Dose-response characteristics of intravenous ketamine on dissociative stereotypy, locomotion, sensorimotor gating, and nociception in male Sprague-Dawley rats

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Abstract

Clinicians administer subanesthetic intravenous (IV) ketamine infusions for treatment of refractory depression, chronic pain, and post-traumatic stress disorder in humans. However, ketamine is administered via the subcutaneous (SC) or intraperitoneal (IP) routes to rodents in most pre-clinical research, which may limit translational application. The present study characterized the dose-response of a subanesthetic IV ketamine bolus (2 and 5 mg/kg) and 1-hr infusion (5, 10, and 20 mg/kg/h) on dissociative stereotypy, locomotion, sensorimotor gating, and thermal nociception in male Sprague-Dawley rats. The secondary aim was to measure ketamine and norketamine plasma concentrations following IV ketamine bolus at 1, 20, and 50 min and at the conclusion of the 1-hr infusion using liquid chromatography/mass spectrometry. The results showed that ketamine bolus and infusions produced dose-dependent dissociative stereotypy. Bolus (2 and 5 mg/kg) and 20 mg/kg/h infusion increased locomotor activity while 5 mg/kg/h infusion decreased locomotor activity. Both 10 and 20 mg/kg/h infusions reduced the acoustic startle reflex, while 5 mg/kg bolus and 20 mg/kg/h infusion impaired pre-pulse inhibition. Ketamine 5 mg/kg bolus and the 10 and 20 mg/kg/h infusions induced significant and prolonged antinociception to the hotplate test. Plasma concentrations of ketamine decreased quickly after bolus while norketamine levels increased from 1 to 20 min and plateaued from 20 to 50 min. The peak ketamine plasma concentrations [ng/mL] were similar between 5 mg/kg bolus [4100] vs. 20 mg/kg/h infusion [3900], and 2 mg/kg bolus [1700] vs. 10 mg/kg/h infusion [1500]. These results support the findings from previous ketamine injection studies and further validate the feasibility of administering subanesthetic doses of IV ketamine infusion to rats for neuropharmacological studies.

Keywords: ketamine, rat, intravenous, locomotor activity, dissociative stereotypy, sensorimotor gating, antinociception

1. Introduction

Ketamine, a non-competitive antagonist at the glutamatergic N-methyl-D-aspartate (NMDA) receptor, is a potent analgesic and dissociative anesthetic with many applications in both rodent behavioral and human psychiatric research. Pre-clinical researchers utilize ketamine in various rodent models of psychosis, depression, addiction, and pain while clinicians administer ketamine for treatment of refractory depression, post-traumatic stress disorder (PTSD), and chronic pain. Ketamine is typically administered via subcutaneous (SC) or intraperitoneal (IP) injection to rodents, but these delivery routes may not translate well to human studies and treatments that primarily use continuous intravenous (IV) ketamine infusions. Previous research has focused on evaluating the effects of IP and SC ketamine on rodent behaviors, but little is known regarding the dose-response characteristics of subanesthetic doses of ketamine administered by the IV route.

Dose-dependent changes in locomotion and stereotypic dissociative-like behaviors in rodents are observed following subanesthetic ketamine injections. At higher doses, ketamine induces dose-dependent hyper-locomotion (Danysz *et al.* , 1994, Hetzler and Swain Wautlet, 1985, Imre *et al.* , 2006) through increased dopamine turnover in limbic-striatal regions (Jentsch *et al.* , 1998, McCullough and Salamone, 1992, Steinpreis and Salamone, 1993). Interestingly, several studies have observed reduced locomotion following low-dose ketamine injections (Becker *et al.* , 2003, Kotermanski *et al.* , 2013, Trujillo *et al.* , 2011). Phencyclidine (PCP) and ketamine not only impact locomotion, but also produce other characteristic changes in rodent

behavior. Commonly described stereotypic behaviors include circling, side to side head motion, reduced rearing, ataxia, and reduced fine motor movements such as grooming (Danysz *et al.*, 1994, Hetzler and Swain Wautlet, 1985, Kotermanski *et al.*, 2013, Sturgeon *et al.*, 1979).

Similar to hyper-locomotion, dissociative stereotypy is mediated through dopaminergic pathways in striatal regions (Castellani and Adams, 1981b, Finnegan *et al.*, 1976, Glick *et al.*, 1976, Nabeshima *et al.*, 1983).

In addition to stereotypy and locomotion changes, ketamine impacts sensorimotor gating in both humans and rodents (Abel *et al.*, 2003, Geyer *et al.*, 2001). Pre-pulse inhibition (PPI), a measure of sensorimotor gating and an indirect measure of information processing, occurs when a weak stimulus precedes a startle stimulus and reduces the acoustic startle reflex (ASR).

Ketamine-induced disruption of PPI is well-described (Geyer *et al.*, 2001) and is used to model schizophrenia-like behaviors in rodent psychosis models; however, the effects of ketamine on the ASR are inconsistent. Most investigators show ketamine to have either no effect or a non-significant reduction in ASR (de Bruin *et al.*, 1999, Imre *et al.*, 2006, Palenicek *et al.*, 2011), while others report an increase in startle response (Cilia *et al.*, 2007).

Ketamine, a potent analgesic in humans (Prommer, 2012), produces dose-dependent antinociception in rodents at high doses (Hoffmann *et al.*, 2003, Smith *et al.*, 1980). Ketamine mediates antinociception via central and peripheral mechanisms primarily through NMDA antagonism and partially through opioid receptor agonism (Gupta *et al.*, 2011, Pacheco *et al.*, 2014). Although researchers have described dose-dependent increases in hotplate and tail-flick latencies in rodents (Sarton *et al.*, 2001, Smith *et al.*, 1980), there are limited reports that describe the duration of antinociception following ketamine administration. The impact of ketamine on nociception over time provides useful information to researchers regarding dose-

response characteristics and the duration required to determine optimum antinociceptive doses in rodents.

Overall, there is an abundance of literature characterizing rodent behaviors, information processing, and antinociception following ketamine injections, but there is little information describing the dose-response characteristics of subanesthetic IV ketamine and corresponding drug plasma concentrations in rodents. Therefore, our primary aim was to characterize the effects of subanesthetic IV ketamine administered as a bolus and continuous infusion on dissociative stereotypy, locomotor activity, ASR, PPI, and nociception in male Sprague-Dawley rats. Our secondary aim was to measure plasma concentrations of ketamine and norketamine, an active metabolite, following IV bolus at 1, 20, and 50 min and at the completion of a 1-h infusion to provide a comparison to previous rodent ketamine studies that used SC or IP injections. Based upon the previous literature, we hypothesized that administration of subanesthetic IV ketamine may dose-dependently increase dissociative stereotypy, locomotor activity, and antinociception while impairing PPI in rats.

2. Materials and Methods

2.1 Animals

Adult male Sprague-Dawley rats (Taconic Biosciences Inc., Hudson, NY) weighing 300 - 350 g were housed individually in clear Plexiglas shoebox cages (reversed 12-h light/dark cycle; testing during the dark cycle) in a climate-controlled environment with food and water *ad libitum*. Animals were handled daily to limit stress-responses during behavioral testing. All procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the Uniformed Services University of the Health Sciences.

2.2 Catheter Surgery

Rats were anesthetized with ketamine/xylazine (80 and 10 mg/kg, IP) and a jugular vein catheter was cannulated as previously described (Choi *et al.* , 2006). Briefly, the right jugular vein was isolated through a neck incision and a 3Fr polyurethane catheter (Instech, Plymouth Meeting, PA) was advanced into the vein. The remaining catheter exited via a back incision. The externalized catheter was made with blunt tip 22-gauge stainless steel cemented into place with bell-shaped dental cement. Catheters were flushed daily with a saline solution (0.2 mL) containing gentamycin (1 mg/ml) and heparin (10 units/ml). Animals were given a 7-day recovery period prior to testing.

2.3 Bolus Experiment

2.3.1 Drug and Dosing. Racemic (+/-) ketamine hydrochloride (100 mg/ml) (Mylan Institutional LLC, Rockford, IL) was diluted in 0.9% sterile saline daily prior to bolus and infusion experiments. A counter-balanced design of ketamine doses (saline, 2 mg/kg, and 5 mg/kg, IV) was used ($n = 8$ per dose). Animals were tested on Days 1 and 5 separated by a 4-day drug clearance. Control group animals received IV saline while experimental groups received ketamine doses 2 or 5 mg/kg IV immediately prior to behavioral testing. All doses were delivered in a 1 mL/kg volume.

2.3.2 Dissociative stereotypy. Following ketamine bolus, individual animals were placed in a clear Plexiglas shoebox cage (42.5 x 20.5 x 20 cm). Five stereotypic dissociative-like behaviors (circling, side to side head motion (weave), rearing, ataxia, and grooming) were video recorded (Sony DCR-SX44) over 10 min following ketamine administration (Figure 1). A study team member blinded to dosing watched the recorded video and tallied the frequency of each behavior and grouped the data into 2-min bins (5 bins total). Dissociative behaviors were chosen

based upon previous ketamine and PCP literature (Kotermanski *et al.*, 2013, Sturgeon *et al.*, 1979).

2.3.3 Locomotion. Following ketamine bolus, individual animals were placed in a clear Plexiglas shoebox cage (42.5 x 20.5 x 20 cm) marked with 9 equal sized floor grid-squares. Locomotion was video recorded (Sony DCR-SX44) over 15-min (Figure 1). A member of the study team blinded to ketamine dosing watched the recordings, counted the total number of square entries, and grouped the data into 3-min bins (5 bins total). A square entry consisted of both front and rear paws entering the square.

2.3.4 Sensorimotor Gating. ASR was measured with a Startle Response Acoustic Test System as previously described (Le *et al.* , 2014) for the bolus and infusion experiments. The ASR system consisted of 4 weight-sensitive platforms in 4 sound-attenuated chambers (Coulbourn Instruments, Columbus, OH). A ventilation chamber fan provided background noise during a 3-min acclimation period and the testing session. After acclimation, animals were exposed to 6 types of stimulus trials: 100 and 110 dB startle pulse (SP), 100 and 110 dB SP with an 84 dB pre-pulse (PP), 84 dB PP alone, and no stimulus. A piezoelectric accelerometer measured the maximum startle amplitude occurring within 200 ms of the onset of the SP. The interval between PP and SP was 100 ms and each trial type was presented 8 times. Those values were analyzed using the ROUTE method for outlier detection before calculating the mean using the GraphPad Prism software (ver 7.0). No animal was removed from the data as an outlier. Trial types were presented in random order with inter-trial intervals ranging from 15 - 25 s to avoid order effects of habituation. A desktop computer with ASR System software was used to present stimuli and record data. PPI was defined as the percentage reduction in mean startle amplitude in the presence of the PP compared to mean startle amplitude in the absence of PP: $100 - [100 \times$

(mean startle amplitude PP + SP trials/mean startle amplitude SP alone trials)]. Baseline ASR and PPI were measured 24-h before the testing. Subsequent ASR and PPI were measured 20 min after bolus dosing (Figure 1).

2.3.5 Thermal Nociception. Nociception was measured using hotplate latency (Omnitech/Accuscan Electronics Analgesia Monitor) for bolus and infusion experiments. The metal hotplate (26 x 26 cm) was enclosed on all sides by clear Plexiglas walls, covered with a removable Plexiglas top, and heated to 51°C. Latencies were measured as the time in seconds from placement on the heated surface until the animal raised and licked the hind paw. Animals that did not lick their hind paws during the 60 s period were removed from the surface. Two cohorts of rats were used to measure nociception. Cohort 1 ($n = 8$) was tested at 20, 30, 40, and 50 min after ketamine bolus while cohort 2 ($n = 8$) was tested at 70 and 90 min. The latencies from both cohorts were combined to show duration data from 20 - 90 min post bolus (Figure 1).

2.3.6 Blood Sampling. A separate group of rats were used to collect blood samples following ketamine administration. Blood was withdrawn (0.2 ml) to clear the catheter of medication and prime with fresh blood. Then 0.4 ml of blood was withdrawn and placed into 1.5 ml Eppendorf tubes with 10 μ L of ethylene-diamine-tetra-acetic-acid (EDTA) additive to prevent blood clotting. Blood samples were centrifuged at 4,000 rpm for 20 min at 4°C (Eppendorf 5415 R). Plasma supernatant (100 μ L) was collected for liquid chromatography/mass spectrometry (LCMS). The blood sample did not exceed volume limits recommended by the Animal Care and Use guidelines of the NIH (National Institutes of Health, 2015). Blood samples were collected at baseline and at 1, 20, and 50 min after ketamine bolus ($n = 6$ per dose) (Figure 1).

“Figure 1 here”

2.4 Infusion Experiment

2.4.1 Drug and Dosing. A separate group of animals from the bolus experiment underwent ketamine infusions in a counter-balanced design separated by a 4-day drug clearance between doses ($n = 16$ per dose). Each infusion trial began with a 5 mg/kg IV ketamine bolus to ensure plasma loading followed by a 1-h subanesthetic IV ketamine infusion at either 5mg/kg/h, 10mg/kg/h, or 20mg/kg/h delivered by syringe pump (Harvard, Holliston, MA). A separate control group not exposed to previous ketamine infusions ($n = 16$) received a 1-h saline infusion. Total 1-h infusion volumes did not exceed 1 mL.

2.4.2 Apparatus. Ketamine infusions were administered in an operant conditioning chamber (Med Associates Inc., St. Albans, VT). Chambers were equipped with a 10 ml plastic syringe and Teflon tubing connected to a fluid swivel (Instech, Plymouth Meeting, PA) encased in a metal spring wire. Rats were freely mobile within the operant chamber during the infusion. The room lights were off and a small red chamber light facilitated behavioral observation.

2.4.3 Dissociative Stereotypy. A study team member blinded to dosing observed and counted the frequency of dissociative stereotypy during the final 10 min of the 1-h infusion period as described in the bolus experiment (Figure 1).

2.4.4 Locomotion. Operant chambers were equipped with photo beams to facilitate counting locomotion during the infusion. The total number of beam breaks were recorded throughout the 1-h infusion period and grouped into 10-min bins (6 bins total) (Figure 1).

2.4.5 Sensorimotor Gating. Baseline ASR and PPI were measured at baseline and 30 min after completion of the 1-h infusion (Figure 1).

2.4.6 Thermal nociception. Hotplate latency was measured at baseline and 60, 80, 100, and 120 min after completion of the 1-h ketamine infusion (Figure 1).

2.4.7 Blood Sampling. Blood samples were collected at the conclusion of the 1-h ketamine infusion in the same manner as described in the bolus experiment (Figure 1).

2.5 Liquid Chromatography Mass Spectrometry

The LCMS protocol was developed based upon previous literature (Parkin *et al.* , 2008). Plasma samples were precipitated with 5 volumes of cold acetonitrile (500 μ L), allowed to stand on ice for 15 min, then spun at 13,000 rpm x 5 min. The supernatant (83%) was removed and dried in a Savant SpeedVac Concentrator. The pellets after drying were dissolved in a volume of 0.2% formic acid equal to the initial serum volume, then further diluted 50X, also with 0.2% formic acid. LCMS injections (5 μ L) were performed on an Agilent 1200 HPLC system feeding a Turbo V ion source on an AB Sciex Q-Trap 4000; a TARGA C18 column (100 X 2.1 mm, 3 μ m) with a flow rate of 200 μ L /min was used to separate ketamine and its metabolites. Multiple Reaction Monitoring transitions for ketamine (238.1 $\rightarrow$ 125.0 Da) and norketamine (224.1 $\rightarrow$ 125.0 Da) were run for 40 ms, and the identity of each analyte was confirmed with Enhanced Product Ion scans run at 238.1 and 224.1 Da, respectively. Peak areas were measured and converted to concentrations using calibration curves run over a range of 0.5 to 400 ng/ml (concentration in 50X dilutions of serum sample) for both analytes.

2.6 Statistical Analysis

All data are presented as means $\pm$ standard error of the mean (SEM) and were analyzed using GraphPad Prism (GraphPad Software, version 7.0) with *p* values < .05 considered significant. Locomotion and nociception data were analyzed with RM two-way analysis of variance (ANOVA), with time and ketamine dose as factors. Total locomotion, dissociative stereotypy, ASR/PPI, and LCMS data were analyzed using one-way ANOVA. Differences were compared with *post-hoc* Tukey's HSD.

3. Results

3.1 Bolus Experiment

3.1.1 Dissociative stereotypy. Dissociative behaviors were observed for 10 min after IV ketamine bolus. There were significant dose-dependent differences in stereotypy (Figure 2). Ketamine induced greater circling $F(2,21) = 36.7$ ($p < .001$), reduced rearing $F(2,21) = 34.1$ ($p < .001$), increased head weave $F(2,21) = 18.9$ ($p < .001$), increased ataxia $F(2,21) = 37.1$ ($p < .001$), and reduced grooming $F(2,21) = 21.3$ ($p < .001$) compared to saline. Ketamine 5 mg/kg bolus induced each dissociative behavior ($p < .001$) while 2 mg/kg reduced rearing and grooming behaviors ($p < .001$) and spared ataxia, circling, and head weave. Circling, ataxia, and head weave resolved within the first 6 minutes after the 5 mg/kg dosing (Figure 2); however, reduced rearing and grooming behaviors persisted throughout the 10 min observation period for both ketamine doses.

“Figure 2 here”

3.1.2 Locomotion. Locomotor activity was measured over 15 min following an IV ketamine bolus. Ketamine significantly increased total locomotor activity, $F(2,21) = 5.23$ ($p < .05$). Post-hoc testing showed that ketamine 5 mg/kg significantly increased total locomotion vs. saline (Figure 3a). The effects of ketamine on locomotion over time were also compared (Figure 3b). There were significant main effects of dose $F(2,69) = 12.19$ ($p < .001$), time $F(4,276) = 66.04$ ($p < .001$), and time x dose interaction $F(8,276) = 33.55$ ($p < .001$). Post-hoc testing showed that ketamine 2 mg/kg induced immediate hyper-locomotion between 0-3 min while the 5 mg/kg dose induced delayed hyper-locomotion between 4-9 min. Interestingly, ketamine

treated rats trended towards hypo-locomotion by 15 min, but did not reach significance. Overall, the stimulatory effects of ketamine were dose dependent as 2 mg/kg caused immediate hyper-locomotion, while 5 mg/kg produced a delayed and extended hyper-locomotion response with greater total activity.

“Figure 3 here”

3.1.3 Sensorimotor gating. The effects of ketamine on ASR and PPI measured 20 min after IV bolus were compared. There were no differences in ASR and PPI results across groups at baseline. Ketamine had no effect on maximum startle amplitude at 100 dB $F(2,21) = 0.212$ ($p = 0.8$) (Figure 3c). However, ketamine impaired the PPI of 100 dB, $F(2,21) = 4.89$ ($p = .018$) (Figure 3d). Post-hoc testing showed ketamine 5 mg/kg significantly impaired the PPI of 100 dB compared to saline ($p < .05$) while 2 mg/kg showed no effect.

3.1.4 Thermal nociception. Hotplate latency was measured from 20 to 90 min after IV ketamine bolus. There were no differences in baseline latencies between groups. There were significant main effects of ketamine dose, $F(2,14) = 14.68$ ($p < .001$) and time, $F(5,70) = 5.265$, ($p < .001$) and no significant dose x time interaction. Post-hoc testing showed ketamine 5 mg/kg produced significant antinociception between 20 - 50 min vs. saline (Figure 4a). Although the 2 mg/kg bolus latencies were elevated between 20 - 40 min, the differences did not reach significance. Overall, ketamine 5 mg/kg produced antinociception up to 50 minutes after a bolus injection.

“Figure 4 here”

3.1.5 Plasma concentration. Ketamine and norketamine plasma concentrations were measured at serial time-points after IV bolus using LCMS (Table 1). Ketamine 5 mg/kg produced significantly greater plasma concentrations of ketamine at each time point compared to 2 mg/kg. As expected, there were no differences between norketamine concentrations at 1 min as ketamine had little time to metabolize. Norketamine concentrations increased from 1 to 20 min and essentially plateaued from 20 to 50 min for both dosages. The 5 mg/kg bolus produced significantly greater norketamine concentrations at 50 min.

“Table 1 here”

3.2 Infusion Experiment

3.2.1 Dissociative stereotypy. Dissociative behaviors were observed over the final 10 min of a ketamine infusion. There were significant dose-dependent differences in circling $F(3,28) = 6.48$ ($p = .002$), rearing episodes $F(3,28) = 14.3$ ($p < .001$), head weave $F(3,28) = 4.87$ ($p = 0.008$), ataxia $F(3,28) = 128$ ($p < .001$), and grooming $F(3,28) = 25.3$ ($p < .001$). Rats that received ketamine at 20 mg/kg/h demonstrated each dissociative behavior (increased circling, reduced rearing, increased head weave, increased ataxia, and reduced grooming), while rats dosed at 5 and 10 mg/kg/h demonstrated reduced rearing and grooming (Figure 5).

“Figure 5 here”

3.2.2 Locomotion. Locomotor activity was measured throughout 1-h ketamine infusions. Significant differences were observed across the ketamine groups in total locomotion, $F(3,60) = 19.03$ ($p < .001$). Post-hoc testing showed ketamine 20 mg/kg/h significantly increased total activity vs. saline ($p < .001$) while 5 mg/kg/h reduced activity ($p < .05$) (Figure 6a). Next, the effects of ketamine on activity over time were measured (Figure 6b). There were significant main effects of dose $F(3,62) = 20.65$ ($p < .001$), time $F(5, 310) = 111.1$ ($p < .001$), and a time x dose interaction $F(15,310) = 4.883$ ($p < .001$). All ketamine doses showed increased activity vs. saline vehicle from 0-10 min ($p < .05$), which was most likely due to the ketamine 5mg/kg loading dose. There were no differences in activity between 10-20 min. However, the ketamine 20 mg/kg/h infusion maintained hyper-locomotion vs. saline from 30 to 60 min. Interestingly, ketamine 5 mg/kg/h had an opposite effect and induced hypo-locomotion vs. saline during the same period of 30 to 60 min. Overall, there was a dose-dependent locomotor response to ketamine infusions. The 20 mg/kg/h infusion increased activity while the 5 mg/kg/h infusion reduced locomotion.

“Figure 6 here”

3.2.3 Sensorimotor gating. ASR and PPI were measured 30 min after completion of 1-h ketamine infusions. There were no differences in ASR and PPI results across groups at baseline. Ketamine reduced ASR at 100 dB, $F(3,60) = 6.27$ ($p < .001$). Post-hoc testing revealed that ketamine infusions at 10 and 20 mg/kg/h significantly reduced ASR at 100 dB compared to saline (Figure 6c). Ketamine significantly impaired the PPI of 100 dB $F(3,60) = 7.338$ ($p < .001$) (Figure 6d). Post-hoc testing showed ketamine 20 mg/kg/h significantly impaired the PPI at 100

dB compared to saline ($p < .001$). Overall, 10 and 20 mg/kg/h subanesthetic ketamine infusions reduced ASR while only 20 mg/kg/h impaired PPI.

3.2.4 Thermal nociception. Hotplate latencies were measured from 60 to 120 min after the completion of a 1-h ketamine infusion. There were no differences in baseline latencies between groups. There were significant main effects of ketamine dose, $F(3,52) = 18.68$ ($p < .001$) but non-significant effects for time, $F(3,156) = 1.831$ ($p = .14$) or dose x time interaction, $F(9,156) = 1.369$ ($p = .21$). Post-hoc testing showed that a ketamine 20 mg/kg/h infusion produced significant antinociception up to 120 min after the infusion (Figure 4b). The ketamine 10 mg/kg/h infusion produced significant antinociception up to 100 min. Low-dose ketamine at 5 mg/kg/h did not produce antinociception. Ketamine infusions at 20 and 10 mg/kg/h produced significant and extended antinociception compared to the low-dose ketamine infusion.

3.2.5 Plasma Concentration. Ketamine and norketamine plasma concentrations were measured at the conclusion of 1-h ketamine infusions (Table 2). There were significant differences in ketamine plasma levels between infusion doses, $F(2,15) = 17.21$ ($p < .001$). Ketamine infusion at 20 mg/kg/h produced greater ketamine plasma concentrations compared to both 5 ($p < .001$) and 10 mg/kg/h ($p < .01$) infusions. Although the 10 mg/kg/h infusion resulted in greater ketamine concentration compared to 5 mg/kg/h, the difference was not significant. Similarly, there were significant differences in norketamine levels after the 1-h ketamine infusion, $F(2, 15) = 37.37$ ($p < .001$). Post-hoc testing showed ketamine infusion at 20 mg/kg/h produced greater norketamine than both 5 and 10 mg/kg/h infusions ($p < .001$). We also compared ketamine plasma concentrations between bolus and infusion doses. The 20 mg/kg/h infusion produced a similar ketamine concentration as the 5 mg/kg bolus ($p > .05$). Additionally, the 10 mg/kg/h infusion and the 2 mg/kg bolus resulted in similar ketamine concentrations ($p >$

.05). The 5 mg/kg/h infusion produced significantly lower ketamine concentration than the 2 mg/kg bolus ($p < .01$). Overall, there was a dose-dependent effect on ketamine and norketamine plasma concentrations.

“Table 2 here”

4. Discussion

Rodent behaviors following SC and IP ketamine injections are well-described, but clinicians typically administer ketamine treatments to humans using the IV route. Therefore, this investigation sought to provide a missing translational linkage between traditional rodent SC/IP injections and human IV ketamine delivery. To our knowledge, the present investigation is the first to measure dose-response characteristics of rodent behaviors following subanesthetic IV ketamine administered as a bolus and continuous infusion. Ketamine administered by injections produce well-described changes in rodent behaviors such as increased locomotion, dissociative stereotypy, impaired PPI, and antinociception. This investigation supported and extended these findings through the characterization of behavioral effects at various IV ketamine dosages, which will facilitate future pre-clinical IV ketamine studies.

High-dose subanesthetic IV ketamine delivered as a bolus and infusion induced hyper-locomotion while low-dose ketamine infusions reduced locomotor activity. These results are similar to previous reports that observed dose-dependent hyper-locomotion following subanesthetic ketamine injections (Danysz *et al.*, 1994, Hetzler and Swain Wautlet, 1985, Imre *et al.*, 2006, Yamamoto *et al.*, 1997). In addition to NMDA receptors, ketamine is known to interact with other receptor types that include dopamine receptors (Kapur and Seeman, 2002, Yamamoto *et al.*, 1997). Previous PCP studies suggest that ketamine induces rodent locomotor

activity at high-doses through increased dopamine turnover in the nucleus accumbens (Carboni *et al.* , 1989, Jentsch *et al.*, 1998, McCullough and Salamone, 1992, Steinpreis and Salamone, 1993). Interestingly, rats given ketamine at 2 and 5 mg/kg IV boluses trended towards hypo-locomotion after 15 min, and lower-dose 5 and 10 mg/kg/h infusions reduced locomotion compared to saline controls (Figure 3 and 6). Previous reports described hypo-locomotion following low-dose subanesthetic ketamine injections, but the mechanism is unclear (Gigliucci *et al.* , 2013, Kotermanski *et al.*, 2013, Palenicek *et al.*, 2011, Trujillo *et al.*, 2011). Clinicians are administering ketamine infusions for treatment refractory PTSD at low-doses that typically do not produce psychotomimetic effects (Feder *et al.* , 2014). It is possible that low-dose subanesthetic ketamine exerts anxiolytic or calming effects (Zhang *et al.* , 2014) while sparing dopamine induced locomotor effects.

Intravenous ketamine produced dose-dependent changes in dissociative stereotypy. High-dose subanesthetic IV ketamine at 5 mg/kg bolus and 20 mg/kg/h infusion induced behaviors such as circling, side to side head movement (head weave), mild ataxia, reduced rearing, and reduced grooming (Figures 2 and 5). These dissociative stereotypic behaviors are well-described in previous reports (Castellani and Adams, 1981a, Hetzler and Swain Wautlet, 1985, Sturgeon *et al.*, 1979) and are mediated by dopaminergic activation in striatal regions (Finnegan *et al.*, 1976, Glick *et al.*, 1976, Nabeshima *et al.*, 1983). Rats that received a low-dose ketamine infusion at 5 mg/kg/h displayed similar behavior to saline controls with the exception of reduced rearing and grooming behavior. A previous investigation reported reductions in fine motor movements and described absent grooming behavior after subanesthetic IP ketamine injections (Kotermanski *et al.*, 2013). The neural circuitry of rodent grooming behavior is complex and involves multiple brain regions (Kalueff *et al.* , 2016). In our study, ketamine at high-doses induced increased

locomotor activity, which may contribute to reduced grooming behavior; however, reduced grooming was also observed at low-doses and reduced locomotor activity. Thus, reduced grooming observed at both high and low doses of ketamine may not result from altered locomotor activity.

Ketamine 5 mg/kg IV bolus and 20 mg/kg/h infusion impaired PPI but only 10 and 20 mg/kg/h infusions reduced ASR. There are many reports describing PPI interruption in rodents after NMDA antagonist injections (Geyer *et al.*, 2001, Imre *et al.*, 2006, Kamiyama *et al.*, 2011), which model sensorimotor gating deficits in schizophrenia patients (Braff *et al.*, 1992, Quednow *et al.*, 2008). This investigation confirmed these reports at high-doses, but low-dose ketamine at 2 mg/kg IV bolus and infusions at 5 and 10 mg/kg/h failed to impair PPI (Figures 3 and 6). Ketamine infusions at 10 mg/kg/h and 20 mg/kg/h reduced ASR while both bolus doses and the 5 mg/kg/h infusion showed no effect (Figures 3 and 6). These results confirm previous reports that also found subanesthetic ketamine injections had either no change or non-significant reductions in ASR (de Bruin *et al.*, 1999, Imre *et al.*, 2006, Palenicek *et al.*, 2011). The ASR reduction following 20 mg/kg/h ketamine infusion was surprising because of its similar plasma ketamine concentration to the high-dose ketamine bolus 5 mg/kg, which had no effect on ASR. It is possible that hyper-locomotive rats throughout the 1-h high-dose ketamine infusion experienced a recovery hypo-locomotive state at the time of ASR measurement, which was 30 min after completion of the infusion. A similar step-wise reduction in ASR was observed for the 5 and 10 mg/kg/h infusions (Figure 6), which showed an inverse relationship to locomotor activity (Figure 6). Ketamine concentrations in brain tissue were most likely very low at ASR measurement as IV ketamine undergoes rapid metabolism in rat (Cohen *et al.*, 1973). Similar to PCP, ketamine has dose-dependent receptor affinities (Johnson *et al.*, 2015) where at low

brain/blood concentrations, ketamine could preferentially bind to receptors that impact either sensory input or motor output responses and reduce ASR (Koch and Schnitzler, 1997).

Intravenous ketamine produced dose-dependent changes in antinociception. The antinociceptive effects of ketamine in various rodent pain models are previously described (Getova and Doncheva, 2011, Gupta *et al.*, 2011, Pacheco *et al.*, 2014, Sarton *et al.*, 2001), but there is little information regarding duration of antinociception. Ketamine administered as a 5 mg/kg IV bolus and 10 and 20 mg/kg/h infusions produced long-lasting antinociception (Figure 4). Extended duration of ketamine analgesia following infusion stoppage is reported in human literature (Jackson *et al.*, 2001) and in a rodent study that measured opioid/ketamine interaction (Gupta *et al.*, 2011). Although unbound IV ketamine undergoes rapid metabolism and clearance (Cohen *et al.*, 1973), bound ketamine may prolong NMDA and opioid receptor activation, which may have extended the antinociception observed in the present study. Another possibility is the antinociceptive effect of norketamine, an active metabolite of ketamine (Domino, 2010). In the present study, norketamine levels increased from 1 to 20 min and essentially plateaued from 20 to 50 min following both IV bolus dosages (Table 1); however, norketamine concentrations were greater for the 5 mg/kg bolus and may have contributed to extended antinociception. As a final point, dissociative stereotypy were not observed at the time of nociception measurement, which suggests limited receptor engagement beyond NMDA receptors at the low plasma ketamine concentrations at 20 and 50 min (Table 1).

Ketamine and metabolite concentrations in blood following parenteral injections are well-characterized in both rodents (Cohen *et al.*, 1973, Eckert *et al.*, 2015, Marietta *et al.*, 1976, Palenicek *et al.*, 2011, Williams *et al.*, 2004) and humans (Peltoniemi *et al.*, 2016). To our knowledge, no studies have described ketamine and norketamine plasma concentrations

following a continuous subanesthetic IV ketamine infusion in rats. The peak ketamine plasma concentration obtained after 5 mg/kg IV bolus administration (4100 ng/mL) is greater than that reported by Eckert et al. (2015) at the same dose (2800 ng/mL) (Eckert *et al.*, 2015). Different sampling time points may have contributed to the discrepancy as the peak ketamine sample was collected at 1 min following bolus dosing. The peak plasma concentrations reported for bolus and infusion doses in this study are also greater than those reported in human studies following 0.5 mg/kg IV ketamine infusions for depression treatment (Zhao *et al.*, 2012). However, there are differences in CYP450 metabolism across species (Martignoni *et al.*, 2006), which is the metabolic pathway for ketamine. These inter-species differences contribute to the larger doses and increased ketamine brain/plasma concentrations required for rodents to yield similar treatment effects compared to humans (Eckert *et al.*, 2015). Overall, it is anticipated that plasma concentrations described in the present study will provide a reference point for future pre-clinical rodent investigations utilizing IV ketamine infusion and a comparison to previous rodent ketamine models that utilized SC or IP injections.

Intravenous ketamine infusion in rodents is a clinically relevant model that is surprisingly absent from pre-clinical literature. Researchers may prefer to administer ketamine via injections to rodents due to convenience and ease of administration compared to an IV route but injections pose challenges for some neurobehavioral studies. First, ketamine undergoes rapid metabolism and clearance following parenteral injections (Giroux *et al.*, 2016, Marietta *et al.*, 1976). Rapid metabolism results in limited brain exposure to ketamine at peak plasma concentration, which is not comparable to the steady state plasma concentration maintained by continuous IV infusions used in clinical practice. Researchers have attempted to mimic ketamine infusions through repeated IP injections (Groeber Travis *et al.*, 2015), but this method results in successive peak

and trough plasma concentrations that resemble a saw-tooth profile. Second, injections stress rodents causing increased corticosterone (Wu *et al.* , 2015) and local tissue necrotic injury possibly due to the acidic pH of ketamine (Wellington *et al.* , 2013). Repeatedly stressing rodents through manual restraint and painful injections could confound behavioral studies. Lastly, complications are associated with IP injections performed without visual confirmation of needle placement. Inadvertent injections can occur into fat, urinary bladder, muscle, or abdominal organs and result in a 10-20% failure rate (Gaines Das and North, 2007). Overall, we suggest that pre-clinical researchers consider using similar ketamine delivery routes used in human treatments, such as IV infusions, to enhance study translation across species.

The present study is not without limitations. Small sample sizes were utilized with rats that underwent repeated exposure to ketamine dosing. For example, ketamine/xylazine anesthesia was used for the indwelling jugular catheter surgery. However, baseline ketamine plasma concentrations were measured 7-days after surgery and no ketamine was detected in the plasma prior to the experiments. Additionally, rats were given a 4-day period between testing to ensure complete ketamine clearance. Repeated ketamine exposure raises concerns for tolerance of ketamine. However, both bolus and infusion groups underwent a counter-balanced exposure to all dosages and did not display tolerance. These rats showed distinct dose-dependent differences in locomotion, stereotypy, ASR/PPI, and antinociception. Despite these limitations, the study methods also provided some advantages. For example, the use of an operant chamber with metal spring wire and rotating swivel allowed the authors to administer a prolonged ketamine infusion to a freely moving rat. Additionally, the indwelling jugular catheter allowed the study team to conduct repeated *in vivo* blood sampling from rats, reducing the number of rats required for repeated blood sampling.

Lastly, this ketamine investigation utilized only male rats. Previous work has demonstrated sex-related differences in response to anesthetic ketamine doses on metabolism (Waterman and Livingston, 1978) and subanesthetic doses on antidepressant-like effects in rats and mice (Carrier and Kabbaj, 2013, Franceschelli *et al.* , 2015). In fact, there has been an overall trend to exclude female rodents in neuroscience and biomedical research mostly due to unsubstantiated concerns about estrus-cycle mediated hormonal changes (Beery and Zucker, 2011, Prendergast *et al.* , 2014). Therefore, the current findings in male rats cannot be generalized to female rats and future investigations regarding the effects of IV ketamine on female animals are warranted.

5. Conclusion

IV ketamine administered by bolus and infusion produced dose-dependent changes in dissociative stereotypy, locomotion, sensorimotor gating, and antinociception. Ketamine and norketamine plasma concentrations were described following IV bolus at 1, 20, and 50 min and at the conclusion of a 1-h infusion. The current ketamine infusion model may provide unique opportunities for future pre-clinical investigations that mirror human ketamine administration. For example, clinicians administer low-dose IV ketamine infusions to treat chronic pain (Prommer, 2012), depression (Xu *et al.* , 2016), and PTSD (Feder *et al.*, 2014) in humans. Pre-clinical researchers could administer low-dose subanesthetic IV ketamine infusions to rodents that will provide a sustained steady state brain/plasma concentration similar to human applications while limiting dissociative stereotypy. Additionally, there is recent work exploring the effects of an active ketamine metabolite, hydroxynorketamine, as a novel depression treatment (Singh *et al.* , 2014, Zanos *et al.* , 2016). Administering ketamine and metabolite

treatments to rodents through an IV infusion model, similar to human investigations, may provide a more clinically relevant route for future translational studies.

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All opinions in this article are those of the authors and do not reflect upon the official policy or position of the Department of the Navy, the Department of Defense, the Uniformed Services University of the Health Sciences, or the United States Government.

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Figure legends

Figure 1. Experimental timeline for ketamine bolus and infusion. KB = ketamine bolus (saline, 2 and 5 mg/kg, IV), B = blood sample, DS = dissociative stereotypy, L = locomotion, ASR/PPI = acoustic startle reflex/pre-pulse inhibition, HP = hotplate latency, Infusion (saline and ketamine 5, 10, and 20 mg/kg/h, IV). Baseline hotplate and ASR/PPI were conducted 24-h prior to first dosing.

Figure 2. Effects of intravenous ketamine bolus on dissociative behaviors. Dissociative behaviors comprised circling, side to side head motion (weave), reduced rearing, ataxia (loss of balance), and reduced grooming. A-B) Ketamine 5 mg/kg significantly increased total circling with most circles occurring from 0-6 min. C-D) Ketamine 2 and 5 mg/kg dose dependently reduced total rearing over 10 min. E-F) Ketamine 5 mg/kg induced significant total head weave behavior with most occurring from 0-4 min. G-H) Ketamine 5 mg/kg induced more ataxia episodes. Ketamine 2 mg/kg induced ataxia over 2 min while 5 mg/kg induced ataxia over 4 min. I-J) Ketamine dose dependently reduced grooming behavior. Ketamine 2 mg/kg reduced grooming over 4 min while 5 mg/kg reduced grooming throughout 10 min. Data are shown as mean $\pm$ SEM. (*/# $p < .05$, **/## $p < .01$, ***/### $p < .001$ vs. saline; $n = 8$ per group).

Figure 3. Effects of intravenous ketamine bolus on locomotor activity and sensorimotor gating at 100dB. A) Ketamine 5 mg/kg bolus increased total activity over 15 min. B) Ketamine 2 mg/kg bolus increased activity from 0-3 min while 5 mg/kg produced a delayed response and increased activity from 4-6 min. C) Ketamine did not alter startle response 20 minutes after bolus dosing.

D) Ketamine 5 mg/kg impaired PPI while 2 mg/kg had no effect. Data are shown as mean $\pm$ SEM. (* $p < .05$; *** $p < .001$ vs. saline; $n = 8$ per group).

Figure 4. Effects of intravenous ketamine bolus and infusion on hotplate latency. A) Ketamine increased antinociception up to 50 min after a 5 mg/kg bolus injection. B) Ketamine 20 mg/kg/h induced significant antinociception through 120 min after the completion of a 1-h infusion. Ketamine 10 mg/kg/h induced antinociception through 100 min. Data are shown as mean $\pm$ SEM. (* $p < .05$, **/## $p < .01$; ***/### $p < .001$ vs. saline; $n = 8$ per bolus group; $n = 16$ per infusion group).

Figure 5. Effects of intravenous ketamine infusion on dissociative behaviors. Dissociative behaviors comprised circling, side to side head motion (weave), reduced rearing, ataxia (loss of balance), and reduced grooming. A) Ketamine 20 mg/kg/h increased circling behavior. B) Ketamine infusions reduced rearing at all doses. C-D) Ketamine 20 mg/kg/h significantly increased head weave and ataxia. E) Ketamine infusions significantly reduced grooming behavior at all doses. Data are shown as mean $\pm$ SEM. (* $p < .05$, ** $p < .01$, *** $p < .001$ vs. saline; $n = 8$ per group).

Figure 6. Effects of intravenous ketamine infusion on locomotor activity and sensorimotor gating at 100dB. A) Ketamine 20 mg/kg/h infusion increased total activity over 60 min while 5 mg/kg/h reduced activity. B) Ketamine 20 mg/kg/h induced hyper-locomotion over the infusion period while 5 mg/kg/h induced hypo-locomotion. C) Ketamine at 10 and 20 mg/kg/h reduced the startle response 30 min after a 1-h infusion. D) Ketamine 20 mg/kg/h impaired PPI while

lower dose infusions had no effect. (*/# $p < .05$; **/## $p < .01$; ***/### $p < .001$ vs. saline; $n = 16$ per group).

Table 1. Plasma concentrations after IV ketamine bolus				
	Ketamine		Norketamine	
	5 mg/kg	2 mg/kg	5 mg/kg	2 mg/kg
1 min	4100 (690)**	1700 (170)	76 (2.5)	77 (11)
20 min	490 (27)*	340 (27)	290 (28)	240 (7.6)
50 min	140 (11)***	73 (7.6)	320 (11)***	230 (7.0)

Table 1. Plasma concentrations of ketamine and norketamine after bolus injections. Ketamine 5 mg/kg IV produced greater ketamine levels at each time point compared to 2 mg/kg.

Norketamine metabolite levels were similar at 1 and 20 min between dosages, but 5 mg/kg produced greater norketamine at 50 min. Data are presented as means (SEM) [ng/ml]. (* $p < .05$,

** $p < .01$, *** $p < .001$; $n = 6$ per group)

Table 2. Plasma concentrations after 1-h IV ketamine infusion		
	Ketamine	Norketamine
5 mg/kg/h	880 (140)	490 (20)
10 mg/kg/h	1500 (120)	740 (43)
20 mg/kg/h	3900 (560)**	1200 (90)***

Table 2. Plasma concentrations of ketamine and norketamine after a 1-h ketamine infusion.

Ketamine 20 mg/kg/h resulted in greater ketamine and norketamine concentrations than both 5 and 10 mg/kg/h. Data are presented as mean (SEM) [ng/ml]. (** $p < .01$; *** $p < .001$; $n = 6$ per group).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6