiment, may indeed be of physiological

relevance. While the value of minimizing or even eliminating the use of cholinesterase inhibitors is without debate, their usage is necessary given the current techniques and goals of this manuscript. Our laboratory, as well as other investigators, have repeatedly reported pharmacological and environmental stimuli-induced *increases* in cortical ACh even when neostigmine concentrations were 10 times or more than those used in the present study (0.5 μ M).

4.2. Role of cortical ACh in attention

While a general increase in cortical ACh efflux may be expected during performance of an attentionally demanding task [44], no changes from baseline ACh levels were observed during performance of either the visual discrimination or the variable interval schedule of reinforcement. This lack of task-related changes in cortical ACh efflux was largely expected, as it is in accordance with previous research demonstrating that manipulations of the cholinergic system do not affect performance in previously learned conditional discrimination tasks [7,9,30,32,38]. The current results support the conclusion that the cortical cholinergic system is not involved in performance of such tasks, which likely involve automatic, habit-based rather than controlled or effortful processing of stimuli or response rules [22,40].

The failure of operant performance to affect cortical ACh efflux may appear to conflict with recent studies involving microdialysis in performing rats. Rats acquiring a simple FR1 operant task for food reward exhibit a transient increase in cortical ACh efflux during the block in which lever pressing begins to increase beyond low-level initial rates [32]. Given the nature of the task, it is impossible to attribute specific behavioral constructs to the rise in ACh efflux. It is interesting in light of the discussion above, however, that there were no increases in ACh efflux once the animals reached stable levels of responding or in animals that had been previously trained in the task. Another study [14] reported increases in cortical and hippocampal ACh efflux in “anticipation” of the opportunity to consume a palatable meal. Again, the nature of the task makes it difficult to dissociate the relative contributions of arousal, activation, motivational and attentional processes. Finally, animals trained in a conditioned fear paradigm exhibit increased cortical ACh efflux in response to presentation of a complex stimulus that had previously been paired with footshock, suggesting that presentation of behaviorally relevant stimuli are sufficient to influence cortical ACh [1]. In light of these findings, one may have expected similar increases during the performance of appetitive operant tasks, at least in the visual discrimination task, where the sensory stimuli function to signal that reinforcement is available. However, given the differences between the events of fear conditioning, in which animals are not required to respond but simply to endure the presentation of aversive stimuli, and the requirements of operant performance, in which animals exert more control

over their behavior, it may not be valid to expect comparable changes in cortical ACh across these types of tasks. Additionally, the experiments by Inglis et al. [14] and Acquas et al. [1] did not explicitly eliminate novelty- and stress-like events or manipulations which per se involve the activation of attentional processes and thus would be expected to correlate with increased cortical ACh efflux. Therefore, the results from these studies may not necessarily be in conflict with the conclusions from the present experiments, which attempted to exclude the confounding effects of manipulations associated with stress and novelty.

Our previous results suggested a correlation between demands on attention and cortical ACh efflux, as indicated by a decline in attentional performance accompanied by an increase in cortical ACh efflux during presentation of a flashing houselight distractor [44]. Since it has been previously demonstrated that *novel* stimuli, including a flashing light, can stimulate cortical ACh efflux [1,13], care was taken in the current study to expose animals to the flashing houselight several times before testing, thus ensuring that any observed changes in ACh efflux would not be due to novel sensory effects. This aspect of the experimental procedure deserves emphasis, as the interpretation of the current results is at risk to suffer from circular logic (i.e., “if a stimulus is not activating ACh, it is concluded to lack novelty- or stress-inducing properties”). Flashing houselight would clearly be predicted to elevate cortical ACh when presented for the first time. However, the animals in the current experiment and those previously trained in the sustained attention task were exposed to this stimulus several times before the dialysis session. Thus, flashing houselight does not affect basic operant performance but does influence sustained attentional abilities, and only the latter situation was associated with increases in cortical ACh efflux. This suggests that the previously observed increases in efflux during presentation of the flashing houselight [44] were associated with its disruptive effects on attentional processing, and not with its sensory characteristics.

Our study also demonstrated that motor activity (associated with lever pressing) and loss of reinforcement (during extinction) did not systematically affect cortical ACh efflux. The effects of locomotor activity on cortical ACh efflux have remained unclear; some studies have presented evidence of a correlation between the two variables [3,17], while others have not [1,6,24]. The relationship between motor activity associated with lever pressing and cortical ACh efflux has received very little attention. In the current study, extremely high rates of lever pressing (variable interval task) were observed in the absence of changes in cortical ACh efflux. In the only other study to examine the relationship between lever pressing and cortical ACh efflux, it was found that high rates of lever pressing were also not associated with increases in ACh efflux [32].

The analyses revealed an overall difference in ACh efflux between the Standard and Extinction sessions of the

visual discrimination task. We do not believe that this difference is being driven by the loss of reinforcement for three reasons. First, there was no interaction between Session Type and Collection Interval. Second, there was no difference between Standard and Extinction sessions of the VI task. Finally, during extinction trials, in which animals experienced a loss of expected reinforcements and subsequently ceased responding, cortical ACh efflux did not change in either task. It is likely that the loss of reinforcements resulted in increased levels of motivation, at least during the first few extinction trials. However, the lack of corresponding changes in ACh efflux suggests that neither motivational factors nor decreases in reward density play a significant role in modulating cortical ACh efflux.

The apparent dissociations between lever pressing activity and cortical ACh efflux, and between reinforcement-induced motivation and ACh efflux, will greatly improve our ability to interpret more clearly the effects on cortical ACh efflux of performance in other tasks, such as those that explicitly tax attentional processing. It is important to acknowledge, however, that there is a necessary confound between response rate and reward in the current experiments; thus, the present experiments did not *independently* manipulate response rate and reward density.

Collectively, the available data support the hypothesis that the increase in ACh efflux associated with distractor-induced impairments in sustained attention [44] was not primarily linked to the loss of reward and/or the changes in motor activity which represent inherent components of such a performance impairment; rather, the increases in cortical ACh release were predominately associated with the distractor-induced increases in the demands on attentional processing. This hypothesis, however, needs to be tested more rigorously in future experiments.

4.3. Future considerations for relating transmitter efflux to cognitive processes

The results of this experiment, in conjunction with recent studies from other laboratories (see [43] for a discussion of this issue), highlight the importance of several paradigmatic issues when attempting to relate changes in transmitter efflux with changes in cognitive functions. *First*, care must be taken to define the appropriate pre-task baseline. The likely occurrence of transfer effects from home cage or dialysis bowl to test chamber can conceivably mask or severely undervalue task-related increases in transmitter efflux. *Second*, animals should be extensively habituated to the testing chamber and any sensory stimuli (i.e., lights, tones, etc.) associated with the task to minimize the consequences of implicit variables of novelty and stress. The presentation of novel stimuli during the task itself may lead to spurious increases in transmitter efflux that have little to do with changing cognitive demands of the task and may confound cognition-associated changes in

ACh release. *Third*, the effects of motor activity (locomotor, lever pressing) on transmitter efflux should be dissociated from the cognitive components of the task. Relatedly, to the extent that motor activity is contributing to changes in efflux, any effects of independent manipulations (drugs, lesions, aging) on motor activity should be considered when interpreting the results. *Finally*, as performance in either appetitive or aversive tasks declines it is necessarily accompanied by changes in reward density. Control experiments, assessing whether changes in reward density are able to affect transmitter efflux independent of the cognitive demands of the task, are critical in properly attributing the relationship between transmitter efflux and cognitive processes.

In summary, the results of the current study identify several factors associated with performance in our operant tasks that do not affect cortical ACh efflux. In future studies characterizing the relationship between performance in our vigilance task and cortical ACh efflux, the effects of manipulations of attentional demand and other factors can be more readily interpreted. Any changes in cortical ACh efflux observed during performance in the vigilance task can be more confidently attributed to attentional factors, rather than such variables as retrieval of response rules, sensory stimulation, motor activity, motivation, and reinforcement properties.

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