

A Neuropsychological Assessment of the Effects of Chronic Ketamine Exposure in a Rodent Model of Drug Abuse

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Abstract- While used as paediatric and veterinary anaesthetic and for pain management, the nonselective NMDA (N-methyl-D-aspartate) antagonist ketamine also remains popular among recreational users. As such, there is legitimate concern about the psychological and physiological consequences associated with chronic abuse of this drug. The purpose of the present investigation was to assess the impact of chronic exposure to ketamine following a period of abstinence in a rodent model of ketamine abuse. In the present experiment, rats were given repeated injections of saline, 5 mg/kg, or 40 mg/kg of ketamine. Beginning at 111 days of age, the animals were tested for retention of an aversive outcome on a step-down avoidance task and assessed for general levels of activity. In addition, the animals were trained on a series of tasks with spatial components of various levels of difficulty, a spatial learning set task, and a nonspatial response learning task. On early trials with water maze tasks of varying difficulty, the ketamine-treated rats were impaired relative to controls, with dose-dependent effects observed on many of the tasks. On probe trials the drug-treated animals spent significantly less time in the target quadrant. In addition, the performance of the drug-treated rats was inferior to that of the control animals on a spatial learning set task, and a response learning task suggesting some difficulty in adapting their responses to changing task demands. The results suggest that chronic exposure to this NMDA receptor antagonist in young adult rats is capable of producing a variety of changes that affect nonspatial learning and memory performance in adulthood, well after the drug exposure period.

Keywords- Ketamine; (\pm)-2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone); Spatial Learning; Learning Set; Response Learning; Avoidance Learning; Morris Water maze; Memory

I. INTRODUCTION

In the United States, ketamine hydrochloride is classified as a Schedule III controlled substance (US Drug Enforcement Administration, 1999) with an appropriate use as a veterinary anaesthetic with dissociative properties. The drug has been considered useful because of its rapid onset when an IV injection is desired and has an elimination half-life of two to three hours (Adams and Werner, 1997; Domino et al., 1984). The drug is effective in creating a disruption of awareness to pain as well as the general environment without the accompanying side effects associated with depression of autonomic reflexes (Dotson, Ackerman, and West, 1995).

The use of ketamine [(\pm)-2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone] for recreational purposes and known by such names as K, Vitamin K, Special K, Cat Valium, or Super Acid (US Drug Enforcement Administration, 1999), can produce powerful hallucinations, including out of body experiences or a phenomenon similar to that described for near death experiences (Jansen, 2004). Ketamine is common at parties and “raves”, where other drugs are present. As a derivative of phencyclidine hydrochloride (PCP), it is often found with other illicit drugs such as marijuana, 3,4-methylenedioxymethamphetamine (MDMA), gamma-hydroxybutyrate (GHB), and methamphetamine (Freese, Miotto, and Reback, 2002). When abused, Ketamine is smoked with tobacco or marijuana, ingested orally or intranasally, as well as the traditional route of introduction, an injection (Meyer and Quenzer, 2013). Like the other drugs mentioned above, the use of ketamine is associated with an enhancement in the frequency of risky sexual behaviours (Mattison, Ross, Wolfson, and Franklin, 2001), including among homo- and bi-sexual males frequenting circuit-parties (Freese, Miotto, and Reback, 2002; Mansergh *et al.*, 2001).

The pharmacological actions of ketamine are complex and appear to influence a number of neurotransmitter systems (Morgan and Curran, 2012). Because ketamine acts as a noncompetitive antagonist at the N-methyl d-aspartate glutamate receptor site, much of the available research has focused on its glutamatergic properties (Haas and Harper, 1992), with considerable interest focused on learning and memory systems (Morgan and Curran, 2012). However, ketamine also appears to exert influences via other sites of action. For example, it appears to potentiate the action of gamma-aminobutyric acid (GABA) synaptic inhibition (Morgan and Curran, 2012) and act as an agonist at μ and σ opioid receptor sites (Sharp, 1997; Smith, Pekoe and, Martin, 1980). Further, many of the subjective reports of the effects of ketamine glutamatergic effects (Deakin *et al.*, 2008) as well as an increase in dopamine release appears to be mediated by a glutamate-dopamine interaction (Absalom and Menon, 2010; Aalto *et al.*, 2005).

Beyond its role as an anaesthetic, first in the Vietnam War, in emergency room procedures (Freese, Miotto, and Reback,

2002) and as veterinary anaesthetic (Morgan and Curran, 2012), ketamine has been reported to offer neuroprotective effects in animal models (Himmelseher and Durieux, 2005). In a review by Himmelseher and Durieux, the evidence reported that the effect is due to impeding the well-known overstimulation of the NMDA receptor and concomitant enhanced Ca^{++} influx associated with neural injury. Last, in humans ketamine remains a common paediatric drug (Kohrs and Durieux, 1998).

Recently, there have been a number of reports linking ketamine to cognitive deficits associated neurodegeneration of the developing brain (Campbell *et al.*, 2011; Gomes, Garcia, Ribamar, and Nascimento, 2011; Huang, Liu, Jin, Ji, and Dong, 2012; Paule *et al.*, 2011; Peng, Zhang, Zhang, Wang, and Ren, 2010; Viberg, Pont  n, Eriksson, Gordh, and Fredriksson, 2008; Zou, Patterson, Sadovova, *et al.*, 2009; Zou, Patterson, Divine, *et al.*, 2009). For example, Huang *et al.* found that rats administered 75 mg/kg for three days beginning on postnatal day (PND) 7 were impaired relative to controls in Morris water maze tests at 60 days of age. Pups administered lower doses were unimpaired. In the CA1 and dentate gyrus areas of the hippocampus, the number of apoptotic cells was higher, but only at the 75 mg/kg level of exposure.

Ketamine is noted as a noncompetitive NMDA receptor antagonist (Haas and Harper, 1992). However, its effects on dopamine D_2 , muscarinic acetylcholine, and opioid receptors (Hustveit, Maurset, and Oye, 1995; Kapur and Seeman, 2001; Smith, Pekoe, Martin, and Coalgate, 1980) and observations that ketamine acts by inhibiting the reuptake of serotonin and the catecholamines dopamine and norepinephrine (Tso, Blatchford, Callado, McLaughlin, and Stamford, 2004) complicate our understanding of the neural and biochemical changes associated with abuse. Nonetheless, much of the research emphasis has focused on the NMDA receptor effects because of the putative role of NMDA in long-term potentiation (LTP) (Collingridge, Kehl, and McLennan, 1983), a phenomenon implicated in learning and memory (Carlson, 2010; Sweatt, 2008). The current evidence suggests that ketamine is capable of producing learning impairments in rodents by adversely affecting hippocampal LTP (Rowland *et al.*, 2005). Given that NMDA receptors are implicated in the regulation of the survival of neurons and synaptogenesis during CNS development (Haberny *et al.*, 2002; Wright, Pearson, Hammond, and Paule, 2007) and synaptic plasticity, concerns about chronic exposure to ketamine become a salient issue.

Unfortunately, what is known so far, while inconclusive, is not encouraging ([Jansen, 1990; Pallares Nadal, Silvestre, and Ferre, 1995; Wesierska, Macias-Gonzalez, and Bure  , 1990). For example, Wright and his colleagues exposed rats via an orogastric gavage to one of two doses of ketamine beginning on PND 27 for a period of 234 days. Here, the authors found that the 100 mg/kg dose of ketamine impaired acquisition on one of a series of operant tests employed by the experimenters, an audio-visual task. Evidence of ketamine-mediated deficits in motivation and motor ability was found. Last, mice exposed to a 5mg/kg dose of ketamine were impaired on a fear conditioning task (Amann *et al.*, 2009). Length of exposure appeared to be a factor as the impairment was found after four weeks of exposure. Conversely, two weeks of exposure did not compromise test performance.

In tests of memory and other cognitive abilities with human subjects, the results appear to be mixed (Morgan and Curran, 2012). In a review of the literature, Morgan and Curran (2006) concluded that chronic ketamine use can lead to deficits of both episodic and semantic memory. The authors noted that the reported effects are similar in type but of greater severity than the episodic and semantic deficits following acute exposure. When administered ketamine, both episodic and semantic memory are impaired (Adler, Goldberg, Malhotra, Pickar, and Breier, 1998; Krystal *et al.*, 1994; Malhotra *et al.*, 1996; Morgan, Mofeez, Brandner, Bromley, and Curran, 2004; Morgan, Rossell, *et al.*, 2006; Newcomer *et al.*, 1999). Ketamine use is correlated with spatial working memory deficits and changes in use over 12 months of testing. Where found, ketamine appears to exert these effects by compromising a number of working and episodic memory processes mediated by the frontal cortices (Morgan and Curran, 2006). Liao *et al.* (2011) found a significant bilateral reduction frontal lobe tissue associated with the duration of ketamine use. However, such impairments may not be permanent as one group of former users tested normally when abstinent for a year (Morgan & Curran, 2012).

As noted earlier, a number of consequences associated with the chronic use of ketamine have been reported, with drug-induced damage observed in areas of the cerebral cortex and hippocampus (Majewski-Tiedeken, Rabin, and Siegel, 2008; Olney, Labruyere, and Price, 1989; Scallet *et al.*, 2004; Wang *et al.*, 2005; Young *et al.*, 2005; Zou, Patterson, Divine, *et al.*, 2009). Owing to the fact that the use of ketamine as a "club drug" has increased and remains popular, such side effects are a public health concern. Unfortunately, much remains to be elucidated about the long-term impact of repeated use of ketamine on neural physiology and brain function. In particular, there is a need to continue to assess the long-term consequences of ketamine exposure, as this area has received less attention or involves confounds such as selection biases and poly-drug use in human studies. These issues are relevant as some effects may be short-lived effects of drug withdrawal rather than a result of permanent changes in brain function (Featherstone, Liang, Saunders, Tatard-Leitman, Ehrlichman, and Siegel, 2012). Therefore, the purpose of the present study was to use a nonhuman model to assess the physiological and cognitive risks associated with the chronic use of ketamine as a drug of abuse.

II. MATERIALS AND METHODS

A. Drug

Ketamine hydrochloride (Ketaset, Fort Dodge, IA) was obtained from Henry Schein (Melville, NY). Doses of 5 mg/kg or 40 mg/kg were chosen after examination of the literature and pilot testing with rats not included in the present study. The

animals in the control group received the injections consisting of an equivalent volume of saline solution. All injections were delivered IP.

B. Subjects

The subjects consisted of 21 male experimentally naive Long-Evans rats (Charles River, Wilmington, MA). One rat died during the course of the experiment and was not included in any of the analyses. The research protocol was approved by the Institutional Animal Care and Use Committee of Palm Beach Atlantic University and the animals were cared for in a manner consistent with the principles of animal care outlined in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Drug exposure began when all of the rats were approximately 60 days of age. All animals received a total of 15 injections, with each injection evenly spaced over a 30-day period. The rats were individually housed in stainless steel suspended cages and maintained on a 12-hr light/12-hr dark cycle with the lights on at 7:00 am. Throughout the experiment, the animals were provided with ad libitum access to water and food (Mazuri Rodent Chow).

C. Apparatus – Morris Water Maze (MWM)

All spatial testing was similar to that described by Compton, Selinger, Westman, and Otero (1997) and occurred in a circular white acrylic plastic swimming pool 183 cm in diameter. Water was filled to a depth of 30 cm and made opaque by the addition of a nontoxic paint (Sargant Art, Hazelton, PA). The pool was located in a testing room approximately 36.88 square meters in size. Using white curtains, a minimum of external stimuli was visible from the surface of the pool. For the cued water maze task, the platform protruded 15 mm above the surface of the water. A 15 cm X 15 cm escape platform painted flat white was located 18 cm from the wall of the swimming pool and submerged a depth of 15 mm below the surface of the water.

Across all phases of training on each trial the rat was released into the pool at one of four release points, north, south, east, or west, and permitted to find the platform. Platform location varied at one of four compass positions – northeast, northwest, southeast, or southwest. All trials were given a ceiling of 60 seconds, at which point the rat was placed on the platform. Swim times to the escape platform were recorded with a stopwatch and errors, operationally defined as crossing one of four quadrants associated with the four compass points, were recorded.

In order to make direct comparisons of swim latencies associated with start locations with different optimal swim path distances, the recorded escape latencies for each of the four start locations were normalized. Normalization was accomplished by computation of the ratio of the minimum swim distance in centimetres for each of the two longer swim paths to the escape platform (e.g., north start location and a southwest goal location) to the minimum swim of the two shorter swim paths (e.g., north start location and a northwest goal location) trials in centimetres.

D. Methods and Experimental Design

All behavioural testing began approximately 21 days after the last period of drug exposure. As noted earlier, the purpose of our behavioural testing regimen was to determine the possibility of permanent cognitive deficits resulting from ketamine exposure and the nature and, if present, the severity of such deficits. Spatial learning and memory was evaluated using variations of Morris water navigation. Nonspatial response learning was examined using a Morris water maze version of the Greek cross task described elsewhere (Compton, Selinger, Westman, & Otero, 2011). In addition, a test of activity and the cued version of the Morris water maze task were administered to not provide a more complete picture of the physical state of the rats.

1) Assessment of General Activity:

General locomotor activity levels were evaluated for 5 minutes in a 24" x 24" chamber consisting of a series of squares (i.e., a checkerboard). General activity was determined by the number of squares crossed during the measurement period. The number of rearings was also tallied. Additional sensorimotor and motivational deficits were explored using a cued version of the Morris water maze task (see below).

2) Step-Down Passive Avoidance Testing:

Step-Down passive avoidance training occurred in a standard operant chamber (Lafayette Model 84022) with a stainless steel electrified grid floor. Located in the centre of a 21 cm x 28 cm chamber was a 10.14 cm x 10.14 cm brick block. A 0.4mA current was delivered to the feet of the rat whenever the animal left the wood block.

3) Water Maze Task:

The water maze protocol we used is representative of protocols to test rodents without the need for food deprivation. For all water maze components of the experiment, the platform either protruded 15 mm above the water's surface (Cued task) or was submerged 15 mm below the surface of the water (the Place & Learning Set tasks). For each trial, the rat was released into the

pool at one of four release points and allowed to find the platform. All trials lasted a maximum of 60 seconds, at which point the rat was placed on the platform.

a) Cued Water Maze Task

The cued water maze navigation task was administered beginning after a recovery period to determine whether nonassociative influences might develop over time and influence performance on subsequent place learning or learning set tasks. Specifically, using a visible platform the cued version of MWM was used to test the presence of sensorimotor (e.g., swimming ability, visual), motivational deficits, and problems of nondeclarative memory ability that could adversely impact performance during the spatial place, learning set, and response learning tasks. Training on the cued water maze navigation task began when the rats were 117 days old, 27 days after the last drug exposure. The rats were given 10 trials during 2 days of testing where each trial involved one of four possible platform locations. Rats were allowed to remain on the platform for 15 seconds after each trial.

b) Place Water Maze Task

Variants of this task are considered tests of spatial reference memory with differing levels of difficulty. The task involved learning the location of a submerged platform that remained the same across all trials within a given phase. Variations in the tasks were employed because only minor deficits (if any) using the standard version of this test are seen (Hartman, Lee, Zipfel, and Wozniak, 2005). Therefore, we used two different place learning protocols (labelled Easy & Difficult) to determine if the difficult version was more sensitive for detecting spatial learning/memory impairments following chronic exposure to ketamine.

b1) the easy version of place learning

The protocol for the easy version of place learning consisted of training the rats for 10 trials per day for a period of 2 days. The rats were allowed to remain on the platform for 15 seconds after each trial. Retention was evaluated with a probe trial on the second day. This consisted of removal of the escape platform and testing the subject on a 60 second “free swim” 2 hours after the last place trial. Both the time spent swimming in the target quadrant and the number of crossings over the former platform location were quantified.

b2) the difficult version of place learning

All rats were trained 4 consecutive trials per day for 5 days. Task difficulty was increased by placing a curtain around the water maze, with the room indirectly lighted by a single 60 watt red light bulb, thus leaving few cues to aid navigation. Rats were allowed to remain on the platform for 10 seconds after each trial. Probe trials were administered 2 hours after the last trial of the daily four-trial series.

c) Learning Set Acquisition Testing

In the learning set acquisition phase, the animals were required to learn a new location of the escape platform on each day of testing. Testing consisted of 5 consecutive days and began on post-drug exposure day 46 and continued through day 50. All animals received 4 consecutive trials per day. The averaged performance on Trial 2 of each day was an index of spatial working (short-term) memory because the animal is required to recall its response on the trial immediately preceding the current one. The rats were allowed to sit on the platform for 15 seconds at the completion of each trial.

4) Nonspatial Response Learning

We employed a Greek-cross response learning task similar to that discussed elsewhere (Compton, Selinger, Westman, and Otero, 2011) to assess nonspatial response learning and perseverative behaviour. Here, the animal was faced with three response alternatives - to turn right, to turn left, or to swim straight ahead. Using a Fellows series (Fellows, 1967), the order in which the animals were placed at one of the two starting points was randomized. Consistent with earlier tests, all trials in this phase began by lowering the animal to the surface of the water while facing the wall of the tank. Therefore, the animal was required to turn 180° and swim towards the choice point, located in either the left- or right-hand arm of the Greek cross.

Within a given set of trials, the configuration of the available allocentric information differed as a function of each trial (McDaniel *et al.*, 1995). In order to master the task, (i.e., “turn left” vs. “turn right”), the animal must learn a rule to turn in a specific direction regardless of the start location. While the goal remained fixed for each animal and reversals were not considered here, the ability to flexibly adjust behaviour as function allocentric cues provided a reasonable test of perseverative behaviour.

III. RESULTS

A. General Activity

As seen in Figure 1, examination of the activity data revealed group differences in the number of squares traversed, $F(2, 18) = 5.41$, $p < .05$, $\eta_p^2 = .389$, but not in the number of rearings, $F(1, 18) = 0.91$, $p > .05$. Post hoc examination of this result

indicated that the 5 mg/kg ketamine-treated rats were significantly more active than either remaining groups, with the latter two not differing significantly.

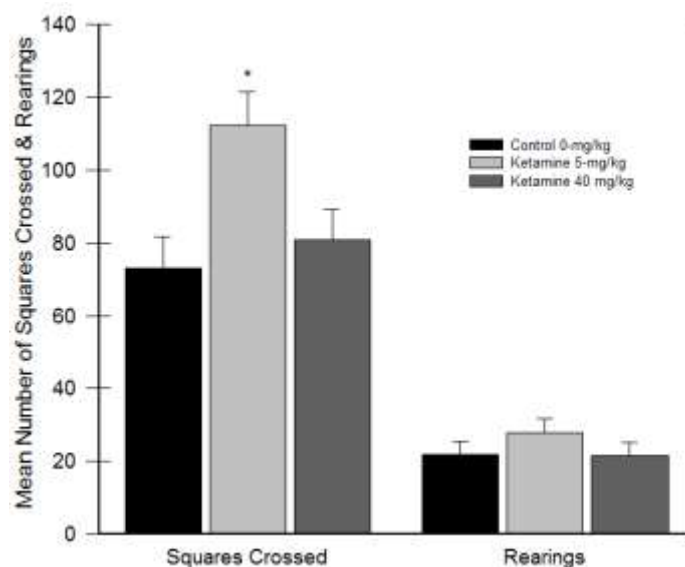


Fig 1 Activity of the two drug treated groups and the control group as defined by number of quadrants crossed and the number of rearings. *significant difference between groups ($p < .05$)

B. Step-Down Passive Avoidance Testing

The step-down avoidance data were analysed using a 3 (groups) X 2 (trials) mixed ANOVA. The results of the step-down passive avoidance test are presented in Figure 2. Step-down latencies as a function of group, $F(2, 18) = 8.75$, $p < .005$, $\eta_p^2 = .493$, and trial, $F(2, 18) = 77.91$, $p < .001$, $\eta_p^2 = .812$, were observed. Post hoc examination of the groups indicated significant differences between the saline- and 40-mg/kg ketamine rats. Latencies for the 5 mg/kg rats were intermediate between the two but not significantly different from either of the other two groups. In addition, a significant group X trial interaction, $F(2, 18) = 10.79$, $p < .001$, $\eta_p^2 = .545$, was found, indicating that group latencies differed as a function of trial. Post hoc examination of the data presented in Figure 1 indicated that step-down latencies were significantly higher for day two in the saline and 5 mg/kg ketamine-treated animals but not in the animals treated with 40 mg/kg of ketamine.

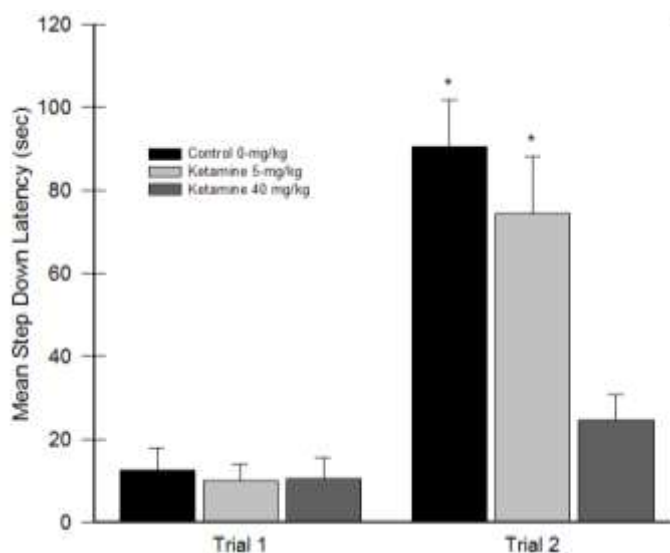


Fig. 2 Step-down passive avoidance learning for the Ketamine and control Groups. *significant change ($p < .05$) in step-down latency on trial 2

C. Cued Water Maze Task

For the assessment of cued navigation, the data were analysed using a 3 (groups) X 4 (blocks) of trials mixed ANOVA. Cued spatial navigation in the Morris water maze presented no difficulty for any of the groups. Only a main effect of blocks of trials was significant, $F(3, 51) = 8.90$, $p < .001$, $\eta_p^2 = .344$, with latencies to the escape platform predictably decreasing as a function of experience.

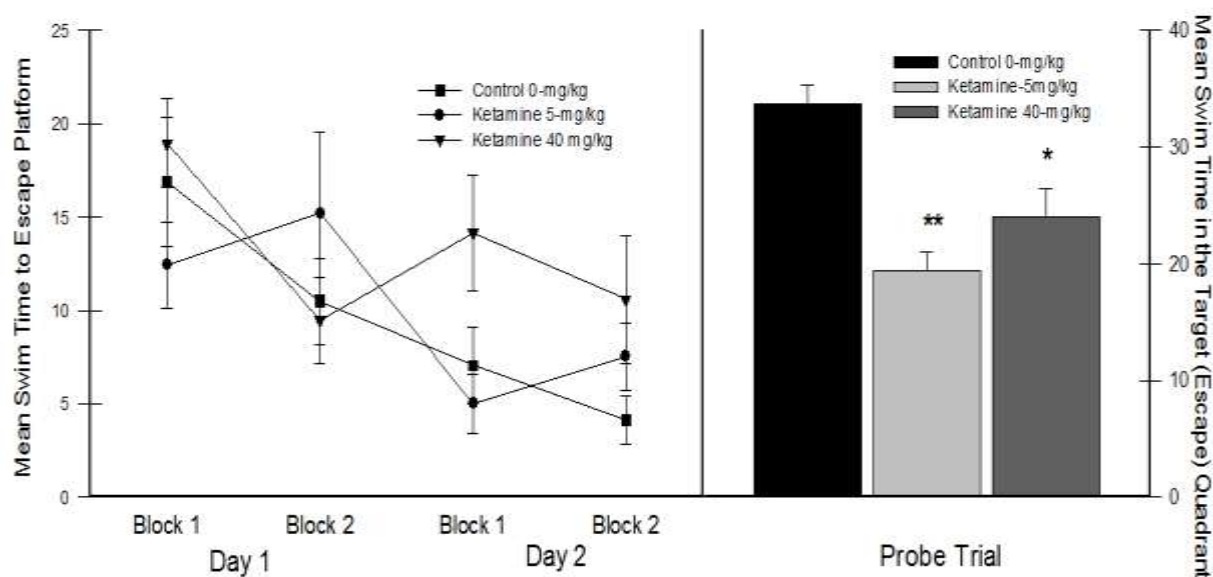


Fig. 3 Easy place learning results. The left panel reflects escape performance over the 4 blocks of 2 test days. The right panel is a presentation of the time spent in the target quadrant during probe testing. Vertical bars represent the standard error of the mean. *significantly different from the control group ($p < .05$). **significantly different from the 40 mg/kg ketamine group

D. The Easy Version of Place Learning

Consideration of the data from the easy place learning task revealed the following. Analysis of the data with a 3 (drug groups) X 4 (blocks) mixed ANOVA indicated a nonsignificant main effect of drug but a significant main effect of blocks of trials, $F(3, 51) = 10.80$, $p < .001$, $\eta_p^2 = .388$, suggesting that swim times generally improved as a function of training. Of greater importance, a significant drug X blocks, $F(6, 51) = 3.01$, $p < .025$, $\eta_p^2 = .262$, interaction was detected. Further consideration of the two-way interactions revealed the following. By blocks three and four, swim times were significantly higher for the 40 mg/kg ketamine-treated rats than for the saline-treated. Swim times for the 5 mg/16.9kg ketamine-treated animals were generally similar (albeit somewhat higher) to those of the saline treated rats, although the 5mg/kg and 40 mg/kg ketamine groups differed significantly on the third block of training.

When the probe trial was considered, a ketamine exposure effect was found, $F(2,17) = 13.58$, $p < .001$, $\eta_p^2 = .615$. Here, the saline-treated animals spent significantly more time in the target quadrant than either ketamine-treated group. Interestingly, the 5 mg/kg ketamine-treated animals spent significantly less time in the target quadrant than the 40 mg/kg ketamine-treated rats. Easy place learning results are shown in Figure 3 below.

E. The Difficult Version of Place Learning

For the assessment of the complex place learning data, the four daily trials were normalized and averaged and the navigation performance was assessed over a five-day period. Using a 1-Between (drug groups), 1-Within (days) ANOVA, the analysis indicated only a main effect of test days, $F(4, 68) = 11.66$, $p < .001$, $\eta_p^2 = .407$, suggesting that collectively the swim times generally decreased across the five-day test period. However, the drug group X test days interaction was nonsignificant, $F(8, 68) = 0.97$, $p = .469$.

Nonetheless, when the probe trial data were considered a quite different behavioural pattern emerged. Although the main effect of drug groups was nonsignificant, a main effect of days was found, $F(4, 68) = 15.90$, $p < .001$, $\eta_p^2 = .483$. More important here, however, was a significant drug group X days interaction, $F(8, 68) = 6.26$, $p < .001$, $\eta_p^2 = .424$. Decomposition of this result with Tukey tests indicated that the saline-treated rats spent more time in the target quadrant on days two through four than either of the two groups, which generally did not differ significantly. However, by day five of probe testing, all groups responded in a similar manner. Different place learning results are shown in Figure 4 below.

F. Set Acquisition Testing

The swim time data associated with the MWM learning set task is presented in Figure 5. Here, data from the first and last days of testing were considered. Although the main effects of drug group and days were nonsignificant, the main effect of trials was significant, $F(3, 45) = 39.82$, $p < .001$, $\eta_p^2 = .701$. Thus, while across trials the swim times for the three groups were comparable and they were able to respond on trial two on the basis of what they learned on the first trial. Specifically, by day five of testing, paired t -tests for each group indicated that swim times were significantly shorter on trial two than trial one, smallest $t(5) = -9.68$, $p < .001$. Visually, a three-way interaction was suggested but was nonsignificant, $F(3, 45) = 3.25$, $p = .064$.

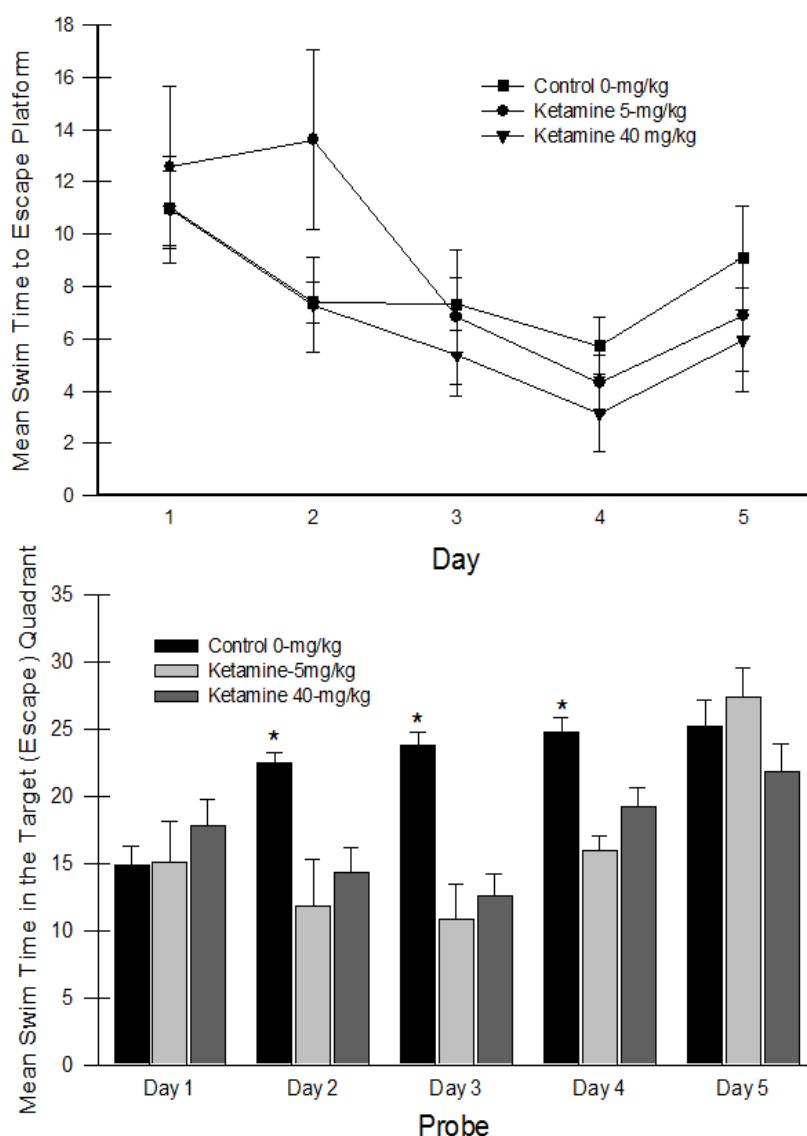


Fig. 4 Difficult place learning results. The top panel reflects escape performance over the 5 test days. The bottom panel is a presentation of the time spent in the target quadrant during probe testing. Vertical bars represent the standard error of the mean. *significant difference between groups ($p < .05$)

G. Learning Set Acquisition Testing

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H. Nonspatial Response Learning

Training in the Greek-cross version of the Morris water maze began immediately following spatial learning set testing and continued five days per week until a criterion of nine errorless escape trials occurred within a single training session or a ceiling of 100 trials was attained. Beginning with the total number of errors, all errors were scored as either reference or working memory errors as defined by others (Kesner, DiMattia, and Crutcher, 1987; McDaniel *et al.*, 1995). Reference memory errors were scored whenever an animal initially entered an incorrect alley. Working memory errors were defined as re-entries into incorrect alleys. Because a given alley has previously been explored within the trial in the Greek cross task, working memory errors as defined here are considered indicative of perseverative behaviour. Using a one-way multivariate analysis of variance (MANOVA), the task errors were analysed in terms of working memory, reference memory, and total errors.

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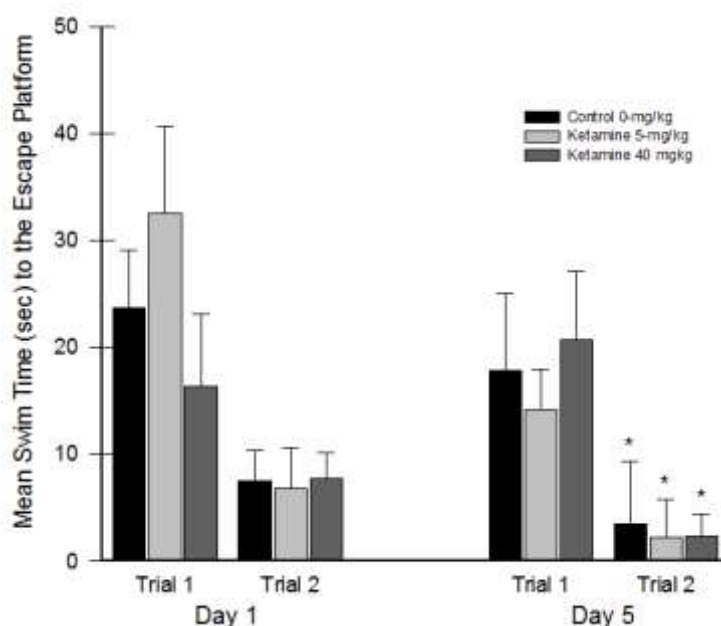


Fig. 5 Bar graphs depicting the mean and standard error of the mean trial one versus trial two performance on the learning set task at the beginning and end of testing

The overall MANOVA was significant, Wilk's $\lambda = .971$, approximate $F(6, 30) = 14.29$, $p < .001$, $\eta_p^2 = .741$. Closer examination of each dependent variable revealed the following. As can be seen in Figure 6 (panel C), a significant drug effect was observed for total errors, $F(2, 17) = 90.56$, $p < .001$, $\eta_p^2 = .914$, as well as for reference, $F(2, 17) = 36.64$, $p < .001$, $\eta_p^2 = .812$, and working memory, $F(2, 17) = 89.64$, $p < .001$, $\eta_p^2 = .913$, errors (see Figure 6, panels A & B). Although a visual examination of the data suggested that the errors appeared higher for both ketamine-treated groups, Tukey tests indicated that all types of errors were significantly higher in only the 40 mg/kg ketamine-treated group (see Figure 6).

IV. DISCUSSION

In the present investigation, we used a number of spatial and nonspatial tasks to provide an assessment of the effects of chronic exposure to ketamine on memory and cognition. The finding that neither the 5- or 40-mg/kg dose of ketamine interfered with escape to the visible platform during the cued learning phase indicates that the above effects are not due to a gross motor deficit or sensory impairment. The results presented here suggest that chronic exposure to ketamine adversely impacts performance on a number of tests of memory and, to some extent, the effects of the exposure is dose-dependent. As such, the present results are consistent with that reported among human ketamine users (Curran and Monaghan, 2001; Curran and Morgan, 2000; Morgan, Muetzelfeldt, and Curran, 2009; Morgan, Perry, Cho, Krystal, D'Souza, 2006; Morgan, Rossell *et al.*, 2006; Narendran *et al.*, 2005; Newcomer *et al.*, 1999). The clinical implications of our results require additional research before clear conclusions can be drawn. Nevertheless, observations linking adult learning anomalies with multiple childhood surgeries (Dimaggio, Sun, and Li, 2011) lend credence to the idea that ketamine can produce long-lasting and perhaps permanent changes in cognitive function and neuronal apoptosis (Huang, Liu, Jin, Ji, and Dong, 2012).

Although there are reports on the effects of the behavioural effects of noncompetitive NMDA antagonists in nonhuman primates, much less is known about the effects of ketamine than other compounds (e.g., PCP; Taffe, Davis, Gutierrez, and Gold, 2002). In one recent report, ketamine decreased response rates in a progressive ratio operant task. The results were similar to previous reports on the effects of PCP, dizocilpine and ketamine (Brady, Balster, Meltzer, and Schwertz, 1980; Byrd, Standish, and Howell, 1987; Paule, 1994; Taffe, *et al.*, 2002). In the Taffe *et al.* investigation, when the subjects were tested on a delayed matching-to-sample procedure, ketamine fuelled an enhanced rate of forgetting in a dose dependent fashion. In sum,

the nonhuman primate research appears to be consistent with that of investigations involving humans (Taffe, Davis, Gutierrez, and Gold, 2002).

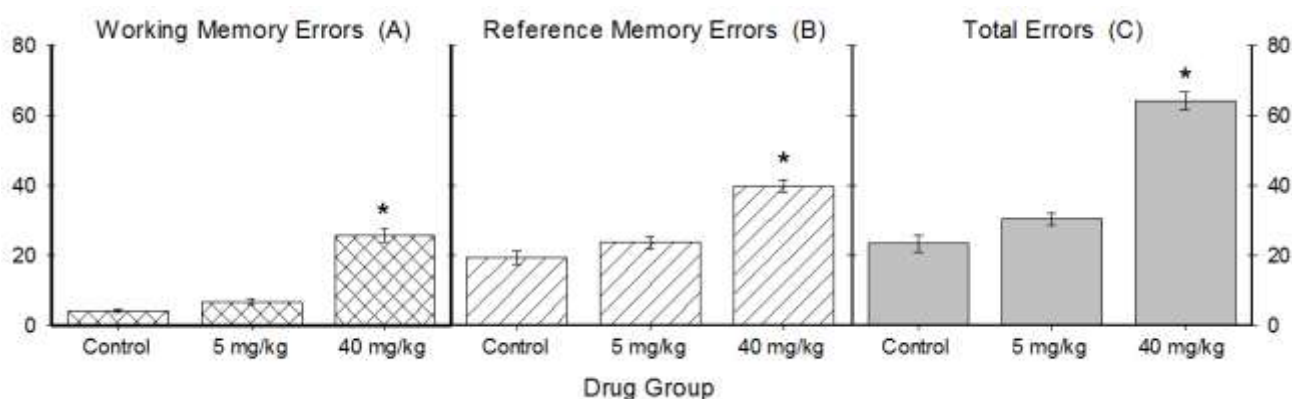


Fig. 6 Bar graphs depicting the mean and standard error of the mean for (a) working memory errors, defined as re-entry errors, (b) reference memory errors, defined as initial alley entrance errors, and (c) total errors. *Significantly different ($p < .05$) from control animals.

As noted earlier, ketamine has an affinity for more than one receptor in the brain. The available evidence suggests that ketamine, at both sub anaesthetic and anaesthetic concentrations, influences the release of a number of neurotransmitters including acetylcholine (Nelson, Burk, Bruno, and Sarter, 2002), histamine (Fell *et al.*, 2010), serotonin (Lindfors, Barati, and O'Connor, 1999), and norepinephrine (Kubota *et al.*, 1999). Ketamine is also capable of promoting a dose-dependent increase in extracellular levels of dopamine within the medial prefrontal cortex (Kamiyama *et al.*, 2011). Such findings are relevant in to our results as the 5-mg/kg rats performed worse than the 40-mg/kg rats on elements of the easy place learning task. As ketamine has been reported to offer neuroprotective effects by acting as an NMDA receptor antagonist (Green and Coté, 2009), it seems reasonable to postulate quite different behavioral and physiological effects as a function of different molecular targets with different affinities (Kotermanski, Johnson, and Thiels, 2013).

Learning experiences and the formation of long-term memory require a number of biochemical and structural synaptic changes at synapses including alterations in gene expression and function (Carlson, 2010). Two types of LTP have been described, an early phase LTP which may last from minutes to about an hour and a late phase LTP which lasts for a significantly longer period of time (Dozmorov *et al.*, 2006; Zorumski and Izumi, 2012). A number of enzymatic and transcription factors appear to drive synaptic plasticity including cAMP response element binding protein (CREB), cAMP/protein kinase A (PKA), mitogen-activated protein-kinase (MAPK), and Ca^{++} /calmodulin-dependent kinase II/IV (CaMKII/IV) (Kandel, 2001; Lisman, Schulman, and Cline, 2002; Malenka and Nicoll, 1999; Miyamoto, 2006; Nguyen and Woo, 2004). NMDA and AMPA glutamate receptors are implicated in LTP (Carlson, 2010). As a consequence, any process (e.g., drugs, aging) that decreases transcription of NMDA and AMPA subunits resulting from a reduction in CREB signaling presumably would directly impact the formation of late phase LTP formation (Hanson and Zhang, 2013). Therefore, the ability of the organism to encode and consolidate new memories and form stable long-term memories would be affected. For example, Bourtschuladze *et al.* (1994) demonstrated that CREB knockdown mice exhibit impaired spatial memory performance in the Morris water maze. In this study, long-term memory was affected by LTP, even though recordings of short-term enhanced activity were normal. Conversely, LTM in mice has been facilitated following CREB overexpression (Brightwell, Smith, Neve, and Colombo, 2007). Thus, the effects of chronic ketamine exposure could very well adversely impact the expression of genes in areas such as the hippocampal formation implicated in learning and in long-term memory formation (Chen *et al.*, 2005; Sakai *et al.*, 2000; Yu *et al.*, 2007) such as CREB (Cammara *et al.*, 2000) and brain-derived neurotrophic factor (bDNF, Lubin, 2011).

Recently, it has been shown that chronic exposure to ketamine produces a significant increase in hyperphosphorylated tau protein (Yeung *et al.*, 2010). In this study, ketamine-treated mice (30 mg/kg) and monkeys (1 mg/kg iv.) were treated for periods up to and including 6 months. Relative to that of controls, hyperphosphorylated tau proteins were detected in the prefrontal and entorhinal cortices of ketamine-treated animals. Of greater importance, many of the identified cells were TUNEL positive suggesting a relationship between the hyperphosphorylation and apoptosis. Associated with neuronal microtubules, hyperphosphorylated Tau proteins are associated with the formation of neurofibrillary tangles seen in Alzheimer's disease (Augustinack, Schneider, Mandelkow, and Hyman, 2002; Huang and Jiang, 2009; Yeung *et al.*, 2010).

Recurrent exposure to NMDA antagonists produces neuronal degeneration in many areas of the brain including cortic limbic areas such as the hippocampus and amygdala as well as the posterior cingulate area (Olney *et al.*, 1991). Persistent deficits in cognitive function are also found (Morgan and Curran, 2012; Wesierska, Macias-Gonzalez, and Bures, 1990). In addition, neurotoxicity has been observed in rats administered repeated doses of ketamine (Olney *et al.*, 1989).

The step-down passive avoidance results stand in contrast to those reported by others (Wang, Fu, Wilson, and Ma, 2006; see also, Uchihashi, Kuribara, Isa, Morita, and Sato, 1994). Unlike the MWM tasks employed in the present study, the step-

down passive avoidance task involves a punished active response and rewarding response inhibition (Maren, 2008). On the basis of studies involving lesions of the amygdala (e.g., Liang *et al.*, 1982; Jellestad and Bakke, 1985), it has been proposed that this structure, especially the central and lateral nuclei mediates the response (Tomaz, Dickinson-Anson, and McGaugh, 1992). As Maren (2008) noted, such observations fit with proposals that the central and lateral nuclei are involved the Pavlovian associations formed during a single-trial passive avoidance experience (see also, Maren and Quirk, 2004). Last, manipulation of hippocampal function via lesion, stimulation, or reversible inactivation impairs acquisition, storage, and retrieval of the memories associated with a passive avoidance task (Kesner and Hardy, 1983; Lorenzini, Baldi, Bucherelli, Sacchetti, and Tassoni, 1996; Ambrogi Lorenzini, Baldi, Bucherelli, Sacchetti, and Tassoni, 1997; Winocur and Bindra, 1976). While the amygdala is an important structure involved in Pavlovian aspects of passive avoidance experiences, the hippocampus is important as well, processing the contextual elements of the conditioning experience (Maren, 2008).

The results presented here alongside of those from other investigators (e.g., Yeung, Rudd, Lam, Mak, and Yew, 2009; Yeung *et al.*, 2010), suggest the possibility that ketamine can act as neurodegenerative agent if misused. The present results provide preliminary and convincing evidence that the chronic use of ketamine can have consequences that include but are not necessarily limited to long-term deleterious effects on learning and memory. Further research should explore the nature of ketamine toxicity, the path of neurodegeneration, and the developmental consequences associated with use at different time points in the lifespan of those exposed to the drug. Because of the variety of neural and neurotransmitter-mediated effects outlined earlier, drugs of abuse such as ketamine should be examined in greater detail for the possible consequences associated with chronic use, especially among a vulnerable teenage population.

V. CONCLUSIONS

The data reported in the present study suggest that chronic exposure to ketamine adversely impacts performance on a number of tests of memory and, to some extent, the effects of the exposure is dose-dependent. As such, the present results are consistent with that reported among human ketamine users. Further research should further explore the nature of ketamine toxicity, the path of neurodegeneration, and the consequences associated with use at different developmental time points in the lifespan of those exposed to the drug.

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