Pergamon

www.neuroscience-ibro.com

PII: S0306-4522(02)00292-0

Neuroscience Vol. 114, No. 2, pp. 451–460, 2002

0306-4522/02 $22.00+0.00

DIFFERENTIAL CORTICAL ACETYLCHOLINE RELEASE IN RATS PERFORMING A SUSTAINED ATTENTION TASK VERSUS BEHAVIORAL CONTROL TASKS THAT DO NOT EXPLICITLY TAX ATTENTION

H. M. ARNOLD, J. A. BURK, E. M. HODGSON, M. SARTER and J. P. BRUNO*

Departments of Psychology and Neuroscience, The Ohio State University, Columbus, OH 43210, USA

Abstract—The present study used microdialysis techniques to compare acetylcholine release in the frontoparietal cortex of rats performing in a task requiring sustained attention with that of rats performing in two control procedures. The two control procedures were a fixed-interval 9-s schedule of reinforcement assessing primarily the effects of operant responding and comparable reward rates, and an operant procedure designed to test the effects of lever extension to prompt responding. These two control procedures involved comparable sensory-motor and motivational variables to those of the sustained attention task, but did not explicitly tax attentional processes. Performance of the sustained attention task was associated with a significant increase in cortical acetylcholine efflux, reaching a maximum of nearly 140%. Performance of the two control procedures was associated with significantly smaller (~50%) increases in cortical acetylcholine release.

This robust dissociation between attentional and control performance-associated increases in cortical acetylcholine release resulted, in part, from the elimination of the pre-task transfer of the animals into the operant chambers and the associated increases in acetylcholine release observed in previous studies. The present results support the hypothesis that demands on attentional performance, as opposed to the frequency of lever pressing, reward delivery and other task-related variables, selectively activate the basal forebrain corticopetal cholinergic system.

Key words: acetylcholine, sustained attention, microdialysis, cortex, basal forebrain, operant performance.

Cortically projecting basal forebrain cholinergic neurons have been hypothesized to mediate attentional functions such as the detection, selection, and processing of environmental stimuli (for reviews see Everitt and Robbins, 1997; Sarter et al., 2001). Consequently, aberrations in the functioning of these basal forebrain corticopetal cholinergic neurons, and in the afferent regulation of their excitability, have been suggested to play an important role in the manifestation of the cognitive symptoms underlying several major neuropsychiatric disorders, including schizophrenia, dementia, and compulsive drug use (Sarter and Bruno, 1999).

The use of operant tasks designed to assess attentional functions in animals has assisted in substantiating theories regarding the role of cortical acetylcholine (ACh) in attentional processing (Everitt and Robbins, 1997; Sarter and Bruno, 1997; Sarter et al., 2001). For example, the necessity of basal forebrain activation and cortical cholinergic transmission for attentional processing was demonstrated by studies showing that performance in the five-choice serial reaction time task is disrupted by blockade of high-affinity choline uptake or excitotoxic lesions of the basal forebrain (Muir et al., 1994; Muir et al., 1995). Similarly, using an operant task designed to measure sustained attention performance, McGaughy et al. (1996) demonstrated that the loss of cortical cholinergic inputs produced by infusions of the selective cholinergic immunotoxin 192 IgG-saporin into the basal forebrain decreases animals' ability to process signal events (McGaughy et al., 1996; McGaughy and Sarter, 1998). Moreover, this impairment in detecting signals can be reproduced by manipulations that decrease the excitability of basal forebrain neurons and attenuate stimulated cortical ACh efflux such as intra-basalis administration of benzodiazepine receptor agonists (Holley et al., 1995; Moore et al., 1995a,b), or *N*-methyl-D-aspartate (NMDA) receptor antagonists (Fadel et al., 2001; Turchi and Sarter, 2001a). Importantly, these lesions and neurochemical manipulations do not affect performance in tasks that are devoid of explicit attentional demands (Himmelheber et al., 2001; Turchi and Sarter, 2001a,b).

Several recent studies, using *in vivo* microdialysis, have begun to address the dynamics of cortical ACh release while rats perform complex operant tasks designed to explicitly assess attention. Experiments examining rats

*Correspondence to: J. P. Bruno, Department of Psychology, The Ohio State University, 31 Townshend Hall, 1885 Neil Avenue, Columbus, OH 43210, USA. Tel.: +1-614-292-1770; fax: +1-614-688-4733.

E-mail address: bruno.1@osu.edu (J. P. Bruno).

Abbreviations: ACh, acetylcholine; ANOVA, analysis of variance; FI, fixed-interval; FR, fixed ratio; ITI, inter-trial interval.

trained in the five-choice serial reaction time task have demonstrated that cortical ACh efflux is greater in rats performing this task than in rats passively exposed to the testing chamber (Passetti et al., 2000) or in rats whose instrumental contingency in the task had been abolished (Dalley et al., 2001). Himmelheber et al. (2001) found that ACh efflux in the frontoparietal cortex of rats performing a sustained attention task, though increased, was not increased beyond that of rats performing a control task that did not explicitly tax attentional processes. However, presentation of a visual distracter to rats in the sustained attention task led to increases in cortical ACh efflux beyond those seen during normal task performance (Himmelheber et al., 2000). Although these studies collectively demonstrate that performance in a behavioral task designed to tax attentional processing leads to increases in cortical ACh efflux, the question remains whether these increases in cortical ACh release are related to the level, or degree, to which animals are required to exercise attentional mechanisms during the task, or whether other aspects of operant performance such as motor activity or reward density are sufficient to increase cortical ACh release.

In the experiments by Himmelheber et al. (2000, 2001), rats were placed in a holding area for several hours following insertion of the microdialysis probe, and then moved to the operant chamber only minutes before task onset. This handling/transfer experience was sufficient to produce marked increases in pre-task cortical ACh efflux, possibly limiting the range of subsequent task-related changes in cortical ACh efflux. The present experiment was designed to avoid this 'transfer effect' (Bruno et al., 1999) by placing animals in the operant chambers during all aspects of the experiment. This procedure removes the pre-task handling and transfer effects on cortical ACh efflux and, thus, may maximize the ability to detect task-related changes in cortical ACh efflux.

The goal of the current experiment was to compare cortical ACh efflux (measured by *in vivo* microdialysis) during performance of the sustained attention task with that of two separate groups of animals performing operant control tasks designed to equate reinforcement density and mimic the sensory and motor components of the sustained attention task, but that did not explicitly tax attentional processes. It was predicted that ACh efflux would increase above basal levels during performance of the sustained attention task. Because increases in cortical ACh efflux have been associated with presentation of conditioned and unconditioned stimuli as well as with anticipation and delivery of reward (Acquas et al., 1996; Inglis et al., 1994; Inglis and Fibiger, 1995) cortical ACh efflux was also expected to increase above baseline during performance of the operant control tasks, which would be consistent with other reports (Dalley et al., 2001; Himmelheber et al., 2000, 2001). However, if the increases in cortical ACh efflux observed during the task are, in fact, related specifically to attentional processing then cortical ACh levels were expected to be augmented in animals performing the sustained attention task relative to levels in animals performing the operant control tasks.

EXPERIMENTAL PROCEDURES

Subjects

Subjects were 19 male Fisher/Brown-Norway F1 hybrid rats that were three to four months of age at the beginning of behavioral training and between eight and 14 months of age at the time of the microdialysis session. Animals were housed individually in a temperature (23°C)-controlled environment on a 12-h light/dark cycle (lights on at 6:30 a.m.). All animals were handled extensively prior to behavioral training, and were water-deprived to approximately 90% of their free-feeding body weight. This was accomplished by allowing animals access to water during the course of task performance and for 8 min immediately following each session. Rats were provided with *ad libitum* access to food throughout the course of the study. 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.

Apparatus

Behavioral training took place in a set of 20 operant chambers (MedAssociates, St. Albans, VT, USA) located inside larger sound-attenuating chambers. Each operant chamber was equipped with an intelligence panel consisting of three lights (2.8 W), two retractable levers, and a water dispenser (40–45 µl water per delivery) all located on the front wall. A houselight (2.8 W) was located near the ceiling on the rear wall. Microdialysis sessions were performed in a separate set of four operant chambers, similar to the chambers described above, with the following modifications: (1) the height of the recessed water delivery area was increased to allow room for rats, with guide cannula secured to the top of their skulls, to drink, and (2) the top of the operant chamber had an opening to allow microdialysis tubing to extend out of the operant and sound-attenuating chambers. Med-PC for Windows software (v. 1.1, MedAssociates) controlled all signal presentations, lever operation, reinforcement delivery, and data collection in each system via a Pentium PC.

Behavioral training

Sustained attention task. Animals were initially shaped to press both levers on a modified fixed ratio 1 (FR-1) schedule for water reinforcement. Following at least three days of 120 reinforced lever presses, animals began training in an operant task previously described and validated as generating a measure of sustained attention in rats (McGaughy and Sarter, 1995). During each session, following a 5-min period of adaptation to the operant chamber, animals were required to discriminate between signal (1.0 s illumination of the central panel light) and non-signal events. Both levers were extended into the chamber 2.0 s after a signal or non-signal event. On trials in which a signal was presented, a press on the left lever was reinforced and termed a 'hit'; a press on the right lever was not reinforced and termed a 'miss'. On non-signal trials (trials in which the central panel light was not illuminated), a press on the right lever was reinforced and termed a 'correct rejection'; a press on the left lever was not reinforced and termed a 'false alarm'. If animals did not respond within 4.0 s the levers were retracted and an error of omission was recorded. If animals did not respond correctly (a hit or correct rejection), a 'correction trial' was presented that was identical to the previous trial. Incorrect responses during a correction trial resulted in a 'forced' trial. During forced trials, the event (signal or non-signal) was repeated but only the correct lever was extended into the chamber. The lever remained available for 90 s, and in the case of a signal trial, the panel light remained illuminated during this period. The inter-trial interval (ITI) was 12 ± 3 s during this stage of training. Signal and non-signal trials were presented in

pseudo-random order, with 81 signal and 81 non-signal trials throughout each session.

Following five consecutive days of responding correctly on $>70\%$ of both signal and non-signal events correction and forced trials were discontinued and the 1.0-s signal was replaced with three different signal lengths of 500, 50, and 25 ms which were equally distributed throughout the task session. Animals were trained until they responded correctly on $>70\%$ of the 500-ms signal trials and $>70\%$ on the non-signal trials.

During the next stage of training, the length of the pre-task adaptation period was increased to 12 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. Selection of trial type (signal or non-signal) and signal length were pseudo-randomized to insure that approximately half of the 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 addition, the event rate was increased by reducing the ITI to 9 ± 3 s. Following at least seven days of stable performance (at least 70% hits to 500-ms signals and 70% correct rejections, and less than 30% omissions) animals began training in the final version of the sustained attention task.

In the final version of the task, the parameters remained identical to those just described for the second training step except that the adaptation period was lengthened to 18 min and the houselight was illuminated throughout the task and the adaptation period. Stable performance in this task was defined as at least seven days with at least 70% hits to 500-ms signals and 70% correct rejections, and less than 40% omissions.

Operant control tasks. As a control for the potential effects of sensory and motor components of the sustained attention task, two additional groups of animals were trained in separate operant tasks. Animals in each of these two groups were initially shaped to lever press on a modified FR-1 schedule for water reinforcement with only a single lever available for responding. Following at least three days of 120 or more reinforced lever presses, animals were assigned to one of two different operant control tasks that did not involve explicit demands on attentional processing.

The first of these tasks required rats ($n=6$) to press a lever on a fixed-interval 9-s (FI-9) schedule for water reinforcement during a 36-min task period (henceforth referred to as the 'FI-9' task). This task was designed to minimize implicit demands on processing capacity that may result from tracking the presentation and removal of the response levers. Subsequently, this task allows rats to press the response lever freely during the ITI. Thus, after a 6.0-min adaptation period in an operant chamber, illuminated by the houselight, a single lever was available for responses; this lever (left or right; counterbalanced across animals) remained extended into the task chamber for the entire length of the 36-min task. After five days of stable performance, defined as rats earning more than 120 reinforcements during a daily session, the task was modified in steps to equate the adaptation period and the number of reinforcements received by animals in this task with those of rats in the sustained attention task. In the next stage of training, reinforcement was delivered only on 85% of the presses that would have been reinforced according to a standard FI schedule and the adaptation period was lengthened to 12 min. Again, after another five days of stable performance (defined above), the percentage of lever presses after the 9-s interval that were reinforced was lowered to 70% and the pre-task interval was lengthened to 18 min. Finally, after five days of stable performance on this version of the FI-9 task (defined as rats earning more than 100 reinforcements during a daily session) the percentage of reinforcement was lowered to 60% of the trials that would otherwise have been reinforced. After at least five stable days on this final version of the task (again, defined as >100 reinforcements earned), training continued in the operant chambers designed for microdialysis.

The second control task required rats ($n=6$) to press a lever for water reinforcement during a 36-min task period (henceforth referred to as the 'retracting lever task') with an ITI of 9 ± 0 s. The retracting lever task was designed to mimic repeated extension of a lever into the chamber as in the sustained attention task, as well as the rate of reinforcement and motoric demands of the sustained attention task. In the initial stage of training, the houselight was illuminated throughout each session and during a 6-min adaptation period to the chamber preceding onset of the behavioral task. During the retracting lever task only a single lever was extended into the box on each trial and the side where the lever was presented (left or right) was counterbalanced across animals. A press on the lever resulted in delivery of 40 µl water reinforcement. Stable performance on this version of the task was defined as rats earning more than 120 reinforcements during the 36-min daily session. After five days of stable performance, the task was modified such that the adaptation period was 12 min and reinforcement was delivered following 85% of lever presses. Again, after another five days of stable performance, the adaptation period was increased to 18 min and the percentage of reinforced lever presses was lowered to 70%. Finally, after five days of stable performance on this version of the retracting lever task (defined as rats earning more than 100 reinforcements during a daily session) the percentage of reinforced lever presses was lowered to 60%. As in the FI-9 task, this was done to equate the number of reinforcements received by animals in this task with those of rats in the sustained attention task and to mimic the partial reinforcement received by animals in the sustained attention task. After stable performance was achieved in this task (again, defined as >100 reinforcements earned) training continued in the operant chambers designed for microdialysis.

Guide cannula implantation

Upon reaching stable performance in the operant chambers (as defined above) in the final version of their respective task, animals continued training in the operant chambers designed for microdialysis. During this time the adaptation period prior to task onset was increased to approximately 4 h daily and the animals remained in the chamber for 30 min after completing the task with the chamber illuminated by the houselight in order to mimic the conditions of the microdialysis session (see below). Animals continued to be trained under these conditions until at least three days of stable performance were achieved at which time animals underwent guide cannula surgery; the mean number of sessions required to reach criterion was 12 ± 5. Animals were anesthetized with ketamine (100.0 mg/kg, i.p.) and xylazine (3.0 mg/kg, i.p.) prior to stereotaxic surgery to implant a chronic microdialysis guide cannula (10.0 mm plastic shaft, o.d. 720 µm, Bioanalytical Systems, West Lafayette, IN, USA) 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 a 45° angle away from the midline (all coordinates according to the atlas of Paxinos and Watson, 1986). Hemisphere was counterbalanced between animals in all three groups. The cannula was affixed to the skull with stainless steel screws and dental cement. A stainless steel stylet that ended flush with the termination of the guide cannula was inserted into the guide cannula to prevent clogging. Animals were given a post-operative injection of amoxicillin (100 mg/kg, s.c.) and allowed to recover in their home cages for three days with food and water *ad libitum*. After recovery, water deprivation levels were reinstated and animals resumed training in the microdialysis operant chambers.

Once animals had regained stable performance in their respective task (mean number of required sessions 15 ± 6), they continued training with the addition of a tethering apparatus attached to a counterbalance arm used in microdialysis experiments to keep tension off the microdialysis tubing.

Microdialysis sessions

Each animal participated in a single microdialysis session. Each microdialysis session began by placing the rat in the operant chamber for 45–60 min prior to the removal of the stylet

and insertion of a concentric microdialysis probe (2.0 mm membrane tip, o.d. 320 μm, BAS) through the guide cannula into the frontoparietal cortex. The microdialysis probe was perfused (2.0 μl/min) with an artificial cerebrospinal fluid (aCSF; pH = 7.1 ± 0.1) with the following composition (in mM): 126.5 NaCl, 27.5 $NaHCO_3$, 2.4 KCl, 0.5 Na_2SO_4, 0.5 KH_2PO_4, 1.2 $CaCl_2$, 0.8 $MgCl_2$, 5.0 glucose, and 0.1 μM of the reversible cholinesterase inhibitor neostigmine bromide (Sigma Chemical, St. Louis, MO, USA). This moderate concentration of neostigmine was originally chosen in order to assure that ACh levels would be detectable in 6.0-min collection intervals and to maintain compatibility of these data with those of Himmelheber et al. (2001). Following a 3-h discard period, to allow ACh efflux to become stable and dependent upon neuronal depolarization (Moore et al., 1992), collection of dialysate samples (every 6.0 min) began. Four baseline collections were taken prior to onset of the operant task. Six collections were taken during performance of the task (corresponding to the six blocks of the task) and, five microdialysis samples were collected following the completion of the task while the animal remained in the operant chamber, illuminated by the houselight. Following probe removal, an *in vitro* estimate of probe efficiency (recovery) was obtained by placing the probe in solution with a known concentration of ACh (100 nM). Probe recoveries averaged 15%; individual data were not corrected for recovery.

ACh analysis

ACh levels in dialysates were determined by high-performance liquid chromatography with electrochemical detection. From each sample collected, 10.0 μl were injected. ACh and choline were separated by a C-18 carbon polymer column (250 × 3 mm; ESA, Chelmsford, MA, USA) using a sodium di-phosphate mobile phase (100.0 mM Na_2HPO_4, 5.0 mM TMACI, 2.0 mM 1-octanesulfonic acid, pH = 8.0). ACh was hydrolyzed on a post-column enzyme reactor (ESA) and converted to hydrogen peroxide (Potter et al., 1983) that was detected using a 'peroxidase-wired' (Huang et al., 1995) ceramic, glassy carbon electrode (ESA) with the potential set at −200 mV. The detection limit for ACh under these conditions was 5.0 fmol/10.0 μl injection.

Histological verification of probe placement

Within five days of the last microdialysis session, animals were given an overdose of sodium pentobarbital and transcardially perfused with 0.2% heparin in 0.9% saline followed by 10% formalin. Brains were stored in 10% formalin at 4° C for at least 24 h and then cryoprotected in 30% sucrose phosphate buffer until sectioning (45 μm) at least three days later. Sections were stained with Cresyl Violet and examined with a VANOX Olympus Research Microscope (Olympus America, Melville, NY, USA) to verify dialysis probe placement.

Statistical analysis

Statistical analyses using a mixed (between groups and repeated measures) analysis of variance (ANOVA) were performed on behavioral and neurochemical data separately. Significant F values were further evaluated with multiple dependent or independent t-tests. All statistical tests were conducted with $\alpha = 0.05$.

Behavioral performance. For rats performing the sustained attention task, the total number of hits, misses, correct rejections, false alarms, and omissions was calculated for each of six task blocks (6 min each) to assess the effects of time-on-task on performance and to correspond with the microdialysis collection intervals. To examine the accuracy of animals in the sustained attention task data on trial outcomes, the relative number of hits (hits/hits+misses) for each signal length and the relative number of correct rejections (correct rejections/correct rejections+false alarms) were calculated. To normalize the relative percentage data, an angular transformation [$X' = 2 \arcsin(X^{1/2})$] was performed (Zar, 1974). Performance of rats in the sustained attention task during the microdialysis session was analyzed with a two-way repeated measures ANOVA on the relative number of hits, with the factors of task block (six levels) and signal length (three levels; 500, 50, and 25 ms). A repeated measures ANOVA was also performed on the relative number of correct rejections over the six task blocks.

To compare performance among all three behavioral groups, a mixed-design ANOVA was conducted over all six blocks of task performance. The groups were compared on the number of reinforcers received during each block of the task and the total number of lever presses made during each task block. For rats in the sustained attention task this included presses on the left and right levers, regardless of response accuracy.

ACh efflux. To compare basal ACh efflux values (pmol/10 μl) among all three behavioral groups, the baseline collections for each group were compared using a mixed two-factor (three groups × four collections) repeated measures ANOVA. Demonstrating that basal efflux for each treatment group is similar permits unbiased expression of the subsequent data as a percent change from basal levels. Thus, for each subject the mean of the four baseline collections was calculated and the remainder of the statistical analyses were performed on data expressed as a percent change from the mean baseline.

To assess changes in cortical ACh efflux in the three groups during and after performance of the behavioral task, data were analyzed using a mixed ANOVA over the entire baseline period, the six collections taken during the task and the five post-task collections (15 time points). To address specific questions regarding the nature of the observed changes in cortical ACh efflux, multiple dependent t-tests were performed to compare ACh efflux within a particular group and independent t-tests were performed to compare ACh efflux between different treatment groups.

RESULTS

All probe placements were located within the boundaries of the frontoparietal cortex, similar to the placements reported in Himmelheber et al. (2001).

Attentional task performance

The detection of the visual signals was highly dependent on the duration of the signal, as revealed by a main effect of signal length on the relative number of hits ($F_{2,12} = 19.258$, $P < 0.001$). Figure 1 illustrates that animals were more accurate in detecting the 500-ms signal than either the 50-ms signal ($t_6 = 4.359$, $P < 0.005$) or the 25-ms signal ($t_6 = 4.786$, $P < 0.003$), and more accurate to the 50-ms signal than to the 25-ms signal ($t_6 = 2.604$, $P < 0.040$). This signal-length dependency was maintained over the six blocks of the sustained attention task ($F_{10,60} = 0.602$, $P = 0.806$). Although the relative number of hits did not change significantly over blocks of time on the sustained attention task ($F_{5,30} = 0.447$, $P = 0.812$), these data are presented for each of the six task blocks in Fig. 1 to correspond with neurochemical data presented in Fig. 4. Figure 1 also illustrates that the relative number of correct rejections remained stable across all six blocks of the task ($F_{5,30} = 0.986$, $P = 0.443$). The number of omissions was low (ranging from 2.9–7.4 per block) and did not vary across blocks ($F_{5,30} = 2.530$, $P = 0.114$).

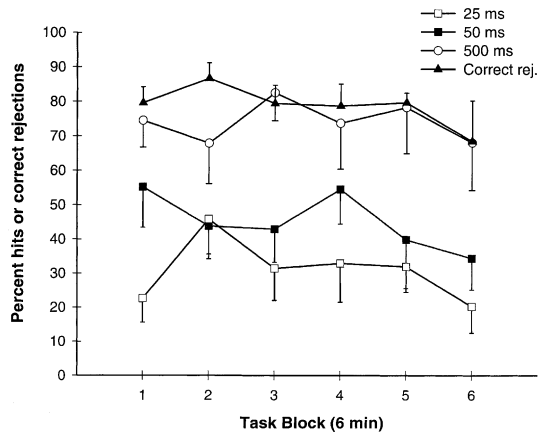

Fig. 1. Behavioral performance for rats ($n=7$) in the sustained attention task during the microdialysis session. The data shown are the mean (±S.E.M.) relative number of hits (hits/hits+misses) for each signal length (500, 50 and 25 ms) as well as the relative number of correct rejections (correct rejections/correct rejections+false alarms) for each of the six task blocks. Each 6-min block consisted of approximately 27–30 trials pseudo-randomly distributed between signal and non-signal trials. While performance on signal trials was dependent on signal length, the relative number of hits and correct rejections did not change across the six blocks of the task.

Between groups analysis of operant task performance

As illustrated in Fig. 2, rats in the FI-9 task pressed the lever much more frequently than rats in the other two task groups ($F_{2,16}=21.070$, $P<0.001$). This was true for the entire session as the number of presses remained stable across the six behavioral task blocks ($F_{5,80}=1.421$, $P=0.226$) and as the effects of block did not differ between groups ($F_{10,80}=0.769$, $P=0.658$). Further analysis of the main effect of group confirmed that animals performing the FI-9 task pressed the lever more often than animals performing the retracting lever task ($t_{10}=-3.927$, $P=0.003$) or the sustained attention task ($t_{11}=-4.393$, $P=0.001$); rats in the retracting lever task

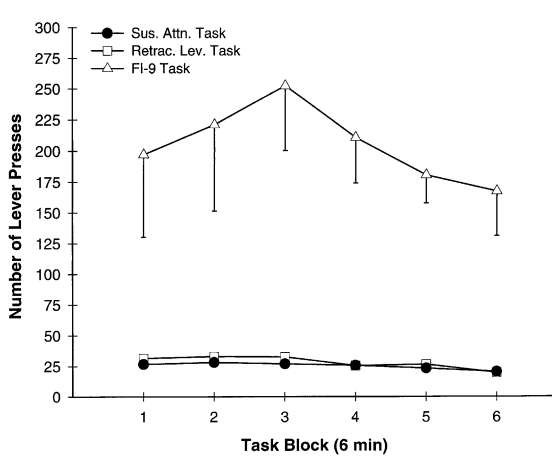

Fig. 2. Mean (±S.E.M.) number of lever presses made during performance in the sustained attention ($n=7$), retracting lever ($n=6$), and FI-9 ($n=6$) tasks. Error bars for the sustained attention and retracting lever task-performing animals are not visible due to the scaling of the figure and the low variability in these data.

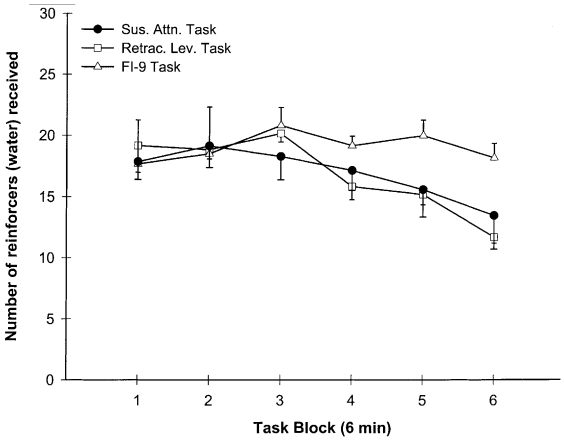

Fig. 3. Mean (±S.E.M.) number of reinforcements (40 µl of water) received during performance in the sustained attention ($n=7$), retracting lever ($n=6$), and FI-9 ($n=6$) tasks.

also pressed the lever more often than rats in the sustained attention task ($t_{11}=-2.599$, $P=0.025$).

As shown in Fig. 3, rats in the sustained attention task did not receive any more (or less) reinforcement during performance in the operant tasks than rats in the other two groups, as the ANOVA did not reveal a main effect of group ($F_{2,6}=1.127$, $P=0.348$), nor a significant interaction between block and group ($F_{10,80}=0.968$, $P=0.477$). The number of water reinforcements received during performance in the operant tasks did vary across the six task blocks ($F_{5,80}=3.664$, $P=0.005$), largely due to the tendency for the number of reinforcers earned to decline in blocks 4–6. The number of reinforcers received tended to increase during the first two to three blocks of task performance, and then declined during the final three blocks of the task.

Basal levels of cortical ACh efflux

Basal efflux of cortical ACh was similar in each group ($F_{2,16}=3.145$, $P=0.07$; sustained attention = 0.051 ± 0.015, retracting lever = 0.101 ± 0.018, FI-9 = 0.104 ± 0.019 pmol/10 µl) and stable across the four baseline collections ($F_{3,48}=0.600$, $P=0.618$); there was no interaction between these factors ($F_{6,48}=0.652$, $P=0.689$). The basal ACh efflux levels averaged across group and time were 0.084 ± 0.011 pmol/10 µl.

Operant task-related changes in cortical ACh efflux

Performance of all three behavioral tasks was associated with significant increases in cortical ACh efflux, as illustrated in Fig. 4. An ANOVA over all 15 microdialysis collection intervals revealed significant main effects across time ($F_{14,224}=15.152$, $P<0.001$) and among the three behavioral groups ($F_{2,16}=15.446$, $P<0.001$), as well as a significant interaction between time and group ($F_{28,224}=3.718$, $P<0.001$). To further characterize the nature of this interaction additional one- and two-way ANOVAs were conducted.

In rats performing the sustained attention task, cortical ACh efflux varied significantly over the 15 collection

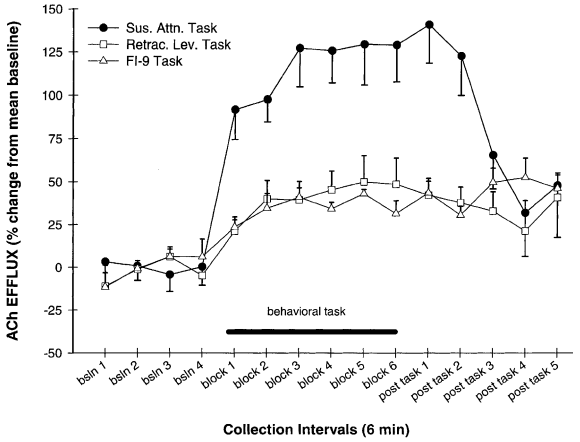

Fig. 4. Mean (±S.E.M.) levels of cortical ACh efflux (% change from baseline) before, during, and after performance of the sustained attention ($n=7$), retracting lever ($n=6$), and FI-9 ($n=6$) tasks. All three behavioral tasks were associated with increases in cortical ACh efflux. However, ACh efflux was significantly higher in rats performing in the sustained attention task than it was in rats performing in the other two tasks.

intervals ($F_{14,84}=9.293$, $P<0.001$). Pairwise comparisons revealed that cortical ACh was significantly increased in each of the six blocks of the behavioral task relative to cortical ACh levels observed during the final baseline collection (bsln 4) (block 1: $t_6=$-4.203, $P=0.006$; block 2: $t_6=-4.654$, $P=0.003$; block 3: $t_6=-4.406$, $P=0.005$; block 4: $t_6=-4.934$, $P=0.003$; block 5: $t_6=-4.182$, $P=0.006$; block 6: $t_6=-4.837$, $P=0.003$).

Similarly, in rats performing the retracting lever task, cortical ACh efflux also varied significantly over the 15 collection intervals ($F_{14,70}=6.556$, $P<0.001$). Pairwise comparisons on these data confirmed that cortical ACh was increased in all six blocks of the behavioral task over the levels obtained during the final baseline collection (bsln 4) (block 1: $t_5=-3.894$, $P=0.012$; block 2: $t_5=-3.860$, $P=0.012$; block 3: $t_5=-3.581$, $P=0.016$; block 4: $t_5=-4.224$, $P=0.008$; block 5: $t_5=-3.340$, $P=0.021$; block 6: $t_5=-3.267$, $P=0.022$).

In rats performing the FI-9 task, cortical ACh varied over time ($F_{14,70}=6.581$, $P<0.001$) as in the other two groups. However, in the FI-9 task, the increase was not significantly higher than the last baseline collection (bsln 4) until the fifth and sixth block of the behavioral task (block 1: $t_5=-1.300$, $P=0.250$; block 2: $t_5=-1.786$, $P=0.134$; block 3: $t_5=-2.476$, $P=0.056$; block 4: $t_5=-2.050$, $P=0.096$; block 5: $t_5=-2.961$, $P=0.031$; block 6: $t_5=-4.263$, $P=0.008$).

To assess changes in cortical ACh among the three treatment groups, a mixed two-factor ANOVA was performed over the six collections taken during performance of the behavioral tasks. This analysis revealed that cortical ACh efflux was significantly different among the three behavioral treatment groups during these six collections ($F_{2,16}=12.253$, $P=0.001$), and varied significantly across the six collections ($F_{5,80}=8.286$, $P<0.001$); there was not an interaction between these two factors ($F_{10,80}=1.478$, $P=0.163$). Subsequent analysis of the main effect of group with t-tests for independent groups revealed that cortical ACh efflux was significantly higher in rats performing the sustained attention task relative to that of rats performing the retracting lever task ($t_{11}=3.393$, $P=0.006$) or the FI-9 task ($t_{11}=4.097$, $P=0.002$). Cortical ACh efflux in rats performing the retracting lever task was not different from rats performing the FI-9 task ($t_{10}=0.522$, $P=0.613$).

Post-task-related changes in cortical ACh efflux

Figure 4 also shows that cortical ACh efflux in rats that had performed the sustained attention task declined over the course of the post-task collection period. Specifically, relative to the last baseline collection (bsln 4), cortical ACh efflux in the first three post-task collections was still significantly elevated (post task 1: $t_6=-4.921$, $P=0.003$; post task 2: post task 1: $t_6=-4.820$, $P=0.003$; post task 3: $t_6=-2.526$, $P=0.045$). However, cortical ACh levels were no longer significantly higher than the last baseline collection during the fourth and fifth post-task blocks (both P's >0.10).

Cortical ACh efflux in rats that performed the sustained attention task remained significantly higher than that of rats in the retracting lever task during the first two post task collections (post task 1: $t_{11}=3.882$, $P=0.003$; post task 2: $t_{11}=3.250$, $P=0.008$) but were no different from this group during the final three post task collections (all P's >0.10). Similarly, cortical ACh efflux in rats that performed the sustained attention task was elevated over that observed in rats that had performed the FI-9 task during the first two post collection intervals (post task 1: $t_{11}=3.815$, $P=0.003$; post task 2: $t_{11}=3.659$, $P=0.004$), but were no different from efflux levels in these rats during the final three post task collections (all P's >0.50). Finally, there were no differences in cortical ACh efflux levels between rats performing the retracting lever task and rats performing the FI-9 task during the any of the five post-task collections (all P's >0.10).

DISCUSSION

The primary findings of this investigation can be summarized as follows. First, ACh efflux in the frontoparietal cortex of rats engaged in a task designed to measure sustained attention was significantly elevated over pre-task baseline levels. In addition, cortical ACh efflux was increased over baseline levels in rats performing in either of two different operant control tasks that were designed to mimic the sensory, motor, and motivational properties of the sustained attention task, but not the explicit demands on attentional processing. Importantly, the increase in cortical ACh efflux observed in rats performing the sustained attention task was significantly greater than the increase observed in rats performing either of the two operant control tasks. This differential increase in cortical ACh efflux may be related to the cognitive aspects of the sustained attention task given that rats performing this task made fewer lever presses

than rats performing the FI-9 control task, and the observation that all groups received equal amounts of water reinforcement during task performance. Thus, these findings suggest that motor activity, and the anticipation and consumption of the reinforcer during the task, cannot fully account for the enhanced increase in cortical ACh release observed in rats performing the sustained attention task. The discussion that follows addresses further the procedural changes from similar previous experiments measuring ACh release in sustained attention task-performing animals, and the implications of these data for theories regarding the role of cortical ACh in attentional processes.

The current finding that cortical ACh release was significantly higher in rats performing the sustained attention task than in rats engaged in the operant control tasks diverges from the observations of Himmelheber et al. (2001), who reported that there was no differential increase in cortical ACh release in rats performing an operant control task and rats performing the sustained attention task. Though the methods of the current study were closely modeled after those reported by Himmelheber et al. (2000, 2001), the current study employed different operant control tasks and, more importantly, eliminated the pre-task handling of the animals.

In both studies by Himmelheber et al. (2000, 2001), basal levels of cortical ACh were determined in a microdialysis bowl adjacent to the operant task chamber. Rats were then moved into the operant chamber 18 min (three 6-min collection intervals) prior to the start of the behavioral task. This pre-task handling and transfer of the animals into the operant chamber was accompanied by a large (~150%) increase in cortical ACh efflux that did not significantly decline over the 18-min pre-task interval. Himmelheber et al. (2000, 2001) attributed this increase in cortical ACh efflux to factors such as movement into a different (though not novel) environment, the anticipation of reinforcement, and the physical handling of the animals; all of which have been demonstrated to increase cortical ACh levels in the cortex (Acquas et al., 1996; Himmelheber et al., 1997; Inglis et al., 1994; Thiel et al., 1998).

To assess operant task-related changes in cortical ACh efflux following transfer, Himmelheber et al. (2000, 2001) appropriately calculated a 'new' baseline for animals, after they were moved into the operant chamber (see Bruno et al., 1999). In their experiments, the average increase in cortical ACh efflux during task performance ranged from approximately 15% to 30% above this new pre-task baseline. Thus, while significant task-related increases in cortical ACh efflux were detected, this increase reflected changes in ACh above and beyond already significantly elevated ACh levels. In one of these studies (Himmelheber et al., 2001), these authors reported similar increases in cortical ACh efflux in rats performing an operant control task which, like the controls reported here, did not place explicit demands on attentional processes.

By eliminating the pre-task handling and transfer of animals just prior to the onset of the behavioral task, and thus, eliminating the large pre-task increase in cortical ACh efflux observed in the studies by Himmelheber et al. (2000, 2001), the current experiment demonstrated a much larger percent increase (~140%) in cortical ACh efflux during the performance of the sustained attention task. Importantly, the absolute level of ACh observed by Himmelheber et al. (2001) during the baseline period and prior to the transfer of the animals (65 fmol/10 µl) was rather similar to the pre-task baseline levels reported here (84 fmol/10 µl). Therefore, it is likely that this large transfer effect limited the ability for Himmelheber et al. (2001) to detect differential increases in cortical ACh efflux between animals performing the sustained attention task and their operant control task. This speculation is supported further by the calculation that, had the changes in cortical ACh reported by Himmelheber et al. (2001) been expressed as changes from the original bowl baseline, the percent change values in cortical ACh would have been approximately 170% above basal levels, very close to the increase in cortical ACh efflux reported here. Furthermore, in experiments examining ACh efflux in rats engaged in the five-choice serial reaction time task, similar increases (150–170%) over basal levels have been reported (Dalley et al., 2001; Passetti et al., 2000) in animals that were not handled or transferred within the hour prior to onset of that behavioral task.

Collectively, the available data suggest that the ability of behavioral manipulations to enhance cortical ACh efflux may be limited to approximately 150–200% beyond basal values. If this is the case, then manipulations that affect the level of pre-task basal release must be considered a critical variable in the power of an experiment to reveal performance-related increases in cortical ACh efflux. However, it should also be noted that a 200% increase in cortical ACh efflux does not represent a ceiling on the release capacity of cortical cholinergic neurons as there are neuropharmacological manipulations that result in far greater increases (400–800%) in ACh efflux (Moore et al., 1995b, 1996).

An additional issue related to performance-related increases in cortical ACh efflux concerns the use of the cholinesterase inhibitor neostigmine in order to ensure the detection of basal levels in relatively short, 6-min collections. In theory, neostigmine could, via its ability to enhance autoreceptor activity (Quirion et al., 1994), artificially limit the responsivity of the cholinergic system. However, the work cited above on ACh efflux during performance of the five-choice serial reaction time task (Dalley et al., 2001; Passetti et al., 2000) indicated very similar ACh efflux values yet the neostigmine concentration was half (0.05 µM) of that employed in the present experiment and that of Himmelheber et al. (2001). In addition, we have recently observed similar increases (150–200%) in cortical ACh efflux during performance of the sustained attention task in animals that are dialyzed without the inclusion of any cholinesterase inhibitor (Arnold et al., unpublished observations). Thus, there is no evidence in support of the possibility that the use of neostigmine in the present study resulted in a level of task-related ACh efflux that was artificially dampened.

The operant control tasks used in the current study

also have implications for the potential relationship between motor activity and cortical ACh release, as manipulations that increase motor activity may also increase cortical ACh release (Acquas et al., 1998; Bruno et al., 1999; Day et al., 1991). Rats performing the FI-9 task made many more presses than did rats in either of the other two tasks, yet ACh efflux in these rats was significantly lower than in rats performing the sustained attention task. If one assumes that lever-pressing rate can be used as an estimate of motor activity, then it is clear that motor activity alone cannot account for the full range of the observed increase in cortical ACh efflux in rats performing the sustained attention task. Lever pressing per se also cannot account for the increases in cortical ACh efflux in rats performing the two operant control tasks, given that rats performing the FI-9 and retracting lever tasks demonstrated large differences in amounts of lever pressing, but had quite similar changes in cortical ACh efflux during task performance. These observations are consistent with the study by Dalley et al. (2001), who reported that, relative to rats performing the five-choice serial reaction time task, rats that had their instrumental contingency removed, but were still active in the task, had significantly lower increases in cortical ACh efflux during the behavioral task. Moreover, in a series of experiments measuring cortical ACh efflux in rats performing in simple operant tasks, cortical ACh efflux did not change during performance of these well-learned simple tasks, despite large experimental variations in reinforcement and lever-pressing rate (Himmelheber et al., 1997).

Because food reward has been shown to elevate cortical ACh efflux (Inglis et al., 1994), the retracting lever task and the FI-9 task were both designed to equate the amount of water reinforcement received (and presumably consumed), with that of rats in the sustained attention task. In addition, the retracting lever task was designed to equate the percentage of lever presses that resulted in reinforcement ($\sim$60%) with that of the rats performing the sustained attention task. Thus, the differential increases in cortical ACh efflux observed among rats in the sustained attention task from those of rats in the operant control tasks cannot be due to simple differences in consummatory behavior or reinforcement density.

Though the increase in cortical ACh efflux was greater in rats performing the sustained attention task than in rats performing the operant control tasks, it should be reiterated that cortical ACh efflux was significantly increased over basal levels during performance of both control tasks reported here. This is consistent with observations regarding animals in the 'non-contingent' condition reported by Dalley et al. (2001). Though not reporting a differential increase in cortical ACh efflux between rats performing the sustained attention task or operant control task, Himmelheber et al. (2001) reported that there was a significant increase in cortical ACh efflux in animals performing an operant task designed not to explicitly tax attention. The operant control task by Himmelheber et al. (2001) required rats to press a lever (left or right) signaled by a cue light over the appropriate lever. Together, these data suggest that reinforcement density and motor activity, though not able to account for the *entirety* of the increase in cortical ACh efflux in rats performing complex cognitive tasks, may contribute to the elevated cortical ACh levels observed in all the tasks reported here. Moreover, these control tasks, though not *explicitly* taxing attention, may have arousing aspects and *implicit* demands on attentional processes that also contribute to the moderate increase in cortical ACh release.

The specific relationship between the extent of attentional processing and the magnitude of increases in cortical ACh release remains unsettled. One suggestion is that the level of *performance* on an attentional task may systematically regulate cortical ACh release. This hypothesis was recently tested by Passetti et al. (2000) using rats trained in the five-choice serial reaction time task. To manipulate behavioral performance on the task, rats were presented with variable lengths of stimulus duration across three different days in the task. Microdialysis measurements of prefrontal cortical ACh efflux suggested that while ACh levels increased during performance on the task, it did not vary with measures of performance, which were significantly affected by the length of the stimulus.

A second possibility regarding the magnitude of cortical ACh efflux and attentional processing is that rather than reflecting the actual level of performance of an attentional task, cortical ACh release may vary according to the cognitive effort that the animal exerts during performance in the task. In other words, increases in cortical cholinergic transmission may be more directly related to increases in attentional effort than to changes in behavioral *performance* per se. To investigate such a possibility, one strategy we have begun to employ is systematically changing the attentional demands within a session by manipulating variables demonstrated and conceptualized as taxing sustained attention performance (Parasuraman et al., 1987). The effects of one such manipulation, the presentation of a visual distracter to increase background noise, was reported by Himmelheber et al. (2000). In that study, the animals responded to this stimulus with a shift in side bias that was interpreted as a 'disengagement from active attentional processing, reflecting a lessening of animals' effort in the sustained attention task' (Himmelheber et al., 2000, p. 323). On the subsequent block of trials, performance returned to a 'standard' level of performance, and ACh levels were significantly elevated above those seen during standard task and performance conditions. Thus, these data are consistent with the interpretation that performance level was maintained by an increase in effortful processing, leading to higher levels of cortical ACh efflux. Obviously, the suggestion that effort rather than performance better reflects the role of cortical ACh will be a challenging one to investigate, given the complexities of separating the cognitive effort exerted by a subject during a task, from typical measures of performance. In many instances performance and effort will necessarily be correlated. However, the challenge to cognitive neuroscience is to tease apart these underlying cognitive pro-

cesses from overt measures of behavior and to dissociate underlying neuronal mechanisms.

CONCLUSION

The current findings extend our analysis of the role of cortical ACh release during performance of a sustained attention task in rats. By eliminating problems associated with pre-task handling of animals, robust increases in cortical ACh efflux in attentional task-performing animals were demonstrated. The present data support the general hypothesis that cortical cholinergic inputs, while not exclusively mediating attentional processes, are exceptionally activated by behavioral situations taxing the animals' attentional capabilities.

Acknowledgements—This research was supported by PHS grants MH57436 and NS37026. The authors thank Anne Marie Himmelheber, Ph.D. for her contributions to this work.

REFERENCES

Acquas, E., Wilson, C., Fibiger, H.C., 1996. Conditioned and unconditioned stimuli increase frontal cortical and hippocampal acetylcholine release: effects of novelty, habituation, and fear. J. Neurosci. 16, 3089–3096.

Acquas, E., Wilson, C., Fibiger, H.C., 1998. Pharmacology of sensory stimulation-evoked increases in frontal cortical acetylcholine release. Neuroscience 85, 73–83.

Bruno, J.P., Sarter, M., Arnold, H.M., Himmelheber, A.M., 1999. *In vivo* neurochemical correlates of cognitive processes: methodological and conceptual challenges. Rev. Neurosci. 10, 25–48.

Dalley, J.W., McGaughy, J., O'Connell, M.T., Cardinal, R.N., Levita, L., Robbins, T.W., 2001. Distinct changes in cortical acetylcholine and noradrenaline efflux during contingent and noncontingent performance of a visual attentional task. J. Neurosci. 21, 4908–4914.

Day, J., Damsma, G., Fibiger, H.C., 1991. Cholinergic activity in the rat hippocampus, cortex and striatum correlates with locomotor activity: an *in vivo* microdialysis study. Pharmacol. Biochem. Behav. 38, 723–729.

Everitt, B.J., Robbins, T.W., 1997. Central cholinergic systems and cognition. Annu. Rev. Psychol. 48, 649–684.

Fadel, J.R., Sarter, M., Bruno, J.P., 2001. Basal forebrain glutamatergic modulation of cortical acetylcholine release. Synapse 39, 201–212.

Himmelheber, A.M., Sarter, M., Bruno, J.P., 1997. Operant performance and cortical acetylcholine release: role of response rate, reward density, and non-contingent stimuli. Cogn. Brain Res. 6, 23–36.

Himmelheber, A.M., Sarter, M., Bruno, J.P., 2000. Increases in cortical acetylcholine release during sustained attention performance in rats. Cogn. Brain Res. 9, 313–325.

Himmelheber, A.M., Sarter, M., Bruno, J.P., 2001. The effects of manipulations of attentional demand on cortical acetylcholine release. Cogn. Brain Res. 12, 353–370.

Holley, L.A., Turchi, J., Apple, C., Sarter, M., 1995. Dissociation between the attentional effects of infusions of a benzodiazepine receptor agonist and an inverse agonist into the basal forebrain. Psychopharmacology 120, 99–108.

Huang, T., Yang, L., Gitzen, J., Kissinger, P.T., Vreeke, M., Heller, A., 1995. Detection of basal acetylcholine in rat brain microdialysate. J. Chromatogr. B 670, 323–327.

Inglis, F.M., Day, J.C., Fibiger, H.C., 1994. Enhanced acetylcholine release in hippocampus and cortex during the anticipation and consumption of a palatable meal. Neuroscience 62, 1049–1056.

Inglis, F.M., Fibiger, H.C., 1995. Increases in hippocampal and frontal cortical acetylcholine release associated with presentation of sensory stimuli. Neuroscience 66, 81–86.

McGaughy, J., Kaiser, T., Sarter, M., 1996. Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. Behav. Neurosci. 110, 247–265.

McGaughy, J., Sarter, M., 1995. Behavioral vigilance in rats: task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. Psychopharmacology 117, 340–357.

McGaughy, J., Sarter, M., 1998. Sustained attention performance in rats with intracortical infusions of 192 IgG-saporin-induced cortical cholinergic deafferentation: effects of physostigmine and FG7142. Behav. Neurosci. 112, 1519–1525.

Moore, H., Sarter, M., Bruno, J.P., 1992. Age-dependent modulation of *in vivo* cortical acetylcholine release by benzodiazepine receptor ligands. Brain Res. 596, 17–29.

Moore, H., Sarter, M., Bruno, J.P., 1995a. Bidirectional modulation of cortical acetylcholine efflux by infusion of benzodiazepine receptor ligands into the basal forebrain. Neurosci. Lett. 189, 31–34.

Moore, H., Stuckman, S., Sarter, M., Bruno, J.P., 1995b. Stimulation of cortical acetylcholine efflux by FG 7142 measured with repeated microdialysis sampling. Synapse 21, 324–331.

Moore, H., Stuckman, S., Sarter, M., Bruno, J.P., 1996. Potassium, but not atropine-stimulated cortical acetylcholine efflux, is reduced in aged rats. Neurobiol. Aging 17, 565–571.

Muir, J.L., Everitt, B.J., Robbins, T.W., 1994. AMPA-induced excitotoxic lesions of the basal forebrain: a significant role for the cortical cholinergic system in attentional function. J. Neurosci. 14, 2313–2326.

Muir, J.L., Everitt, B.J., Robbins, T.W., 1995. Reversal of visual attentional dysfunction following lesions of the cholinergic basal forebrain by physostigmine and nicotine but not by the 5-HT3 receptor antagonist, ondansetron. Psychopharmacology 118, 82–92.

Parasuraman, R., Warm, J.S., Dember, W.N., 1987. Vigilance: taxonomy and utility. In: Mark, L.S., Warm, J.S., Huston, R.L. (Eds.), Ergonomics and Human Factors: Recent Research. Springer, New York, pp. 11–32.

Passetti, F., Dalley, J.W., O'Connell, M.T., Everitt, B.J., Robbins, T.W., 2000. Increased acetylcholine release in the rat medial prefrontal cortex during performance of a visual attentional task. Eur. J. Neurosci. 12, 3051–3058.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic coordinates, 2nd ed. Academic Press, New York.

Potter, P.E., Meek, J.L., Neff, N.H., 1983. Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J. Neurochem. 41, 188–193.

Quirion, R., Richard, J., Wilson, A., 1994. Muscarinic and nicotinic modulation of cortical acetylcholine release monitored by *in vivo* microdialysis in freely moving adult rats. Synapse 17, 92–100.

Sarter, M., Bruno, J.P., 1997. Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. Brain Res. Rev. 23, 28–46.

Sarter, M., Bruno, J.P., 1999. Abnormal regulation of corticopetal cholinergic neurons and impaired information processing in neuropsychiatric disorders. Trends Neurosci. 22, 67–74.

Sarter, M., Givens, B., Bruno, J.P., 2001. The cognitive neuroscience of sustained attention: where top-down meets bottom-up. Brain Res. Rev. 35, 146–160.
Thiel, C.M., Huston, J.P., Schwarting, R.K.W., 1998. Cholinergic activation in frontal cortex and nucleus accumbens related to basic behavioral manipulations: handling, and the role of post-handling experience. Brain Res. 812, 121–132.
Turchi, J., Sarter, M., 2001a. Bidirectional modulation of basal forebrain NMDA receptor function differentially affects visual attentional but not visual discrimination performance. Neuroscience 104, 407–417.
Turchi, J., Sarter, M., 2001b. Antisense oligonucleotide-induced suppression of basal forebrain NMDA-R1 subunits selectively impairs visual attentional performance in rats. Eur. J. Neurosci. 14, 103–117.
Zar, J.H., 1974. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.

(*Accepted* 22 *February* 2002)