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Normal spatial and contextual learning for ketamine-treated rats in the pilocarpine epilepsy model

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Abstract

Cognitive impairments frequently accompany epileptic disorders. Here, we examine two neuroprotective agents, the noncompetitive NMDA antagonist ketamine and the dopaminergic antagonist acepromazine, for their efficacy in attenuating cognitive impairments in the lithium–pilocarpine (LI–PILO) model of rat limbic epilepsy. Declarative-like cognitive behaviors were assessed in a Morris water maze task that consisted successively of spatial and nonspatial (cued platform) training. Whereas the ketamine-treated (Ket) LI–PILO rats performed equally in all respects to nonseized control rats for the spatial and nonspatial components of the water maze task, the acepromazine-treated (Ace) LI–PILO rats failed to demonstrate learning in either the hidden or cued platform variants of the task and did not demonstrate any place learning in the platform-removed probe trials. We further assessed nondeclarative (associative) cognitive behaviors with a standard contextual fear-conditioning protocol. LI–PILO rats treated with acepromazine failed to learn the Pavlovian relationship; Ket LI–PILO rats performed equivalently to nonseized controls. Cumulatively, these data suggest robust cognitive sparing for LI–PILO rats with pharmacological NMDA receptor antagonism following induction of status epilepticus (SE). This cognitive sparing occurs despite earlier findings that the mean amount of total brain damage with LI–PILO is equivalent for Ket and Ace rats.

Keywords: Acepromazine; Contextual fear conditioning; Lithium–pilocarpine epilepsy; Ketamine; Water maze

1. Introduction

The pilocarpine (PILO) and lithium–pilocarpine (LI–PILO) models have been rigorously explored as animal models of temporal lobe epilepsy (TLE). PILO and LI–PILO qualitatively reproduce in rodents many of the sequelae noted in human populations of TLE, including behavioral features [cognitive deficits, spontaneous recurrent seizures (SRSs)], electroencephalographic (EEG) characteristics, and the pattern of neuroanatomical damage (Olney et al., 1983; Turski et al., 1983, 1989; Persinger et al., 1988). In the rat, status epilepticus (SE) can be experimentally evoked by a single systemic injection of lithium chloride followed 4 or 24 h later by the muscarinic agonist PILO (Honchar et al., 1983; Persinger et al., 1988). This combination of drugs induces electrographic and overt behavioral seizures within 30 min of PILO administration with evolving accumulation of calcium aggregates (Lafreniere et al., 1992), and necrotic (Fujikawa et al., 1999) and apoptotic (Fujikawa et al., 2000) death of neurons that begins almost immediately. During the days following the induction of SE, a silent period begins and is characterized by normal EEG activity and behavior of the rodent; over the next several weeks, the rodent enters a chronic period characterized both behaviorally and electroencephalographically by the appearance of SRSs (Cavalheiro et al., 1991). Anatomical studies have discerned that the pattern and extent of brain damage asymptotes approximately 60 days following SE induction (Peredery et al., 2000).

Clinical evidence, complemented by laboratory findings in animals, has suggested pervasive cognitive deficits following SE, as inferred by measures of learning or memory (Liu et al., 1994; Holmes, 1997). In particular, compromised functions of both declarative and associative (nondeclarative) memory subsystems have been reported. Experiments direct-

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ed towards identifying therapeutics to improve cognitive outcome following SE are thus indicated. Several recent investigations and reviews have examined the efficacy of specific antiepileptic drugs (AEDs) on protecting the declarative memory system when assessed in rodents that entered into Morris water maze protocols (Rice et al., 1998; Wu et al., 2001; Cha et al., 2002; Hung et al., 2002; Leite et al., 2002; Rutten et al., 2002). This task requires the rat to learn the location of a hidden platform using only extra maze visual cues (Morris, 1984), is thought to assess the cognitive function of a rodent in a manner that may be equated with declarative memory in a human (Squire, 1984), and is a well-established cognitive model sensitive to the damages incurred by PILO treatment (Liu et al., 1994). Importantly, pharmacological antagonism of the NMDA receptor, using either ketamine or MK-801, has been reported to ameliorate the cognitive outcome of epilepsy for this task (Rice et al., 1998; Hort et al., 1999). However, these previous experiments have incorporated the benzodiazepine termination of acute SE into their model, and thus, the exact contribution of post-SE NMDA antagonism remains unclear.

Several investigations completed in our laboratory have documented changes in the ability of rodents to formulate memories of associative relationships between conditioned and unconditioned stimuli following SE. Our initial studies examined the effects of post-SE administration of the neuroleptic dopaminergic antagonist acepromazine on the outcome of ethologically relevant associative memory tasks, particularly conditioned taste aversion (CTA) learning (Venugopal and Persinger, 1988; Persinger et al., 1993, 1994). Despite the success we have had with this agent in eliminating cardiac instability and reducing mortality following typical untreated LI–PILO seizures (Harrigan et al., 1994), no sparing of associative learning was noted. However, ketamine administration immediately following SE onset results in CTA learning indistinguishable from nonseized control rats (Stewart and Persinger, 2001). Additionally, rats treated in this manner are able to learn the association between novel contextual cues and aversive electrical footpad stimulation and generate an adaptive physiological (analgesic) response (Stewart and Persinger, 2001).

In the present study, we compared the declarative and nondeclarative cognitive outcomes for LI–PILO rats given post-SE ketamine or acepromazine treatment in a spatial (place learning) water maze task and a context-dependent conditioned fear (emotional learning) task, respectively. Here, we report the behavioral equivalence of ketamine-treated (Ket) LI–PILO rats to nonseized rats for both place learning and emotional learning, suggesting the potent cognitive sparing of this NMDA antagonist when applied as the only pharmaceutical following SE induction. Rats treated with acepromazine following SE induction were markedly impaired in both tasks. The nature of the deficits suggested that post-SE dopaminergic antagonism was insufficient to prevent either the LI–PILO-evoked cognitive impairments or the emergence of specific sensorimotor problems.

2. Methods

2.1. Subjects

Male Wistar rats were obtained from Charles River (Quebec) at approximately 70 days of age. Rats were housed (three per cage) in standard colony conditions until the onset of the study approximately 3 weeks later. The light–dark cycle was 12:12, with photophase onset at 0730 h local time; ambient temperature was maintained between 20 and 21 °C. Food and water were available ad libitum throughout the entire study. Water maze trials were conducted during the early to mid photophase, and all fear-conditioning training and testing sessions took place during the mid photophase. The experimenter was blind to the control versus Ket LI–PILO condition of the rats. Given the exaggerated startle reflex and experimenter-directed aggression of the acepromazine-treated (Ace) LI–PILO rats, it was not possible for the experimenter to be blinded to the treatment condition of this latter group. All procedures were approved by the local Animal Care Committee and were compliant with regulations established by the Canadian Council for Animal Care.

2.2. Induction of epilepsy

At 90 days of age, rats were injected subcutaneously with physiological (0.9%) saline ($n=5$) or were administered 3 mEq/kg lithium chloride ($n=17$). Twenty-four hours later, saline-injected rats were administered saline again; lithium-chloride-pretreated rats were injected with 30 mg/kg PILO sc. Within minutes following a Racine Level V seizure (rearing and falling with convulsions; Racine, 1972), rats were injected with either 100 mg/kg sc of the noncompetitive NMDA antagonist ketamine ($n=8$) (Fujikawa, 1995) or 25 mg/kg sc of the dopaminergic antagonist acepromazine ($n=7$) (Harrigan et al., 1994). All drugs were obtained from Sigma (St. Louis, MO) and administered at a volume of 1 cc/kg.

Following post-SE intervention with either therapeutic, the rats continued to have multiple Level V seizures over the next 30 to 60 min, after which time, overt displays of tonic–clonic convulsions ended (and were not observed again for several days). Two rats failed to display an overt seizure. These rats were indistinguishable in all respects from saline-treated controls during the behavioral experiments, and thus, the data was pooled (therefore, total $n=7$ for control group). All 15 LI–PILO-seized rats survived due to post-SE intervention. During the weeks following SE induction, all Ace LI–PILO rats displayed SRSs; only one Ket LI–PILO rat was observed to seize and only on a single occasion. We did not include a PILO-seized group without post-SE treatment (vehicle-treated control) as mortality when post-SE intervention is withheld is greater than 95% (Harrigan et al., 1994). Thus, from an animal care perspective, an unacceptably large number of rats would be required to generate this control group.

After seizing, animals were singly housed and placed on a mush diet (rat chow mixed in water (Persinger et al., 1993)) for 3–4 days to facilitate recovery. For the next 90 days, rats were checked daily but otherwise left unhandled—a duration chosen to ensure an asymptote in the temporal progression of neuronal dropout (Peredery et al., 2000). To avoid diluting the individual contribution for any postseizing pharmacological treatment by providing environmental enrichment, a factor known to markedly decrease the cognitive deficits of (at least) weanling rats rendered epileptic with the PILO model (Rutten et al., 2002), we ensured that all rats in the study were maintained with the minimum enrichment permitted by animal care guidelines.

2.3. Water maze procedure

The custom-constructed water maze measured 180 cm in diameter by 50 cm in depth and was filled to a depth of 35 cm. A 10-cm-diameter platform was located 1.5 cm below the surface of the water. Nontoxic blue paint (powdered) was added to the water to visually obscure the location of the platform. The pool had been divided into four quadrants of homogeneous size; the platform was located in the center of one of the quadrants, halfway between the center and the wall of the pool. Various geometric shapes were constructed from Bristol board and placed on the walls of the room to serve as spatial cues. All swim latencies were recorded with a manual stopwatch—a technique routinely employed by others (Hort et al., 1999).

The water maze task consisted of 12 sessions conducted once daily over 12 successive days. Each session consisted of four trials separated by approximately 30 s. These parameters were utilized on the basis of pilot studies employing Wistar strain rats of comparable ages. Rats were placed manually into the pool, facing the pool wall in the center of one of the quadrants that did not contain the platform. Each rat was trained with a unique release schedule that was randomized between sessions and between trials. For any given rat, the location of the platform remained fixed across all trials and all sessions. The latency to find the platform was recorded as the time from release into the pool until the rat had reached the platform. A maximum of 120 s was allowed for each trial. Rats not reaching the platform within 120 s were guided to the platform; a score of 120 s was recorded for each of these experimenter-terminated trials. The rat was allowed to remain on the platform for the duration of the intertrial interval.

A 120-s probe test (platform removed from pool) was conducted on the 13th day of the study (24 h after the last hidden platform session). Rats were released into the pool in the quadrant opposite to that previously associated with the escape platform. A manual time-sampling procedure (one measurement per second) was utilized to record the swimming bias of the rat in each of the four quadrants of the pool.

Nonspatial (cued platform) testing began the day following probe testing. For this measure, dark curtains were drawn around the perimeter of the pool, effectively obscuring all visual cues. The platform was moved to a novel location and was raised 2 cm above the surface of the water. Contrast of the platform from the water was enhanced by 2.5-cm-wide black stripes, each spaced 1 cm apart. Training was conducted for three successive days (sessions) with four trials per day; release sites were randomized on each trial, but for any given rat, the position of the platform remained fixed across trials and sessions.

2.4. Contextual fear-conditioning procedure

Conditioned fear training began 1 week after the end of the water maze procedure. The footshock chamber was a $28 \times 20 \times 20$-cm modified operant chamber with aluminum side walls and Plexiglas front, back, and hinged ceiling. The floor consisted of 18 steel rods, each spaced 1.5 cm apart. This apparatus was connected to an A-615-C Master Shocker (Lafayette Instruments, Lafayette, IN, USA) programmed to deliver scrambled footshocks. A custom-constructed nonconducting frame whose dimensions approximated the inside of the chamber was utilized to facilitate removal of the animals subsequent to conditioning (training) and testing sessions.

Rats were placed singly in the chamber and were given 3 min to habituate to contextual cues. To evaluate preshock motoric activity, midline chamber crossovers, defined as forward movements of the whole animal across the midline of the chamber, were recorded during this 3-min period. Rats then received three unsignaled footshocks (2-s duration, 0.5-mA intensity) at 60-s intervals. Postshock freezing, defined as the absence of all movement except that strictly required for respiration (a species-specific defensive response to fear-evoking stimuli; Bolles, 1970) was scored using a time-sampling procedure (Maren et al., 1994) of one observation per 8 s (seven observations total per 1-min postshock period). Sixty seconds after the last footshock, rats were removed from the chamber and returned to their home cages. The chamber was wiped down with a solution of 10% acetic acid before each trial.

Twenty-four hours after conditioning, rats were returned to the chamber for 8 min and scored for defensive freezing during a contextual retention test. The time-sampling procedure employed consisted of one observation every 8 s, for a total of 60 observations. The percentage of time during the retention test spent freezing was calculated and entered as the dependent measure into the analyses.

2.5. Statistical analyses

Analyses were completed on a VAX 4000 computer employing SPSS software. The primary statistical tool for analyzing escape latency water maze data was three-way multivariate analysis of variance (MANOVA) with two

levels repeated (four trials per session; 12 sessions for spatial training or 3 sessions for nonspatial training) and one level not repeated (group: control vs. Ket LI–PILO vs. Ace LI–PILO). Analysis of quadrant preferences during probe testing was completed using two-way MANOVA with one level repeated (four quadrants) and one level not repeated (seized group). One-way analyses of variance (ANOVAs) were employed to examine fear-conditioning data. Criterion for statistical significance was set at $P < .01$.

Given that the appearance of a seizure during the period immediately preceding testing, and especially during testing, would be expected to negatively impact a rat's performance, we decided a priori to eliminate the data for any rat exhibiting a spontaneous seizure during these intervals. As the majority of seizures and the most severe seizures are displayed during the mid scotophase (0000 to 0400 h local time;Michon and Persinger, 1997; Stewart et al., 2001), they are much less likely to occur during the photophase period prior to our daily testing sessions. To be rigorous, the experimenter, along with technicians trained to identify and record seizure activity, routinely monitored the room housing the epileptic rats prior to the experiments. No overt displays of seizure activity were noted during the pretesting intervals. One rat, however, was removed from the fear-conditioning analysis due to a spontaneous seizure during the testing session.

3. Results

3.1. Hidden platform water maze testing

MANOVA, with two levels repeated (12 daily sessions with four trials per session) and one level not repeated (treatment group), discerned a statistically significant difference between groups for mean latency to escape from the water when averaged across all 12 sessions [$F(2,19) = 43.74$, $P < .001$, $\text{eta}^2 = 0.82$]. A statistically significant difference between sessions, indicative of a learning curve, was noted [$F(11,209) = 23.76$, $P < .001$, $\text{eta}^2 = 0.56$]. The statistically significant interaction between session and treatment group [$F(22,209) = 5.48$, $P < .001$, $\text{eta}^2 = 0.37$] was explained by the marked decrease in mean escape latency across sessions for nonseized and Ket limbic epileptic rats (which did not differ significantly from one another), whereas Ace rats did not improve significantly across sessions (Fig. 1). The latency required to escape from the water decreased across trials [$F(3,57) = 5.95$, $P = .001$, $\text{eta}^2 = 0.24$], suggesting the presence of a moderate learning curve within each session (data not shown); the interaction between treatment group and trials was not significant statistically [$F(6,57) = 0.75$, ns].

Our observations indicated that the seized animals given acepromazine posttreatment appeared to swim faster than all other groups. This observation has been reported previously (Rice et al., 1998) and has been interpreted to mean that deficits in water maze performance cannot be attributed exclusively to generalized motor impairments, such as an inability to swim, but must include an additional component reflecting a cognitive impairment. Ace LI–PILO rats showed a marked propensity for thigmotaxis. Whereas nonseized and Ket LI–PILO rats never spent more than one third of their time circling the pool, Ace LI–PILO rats often spent 90–100% of their time engaged in these circling behaviors during both spatial and nonspatial testing. This thigmotaxic propensity may have interfered with our ability to accurately assess the spatial memory abilities for this group of rats.

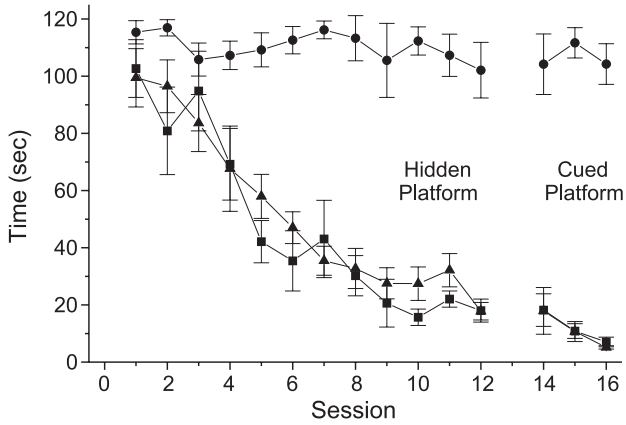

Fig. 1. Mean (± S.E.M.) escape latency (in seconds) per session (average of four daily trials) for nonseized rats (squares) and Ket (triangles) and Ace (circles) LI–PILO rats trained for 12 days in a hidden platform Morris water maze task and 3 days in a cued platform reversal task.

MANOVA, with one level repeated (four quadrants) and one level not repeated (treatment groups), discerned a statistically significant interaction between treatment group and pool quadrant during transfer testing [$F(6,57) = 6.65$, $P < .001$, $\text{eta}^2 = 0.41$]. The source of this interaction (correlated t tests within each group) was the significantly greater percentage of times spent swimming in the training quadrant relative to all other quadrants (which did not differ significantly from one another) for nonseized and Ket limbic epileptic rats. Ace limbic epileptic rats failed to display a training quadrant preference (Fig. 2).

3.2. Cued platform water maze testing

MANOVA with one level repeated (three cued sessions) and one level not repeated (treatment groups), discerned a statistically significant difference between seized groups for latency to escape to the platform during cued platform trials [$F(2,19) = 120.54$, $P < .001$, $\text{eta}^2 = 0.93$]. The source of the effect was the significantly longer durations required to escape to the platform for Ace LI–PILO rats, relative to nonseized and Ket LI–PILO rats, which did not differ significantly from one another (Fig. 1). Correlated t tests were employed to contrast the mean escape latency for the last 3 days of hidden platform trials to the mean escape latency for the 3 days of cued platform trials. Decreases in escape latencies were noted for both nonseized and Ket

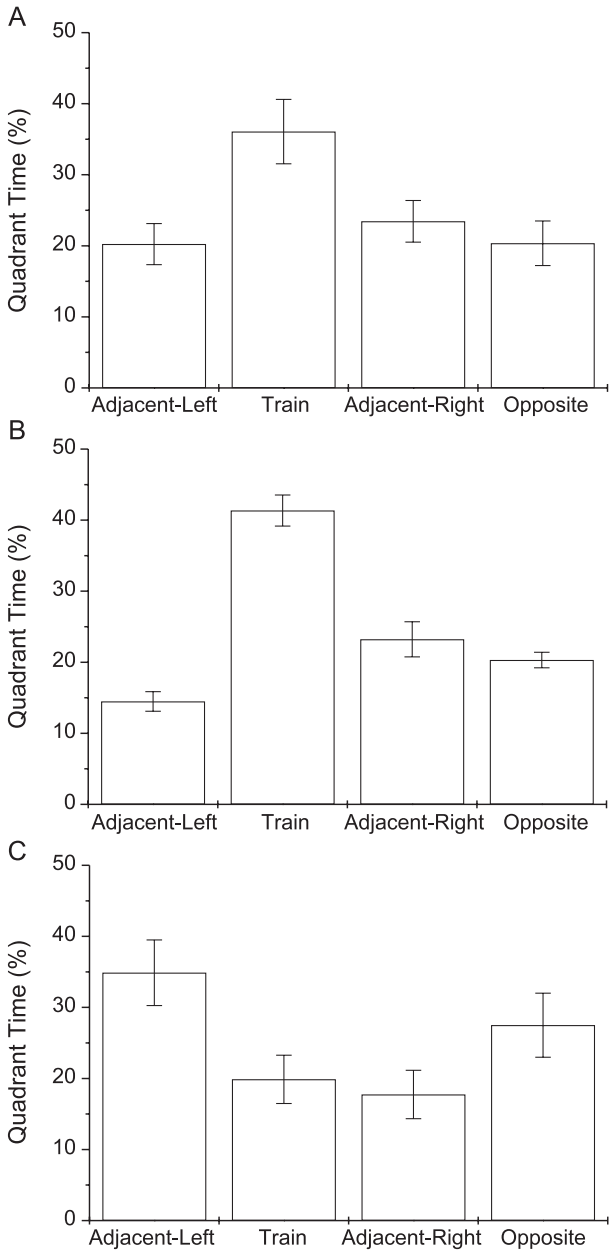

Fig. 2. Mean (± S.E.M.) percent time searching in adjacent–left, training, adjacent–right, and opposite quadrants during probe (transfer) tests for nonseized rats (A), and Ket (B) and Ace (C) LI–PILO rats.

groups during cued platform trials relative to hidden platform trials; escape latencies for Ace rats did not change between hidden and cued platform trials.

3.3. Contextual fear conditioning

Midline chamber crossovers during the 3-min period prior to context/shock pairings, and mean postshock freezing (averaged across three postshock periods) are presented in Table 1. ANOVA discerned a statistically significant difference in the numbers of midline chamber crossovers between Ace LI–PILO rats and nonseized control and Ket LI–PILO rats [which did not differ significantly from one another and were both greater than the motoric activity of the Ace LI–PILO rats; $F(2,19) = 7.06$, $P < .01$, $\text{eta}^2 = 0.43$]. Statistically significant differences between the three treatment groups for the mean duration of freezing following context/shock pairings on the conditioning day were not evident [$F(2,19) = 1.09$, ns], suggesting that all rats were able to respond to the aversive footpad stimulation in the typical species-specific manner, irrespective of their pharmacological treatments. Spontaneous freezing during the preshock (baseline motor) period was not evident.

Table 1
Mean number of preconditioning midline chamber crossovers and mean percent duration of freezing following context/shock pairings

Group	Midline crossovers		Post context/shock freezing	
	Mean	S.E.M.	Mean	S.E.M.
Nonseized	5.29[a]	0.99	57.14	5.60
Ketamine	6.50[a]	0.71	47.02	8.56
Acepromazine	2.29[b]	0.75	61.90	7.20

[a] vs. [b] post hoc $P < .05$.

The mean duration of freezing during the 8-min extinction tests was found to be significantly greater for nonseized controls and Ket rats (which did not differ significantly from one another) relative to Ace rats [$F(2,18) = 19.93$, $P < .0001$, $\text{eta}^2 = 0.69$; Fig. 3]. One Ket rat was excluded from this latter analysis, as it spontaneously seized at the end of its extinction test. Because baseline motor activity, as inferred by midline chamber crossovers, differed between groups prior to conditioning trials, a subsequent ANOVA was completed for freezing to contextual stimuli during extinction testing after first covarying out the variance attributed to baseline motor activity levels. Although the covariate was statistically significant [$F(1,17) = 9.54$, $P < .01$], differences between the treatment groups were still apparent [$F(2,17) = 15.93$, $P < .001$]. The proportion of variance explained in defensive freezing as a function of treatment group after analysis of covariance increased to 90%.

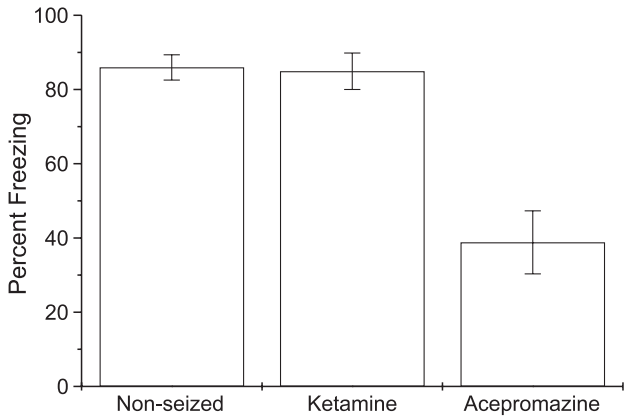

Fig. 3. Mean percent duration of freezing to context during extinction tests for nonseized rats and Ket and Ace LI–PILO rats. Error bars indicate S.E.M.s.

4. Discussion

Single-lesion studies suggest that optimal maze learning or emotional learning abilities may be abolished through discrete lesions to any of several key memory structures (Brandeis et al., 1989; Fendt and Fanselow, 1999); arguably, multifocal brain damage should result in a more pervasive compromise of behavior. However, repeated histological assessments in our laboratory have discerned complete sparing of a broad spectrum of learning- or memory-related behaviors in Ket limbic epileptic rats despite the presence of multifocal damage (Santi et al., 2001; Stewart and Persinger, 2001; Vaillancourt and Persinger, 2001). In fact, the magnitude of neuronal damage when averaged across all brain structures is equivalent between the two pharmacological interventions. The present work extends the known behaviors spared by ketamine posttreatment to include rodent models of declarative memory (place learning) and emotional learning (conditioned fear).

Several key areas implicated in learning or memory, for both declarative and nondeclarative tasks, are damaged in the LI–PILO model despite the behavioral sparing noted with ketamine treatment. Although a histopathological examination of the LI–PILO brains were not completed in the present study, our previous studies have discerned that the damage scores for over 100 Paxinos and Watson structures (Paxinos and Watson, 1986) are extremely consistent both between experiments and between experimenters (Persinger et al., 1988; Harrigan et al., 1991; Lafreniere et al., 1992; Peredery et al., 1992, 1993, 1994; Santi et al., 2001; Stewart and Persinger, 2001; Vaillancourt and Persinger, 2001). Our damage scale assigns a nominal rating of damaged (e.g., evidence of neuronal dropout or gliosis) versus undamaged to 10 successive 10-ìm tissue sections (slides) through each nucleus. The percent damage score for each nucleus is then calculated as the number of damaged sections out of 10, multiplied by 100%. This technique is correlated r=.86 (P<.001) with high-magnification neuronal cell counts for the same nuclei (Peredery et al., 2000). Damage scores in Ket and Ace rats for structures (nuclei) implicated in the memory circuits required by the present behavioral experiments or for structures necessary for the transduction of task-specific peripheral stimuli are the following: entorhinal cortex (Ket: 76%, Ace: 34%), CA fields of hippocampus including dentate gyrus (Ket: 22%, Ace: 55%), basolateral amygdala (Ket: 90%, Ace: 61%), central amygdala (Ket: 34%, Ace: no damage), somatosensory (ventral posterior) thalamic nuclei (Ket: 40%, Ace: 37%), affective (intralaminar) thalamic nuclei (Ket: 44%, Ace: 33%), pyriform cortex (Ket: 94%, Ace: 76%), and parietal cortex (Ket: 29%, Ace: 54%).

A priori, one might hypothesize that 90% destruction of the basolateral amygdala, considered the ‘coincidence detector’ for the integration of somatosensory and emotional events (Fanselow and LeDoux, 1999), would abolish conditioned fear to contextual stimuli for Ket LI–PILO rats. Such a hypothesis would seem even more certain given that the entorhinal cortex, whose integrity is critical for the acquisition of contextual fear stimuli (Maren and Fanselow, 1997), is three-quarters destroyed in the LI–PILO model. Similar arguments can be made for the pattern of brain damage routinely observed in Ket LI–PILO rats, as it would pertain to the acquisition or memory of a place-learning water maze task; yet, behavioral sparing for all aspects of the water maze task is recorded. These results suggest that inhibition of the NMDA receptor following LI–PILO injections abrogates the cognitive deficits that persist with other interventions. This conclusion would not be predicted by the magnitude of multifocal brain damage that occurs in these animals. Interestingly, the nonlinear correlation between measures of hippocampal damage and learning and memory have been recently reported, convincingly demonstrating that significant brain damage does not necessarily correspond to a marked behavioral deficit (Mohajeri et al., 2003). Of potential therapeutic importance is the finding that behavioral sparing is achieved with NMDA blockade during the immediate post-SE interval—a clinically tenable period.

There are several reasons why a discrepancy may exist between overtly normal measures of cognitive behavior and the brain damage known to follow the induction of SE. First is the concept of a physiological safety threshold, wherein a critical number or percentage of neurons within a given structure or circuit must be damaged before changes in behavior are evident. Such a threshold has been observed in Parkinsonism wherein upwards of 80% of the cells in the pars compacta division of the substantia nigra must be destroyed before the symptoms of Parkinsonism are evident. A second possibility emerges given that the mean amount of total brain damage does not differ between post-SE NMDA or dopaminergic blockade, but the pattern of structures damaged does differ significantly between the two post-SE interventions (Santi et al., 2001; Vaillancourt and Persinger, 2001). That the exact pattern of damage, and thus also the pattern of whole-brain remodeling postulated to occur following SE, is important would be consistent with the concept of serial versus parallel circuits for information processing within the brain (Squire, 1987). In fact, there may be considerable redundancy in the neural pathways capable of supporting a given behavior. For instance, during fear conditioning, information concerning the conditioned stimulus must advance from the thalamus to the amygdala. However, it has been established that the thalamo–cortico–amygdaloid route is sufficient to support conditioned fear to auditory stimuli if the direct thalamo–amygdaloid pathway is compromised (Iwata et al., 1986). A third possibility is that rats given post-SE NMDA antagonism use unique maze-learning strategies that we and others (Liu et al., 1994; Rice et al., 1998; Hort et al., 1999) have not been able to behaviorally differentiate from control strategies. If this were true, it would suggest that the water maze task

might measure behavioral constructs other than, or minimally in addition to, the intended learning and memory constructs. Of the three typical maze-learning strategies employed by rodents (place learning, cue learning, and praxic learning), we have determined in the present study that for water maze tasks, Ket limbic epileptic rats rely upon spatial (place-learning) strategies as inferred by a marked preference for the goal quadrant during platform-removed probe (transfer) tests. However, such a determination of maze-learning strategies is not as straightforward for other maze-learning tasks, particularly the radial maze task, for which Ket LI–PILO rats are also known to perform indistinguishably from controls (Santi et al., 2001; Vaillancourt and Persinger, 2001).

Ace LI–PILO rats failed to learn the location of the hidden platform in the water maze. However, it is difficult to interpret this deficit as a failure of learning or memory. While in the water maze, Ace LI–PILO rats swam faster than either Ket rats or nonseized controls (these latter two groups were qualitatively indistinguishable from one another for swimming speed), ruling out a motor impairment intrinsic to this treatment group (Rice et al., 1998; Hort et al., 1999). Within the water maze, the Ace LI–PILO rats displayed a marked propensity for thigmotaxis. On the few occasions where swimming was directed towards the middle of the pool and the platform was encountered, these rats either bumped into the platform and immediately veered away, or climbed onto the platform and immediately jumped off. This latter behavior has been previously reported for PILO rats in the water maze task, although unlike the present experiments, earlier experiments noted the dissipation of this behavior after several days of testing (Rice et al., 1998; Hort et al., 1999). Failure to remain on the platform may reflect either a sensorimotor problem or, alternatively, may represent an inability of these rats to recognize the putative reward value of the platform. Interestingly, this is reminiscent of the perseverative motor behaviors observed in radial maze tasks, wherein Ace LI–PILO rats will often run down an arm and, failing to stop at the end of the arm, jump clear out of the maze (unpublished observations). Alternatively, these rats may make dozens of very rapid consecutive entries to the same arm of the maze. Within the fear-conditioning testing chamber, the heightened startle reflex of these rats and their tendency to spontaneously (but only intermittently) exhibit wet dog shakes and other low amplitude jerking movements may confound the measurement of the memory construct, which, for this task, assumes that memory equals immobility. However, the ability of the Ace rats to respond with control-level freezing behavior to the presentation of the footshock stimuli suggests that global impairments in sensorimotor integration were not present, and thus, the deficits in memory are at least partly attributable to cognitive impairments. In fact, the freezing behavior of the Ace LI–PILO rats during retention testing (approximately 40%) is significantly greater than their spontaneous freezing level during conditioning sessions (which was virtually absent), suggesting that some memory of the context/shock pairings had been retained.

Collectively, the impairments in the Ace LI–PILO group suggest some contribution from a sensorimotor deficit. The origin of this deficit, be it due strictly to LI–PILO treatment or to the combination of LI–PILO with post-SE acepromazine, cannot be elucidated from the present study, as a vehicle-treated LI–PILO group was not included for ethical reasons. However, a previous water maze study has reported for PILO-treated rats with clonazepam termination of seizures as the only post-SE therapeutic that gross impairments in maze learning were also noted (Hort et al., 1999). That clonazepam (a benzodiazepine) and acepromazine (a dopaminergic antagonist) have very different pharmacologies yet resulted in similar behavioral deficits suggests that it is the PILO factor that underlies the most significant component of the behavioral deficit, rather than a nonspecific toxic effect of the post-SE intervention. Neither of these agents is known to be toxic to cognition measured at significant latencies following administration.

Within the population of Ace rats, but not Ket rats, SRSs were noted during the chronic period. The emergence of seizures during the chronic period corresponded to the testing period in the present study and may in part account for the behavioral deficit of the Ace rats. However, these seizures cannot entirely underlie the deficits, as Hort et al. (1999) have reported that their PILO-treated rats given ketamine 120 min post-SE displayed SRSs but had no measurable cognitive impairment. The procedures of these authors do not fully align with our own, however, as there are both strain differences (Long–Evans vs. Wistar) and differences in the SE induction protocol (PILO vs. LI–PILO), which may contribute to slightly different behavioral and neuroanatomical phenotypes following post-SE intervention (Ormandy et al., 1989; Sofia et al., 1993; Bureau et al., 1994; Hort et al., 2000).

NMDA antagonism following induction of SE in the PILO model has been reported to spare cognitive outcome in rodents assessed with water maze tasks (Rice et al., 1998; Hort et al., 1999). However, both of these studies differed from the present work in that they employed pre-SE peripheral cholinergic block and, importantly, post-SE termination of seizures with a benzodiazepine. This latter intervention left open the possibility that the termination of the ictal event with the benzodiazepine biased the effects of NMDA antagonism towards an ameliorated outcome. Our present results would negate this possibility, as we have demonstrated that ketamine treatment alone provides robust cognitive sparing. Furthermore, the study employing ketamine as the post-SE treatment employed a 5-day pretraining phase in the water maze prior to induction of SE, with assessment of maze-learning abilities over the days and few weeks following SE (Hort et al., 1999). Following the demonstration that rats familiarized with the task demands of the water maze are able to acquire a similar water maze task during NMDA antagonism and full blockade of long-term potentiation

(Saucier and Cain, 1995), pre-SE water maze exposure may have minimized the salience of the ketamine intervention. Our results are significant in this context by demonstrating excellent spatial memory in Ket LI–PILO rats despite the lack of pre-SE task familiarization.

In summary, we have demonstrated that the cognitive outcome of LI–PILO seized rats treated with ketamine is indistinguishable from nonseized control rats. These findings were evident in rats seized as adults and tested several months following SE induction. Significantly, this cognitive sparing occurred despite the lack of benzodiazepine termination of the seizures. Given the extensive brain damage that occurs in this model, the cognitive sparing that we and others have reported questions the entire concept of an unambiguous link between multifocal brain damage and degree of impairment.

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