ORIGINAL INVESTIGATION

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Effects of acute and repeated systemic administration of ketamine on prefrontal acetylcholine release and sustained attention performance in rats

Abstract *Rationale*: The effects of non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists model aspects of schizophrenic symptomatology. Because effects on both cortical cholinergic transmission and attentional processes have been hypothesized to represent components of the properties of psychotogenic drugs, the present study investigated the effects of ketamine on the activity of cortical cholinergic inputs and attentional performance. *Objective*: To determine the effects of acute and repeated ketamine administration on cortical acetylcholine release and performance of rats in an operant task designed to assess sustained attention performance. *Methods*: Experiment 1 assessed the effects of ketamine (2.0–20.0 mg/kg, i.p.) on medial prefrontal acetylcholine release using in vivo microdialysis. In experiment 2, animals were pretreated with 2.0 mg/kg or 25.0 mg/kg ketamine for 7 days. Cortical acetylcholine release was assessed in these rats following the subsequent administration of a 'challenge' dose of 2.0 mg/kg on days 1, 8, and 15 following completion of the pretreatment regimen. Experiment 3 assessed the effects of acute ketamine administration (2.0, 4.0, and 8.0 mg/kg, i.p.) on sustained attention performance. In experiment 4, animals trained in the sustained attention task were pretreated with 25.0 mg/kg ketamine or vehicle for 7 days. In these animals, the performance effects of 2.0 mg/kg ketamine administered 1, 8, or 15 days after completion of the pretreatment regimen were assessed. *Results*: The acute administration of ketamine dose dependently increased cortical acetylcholine release by up to 250% above baseline and for over 40 min following the highest dose of ketamine. Pretreatment with 2.0 mg or 25.0 mg/kg did not robustly alter the effects of subsequent ketamine administration on cortical acetylcholine release. In animals performing the sustained attention task, administration of the highest dose of ketamine resulted in high levels of errors of omission, while the administration of the two smaller doses did not affect performance. Pretreatment with 25.0 mg/kg disrupted the attentional performance during the pretreatment period, but it did not affect the baseline performance thereafter. Furthermore, ketamine pretreatment did not systematically alter the performance effects of subsequent ketamine administration. *Conclusions*: The robust stimulation of cortical acetylcholine release represents a potent component of the pharmacological effects of ketamine. The effects of acute ketamine on attentional performance were limited to high rates of omissions. Repeated ketamine administration 'sensitized' neither cortical acetylcholine release nor attentional performance. These effects of repeated ketamine differ substantially from those of another major psychotogenic drug, amphetamine, and thus support the view that ketamine and amphetamine model fundamentally different aspects of schizophrenia.

Keywords Ketamine · Prefrontal cortex · Acetylcholine release · Attention · Schizophrenia

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Introduction

The effects of systemically administered non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists have been proposed to model aspects of the "positive" and "negative" symptomatology of schizophrenia (Olney and Farber 1995; Jentsch and Roth 1999; Newcomer et al. 1999). The non-competitive NMDA receptor antagonist ketamine represents a particularly useful compound in this context, as it has the potential, at sub-anesthetic doses, for use in both human (Krystal et al. 1994, 1999; Lahti et al. 1994, 1995; Malhotra et al. 1996, 1997; Adler et al. 1998; Oranje et al. 2000) and animal studies (Lindefors et al. 1997; Kim et al. 1999).

In intact humans, the psychotogenic effects of ketamine have been largely equated with those of phencyclidine (PCP), and they include brief and reversible posi-

tive and negative symptoms, and impairments in memory (Krystal et al. 1994; Adler et al. 1998; Newcomer et al. 1999). In schizophrenics, the magnitude of the psychotogenic response to ketamine appears generally comparable to that in normal volunteers, although schizophrenics report more positive symptoms than normal volunteers, particularly hallucinations and delusions (Lahti et al. 1999). The effects of ketamine are attenuated by the administration of typical and atypical antipsychotic drugs (Malhotra et al. 1997; Krystal et al. 1999), lending predictive validity to this pharmacological model. Chronic ketamine use in humans appears to result in persistent schizotypal and cognitive symptomatology (Jansen 1990; Curran and Morgan 2000).

The psychotogenic effects of NMDA receptor antagonists have been attributed to the stimulation of prefrontal cortical glutamate transmission, mediated via multi-synaptic mechanisms and yielding excessive non-NMDA receptor stimulation (Moghaddam and Adams 1998; Krystal et al. 1999). The question of whether the psychotogenic effects of ketamine and those of another major psychogenic compound, amphetamine, are mediated via overlapping neuronal circuits continues to be debated (Krystal et al. 1999). For example, evidence supports the possibility that the effects of ketamine or PCP, similar to amphetamine, are associated with increases in striatal dopamine release (Tsukada et al. 2000) and that these compounds cross-sensitize striatal dopamine release (Kegeles et al. 2000).

Jentsch et al. (1998) reported that the acute administration of PCP in rats increases cortical acetylcholine (ACh) release measured by means of microdialysis. Similarly, Kikuchi et al. (1997) reported substantial increases in frontal cortical ACh release following ketamine administration. These data, in concert with the finding that amphetamine also increases and, following repeated administration, sensitizes increases in cortical ACh release (Nelson et al. 2000), suggest that increases in cortical cholinergic transmission may represent a common effect of psychotogenic treatments (see also Sarter et al. 2001).

Persistent abnormal increases in cortical cholinergic transmission have been proposed to represent an essential component of the neuronal circuits mediating the symptoms of schizophrenia (Sarter and Bruno 1997, 1999). The functional implications of changes in cholinergic transmission for schizophrenia remain unsettled (Perry and Perry 1995; Sarter and Bruno 1999; Tandon et al. 1999; Crook et al. 2001). Based on the evidence linking cortical cholinergic transmission to attentional functions, we have hypothesized that the attentional impairments in schizophrenics, defined by their limited ability to filter irrelevant stimuli and associations from further cognitive processing, are mediated via abnormal increases in cortical cholinergic transmission (Sarter 1994; Sarter and Bruno 1999, 2000). Such increases in cortical ACh release may represent, at least in part, a trans-synaptic consequence of increases in striatal, particularly nucleus accumbens, dopaminergic transmission (Moore et al. 1999).

The present study was designed to determine the effects of acute and repeated systemic ketamine administration on both cortical ACh release and sustained attention performance in rats. Ketamine was expected to stimulate cortical ACh release and, similar to amphetamine, possibly yield augmented increases following repeated administration, indicative of repeated ketamine-induced sensitization of cortical ACh efflux. Furthermore, in animals trained in a task designed to assess sustained attention performance (McGaughy and Sarter 1995), administration of ketamine was expected to produce a specific impairment in performance characteristic of drugs that stimulate cortical cholinergic transmission (Holley et al. 1995; Deller and Sarter 1998; Turchi and Sarter 2001). Such an impairment is characterized by increases in the number of false alarms, that is 'claims' for signals during non-signal trials. Furthermore, such an impairment contrasts with the impairments in the detection of signals that result from lesions of cortical cholinergic inputs (McGaughy et al. 1996) or from the administration of drugs that decrease cortical cholinergic transmission (Turchi and Sarter 2001). Repeated pretreatment of ketamine was expected to yield persistent impairments in performance of this task, or at least augmented (or 'sensitized') impairments following subsequent ketamine 'challenges'. The pretreatment regimen for ketamine was adopted from a study by Lindefors et al. (1997) which documented lasting effects on prefrontal dopaminergic transmission.

Materials and methods

Subjects

Adult (n=38 total animals) male Fischer-344/Brown Norway F1 hybrid rats (Harlan Inc., Indianapolis, Ind.), weighing between 250 g and 350 g, were used for all studies. Animals were housed in a temperature-controlled room with a 12-h/12-h light/dark cycle (lights on 0630 hours). For animals in the microdialysis experiments, food and water were available ad libitum. For animals training in the sustained attention task, food was available ad libitum and water was available during the task and for 8 min immediately following daily training. All procedures were performed in accordance with protocols approved by the Ohio State University Animal Care and Use Committee with the U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals.

Experiments 1 and 2: effects of acute and repeated ketamine on cortical ACh efflux

Cannula implantation

Animals were handled and habituated to testing conditions for 4 days prior to surgery. On the morning of surgery, animals were anesthetized with isofluorane and placed in a stereotaxic apparatus. Anesthesia was administered via a SurgiVet machine (Anesco/Surgivet, Waukesha, Wisc.). The carrier gas was oxygen, and delivery was 2% isoflurane at a flow rate of 0.6 l/min (oxygen). Guide cannulae (SciPro Inc., North Tonawanda, N.Y.) were implanted into the medial prefrontal cortex (mPFC: coordinates from bregma: 3.0 mm anterior, 0.8 mm lateral, 1.1 mm below dura; Paxinos and Watson 1986). Cannulae were fixed using stainless-steel screws and dental cement. Animals were returned to their home cages and allowed 3 days of recovery prior to microdialysis.

Microdialysis procedure

On microdialysis days, animals were placed in bowls 30 min prior to microdialysis sessions. Concentric probes (3.0-mm membrane tip, SciPro, Inc.) were inserted into the mPFC and perfused with artificial cerebrospinal fluid (aCSF) containing (in mM) NaCl 166.5, NaHCO$_3$ 27.5, KCl 2.4, CaCl$_2$ 1.2, Na$_2$SO$_4$ 0.5, KH$_2$PO$_4$ 0.5, glucose 1.0, pH 6.9. Probes were attached to a dual-channel liquid swivel (Instech, Plymouth Meeting, Pa.) and perfused at a rate of 1.25 µl/min. No cholinesterase inhibitors were added to the aCSF. Following a 3-h discard period, four baseline collections were taken at 12-min intervals. Ketamine hydrochloride (Butler, Fort Dodge, Iowa) diluted in saline or saline alone was then administered, and collections proceeded for 11 collection intervals post-drug. ACh dialysates were stored at –80°C prior to analysis.

Acute administration

Animals (*n*=6) were subject to four microdialysis sessions on alternating days in counterbalanced order. The four sessions were: (1) saline vehicle; (2) 2.0 mg/kg ketamine i.p.; (3) 10.0 mg/kg ketamine i.p.; and (4) 20.0 mg/kg ketamine i.p. Following the conclusion of a particular session, animals were returned to their home cages. Animals were placed in the testing environment on all non-microdialysis days prior to the conclusion of the experiment.

Repeated administration

Following recovery from surgery, animals were placed in the testing environment daily and treated with ketamine (2.0 mg/kg or 25.0 mg/kg i.p.; *n*=6 per dose) once daily for a total of 7 days. This pretreatment regimen was adopted from a study by Lindefors et al. (1997) which demonstrated a twofold increase in basal prefrontal dopamine levels following the pretreatment with 25.0 mg/kg for 7 days and challenge with the same dose. Microdialysis occurred during the initial administration of drug. Following the pretreatment period, the effects of subsequent "challenge" administrations of 2.0 mg/kg ketamine (i.p.) were tested 1, 8, and 15 days following the completion of the pretreatment period. This dose was selected based on the acute effects of ketamine in experiment 1 (see Fig. 2); as the acute effect of this dose did not reach significance, the demonstration of significant increases following pretreatment would have represented a robust result. Microdialysis was performed on all challenge days. Animals were left undisturbed in their home cages between challenge sessions.

ACh analysis

ACh levels in dialysates were determined using high-performance liquid chromatography with electrochemical detection. A volume of 14.0 µl was injected by autosampler (ESA, Inc., Chelmsford, Mass.) and ACh and choline were separated (0.5 ml/min) by a C-18 carbon polymer column (ESA, Inc.; dimension 250×3 mm) using a sodium phosphate mobile phase (in mM: Na$_2$HPO$_4$ 100.0, TMACl 0.5, 1-OSA 2.0, 0.005% antimicrobicide MB, pH 8.0). ACh and choline were hydrolyzed on a post-column enzyme reactor (ESA, Inc.), converted to H$_2$O$_2$ (Potter et al. 1983), and measured using a peroxidase-wired (Huang et al. 1995) glassy carbon electrode (ESA, Inc.). The detection limit was 5.0 fmol/14 µl injection. Histological analysis (Cresyl violet staining) was used to confirm probe placements at the conclusion of the study.

Statistical analysis

Statistical analyses were conducted using within-subject analyses of variance (ANOVAs). Acute studies began with initial analysis (two-way ANOVA) evaluating whether basal ACh efflux varied over time (0–48 min) or across session (1–4) or treatment (vehicle, 2.0 mg/kg, 10.0 mg/kg, or 20.0 mg/kg ketamine) for the four baseline collections taken during each session. Concerning the analysis of the effects of repeated ketamine administration, an initial analysis (two-way ANOVA) evaluated whether basal ACh efflux varied over time (0–48 min) or across session (initial ketamine, challenge days 1, 8, 15) for the four baseline collections taken during each session. Data from each session were subsequently expressed as percentage change from mean baseline. For acute studies, an overall ANOVA with treatment (vehicle, 2.0 mg/kg, 10.0 mg/kg, or 20.0 mg/kg ketamine) and time (48–180 min) was conducted to determine overall significance for all sessions and time points. To analyze the effects of repeated ketamine administration, first, a one-way ANOVA was conducted to determine the significance of the effects of the initial ketamine administration; second, an overall ANOVA was conducted to determine the effects of session (initial 2.0 mg/kg ketamine, challenge days 1, 8, 15) and time (48–180 min). Subsequent two-way ANOVAs were conducted when warranted to determine effects in specific session comparisons. Finally, for experiment 2, truncated "baseline to baseline" analyses were conducted on session (initial 2.0 mg/kg ketamine, challenge days 1, 8, 15) and time (48–96 min) on a portion of the data to minimize type-II errors. Significance was defined as $\alpha < 0.05$.

Experiments 3 and 4: effects of acute and repeated ketamine on sustained attention performance

Apparatus

Training occurred in eight operant chambers (Med Associates, East Fairfield, Vt.). Each chamber contained two retractable levers, three panel lights and a house light above the central panel light in the front of the chamber. A water dispenser in the back of the chamber delivered reinforcement (40 µl). Each chamber was enclosed within a sound-attenuating box equipped with a fan that provided ventilation and background noise. A PC clone using MED-PC software (v. 1.10; Med Associates) was used to collect data and control execution of programs.

Operant training

After being shaped to press levers for water, animals were trained to perform the sustained attention task. In the sustained attention task, trials were initiated with either a signal (1-s illumination of the central panel light) or a non-signal (no illumination of the central panel light). Two seconds after a signal or a non-signal, the levers were extended into the chamber. For half of the animals, after a signal, a press on the left lever was reinforced and termed a hit, and a press on the right lever was not reinforced and termed a miss. For these animals, after a non-signal, a press on the right lever was reinforced and termed a correct rejection, and a press on the left lever was not reinforced and termed a false alarm. For half of the animals, the rules were reversed (i.e., correct responses were a right lever press during signal trials and a left lever press for non-signal trials). During this stage of training, an incorrect response was followed by a correction trial that was identical to the previous trial. A forced trial was introduced if three consecutive errors occurred during correction trials. On a forced trial, only the correct lever extended into the chamber and, if the errors were during a signal trial, the central panel light was illuminated. The inter-trial interval (ITI) was 12±3 s during this stage of training. Each session consisted of 162 trials and lasted approximately 40 min. Criterion performance during this stage of training was defined as 70% accuracy during signal and non-signal trials for three consecutive sessions. After reaching criterion, the task was changed in two ways. Briefer signals (500, 50, or 25 ms) were introduced, and correction and forced trials were eliminated. Criterion performance during this stage of training was 70% accuracy during 500-ms signal and non-signal trials with fewer than 38 omissions (failure to press the levers within 4 s after they were ex-

Fig. 1 Coronal, Nissl-stained sections from four different rats exemplifying the placement of the microdialysis probes in the medial prefrontal cortex. A 200-μm scale is superimposed on all four sections. The damage produced by the 3-mm probes typically ranged from the ventral cingulate cortex through the prelimbic area into the dorsal infralimbic region. The diameter of the damage corresponds well with the outer diameter of these concentric probes (0.2 mm)
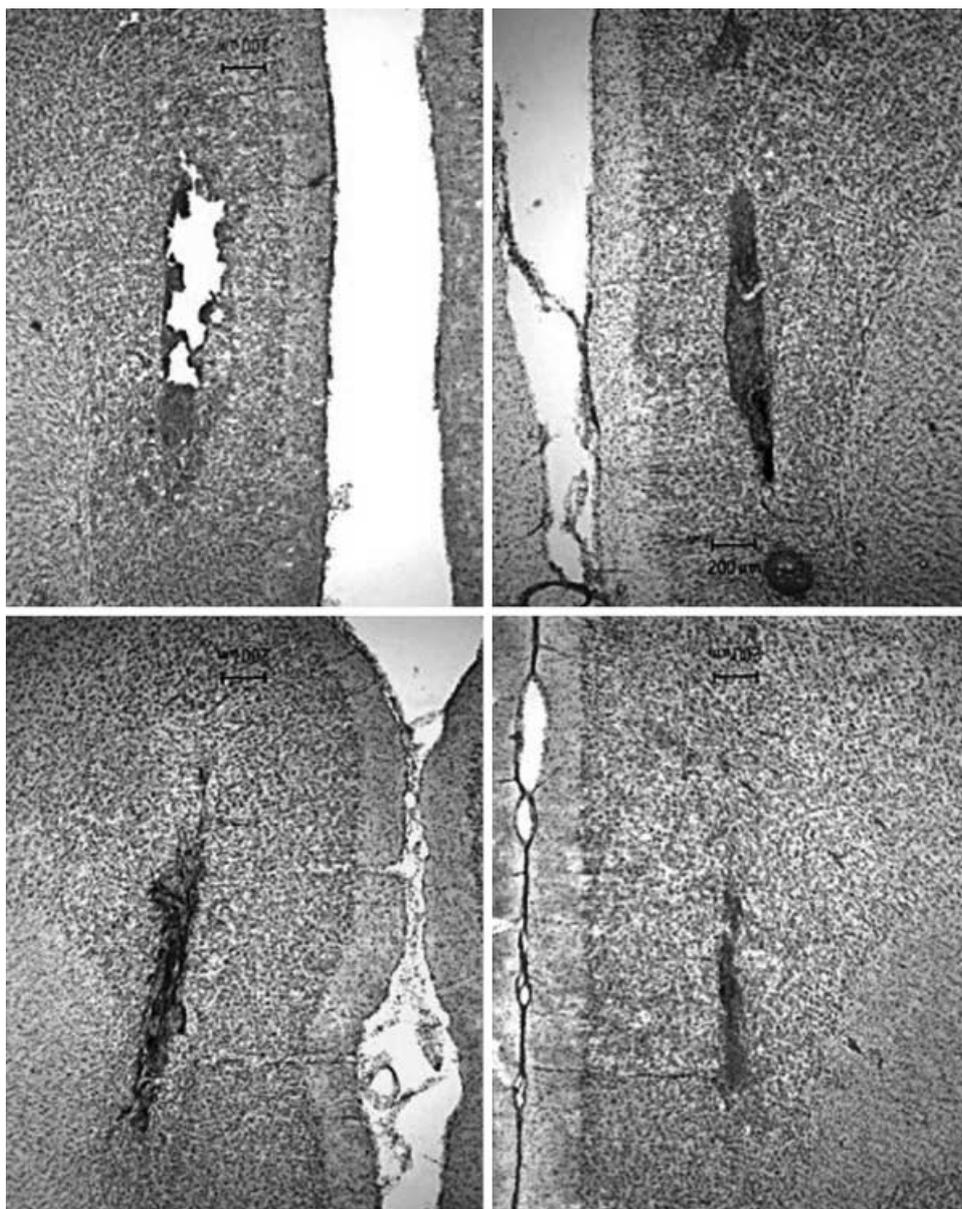
tended into the chamber) for three consecutive sessions. After reaching criterion, animals were trained in the final version of the task with the house light illuminated throughout a session and the ITI decreased to 9±3 s. Animals were required to reach a criterion of 70% accuracy to 500-ms signals and to non-signals and fewer than 38 omissions for seven consecutive sessions prior to any drug treatment in the acute study. The animals of experiment 4 were pseudo-randomly assigned to a drug condition (saline or 25.0 mg/kg ketamine, see below) after reaching criterion performance. In both experiments, half of the animals were trained in accordance with one set of task rules and half with the alternative set of rules.

Effects of acute administration

Rats (n=8) were given injections (i.p.) of saline, 2.0, 4.0, or 8.0 mg/kg ketamine in a counterbalanced order. This dose range was selected based on the initial observation that administration of 2.0 mg/kg and 10.0 mg/kg stimulate cortical ACh release (Fig. 1)

and on evidence suggesting that this dose range produces specific behavioral and cognitive effects in rats (Wesierska et al. 1990). In accordance with this suggestion, preliminary data indicated that rats treated with doses above 8.0 mg/kg omitted a prohibitively high number of trials (>90% of all trials). All injections were made in a volume of 1.0 ml/kg and 10 min prior to onset of the task. Animals spent these 10 min in the operant chambers.

Effects of repeated administration

After reaching criterion in the sustained attention task (see above), animals received i.p. injections of either 25.0 mg/kg ketamine or saline (n=6 per group) for seven consecutive sessions 10 min prior to their training session in the sustained attention task. Furthermore, animals were given a challenge injection of 2.0 mg/kg ketamine (i.p.) 1, 8, and 15 days after completion of the pretreatment regimen. Note that this treatment regimen parallels the design of the microdialysis experiment described above.

Fig. 2 Mean (±SEM) acetylcholine (ACh) efflux (percentage change from baseline) in the medial prefrontal cortex (mPFC) of animals receiving acute ketamine (2.0, 10.0, 20.0 mg/kg, i.p.) or saline. All animals received all treatments, and sessions were counterbalanced. Following baseline collections (0–48 min), ketamine or saline was administered, and collections were taken through 180 min. 2.0 mg/kg (*squares*) increased cortical ACh efflux above saline (*circles*). 10.0 mg/kg and 20.0 mg/kg (*triangles* and *diamonds*, respectively) increased cortical ACh efflux to equivalent levels initially, with the duration of the 20.0-mg/kg effect lasting approximately 36 min longer
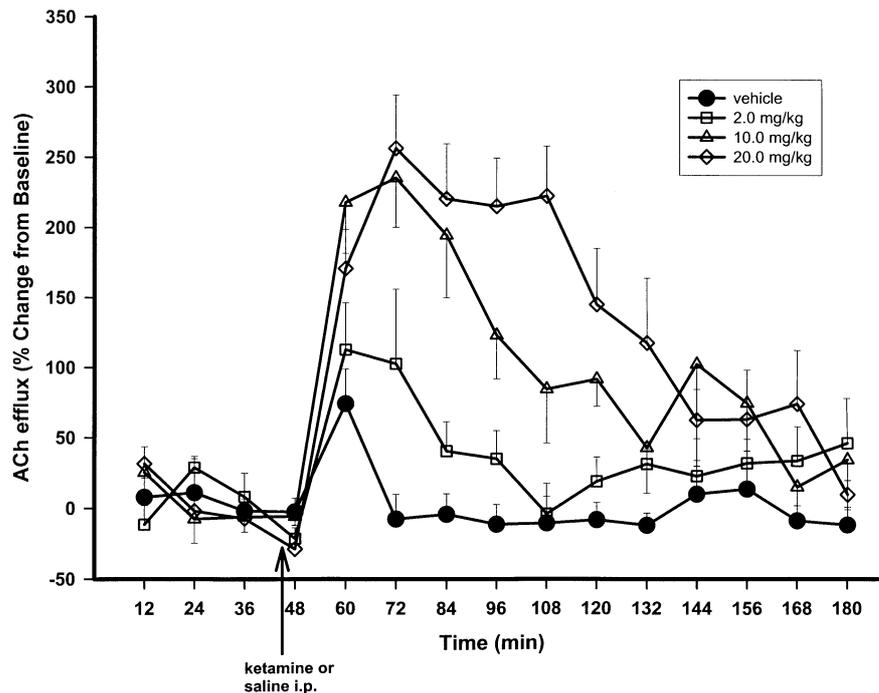
Behavioral measures and statistical analyses

For each session and for each block of 54 trials, the total number of hits (h), misses (m), false alarms (fa), correct rejections (cr), and omissions were calculated. Based on these values, the relative number of hits [h = h / (h+m)] and of correct rejections [cr = cr / (cr+fa)] were calculated. For the acute sustained attention experiment, the number of omissions was relatively high following 8.0 mg/kg ketamine (see below) and, hence, there was not a sufficient number of trials to analyze measures of accuracy. The relative number of hits and correct rejections were angularly transformed ($X' = 2 \times arcsin\ X^{1/2}$; Zar 1974). The transformed hits and correct rejections were analyzed using ANOVAs that included dose (0.0, 2.0, 4.0 mg/kg), signal length (where appropriate), and block (3 blocks with 54 trials per block). For experiment 4, performance for days 6 and 7 after the pretreatment, that is the two sessions prior to the second challenge session, was averaged and analyzed to test whether repeated ketamine pretreatment had persistent effects on (unchallenged) attentional performance. The effects of pretreatment (saline and 25.0 mg/kg ketamine) were then tested separately for each challenge session (days 1, 8, or 15 following completion of the pretreatment regimen), prior to which all animals were administered 2.0 mg/kg ketamine.

Results

Experiment 1: effects of acute ketamine on cortical ACh efflux

As illustrated in Fig. 1, microdialysis probes were placed into the medial prefrontal cortex, primarily into the prelimbic area. In most animals, the dorsal-ventral placement of the membrane included the ventral cingulate cortex and the dorsal infralimbic region (Fig. 1). Minor differences in probe placement were not associated with any systematic variations in basal or ketamine-induced ACh release.

The acute administration of ketamine resulted in dose-dependent increases in cortical ACh efflux (Fig. 2). A two-factor ANOVA revealed that baseline ACh efflux did not differ by treatment group ($F_{3,15} = 1.15$, $P = 0.36$) or over time during the four baseline collections ($F_{3,15} = 1.78$, $P = 0.19$), nor was there an interaction between the two factors ($F_{9,45} = 1.65$, $P = 0.13$). Mean basal values for each of the four treatment conditions were (pmol/14 μl±SEM): vehicle 0.008±0.001; 2.0 mg/kg ketamine 0.008±0.001; 10.0 mg/kg ketamine 0.010±0.001; and 20.0 mg/kg ketamine 0.010±0.001. Likewise, basal ACh efflux did not differ across sessions ($F_{3,15} = 0.09$, $P = 0.96$) or over time during the four baseline collections ($F_{3,15} = 3.10$, $P = 0.06$), nor was there an interaction between the two factors ($F_{9,45} = 1.10$, $P = 0.38$). As a result, all subsequent analyses were conducted using percentage change from the mean baseline values.

An ANOVA across all treatment conditions revealed significant effects of treatment ($F_{3,15} = 10.03$, $P = 0.006$), time ($F_{11,55} = 17.46$, $P = 0.000$) and a significant interaction ($F_{33,165} = 4.56$, $P = 0.000$). Subsequent two-way ANOVAs were conducted to determine the differences between the effects of saline and the individual doses of ketamine. Cortical ACh efflux following administration of 2.0 mg/kg ketamine administration was not significantly higher than that following saline administration ($F_{1,5} = 5.95$, $P = 0.059$). While there was an effect of time ($F_{11,55} = 4.27$, $P = 0.001$), this effect did not significantly interact with dose ($F_{11,55} = 1.53$, $P = 0.21$), reflecting the general effect of injection (Fig. 2). Administration of 10.0 mg/kg ketamine produced significantly higher levels of ACh efflux than saline ($F_{1,5} = 33.49$, $P = 0.002$). The effects of time ($F_{11,55} = 6.17$, $P = 0.000$) significantly interacted with the effects of this dose ($F_{11,55} = 4.06$, $P = 0.02$),

indicating the relatively enduring increase in ACh release when compared with the effects of vehicle. This dose of ketamine (10.0 mg/kg) also produced significantly greater levels of cortical ACh efflux than 2.0 mg/kg ketamine ($F_{1,5}=30.52$, $P=0.003$) but not over time (time × dose $F_{11,55}=1.78$, $P=0.15$; time $F_{11,55}=8.56$, $P=0.000$). The increases in ACh efflux produced by 10.0 mg/kg ketamine and 20.0 mg/kg ketamine did not differ significantly (dose $F_{1,5}=0.95$, $P=0.38$), but the effects of dose and time interacted significantly (time $F_{11,55}=15.91$, $P=0.000$; dose × time $F_{11,55}=2.79$, $P=0.02$), reflecting the longer duration of the increase in ACh efflux produced by the higher dose (Fig. 2). Thus, collectively, these data indicate that acute administration of ketamine resulted in dose-dependent increases in cortical ACh release.

Experiment 2: effects of repeated ketamine administration on cortical ACh efflux

Pretreatment and challenge with 2.0 mg/kg

Baseline analysis revealed that basal cortical ACh efflux did not differ over session ($F_{3,15}=1.62$, $P=0.23$) or over time ($F_{3,15}=1.35$, $P=0.30$; session × time $F_{9,45}=1.59$, $P=0.15$). The mean baseline values for the sessions were (in pmol/14 μl±SEM): initial 2.0 mg/kg ketamine 0.019±0.005; day-1 challenge 0.011±0.001; day-8 challenge 0.016±0.003; day-15 challenge 0.011±0.003. Therefore, all subsequent data were expressed as percentage change from mean baseline.

Repeated pretreatment with this dose of ketamine did not alter the effects of subsequent 'challenge' administrations of this dose on cortical ACh efflux. An overall ANOVA revealed the absence of an effect of session ($F_{3,15}=2.41$, $P=0.15$), an effect of time ($F_{11,55}=7.88$, $P=0.000$), but no significant session × time interaction ($F_{33,165}=1.96$, $P=0.07$). The effect of time reflects the general injection-induced increase in ACh efflux that remained unchanged after repeated pretreatment and subsequent 'challenge' administrations of this dose of ketamine.

As illustrated in Fig. 3, microdialysis collections continued for several collections following the return of cortical ACh efflux to basal levels. Therefore, to minimize a type-II error, a post-hoc analysis focused on the data from last baseline (48 min) through 96 min (four post-drug collections; Fig. 2). The initial administration of ketamine (Fig. 3) significantly increased cortical ACh release (time $F_{4,20}=5.44$, $P=0.005$). The overall analysis failed to reveal an effect of session ($F_{3,15}=3.18$, $P=0.10$) but did yield an effect of time ($F_{4,20}=12.31$, $P=0.000$) and a session × time interaction ($F_{12,60}=2.21$, $P=0.03$). Figure 3 suggests that this interaction was due to a greater and more enduring increase in ACh efflux following the day-15 challenge administration when compared with the effects of day-1 and day-8 challenges; multiple comparisons supported the former (day 1 vs day 15, session × time $F_{4,20}=4.05$, $P=0.01$) but not the latter observation (day 8 vs day 15, session × time $F_{4,20}=2.63$, $P=0.08$). Thus, this post-hoc analysis suggested a greater effect of ketamine after the day-15 challenge, but this was not the case in comparison with the effects of the first administration of ketamine (first vs day 15, session × time $F_{4,20}=1.25$, $P=0.32$) but, instead, with the effects of the day-1 challenge (which itself did not differ from the effects of the first administration of 2.0 mg/kg; first vs day 1: session × time $F_{4,20}=2.43$, $P=0.08$). Thus, even this "aggressive" post-hoc analysis did not yield results that would provide robust support for the hypothesis that repeated pretreatment with 2.0 mg/kg sensitizes the effects of subsequent challenges with this dose.

Pretreatment with 25.0 mg/kg and challenge with 2.0 mg/kg

As mentioned in the Methods, analyses were carried out as within-subjects comparisons, using the effects of the first administration of 2.0 mg/kg ketamine (above) for comparison to the effects of this dose given subsequently to the pretreatment regimen with 25.0 mg/kg. Note again that animals were challenged with 2.0 mg/kg in order to maintain symmetry with the behavioral experiments (below).

Baseline analysis revealed that cortical ACh efflux did not differ across sessions ($F_{3,15}=1.11$, $P=0.38$) nor was there a time × session interaction (time $F_{3,15}=1.50$, $P=0.26$; $F_{9,45}=0.61$, $P=0.78$). Therefore, all subsequent data are expressed as percentage change from mean

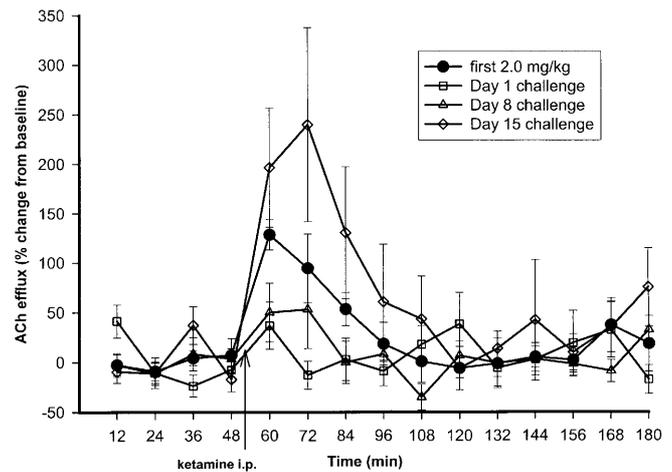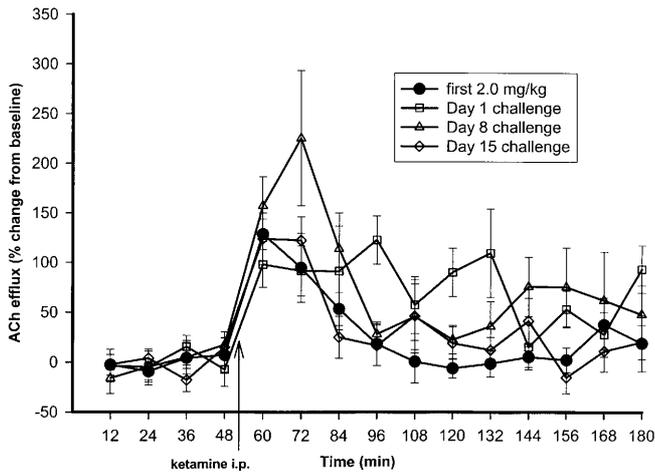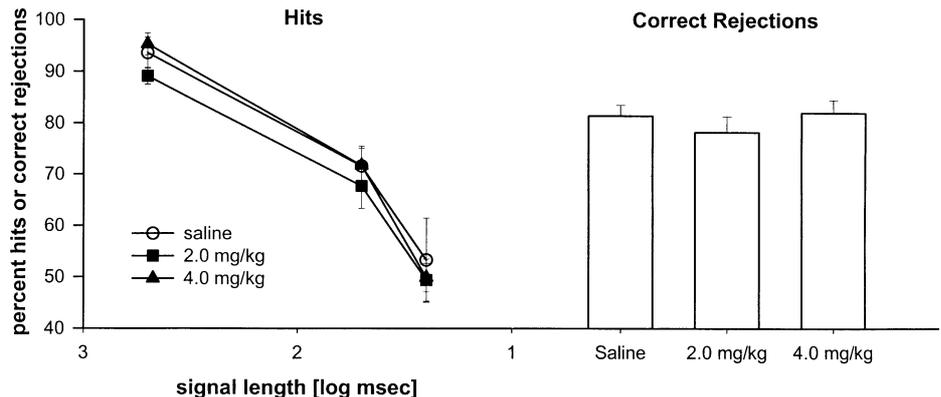**Fig. 3** Mean (±SEM) acetylcholine (ACh) efflux (percentage change from baseline) in the medial prefrontal cortex (mPFC) of animals pretreated with 2.0 mg/kg ketamine. Animals were pretreated once per day for 7 days. Microdialysis was performed on the initial administration day (*circles*) and following subsequent challenge administrations. For all sessions, baseline collections (0–48 min) were followed by ketamine administration (2.0 mg/kg, i.p.), and collections continued through 180 min. Challenge administrations of ketamine (2.0 mg/kg) were administered on day 1 (*squares*), day 8 (*triangles*), and day 15 (*diamonds*) post-regimen. None of the challenge administrations produced cortical ACh efflux significantly greater than the initial administration

Fig. 4 Mean (±SEM) acetylcholine (ACh) efflux (percentage change from baseline) in the medial prefrontal cortex (mPFC) of animals pretreated with 25.0 mg/kg ketamine. Animals were pretreated once per day for 7 days. As a basis for comparison, the initial administration of 2.0 mg/kg ketamine from the other pre-treated group is used here. For all sessions, baseline collections (0–48 min) were followed by ketamine administration (2.0 mg/kg, i.p.), and collections continued through 180 min. Challenge doses of ketamine (2.0 mg/kg) were administered on day 1 (*squares*), day 8 (*triangles*), and day 15 (*diamonds*) post-regimen. None of the challenge administrations produced cortical ACh efflux significantly greater than the initial administration

baseline. The mean basal values for the four sessions were (in pmol/14 µl±SEM): initial 2.0 mg/kg ketamine 0.019±0.005; day-1 challenge 0.011±0.002; day-8 challenge 0.014±0.004; day-15 challenge 0.017±0.003.

An overall ANOVA revealed a significant effect of time ($F_{11,55}=10.642$, $P=0.000$) that reflects the general, transient increase in ACh efflux that resulted from the injection procedure. However, ACh efflux did not differ between the sessions ($F_{3,15}=2.43$, $P=0.11$), and the effects of session and time did not interact ($F_{33,165}=1.79$, $P=0.07$; Fig. 4).

As in the previous experiment, a post-hoc analysis focused on the data from last baseline (48 min) to 96 min (four post-drug collections) to avoid a type-II error. This analysis likewise did not reveal an effect of session nor a session × time interaction (all P values >0.09). Thus,

pretreatment with 25.0 mg/kg ketamine did not alter the effects of subsequent challenges with 2.0 mg/kg.

Experiment 3: effects of acute ketamine administration on sustained attention performance

Baseline performance was characterized by a signal length-dependent hit rate ($F_{2,14}=127.9$, $P=0.000$; 500 ms 94.4±1.2%; 50 ms 75.1±3.3%; 25 ms 51.6±3.6%) and a correct rejection rate of 78.0±2.2% (Fig. 4). Animals omitted more trials during later blocks within each session ($F_{2,14}=7.14$, $P=0.009$; block 1 4.63±1.17 omits; block 2 6.13±0.91; block 3 9.04±0.92) and overall omitted 12.2% of the trials.

One animal did not maintain stable performance and was removed from all subsequent analyses. Furthermore, administration of the highest dose of ketamine (8.0 mg/kg) substantially increased omissions ($F_{3,18}=25.3$, $P=0.001$) from 25.9±6.8 omissions (of 162 trials) following vehicle to 119.4±20.2 omissions following 8.0 mg/kg. Therefore, the number of completed trials per block remained too small to support a meaningful analysis of the effects of ketamine on hits or correct rejections following this dose.

Administration of 2.0 mg/kg or 4.0 mg/kg ketamine did not significantly affect the relative number of hits ($F_{2,12}=2.76$, $P=0.10$; Fig. 5). Furthermore, the effects of dose, signal length, and block did not interact significantly (all P values >0.08). Ketamine administration did not affect the relative number of correct rejections ($F_{2,12}=0.68$, $P=0.53$). Thus, collectively, the acute administration of 2.0 mg/kg or 4.0 mg/kg ketamine did not affect sustained attention performance, and the administration of a higher dose substantially increased omissions.

Experiment 4: effects of repeated ketamine on sustained attention performance

Similar to the second part of experiment 2, animals were pretreated with 25.0 mg/kg and subsequently challenged with 2.0 mg/kg. Prior to the pretreatment regimen, the

Fig. 5 Effects of acute ketamine (2.0 mg/kg and 4.0 mg/kg) on relative number of hits (*left part*) and correct rejections (*right part*). Administration of 8.0 mg/kg resulted in high levels of omissions, precluding an analyses of hits and correct rejections. Administration of 2.0 mg/kg and 4.0 mg/kg ketamine did not significantly affect hits or correct rejections

Fig. 6 Effects of pretreatment with ketamine (25.0 mg/kg or saline for 7 days) on the subsequent attentional performance, at baseline and following challenge administration of ketamine (2.0 mg/kg). The figure depicts performance during challenge sessions at 1 (*top right*), 8 (*bottom left*), and 15 (*bottom right*) days following completion of the pretreatment phase. Furthermore, the average performance during days 6 and 7 (no injections on these days) following chronic administration are also presented (*top left*). Pretreatment with ketamine did not produce long-lasting effects on baseline performance, nor did it alter the effects of subsequent challenge administrations of ketamine
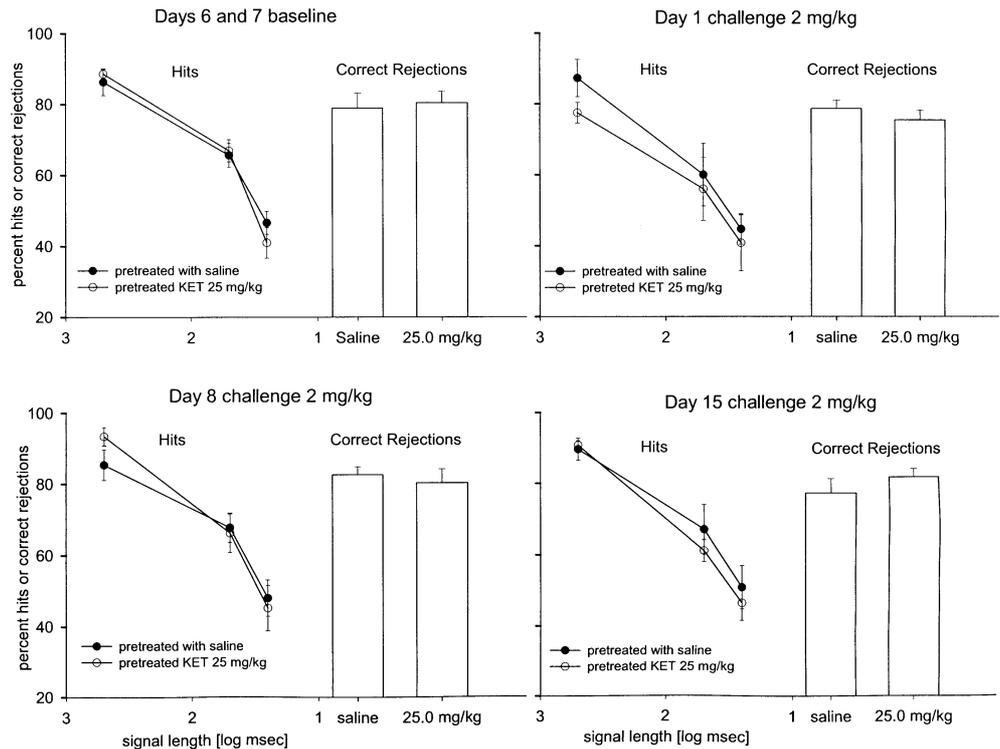
performance of the animals to be pretreated with saline and ketamine (25.0 mg/kg) did not differ (relative number of hits $F_{1,10}=0.15$, $P=0.70$; relative number of correct rejections $F_{1,10}=0.67$, $P=0.43$; omissions $F_{1,10}=0.01$, $P=0.923$).

During the pretreatment phase, the administration of 25.0 mg/kg ketamine resulted in a high number of omissions (>158 omissions). However, subsequent performance, even after the day-1 challenge administration of 2.0 mg/kg, was not characterized by systematic effects of the pretreatment regimen (Fig. 6).

Pretreatment effects on subsequent baseline performance

In order to test whether the effects of repeated ketamine pretreatment resulted in persistent effects on the (unchallenged) performance of the animals, data from days 6 and 7 (i.e., the two sessions prior to the day-8 challenge session) were analyzed. The relative number of hits did not differ between ketamine- and saline-pretreated animals ($F_{1,10}=0.01$, $P=0.91$; all interactions involving drug condition: all P values >0.42). Likewise, the relative number of correct rejections did not differ between the two groups ($F_{1,10}=0.06$, $P=0.81$; pretreatment × block $F_{2,20}=0.14$, $P=0.87$). Finally, the number of omissions did not differ between the groups ($F_{1,10}=0.37$, $P=0.56$) nor did this measure interact with the effects of block ($F_{2,20}=0.79$, $P=0.47$).

Day-1 challenge

The administration of 2.0 mg/kg 1 day after the completion of the pretreatment regimen, compared with saline-pretreated animals, did not significantly affect the relative number of correct rejections ($F_{1,10}=0.872$, $P=0.372$), nor did the effects of the pretreatment interact with block (block $F_{2,20}=1.64$, $P=0.218$; block × pretreatment $F_{2,20}=2.38$, $P=0.118$). Likewise, pretreatment with ketamine did not alter the effects of the challenge dose on the relative number of hits ($F_{1,10}=0.893$; all two-way interactions involving pretreatment: all P values >0.28). However, the effects of pretreatment, signal length and block interacted significantly ($F_{4,40}=2.78$, $P=0.04$). Post-hoc analyses on the effects of signal length or block on the performance of saline- or ketamine-pretreated animals did not identify the source for this interaction (all P values >0.28). Finally, administration of the day-1 challenge dose resulted in a higher number of omissions by rats pretreated with 25.0 mg/kg ketamine (42.50±6.43 omissions/session) when compared with saline-pretreatment (21.16±6.72; $F_{1,10}=5.26$, $P=0.045$).

Day-8 challenge

The ketamine challenge administration on day 8 did not affect the relative number of correct rejections ($F_{1,10}=0.086$, $P=0.776$) or the relative number of hits ($F_{1,10}=0.622$, $P=0.449$). However, the effects of pretreatment on correct rejections interacted with the effects of block (block $F_{2,20}=0.30$, $P=0.75$; block × pretreatment

$F_{2,20}$=5.14, P=0.016). Post-hoc one-way ANOVAs revealed that the relative number of correct rejections significantly differed between ketamine- and vehicle-pretreated rats during block 3 ($F_{1,10}$=6.11, P=0.03), but not during block 1 ($F_{1,10}$=2.07, P=0.18) or block 2 ($F_{1,10}$=0.09, P=0.78). Saline-pretreated rats showed a higher correct rejection rate (86.8±3.2%) than ketamine-pretreated rats (73.7±4.6) during the third block of the task. No such interactions were found in the analysis of the relative number of hits (all P values >0.20). Finally, administration of the day-8 challenge dose did not differentially affect the omission rate in rats pretreated with ketamine or saline ($F_{1,10}$=1.4, P=0.26; pretreatment × block $F_{2,20}$=0.73, P=0.49).

Day-15 challenge

The performance of ketamine- and saline-pretreated rats was not differentially affected by the administration of 2.0 mg/kg ketamine 15 days after the completion of the pretreatment regimen. Pretreatment did not significantly affect the relative number of correct rejections ($F_{1,10}$=0.95, P=0.35; block $F_{2,20}$=3.05, P=0.07; block × pretreatment $F_{2,20}$=0.98, P=0.39). Likewise, pretreatment with ketamine did not alter the effects of the challenge dose on the relative number of hits ($F_{1,10}$=0.33, P=0.58; all interactions involving pretreatment: all P values >0.16). Finally, administration of the day-15 challenge dose did not yield differences in the number of omissions between the groups ($F_{1,10}$=1.44, P=0.26; pretreatment × block $F_{2,20}$=3.23, P=0.08).

Discussion

The results from these experiments can be summarized as follows:

1. The neurochemical experiments demonstrated that acute administration of ketamine dose dependently increased prefrontal ACh release. Following the administration of the highest dose (20.0 mg/kg), ACh release increased by 250%, and this increase lasted for over 40 min.
2. Repeated pretreatment with ketamine (2.0 mg/kg or 25.0 mg/kg/day for 7 days) did not systematically alter the effects of subsequent challenges with 2.0 mg/kg when compared with the effects of the first administration of this dose.
3. The acute administration of ketamine in animals performing the sustained attention task remained without effects at lower doses (2.0, 4.0 mg/kg) but resulted in a substantial proportion of trials (>70%) that were omitted following the administration of 8.0 mg/kg.
4. In animals pretreated with ketamine (25.0 mg/kg/day for 7 days) or saline, the subsequent administration of 2.0 mg/kg did not yield systematic differences in attentional performance.

Thus, while the acute administration of ketamine robustly increased cortical ACh release and disrupted performance, the present data indicate that repeated pretreatment does not alter systematically the cholinergic and attentional response to subsequent ketamine administration. The discussion below focuses on the potential relationships between the acute effects of ketamine on ACh release and performance, on the absence of sensitized ketamine effects, and on the implications of the present results for the use of this compound in preclinical and clinical research on schizophrenia.

Effects on cortical ACh release

The acute administration of ketamine dose dependently increased cortical ACh release. The increase produced by the highest dose (20.0 mg/kg) corresponds with the effects of 25.0 mg/kg ketamine reported by Kikuchi et al. (1997), despite several differences between microdialysis methods. These data, together with the increases in cortical ACh release produced by PCP (Jentsch et al. 1998), suggest that potent increases in cortical ACh release represent a common property of systemically administered, psychotogenic NMDA receptor antagonists.

It is noteworthy that the similarities between ketamine and PCP in stimulating cortical ACh release contrast with substantial pharmacological differences between these two compounds. Compared with PCP, the potency of ketamine to block NMDA receptors is only one-tenth. Furthermore, although ketamine has a relatively short plasma half-life that is assumed to be responsible for the rather brief psychotogenic effects of this drug in humans (Benet and Sheiner 1985), ketamine and PCP (Jentsch et al. 1998) produce relatively lasting (>40 min) activation of cortical cholinergic inputs in rats. As the administration of ketamine did not alter baseline release in subsequent sessions, the present neurochemical (and behavioral) data also support the notion that sub-anesthetic doses of ketamine lack neurotoxic properties (Carpenter 1999).

The present results do not suggest that repeated pretreatment with ketamine sensitizes cortical ACh release. Although this finding, once again, corresponds with the lack of repeated PCP pretreatment on the ability of PCP challenges to stimulate ACh efflux (Jentsch et al. 1998), it cannot be excluded that a test of the effects of different pretreatment regimens and different challenge conditions, particularly higher challenge doses, would reject this conclusion. The literature reflects the complexity of this issue, particularly when taking into account the possibility that some effects reported after a large number of pretreatments with high doses of ketamine (Lannes et al. 1991) may in part have been due to neurotoxic mechanisms (Ellison 1995).

Effects on attentional performance

The main effect of acutely administered ketamine in attentional task-performing animals was a substantial increase in omissions following 8.0 mg/kg. Increases in omissions typically are difficult to interpret, as they may reflect motivational and sensorimotor mechanisms, but may also be due to a disruption of the processing of the task rules, or to other cognitive effects that interfered essentially with task performance. Other data indicate that repeated ketamine administration, even after more extensive pretreatment regimens, does not affect the animal's basic capability to perform operant tasks (Rocha et al. 1996). Likewise, the effects of ketamine on water maze performance did not suggest any major disruption of the animal's behavioral repertoire (Wesierska et al. 1990). Furthermore, ketamine (5.0 mg/kg) did not affect the performance of rats in an operant procedure assessing contextual information processing (Maes et al. 2001), or of rats performing a delayed non-matching-to-position task (Robinson and Crawley 1993). Thus, these data allow the intriguing speculation that the increase in omissions observed after the highest dose of ketamine was due to the disruption of the processing of cognitive aspects of the present task. However, in contrast with the increases in the false alarm rate observed following repeated amphetamine exposure (Deller and Sarter 1998), or other treatments known to stimulate cortical ACh release (Turchi and Sarter 2001), the present data indicate that ketamine does not result in specific, and thus more interpretable, attentional impairments in rats tested in this task.

Similar to the discussion of the effects of repeated ketamine on cortical ACh release, it cannot be excluded that more extensive pretreatment regimens may reveal persistent and specific consequences of ketamine pretreatment on attentional performance. Note again that an informative test of the attentional effects of higher 'challenge' doses of ketamine is limited by high omission rates (above). The literature reports functional consequences of extensive pretreatment regimens (Micheletti et al. 1992; Rocha et al. 1996). Therefore, the present results indicate only that distinctive consequences of ketamine pretreatment on attentional performance cannot be demonstrated using the present pretreatment parameters.

Implications for ketamine as a pharmacological model

The psychotogenic effects of ketamine in normal humans and in schizophrenics clearly support the usefulness of this drug as a pharmacological tool to produce schizophrenic symptomatology and to probe underlying neuronal systems (Krystal et al. 1994, 1999; Carpenter 1999; Lahti et al. 1999). It is also evident that neurochemical and cognitive effects differentiate the ketamine model from amphetamine-induced psychosis (Krystal et al. 1999; but see Kegeles et al. 2000). This discussion is complicated further by the possibility that ketamine, at relevant concentrations, may exhibit dopamine D2 receptor affinity (Kapur and Seeman 2001).

Furthermore, in contrast to amphetamine, ketamine appears not to produce robust effects on sensorimotor gating (Van Berckel et al. 1988; Duncan et al. 2001) and attentional performance (Adler et al. 1998; Newcomer et al. 1999; Oranje et al. 2000) in normal humans. These data, which may correspond with the present findings on the effects of ketamine on attentional performance in rats, further support the possibility that NMDA receptor antagonists and amphetamine model different aspects of psychosis (see also Rosse et al. 1994). In contrast to ketamine, repeated amphetamine produces sensitized impairments in attentional performance in rats (Deller and Sarter 1998) that, similar to the psychotogenic effects in humans, may be mediated via a sensitized mesolimbic dopaminergic system (Lieberman et al. 1990, 1997; Strakowski et al. 1997; Laruelle and Abi-Dargham 1999; Laruelle 2000) and possibly an associated sensitized cortical cholinergic input system (Nelson et al. 2000). Conversely, repeated administration of NMDA receptor antagonists reduce mesocortical dopaminergic activity (Jentsch and Roth 1999), and the behavioral and cognitive consequences of chronic exposure to NMDA receptor antagonists may be unrelated to sensitization of the mesolimbic dopaminergic system. The present data, in conjunction with previous findings on the effects of repeated amphetamine on cortical ACh release (Nelson et al. 2000) and attentional performance (Deller and Sarter 1998) confirm and extend the view that the effects of non-competitive NMDA receptor antagonists and amphetamine model distinct aspects of schizophrenia.

Conclusion

The present findings on the cortical cholinergic and attentional effects of acute and repeated ketamine administration, when compared with our previous findings on the effects of repeated amphetamine, indicate substantial differences between the effects of the two psychotogenic compounds. The limited attentional effects of acute ketamine exposure, and the absence of lasting effects of repeated ketamine administration on cortical ACh release and attentional performance, contrast with the sensitized effects of repeated amphetamine on cortical ACh release and attentional performance observed in previous experiments using similar methods. While the role of cortical cholinergic activity and attentional impairments in the development of schizophrenic symptoms requires more study (Tandon et al. 1999; Crook et al. 2001), the present data support the general view that the neurochemical and behavioral/cognitive effects modeled by non-competitive NMDA receptor antagonists and psychostimulants differ fundamentally.

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