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Ketamine administration diminishes operant responding but does not impair conditioned fear

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ABSTRACT

While not well understood, the NMDA (*N-methyl-$_D$-aspartate*) antagonist ketamine, a dissociative anesthetic, has been reported to be efficacious in depression and related psychological disorders. Conditioned fear is a normal emotional conditioning process that is known to become dysfunctional in individuals suffering from Post-Traumatic Stress Disorder (PTSD) and related stress disorders. We examined the effects of ketamine to determine the potential modulation of the acquisition and extinction of a conditioned fear using a conditioned suppression procedure. Rats were trained on a variable interval (VI), food maintained, operant conditioning task to establish a general measure of performance. Rats were exposed to inescapable shock (IES, unconditioned stimulus) paired (×20) with an audio/visual conditioned stimulus (CS) to establish conditioning. Conditioning was quantified by measuring response suppression following CS presentation during subsequent extinction trials where the CS alone was presented. Ketamine or vehicle was administered either after initial conditioning or after each of the subsequent extinction trials. For each regimen, a series of four injections were administered 60 min apart (100, 50, 50, 50 mg/kg, respectively) in order to sustain a ketamine effect for a minimum of 4 h. Ketamine produced a general decrease in responding on the VI, relative to baseline, as response rates were slower on the operant task when tested 24 h later and longer. Ketamine did not affect the acquisition of the conditioned fear when the regimen was administered shortly after the initial pairings of IES and CS. Ketamine did not alter extinction to the conditioned fear when the regimen was administered following each CS only presentation following initial conditioning. Our conclusion from these findings is that while ketamine alters behavior on an appetitively motivated operant task it does not, however, appear to directly modulate learning and memory processes associated with conditioned fear.

1. Introduction

The NMDA (*N-methyl-$_D$-aspartate*) receptor antagonist, ketamine, is a short-acting dissociative anesthetic that has been shown to have transient cognitive effects, including amnesic effects (Mickley et al., 1998; Moosavi et al., 2012; Wang et al., 2006). It is used as a veterinary tranquilizer (Carter and Story, 2013), human anesthetic in special cases (Haas and Harper, 1992), in post-operative and battlefield pain management (Jouguelet-Lacoste et al., 2015; Plunkett et al., 2012), and as a treatment for depression (Dutta et al., 2015). Due to its anesthetic and antinociceptic effects, ketamine is used in procedure-related pain or trauma and can be beneficial for postoperative analgesia (Jouguelet-Lacoste et al., 2015). It has become a first-line treatment in burn patients not only because of its sedative analgesic effect, but it also allows for preservation of the airway reflexes, and does not affect the heart rate or blood pressure (Richardson and Mustard, 2009; White et al., 1982). Injuries on the battlefield, often of polytrauma nature, can rapidly become lethal if there is cardiorespiratory depression, however, ketamine allows analgesia without the adverse effect that are seen with opioids (e.g., morphine), such as hypotension and respiratory depression (Plunkett et al., 2012). Use of ketamine can lead to a reduction of opioid consumption following surgery (Bell et al., 2006) and in individuals with extreme opioid tolerance (Cohen and DeJesus, 2004).

Ketamine has also been used as a treatment for various types of depression and is reported to provide rapid symptom relief and antidepressant effect with a single dose (Berman et al., 2000; Diazgranados et al., 2010; Zarate et al., 2006) or repeated doses (Correll and Futter, 2006; Murrough et al., 2013); however, symptom reduction was short-lived and returned to baseline levels shortly after treatment within hours or days. Ketamine has been reported to produce a rapid reduction in suicidal ideations (Larkin and Beautrais, 2011; Price et al., 2009). Ketamine has also been tested for therapeutic efficacy in chronic PTSD, resulting in significant and rapid reduction of the PTSD symptoms and comorbid depressive symptoms (Feder et al., 2014). Other pharmacotherapies, specifically SSRIs (selective serotonin reuptake inhibiters) and Tricyclic antidepressants, have been reported to provide a therapeutic effect on PTSD symptom severity including comorbid depression and anxiety symptoms in combat veterans (Puetz et al., 2015). On the

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other hand, ketamine has abuse liability and frequent, long-term ketamine users showed persistent psychopathology that included impairments in working memory, episodic memory, executive functioning, and psychological well-being (Morgan et al., 2009). Additionally, ketamine abuse has been reported to have residual effects for multiple days after ketamine use has ended (Curran and Morgan, 2000).

Verbal memory in humans is modifiable by ketamine (Krystal et al., 1994; Newcomer et al., 1999; Parwani et al., 2005). Ketamine can block the formation of conditioned taste aversion (CTA) in fetal and neonate rats (Jackson and Sanger, 1989; Mickley et al., 1995, 1998), altering acquisition and expression of aversion but it does not appear to alter the animals' ability to sense taste (e.g., sweetness of saccharine and malaise of lithium chloride; Mickley et al., 2002; Welzl et al., 1990). In the water maze task, ketamine impaired memory acquisition and retrieval at subanesthetic (15 mg/kg) and anesthetic (100 mg/kg) doses, but only anesthetic (100 mg/kg) doses affected consolidation (Liu et al., 2014; Moosavi et al., 2012). Memory reconsolidation was disrupted in the T-maze by anesthetic (125–150 mg/kg), but not subanesthetic (5, 10, 20, 50 mg/kg) doses of ketamine (Wang et al., 2006). Anesthetic (100 mg/kg) ketamine dosing disrupts rodent performance on object location (Pitsikas and Boultadakis, 2009) and object recognition (Boultadakis and Pitsikas, 2011; Goulart et al., 2010), which is also affected by a subanesthetic (20 mg/kg) dose (Goulart et al., 2010).

Ketamine's role in the disruption of memory acquisition, consolidation, and retrieval is still being studied but appears to be dose and task, or potentially brain region, dependent. These effects are believed to be due to NMDA receptor antagonism, as NMDA receptors play an important role in long-term potentiation (LTP; e.g., Harris et al., 1984; Volianskis et al., 2015). NMDA receptor activation has been shown to play a role in fear extinction learning (e.g., Santini et al., 2001) and has been suggested to potentially play a role in intrusive memory formation (Perugini et al., 2012), thus NMDA receptors are an important target for PSTD pharmacotherapy. It is notable, however, that ketamine has multiple non-NMDA effects, including nicotinic and muscarinic cholinergic, monoaminergic and opioid receptor activity (Kohrs and Durieux, 1998).

We further evaluated the behavioral effects of ketamine in rats. We were interested in determining if ketamine would disrupt the consolidation or reconsolidation of events associated with conditioned fear. In addition to evaluating potential amnestic effects of ketamine in general, we were specifically interested in evaluating ketamine in the context of emotional conditioning process known to become dysfunctional in PTSD and other stress disorders. We used a well-defined rodent model of conditioned fear, using the conditioned suppression method (Estes and Skinner, 1941). The procedure allows for the concurrent evaluation of general performance effects on an operant conditioning task and also provides an objective measure of conditioned fear. A ketamine regimen was administered either after initial conditioning or after subsequent extinction. Conditioned suppression is a superset of the conditioned freezing model and has been shown to involve additional conditioning processes (Amorapanth et al., 1999; Lee et al., 2005; McDannald, 2010; McDannald and Galarce, 2011; Pickens et al., 2010).

2. Materials and methods

2.1. Subjects

60 adult male Sprague–Dawley rats were used from Charles River Laboratories (CRL, Wilmington, MA). Rats were individually housed in an environmentally controlled room with a 12h:12h light:dark cycle. Subjects were placed on mild food restriction and maintained at a body weight of approximately 330 g; access to water was unrestricted. Supplemental food was administered during the experimental sessions as the behavioral procedure requires appetitive motivation.

All procedures were reviewed and approved by the WRAIR Institutional Animal Care and Use Committee, and performed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition.

2.2. Experimental design and pharmacological treatment

Rats were injected with ketamine or vehicle with specific regimens and timeline of administration dependent upon the treatment group to which the rats were assigned (Table 1). The experimental timeline of the study is outlined in Fig. 1. On treatment days, rats received four injections of ketamine hydrochloride (Ketaset®, 100 mg/mL) at a dose regimen of 100, 50, 50, 50 mg/kg or vehicle (isotonic sterile saline), with injections spaced at 60 min intervals (1/h × 4).

The injections of ketamine were intended to sustain a substantial sedative effect of ketamine activity, but not to produce a continuous state of deep anesthesia. Injections were spaced to keep the rat affected for several hours to optimize the appropriate blockage of consolidation and reconsolidation of a conditioned fear, reflecting the estimated plasma half-life of ketamine in Sprague–Dawley rats of approximately 60 min (White et al., 1976). Consolidation of memory is believed to occur within the first 4 hours after an event (McGaugh, 2000). This dose regimen was adapted from a previous study using high and low doses of ketamine (Genovese et al., 2014). Injections were administered using intramuscular (i.m.) and intraperitoneal (i.p.) routes, in a volume not exceeding 1.0 mL per kg body weight. In all regimens, the first injection was administered i.m. in a split concentration into the caudal thigh/biceps muscle (half dose in each leg) and the three subsequent injections were administered i.p. on alternating sides of the body.

2.3. Operant conditioning

All rats were trained on a variable interval 32 second (VI32) schedule of reinforcement. Rodent operant conditioning chambers (internal testing area: 12″L × 9.5″W × 18.25″H; Med Associates Inc., St. Albans, VT) which were housed inside ventilated, sound-and-light attenuating cabinets (25″L × 15.5″W × 16.5″H) were used for conditioning sessions. The inner chambers contained two response levers and a food trough, all attached to a food dispenser that delivered 45 mg food pellets (Bio-Serv, Flemington, NJ). Additional features of the chamber included a house light mounted near the top, stimulus lights mounted above each of the response levers, and a Sonalert tone generator (2,900 Hz).

There were two response levers in each chamber, though only one lever was designated as the active lever, which was programmed to produce food reinforcement via delivery of a single 45 mg food pellet. The design was counterbalanced so that an equal number of boxes possessed the active lever on the left and on the right. Rats were initially trained on a continuous schedule of reinforcement, and once the active lever association was developed, the schedule of reinforcement was changed to the VI32. A single active lever press following a specified interval, on average 32 s, produced food reinforcement of a single food pellet. Interval values for the schedule were chosen pseudo-randomly, without replacement, from normal distributions (range = 2.44–198.23 s). Sessions lasted 30 min and were conducted daily (Monday–Friday).

VI32 training continued until response rates became stable, as determined by no more than a 20% deviation in the 5-day moving average over 5–10 consecutive sessions and by inspection of the individual cumulative response records. Rats were assigned to treatment groups balanced for response rate, such that there was a similar average rate of responding, before treatment, for each group. A minimum of 35 training sessions were conducted by each subject.