Different dosing regimens of repeated ketamine administration have opposite effects on novelty processing in rats

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ABSTRACT

Repeated exposure to sub-anesthetic doses of ketamine in rats has been shown to induce cognitive deficits, as well as behavioral changes akin to the negative symptoms of schizophrenia, giving much face validity to the use of ketamine administration as a pharmacological model of schizophrenia. This study sought to further characterize the behavioral effects of two different ketamine pre-treatment regimens, focusing primarily on the effects of repeated ketamine administration on novelty processing, a capacity that is disrupted in schizophrenia. Rats received 5 or 14 intra-peritoneal injections of 30 mg/kg ketamine or saline across 5 or 7 days, respectively. They were then tested in an associative mismatch detection task to examine their ability to detect novel configurations of familiar audio-visual sequences. Furthermore, rats underwent a sequential novel object and novel object location exploration task. Subsequently, rats were also tested on the delayed matching to place T-maze task, sucrose preference task and locomotor tests involving administering a challenge dose of amphetamine (AMPH). The high-dose ketamine pre-treatment regimen elicited impairments in mismatch detection and working memory. In contrast, the low-dose ketamine pre-treatment regimen improved performance of novelty detection. In addition, low-dose ketamine pre-treated rats showed locomotor sensitization following an AMPH challenge, while the high-dose ketamine pre-treated rats showed an attenuated locomotor response to AMPH, compared to control rats. These findings demonstrate that different regimens of repeated ketamine administration induce alterations in novelty processing in opposite directions, and that differential neural adaptations occurring in the mesolimbic dopamine system may underlie these effects.

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1. Introduction

Ketamine is a non-competitive antagonist of the NMDA receptor (Martin and Lodge, 1985), traditionally known for its anesthetic properties, as well as its strong psychotomimetic effects in humans and rodents (Malhotra et al., 1996; Becker et al., 2003). Repeated administration of subanesthetic doses of ketamine has been widely used as a pharmacological model for schizophrenia, with superior face validity to other models such as phencyclidine (PCP) and d-amphetamine (AMPH) exposure, due to the fact that repeated ketamine administration induces behavioral alterations that are reminiscent of cognitive, negative, as well as positive symptoms observed in schizophrenia (Stefani and Moghaddam, 2002; Becker et al., 2003; Enomoto and Floresco, 2009; Rushforth et al., 2011; Gama et al., 2012). Notably, subchronic administration of ketamine

has been found to produce disruptions in spatial working memory (Enomoto and Floresco, 2009; Venancio et al., 2011), attentional processes (Nikiforuk and Popik, 2012, 2014) and decreased social interaction (Becker et al., 2003).

More recently, ketamine has garnered increasing clinical attention for its antidepressant properties (Trullas and Skolnick, 1990). Acute, intravenous administration of this NMDAR antagonist significantly improves depressive symptomology without the lag of onset seen in conventional antidepressants (Berman et al., 2000; Zarate et al., 2006). Furthermore, repeated ketamine administration has been reported to produce response and remission rates of even greater efficacy and longevity than those seen in acute doses (Aan het Rot et al., 2010). In considering the underlying neurobiological substrates of the depression-alleviating property of ketamine on the one hand, and its schizophrenia-inducing properties on the other, neural adaptations occurring in the midbrain dopaminergic systems emerge as a strong candidate to explain ketamine's mechanism of action. Despite the fact that ketamine's primary mode of action is mediated by the glutamatergic system (Moghaddam et al., 1997; Kim et al., 2011), growing evidence suggests that ketamine's antidepressive effect is, at least partly,

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regulated by the mesolimbic dopamine system (Belujon and Grace, 2014). Moreover, there are reports of repeated ketamine administration inducing marked neuroadaptations in the wider mesocortico-limbic dopaminergic system. For instance, significant increases in basal dopamine levels and increased density of DA transporters have been reported in the prefrontal cortex, hippocampus and striatum following repeated ketamine administration in rats and mice (Lindefors et al., 1997; Chatterjee et al., 2012; Becker et al., 2003).

Novelty detection is a vital process that leads to the allocation of salience/arousal and attentional resources to novel, unexpected, or incongruent stimuli, and is a capacity that is impaired in schizophrenia patients (Weiss et al., 2004). Much evidence implicates cortico-limbic-striatal areas and associated dopamine pathways in novelty processing. Thus, the detection of spatial novelty, or mismatch in learned associations is highly sensitive to manipulations of the hippocampus (Honey et al., 1998; Lee et al., 2005) and medial prefrontal cortex (Dias and Honey, 2002). Furthermore, novelty preference is impaired as a result of forebrain DA depletion (Pierce et al., 1990), and novel events and social stimuli induce increases in DA levels in the nucleus accumbens shell, hippocampus and medial prefrontal cortex (Horvitz, 2000; Lisman and Otmakhova, 2001; Li et al., 2003; De Leonibus et al., 2006). Taken together, novelty processing may rely on the integrity of the same mesocortico-limbic DA pathway through which ketamine may exert its influence, raising the question as to whether repeated ketamine administration modulates novelty processing through alterations of mesocorticolimbic dopamine pathways.

The objectives of the present study were twofold: Firstly, to assess whether repeated ketamine exposure has an effect on novelty processing that is linked with alterations in the function of the mesocorticolimbic DA system. Secondly, to see if there are dose regimen-dependent effects of repeated ketamine administration upon novelty processing, given that there is, at present, no standard dosing regimen for repeated ketamine administration in pre-clinical research. To this end, we selected two different dosing regimens that have previously been used, and found to induce behavioral and neurochemical alterations akin to schizophrenia symptoms (Becker et al., 2003; Floresco et al., 2009; Jacklin et al., 2012). The first regimen involved daily injections of 30 mg/kg ketamine for 5 days and was previously shown to disrupt latent inhibition and social interaction, and to cause neurochemical adaptations indicative of increased mesolimbic DA function (Becker et al., 2003). The second regimen involved the injections of the same dose of ketamine twice daily, for 7 days, and was previously shown to disrupt a number of cognitive processes (5- or 10-day regimen: Enomoto and Floresco, 2009; Floresco et al., 2009; Jacklin et al., 2012). We hypothesized that novelty processing and other indices of cognition (working memory) would be impaired following the second (higher cumulative dose) regimen, in keeping with previous data. In contrast, we hypothesized that novelty processing may be enhanced following the lower cumulative dose regimen, on the basis of previous data demonstrating enhanced mesolimbic dopamine neurotransmission following the use of the same dosing regimen in rats (Becker et al., 2003).

2. Methods

2.1. Animals

81 male Long Evans rats were used (Charles River, QC, Canada), weighing 300–350 g at the start of the injection regimen. All rats were pair-housed with a constant room temperature of 22 °C and a 12 h light/dark cycle. All animals had free access to water but were food restricted during behavioral testing to maintain their body weight at 85% of their free feeding weight. All behavioral testing took place during the light cycle, in accordance with the ethical and legal requirements under Ontario's Animals for Research Act, the federal Canadian Council on Animal Care, and approval of the University of Toronto Scarborough Local Animal Care Committee.

2.2. Drug administration

2.2.1. Low-dose repeated ketamine administration

Rats were given daily intraperitoneal (IP) injections of 30 mg/kg ketamine (Ketaset, CDMV, QC, Canada) or 0.9% saline over five consecutive days, followed by a 10-day washout period prior to behavioral testing, to ensure that the observed effects were dissociable from the effects of acute withdrawal from the drug (Becker et al., 2003; Enomoto and Floresco, 2009; Jacklin et al., 2012). A total of 28 ketamine and 32 saline pre-treated animals were tested in 4 batches, within 5 weeks of the first day of the injection regimen, in keeping with the timeline used in previous procedures (Becker et al., 2003; Chindo et al., 2012): Batch 1 (8 ketamine, 8 saline) performed the associative mismatch detection, sucrose preference, neophagia and elevated plus maze (EPM) task. Batch 2 (9 ketamine, 8 saline) performed the novel object detection task, sucrose preference, neophagia and EPM task. Batch 3 (4 ketamine, 8 saline) performed the associative mismatch detection, novel object detection task, neophagia and EPM task. Finally, Batch 4 (7 ketamine, 8 saline) performed the T-maze working memory task. In addition, batches 1–3 underwent a locomotor activity test. The order in which the behavioral tests were administered was counterbalanced across the different batches, with the exception of the locomotor test, which was always administered last.

2.2.2. High-dose repeated ketamine administration

Rats received IP injections of 30 mg/kg ketamine or 0.9% saline, twice daily over seven consecutive days, followed by a 10 day washout period before the commencement of behavioral testing. All behavioral testing took place within 8 weeks of the first day of the injection regimen. Two batches of 13 ketamine-pretreated and 8 saline rats performed all of the tests described below, in counterbalanced order.

2.3. Behavioral procedures

2.3.1. Associative mismatch detection

Behavioral training was conducted in one oversized operant chamber (30.5 cm L × 24.1 cm W × 29.2 cm H, Med Associates, VT, USA) contained within a sound attenuating box, illuminated by a house light, equipped with a tone generator and a light stimulus on one side of the chamber. The rat was trained to learn a combination of two audiovisual sequences across three stages (adapted from Honey et al., 1998). In the first stage, animals were habituated to the operant chamber for 30 min on two days without any stimuli presentation. In the next stage, they were trained to learn two different pairs of audiovisual sequences over 20 trials (10 trials per sequence) on each training day for four days. Rats were divided into two groups and received presentations of either sequence group A or B. In sequence group A, the first audiovisual sequence consisted of the presentation of a 10 s continuous tone followed immediately by a 10 s constant light, whereas the second sequence consisted of a 10 s intermittent tone presentation followed by a 10 s flashing light presentation. In contrast, sequence group B consisted of two audiovisual sequences with a continuous tone followed by a flashing light and an intermittent tone followed by a constant light. During the final stage (mismatch test), the rats received 12 trials of familiar training sequences, followed by 10 more sequence presentations consisting of equal numbers of 'match' and 'mismatch' trials. Half of the 10 trials involved presentation of the sequences the animal had been trained with (match — e.g., Auditory Stimulus $(A)_1 \rightarrow$ Visual Stimulus $(V)_1$, Auditory Stimulus $(A)_2 \rightarrow$ Visual Stimulus $(V)_2$) and the other five trials involved presentation of novel sequences (mismatch of two learned audiovisual sequences — e.g., $A_1 \rightarrow V_2$, $A_2 \rightarrow V_1$). Throughout training, audiovisual sequences were presented in a pseudorandom order with no more than two consecutive presentations of the same pair.

The measure of interest was an orienting response to the visual stimulus during its presentation, defined as the nose of the rat pointing towards the corner of the chamber containing the light source during

the presentation of the light. The orientation response to the visual stimulus was summed over four blocks of five trials for each training session to generate a percentage of orienting in each block. For the mismatch testing, the percentage of orienting responses emitted in match trials and mismatch trials was calculated.

2.3.2. Novel object and location exploration

The novel object and object location exploration task was conducted in a plus maze composed of black Perspex, with a central platform (11 cm L × 11 cm W) that connected four identical arms (15 cm L × 11 cm W × 18 cm H). The maze contained four different objects (glass of various shapes) at the end of each arm. The task was divided into habituation and testing sessions, with the external maze cues fixed in the same locations for the duration of the experiment. Habituation consisted of rats being allowed to explore the plus maze for 3 × 5 min (with an inter-trial interval (ITI) of 1 min) without any objects inside the maze, for two days. On the test day, the rat was administered eight trials (5 min each) with an ITI of 2 min. During the first trial, the rat was habituated to the maze without objects. For each of the next three trials, four different objects were placed in the maze arms, and the rat was allowed to explore the objects for 5 min. In the fifth trial, animals were divided into two groups, with group A being exposed to a novel object that replaced one of the four familiar objects, and group B undergoing a spatial location switch by swapping objects in arm 1 and 3 or arm 2 and 4, respectively. Rats received two more trials with this new configuration of objects. During the final (8th) trial, rats in group A underwent the spatial switch of two objects, while group B experienced the introduction of a new object in place of a familiar object. The time spent interacting with each object was measured in all trials.

2.3.3. T-maze working memory task

Rats were trained in a delayed matching to place T maze task, to assess working memory performance. The task was performed on an elevated plus maze composed of black Perspex (57 cm L × 11 cm W × 18 cm H) with one arm permanently blocked off to create the shape of a T, with a start arm and two goal (choice) arms (see Ito and Canseliet, 2010). Rats were given two 5 min habituation sessions, one in which they were allowed to explore the T maze apparatus without reward, and another in which 0.5 ml 20% sucrose solution was placed on both ends of the goal arms. Subsequently, rats were subjected to 6 trials of training per day/session for 15 consecutive days. Each trial consisted of a sample and choice phase, separated by a 60 s delay period. During the sample phase, the rat was placed in the start arm (facing the experimenter), and allowed into one of the goal arms (left or right) to consume the sucrose reward. Following consumption of the reward, rats were placed back in the start arm and kept there for 60 s using a Perspex divider. During the choice phase, rats were given access to both goal arms, and rats were rewarded for entering the goal arm that had previously been rewarded in the sample phase (matching to place). Rats were given an equal number of Left and Right sample arms, with no more than two consecutive trials on the same side. The rats' choice of goal arms during the choice phase was recorded, with the matching to place response counting as the correct response.

2.3.4. Sucrose preference

Two sucrose preference tests were administered to assess anhedonia, and the contribution of novelty to sucrose preference (novel (first test) vs. familiar (second test)). For each test, rats were single-housed over a time period of 16 h, and presented with two bottles, one containing tap water and the other 1% sucrose solution. The bottles were weighed before, and after the test to measure the amount of liquids consumed. The second preference test was performed two weeks after the initial test.

2.3.5. Elevated plus maze

This anxiety test was performed in an elevated plus maze that contained a central platform (10 cm L × 10 cm W) that connected four arms (40 cm L × 10 cm W × 22 cm H), with two open arms and two arms that were enclosed by walls (closed arms). The rat was placed in the central compartment of the maze facing an open arm at the start of the session and was thereafter allowed to explore the maze for 10 min. The entries into open and closed arms as well as the time spent in the arms were measured.

2.3.6. Neophagia

A plastic jar containing corn, a novel food to the rat, was placed at one end of a plastic cage (47.4 cm L × 26.4 cm W × 20.5 cm H), and the animal was placed on the other end of the cage at the start of the 15 min session, and allowed to explore the cage and approach/consume the food. Latency to approach the food for the first time (approach), and the latency to start consuming the food were recorded.

2.3.7. Locomotor activity test

Rats were administered with an acute dose of amphetamine (2 mg/kg) to test for the presence of locomotor sensitization in ketamine pre-treated rats. Animals were first given a 2 h habituation session in a locomotor chamber (plastic cage: 47.4 cm L × 26.4 cm W × 20.5 cm H). On the following day, the rat was placed into the same chamber for 2 h and its baseline locomotor activity was recorded using a camera and EthoVision XT software (Noldus Information Technology, ON, Canada). After 2 h had elapsed, the animal received a single IP injection of 2 mg/kg AMPH (Sigma-Aldrich, ON, Canada), and was immediately placed back into the chamber for locomotor activity recording for a further 2 h. The locomotor activity in both two-hour sessions was measured as the distance traveled in 10-minute bins.

2.4. Data analysis

Data were analyzed using the SPSS statistical package version 21.0 (IBM, ON, Canada). Analysis of variance (ANOVA) was applied to all experimental data. The "Treatment group" (low-dose ketamine, high-dose ketamine, saline) was set as the between-subjects factor, while the within-subjects factor varied according to the dependent measures used in the different procedures, as described for each test individually in the Results section. Furthermore, significant main within subjects effects, thee-way or two-way interactions were further explored using simple effect analyses and post-hoc comparisons (performed with Bonferroni correction).

3. Results

3.1. Associative mismatch detection

All rats demonstrated habituation to the presentation of the audio-visual stimuli presentation (Fig. 1a, b) in the form of significant decreases in the percentage of Orientation Response (OR) emitted towards the visual stimulus over days (Day: $F_{(3,138)} = 18.36$, $p < 0.0001$), and within a session (Bin: $F_{(4,184)} = 64.12$, $p < 0.0001$). There was no significant difference in the rate of habituation/learning between treatment groups (no significant interactions), but ANOVA revealed a significant difference in the overall percentage of OR emitted by the treatment groups (Treatment group: ($F_{(2,46)} = 3.52$, $p < 0.05$). Post-hoc analyses revealed this effect to be due to a significant difference between the overall percentage of OR between the high-dose (HD) ketamine pre-treated group and the saline control group ($p < 0.05$).

ANOVA of the mismatch test data (Fig. 1c) revealed that overall, rats made significantly more ORs to the presentation of a mismatch sequence, than to a match sequence (Trial (match/mismatch): ($F_{(1,46)} = 6.91$, $p < 0.02$). However, there were significant differences in the performance
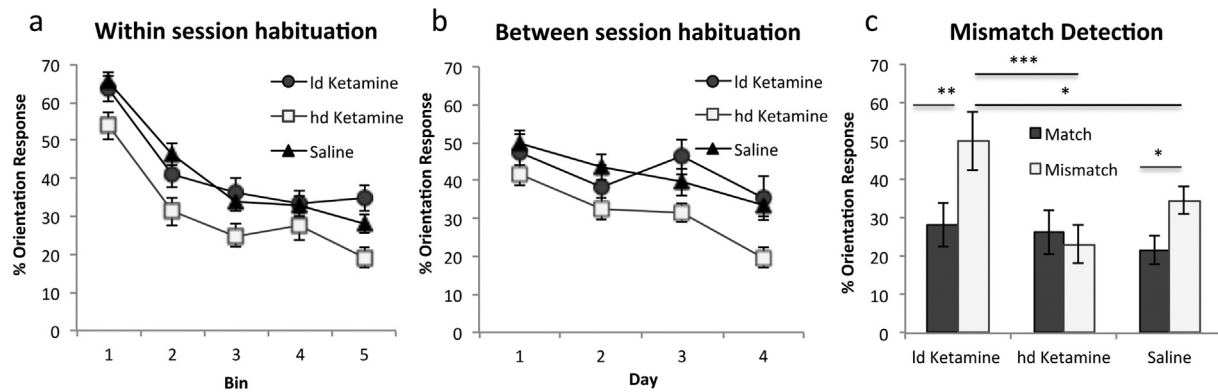
Fig. 1. Associative audio-visual sequence training performance (a & b), expressed as the mean percentage (± SEM) of trials with an orientation response to the presentation of 2 audio-visual sequences (out of 20 trials) within session (a) and across sessions (b) for rats pre-treated with a high-dose (hd) ketamine (n = 13), low-dose (ld) ketamine (n = 12) or saline (n = 24). Performance of the mismatch detection test (c) is expressed as the mean percentage (± SEM) of trials with an orientation response to the presentation of 1) familiar (match) audio-visual sequences (5 trials) and 2) novel (mismatch) audio-visual sequences (5 trials). *p < 0.05, **p < 0.01, ***p < 0.001.

of the treatment groups (Treatment group (F(2,46) = 4.36, p < 0.02); Trial × Treatment group interaction (F(2,46) = 3.37, p < 0.05)). Simple effects analyses revealed the significant interaction effect to be due to a significant simple effect of Treatment group in the level of ORs emitted in the mismatch trials (F(2,46) = 8.01, p < 0.001). Pairwise comparisons revealed the percentage of orienting in the mismatch trials to be higher in the low-dose (LD) ketamine group compared to saline control rats (p < 0.02) and HD ketamine rats (p < 0.001) indicating enhanced performance of mismatch detection in the LD ketamine group. Furthermore, simple effects analyses revealed significant differences in the percentage of OR between the match and mismatch trials in the LD ketamine pre-treated group (F(1,46) = 10.28, p < 0.01) and saline group (F(1,46) = 4.12, p < 0.05), but not in the HD ketamine pre-treated group (F(1,46) = 0.169, p = 0.68). Thus, the LD ketamine pre-treated and saline control rats successfully reinstated their orienting response to novel combinations of audio-visual sequences, but the HD ketamine pre-treated rats failed to detect novel sequences.

3.2. Novel object and object location exploration

In the novel object exploration phase (Fig. 2a), all rats showed increased exploration of the novel object, compared to the exploration

of familiar objects (Object: F(1,45) = 51.28, p < 0.00001). However, there was a significant difference in the overall level of exploration of the objects between the treatment groups (F(2,45) = 7.16, p < 0.01). Post-hoc analyses revealed that the LD ketamine group showed greater exploration of both novel and familiar objects compared to the saline control (p < 0.05) and HD ketamine pre-treated group (p < 0.01). When the spatial locations of two familiar objects were switched (Fig. 2b), rats showed a preference for interacting with the spatially novel objects (Object location: F(1,45) = 21.89, p < 0.0001), but there was a significant difference in the degree to which the treatment groups explored the objects in novel spatial locations (Treatment group × Location interaction; F(2,45) = 3.51, p < 0.05, Treatment F(2,45) = 2.11, p = 0.13, ns). Subsequent simple effects analyses confirmed a significant simple effect of Treatment group in the exploration of the novel spatial locations only (Novel locations: F(2,45) = 5.85, p < 0.01, Familiar locations F(2,45) = 0.10, p = 0.91). Pairwise comparisons revealed the time spent exploring the spatially novel objects to be significantly higher in the LD ketamine pre-treated group, compared to the saline control group (p < 0.01). Furthermore, the exploration time of the spatially novel object locations was significantly higher than the that of the familiar object locations in the LD ketamine pre-treated group (F(1,45) = 20.80, p < 0.0001) but missed significance in the HD
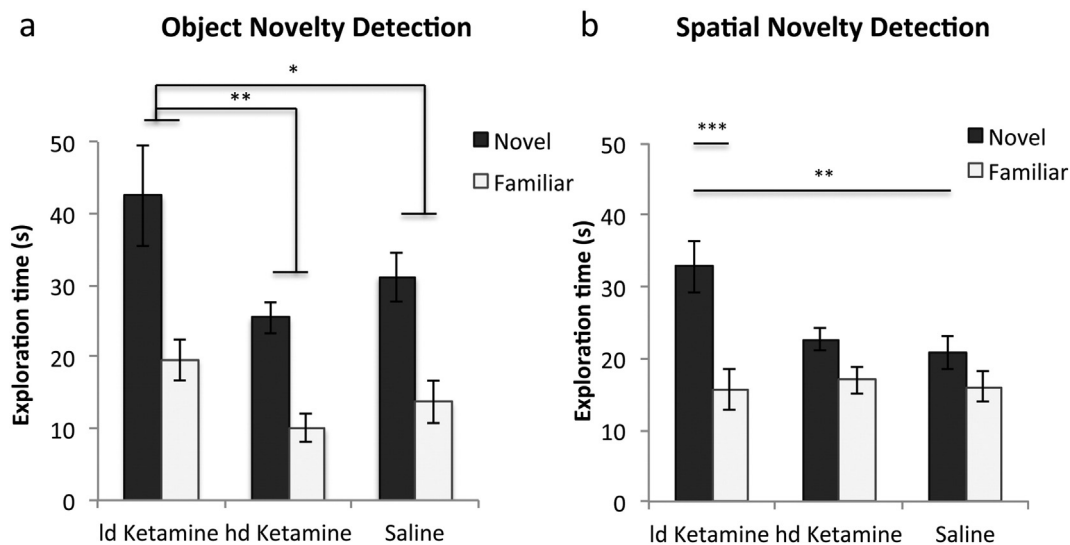

Fig. 2. Object and spatial novelty exploration/detection performance, expressed as the mean time spent (± SEM) exploring the novel object (a) or novel spatial locations of objects (b) as compared to the time spent exploring familiar objects or object locations in rats pre-treated with a high-dose ketamine (n = 13), low-dose ketamine (n = 13) or saline (n = 22). Data from two rats in the saline group were excluded due to the fact that the total amount of time exploring the 4 objects amounted to less than 5 s for both novel object and spatial novelty exploration. *p < 0.05, **p < 0.01, ***p < 0.001.

ketamine pre-treated group (F(1,45) = 3.29, p = 0.08) and saline control group (F(1,45) = 2.48, p = 0.12). Thus, LD ketamine pre-treatment significantly enhanced exploration of novel spatial locations of familiar objects.

3.3. T-maze based working memory

ANOVA of the T maze performance (Fig. 3) revealed significant main effects of Bin (F(14,490) = 41.20, p < 0.0001), and Treatment group (F(2,35) = 8.17, p < 0.001). Post-hoc analyses revealed that the overall percentage of correct responses in the HD ketamine pre-treated group was lower than that of the saline control group (p < 0.001). Additionally, there was a significant Bin × Treatment group interaction (F(28,490) = 3.49, p < 0.0001). Subsequent multiple comparisons revealed this to be attributable to the percentages of correct responses generated by the HD ketamine pre-treated group to be significantly lower than the saline control group in Bins 7, 9–15 (p < 0.0001–0.05), and compared to the LD ketamine pre-treated group in Bins 11, 13 and 15 (all p < 0.05). Thus, the HD ketamine pre-treated rats reached a significantly lower asymptotic level of performance (barely above the 50% chance level) compared to the LD ketamine pre-treated and saline control rats.

3.4. Sucrose preference

A three way ANOVA of the total amount of water and sucrose (1%) solution consumed by the rats during a 16 h period in Tests 1 and 2 (Fig. 4a) revealed a significant main effect of Solution (F(1,52) = 220.51, p < 0.0001), Test (F(1,52) = 20.23, p < 0.0001), and a significant Test × Solution interaction (F(1,52) = 31.49, p < 0.0001) indicating that there was a significant preference for the sucrose solution over water in the 16 h period overall, and a selective increase in consumption of the sucrose solution combined with a decrease in water consumption in the second Test (Simple effect of Test for Sucrose: F(1,52) = 29.80, p < 0.0001, Water: F(1,52) = 6.89, p < 0.01). Additionally, there was a significant Treatment group × Test × Solution interaction (F(2,52) = 3.69, p < 0.05), due to a number of factors. Firstly, simple main effects analyses revealed a significant simple effect of Treatment group in the consumption of the sucrose consumption in Test 1 (F(2,52) = 5.64, p < 0.01) that was due to the sucrose consumption of LD ketamine pre-treated rats being higher than that of the saline control group (p < 0.01). Secondly, the consumption of sucrose solution significantly increased in Test 2 in the saline control (p < 0.0001), and HD ketamine pre-treated groups (p < 0.0001), but not in the LD ketamine pre-treated

group (p = 0.36). Thus, the LD ketamine pre-treated group consumed significantly more sucrose solution than the saline control rats during Test 1.

3.5. Elevated plus maze

All three animal groups made significantly more entries into the closed arms compared to the open arms (Fig. 4b; Arm: F(1,55) = 247.21, p < 0.001, no significant main effect of treatment group: F(2,55) = 0.49, p = 0.61, no Arm × Treatment group interaction F(2,55) = 0.50, p = 0.61). However, ANOVA of the time spent data revealed a significant Arm × Treatment group interaction (F(2,55) = 6.08, p < 0.01) and a significant main effect of Arm (F(2,55) = 168.38, p < 0.0001) and Treatment group (F(2,55) = 4.74, p < 0.02). Simple effects analyses revealed a significant effect of Treatment group in the time spent in the open arms (F(2,55) = 4.59, p < 0.02) and closed arms (F(2,55) = 5.21, p < 0.01). Pairwise comparisons revealed that these effects were due to the HD ketamine pre-treated rats spending less time in the closed arms compared to LD ketamine rats (p < 0.05) and saline control rats (p < 0.01), and the HD ketamine pre-treated rats spending more time in the open arms than the LD ketamine pre-treated group (p < 0.05).

3.6. Neophagia

LD as well as HD ketamine rats and saline control rats did not show any significant differences in their performance in the neophagia task (Fig. 4c). All rats showed similar latencies to approach (F(2,66) = 1.99, p = 0.14) and to start consuming (F(2,66) = 0.35, p = 0.71) the novel food.

3.7. Locomotor activity

Three way ANOVA revealed that the acute amphetamine (AMPH) challenge significantly increased locomotor activity in all three groups of animals (Fig. 5; Condition: F(1,63) = 416.31, p < 0.0001), but there were significant differences in baseline locomotor activity and locomotor response to the AMPH challenge between treatment groups (Condition × Bin × Group interaction, F(11,693) = 2.35, p < 0.0001; Condition × Group interaction F(2,63)–14.07, p < 0.0001, Bin × Group interaction F(22,693) = 2.29, p < 0.001, main effect of Group(2,63) = 5.20, p < 0.01). Separate two way ANOVAs were subsequently conducted to further analyze these significant interaction effects. ANOVA of baseline locomotor activity data revealed a significant reduction in the distance traveled as the animals habituated to the locomotor chamber (Bin: F(11,693) = 43.48, p < 0.0001). While there was no significant difference in the overall level of locomotor activity across the three groups (Group effect: F(2,63) = 1.31, p = 0.28, ns), there was a significant Bin × Group interaction (F(22,693) = 2.47, p < 0.0001), which was due to significant group differences in locomotor activity in Bin numbers 4 (F(2,63) = 4.27, p < 0.02) and 8 (F(2,63) = 3.67, p < 0.04). In both cases, locomotor activity in the LD ketamine pre-treated group was significantly lower than that in the saline control group (p < 0.05). Two way ANOVA of the locomotor response to the AMPH challenge also revealed a significant effect of Bin (F(11,692) = 16.72, p < 0.0001), significant Bin × Group interaction (F(22,693) = 2.25, p < 0.001) and a significant effect of Group (F(2,63) = 9.35, p < 0.0001). Post-hoc tests on the overall level of locomotor response attributed the significant Group effect to be due to the LD ketamine pre-treated group exhibiting higher locomotor activity compared to the saline controls (p < 0.001) and HD ketamine pre-treated group (p < 0.0001). Simple effects analyses further revealed significant group differences in post-amphetamine locomotor response in Bins 3–12 (Bins 3 & 4, p < 0.05; Bins 5 & 12, p < 0.01, Bins 6–11, p < 0.0001). Multiple comparisons revealed significantly elevated locomotor activity in the LD ketamine pre-treated group compared to saline control rats in
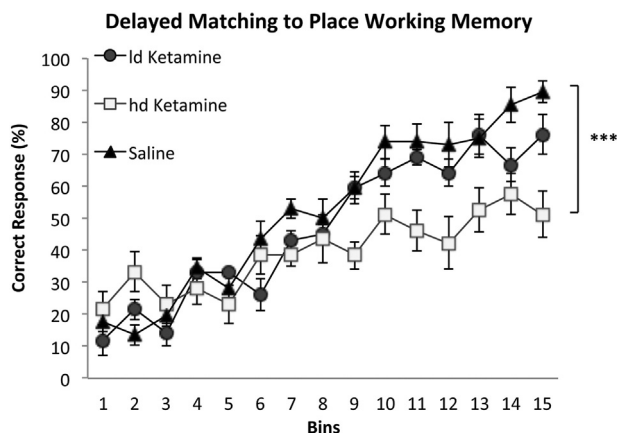

Delayed Matching to Place Working Memory

Fig. 3. Delayed matching to place T maze working memory task performance, expressed as the mean percentage of correct responses (± SEM) made over 15 bins of 6 trials each, in rats pre-treated with high-dose ketamine (n = 13), low-dose ketamine (n = 7) or saline (n = 16). There was a significant difference in the overall percentage of correct responses between the high-dose ketamine pre-treated group and saline control group. ***p < 0.001.
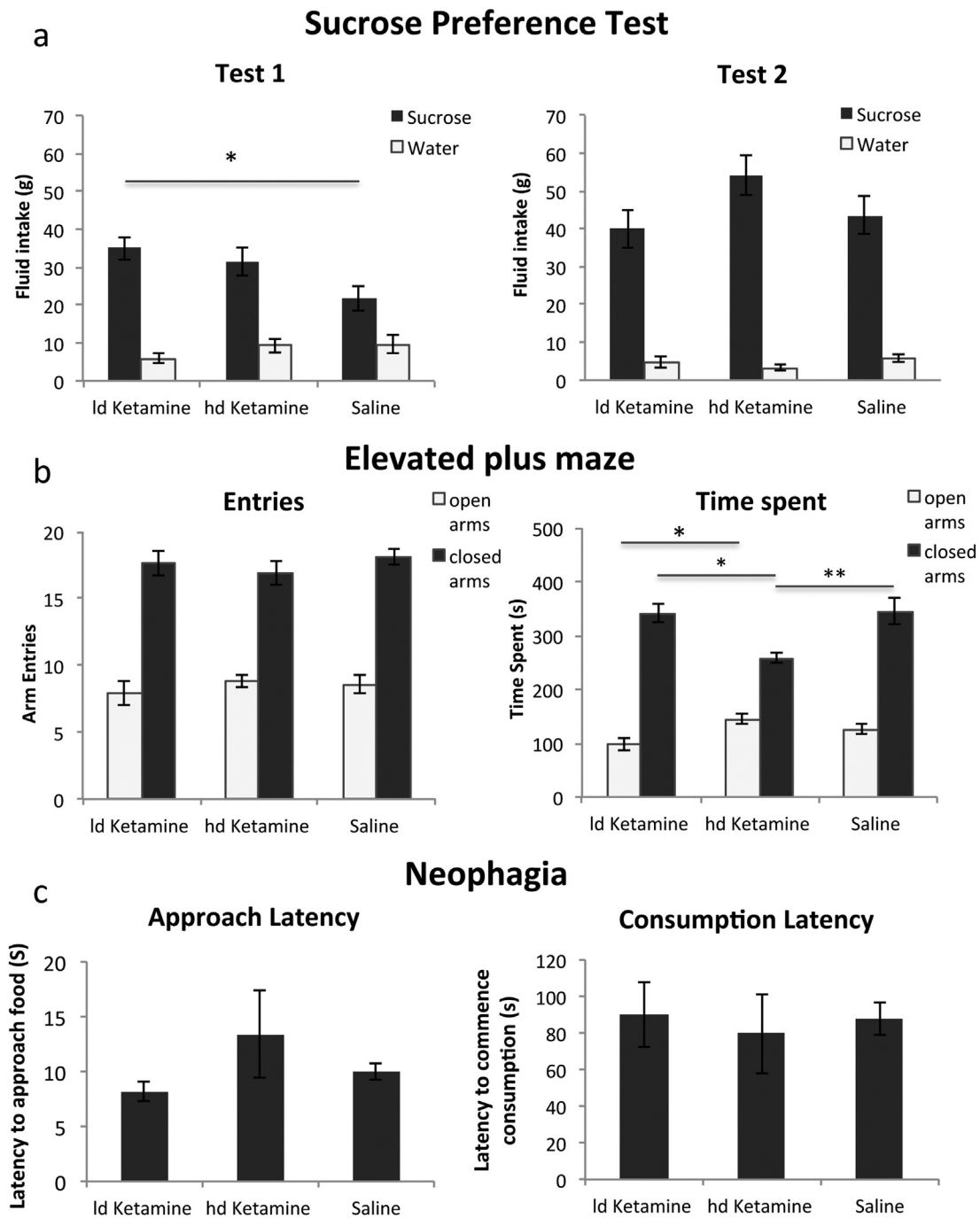
Fig. 4. Performance of sucrose preference (a), elevated plus maze (b) and neophagia (c) in rats pre-treated with high-dose ketamine (n = 13 for all tests), low-dose ketamine (n = 17 for sucrose preference, 21 for the other tests) or saline (n = 24 for first two, 32 for last test). Two sucrose preference tests were conducted, and the mean quantities (± SEM) of 1% sucrose solution and water consumed during a 16 h period are shown. *p < 0.05. In the elevated plus maze, the mean number of entries into (± SEM), and the mean total time spent in the open and closed arms were measured. In the neophagia test, the mean latencies (± SEM) to approach, and to initiate the consumption of the novel food were recorded. *p < 0.05.

all bins (p < 0.05, 0.01, 0.001) and compared to HD ketamine pre-treated group in Bins 4–12 (p < 0.05, 0.01, 0.001). In addition, HD ketamine pre-treated rats exhibited lower locomotor activity, compared to the control saline group in Bins 7–9 (p < 0.05). The reduced locomotor activity in the HD ketamine pre-treated rats was not accompanied by stereotypy.

4. Discussion

The present study provides evidence that repeated subanesthetic doses of ketamine administration in rats induces marked changes in novelty processing in a dose regimen-dependent manner. Pre-treatment with a cumulative dose of 150 mg/kg of ketamine (low-dose − LD) over the course of 5 days significantly *enhanced* novelty/mismatch detection, while pre-treatment with a total dose of 420 mg/kg of ketamine (high-dose − HD) over 7 days impaired mismatch detection, and performance on other cognitive assays such as spatial working memory. In addition, a single acute dose of amphetamine caused a sensitized locomotor response in rats pre-treated with the LD ketamine regimen, in contrast to an attenuated locomotor response in rats pre-treated with a HD ketamine regimen, indicating that the two regimens of ketamine administration induce differential
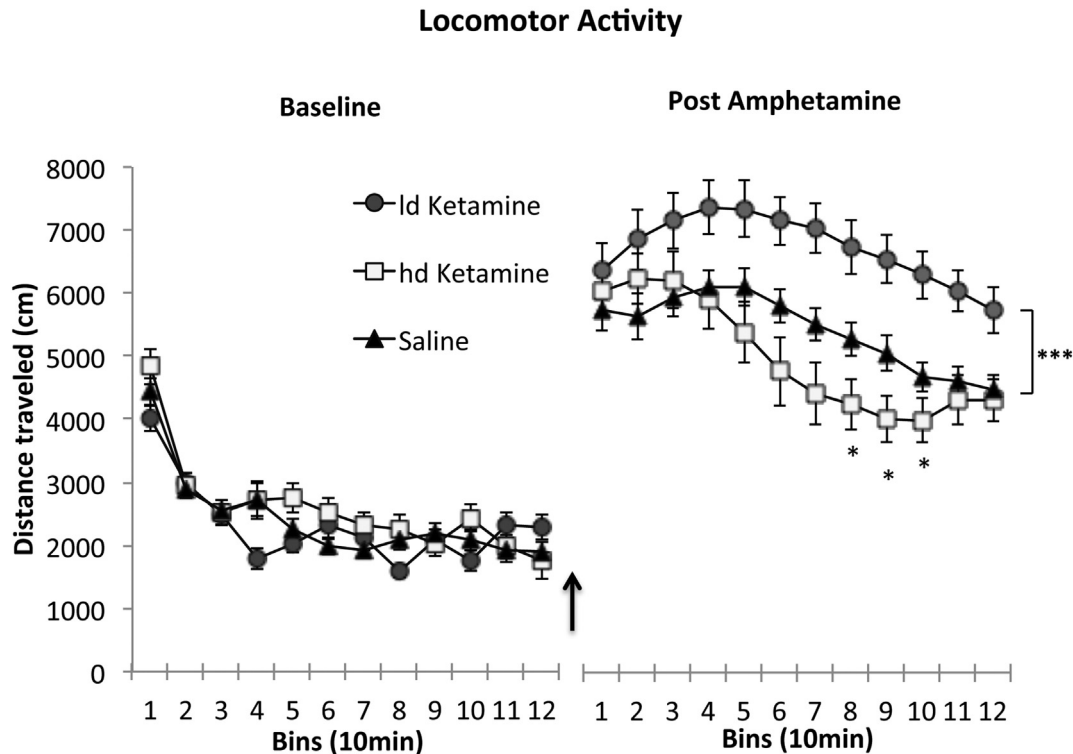
Locomotor Activity

Fig. 5. Locomotor activity expressed as the mean total distance traveled (± SEM) prior to (baseline), and after (post-amphetamine) a challenge dose of d-amphetamine (2 mg/kg) in rats pre-treated with high-dose ketamine (n = 13), low-dose ketamine (n = 21) or saline (n = 32). *p < 0.05, ***p < 0.0001.

neurochemical adaptations that most likely involve the mesolimbic dopamine system.

4.1. Effects of repeated ketamine administration on novelty processing

The major finding of the present study was that different dosing regimens of repeated ketamine administration induced opposite effects on some forms of novelty processing. Associative mismatch detection and the exploration of novel spatial locations of familiar objects were *enhanced* following a LD pre-treatment regimen of ketamine, while mismatch detection was impaired following a HD pre-treatment regimen of ketamine. The associative mismatch detection task assessed the ability of rats to learn stimulus-stimulus associations (two sequences of audio-visual stimuli), and to subsequently detect and direct attention to a novel audio-visual stimulus sequence (Honey et al., 1998). All rats, irrespective of the dose of ketamine pre-treatment, demonstrated successful learning of the two audio-visual sequences, as evidenced by the decrease in orienting responses (OR) to the visual (light) stimulus across acquisition sessions. The HD ketamine pre-treated rats, however, showed an overall reduction in the number of ORs elicited by the presentation of the audio-visual sequences, indicating that they may have had an underlying attentional deficit, which is consistent with previous work demonstrating that repeated administration of ketamine (10 days, 30 mg/kg) induces deficits in the ability to sustain attention throughout a five-choice serial reaction time task session (Nikiforuk and Popik, 2014). However, the HD ketamine pre-treated rats failed to reinstate their OR to the presentation of novel audio-visual sequences in the mismatch test, this time in the *absence* of a reduction in the overall percentage of ORs elicited. Thus, the failure of the HD ketamine pre-treated rats to show an increased level of orienting to novel audio-visual sequences, as compared to familiar audio-visual sequences is likely to reflect deficits in mismatch/novelty detection. In contrast, the LD subchronic ketamine administration selectively increased the elicitation of OR by mismatched sequences to a greater degree than was seen in the saline

control rats. Rats pre-treated with the LD regimen also demonstrated superior performance in the exploration of spatially displaced objects compared to spatially familiar objects, in a task that the saline control rats performed suboptimally. Together, these data provide the first demonstration, to our knowledge, of a dosing regimen of subchronic ketamine that leads to an enhanced faculty (novelty processing).

It is of note, however, that the exploration of novel objects and neophagia (novelty-induced suppression of feeding) were unaffected by either regimen of repeated ketamine pre-treatment, posing a question as to the exact conditions under which novelty processing becomes sensitive to repeated ketamine pre-exposure. In a previous study, Jacklin et al. (2012) reported deficits in novel object recognition in rats that had undergone subchronic ketamine exposure (2 × 30 mg/kg × 10 days) under specific conditions in which there was a significant mnemonic demand (ITI > 5 min), and when the novel object recognition involved tactile-to-visual cross modal integration (even with zero delay). The notion that tasks which place a substantial mnemonic load on the animals may be more vulnerable to disruption by ketamine exposure is highly consistent with the present finding of seeing marked deficits in the delayed-match-to place T maze working memory task in the HD repeated ketamine administration regimen group, and with previous reports of acute and subchronic ketamine administration impairing spatial working memory and behavioral flexibility (Verma and Moghaddam, 1996; Enomoto and Floresco, 2009; Venancio et al., 2011). The present results of a selective deficit in the detection of novel audio-visual sequences, but not novel object exploration in rats that had undergone the HD pre-exposure regimen, is also consistent with the idea that more complex novel object recognition paradigms that rely on a greater degree of integration of information (multisensory), are sensitive to these doses of subchronic ketamine pre-exposure. Taken together, these findings indicate that HD subchronic ketamine exposure induces a plethora of cognitive deficits, which include attentional and novelty processing, multisensory integration and spatial working memory. Furthermore,

the cognitive and novelty processing deficits observed in the present study are likely to reflect impaired functions of the medial prefrontal cortex and hippocampus. Indeed, performance of the associative mismatch detection task and delayed matching-to-place T maze task is highly sensitive to excitotoxic lesions of the medial prefrontal cortex (mPFC, Dias and Aggleton, 2000; Dias and Honey, 2002), and hippocampus (Honey et al., 1998). Both of these brain regions are known to undergo significant neurobiological alterations as a result of repeated ketamine administration, including the loss of the influence of GABAergic interneurons and resultant disinhibition of glutamatergic pyramidal neurons, and increased glutamate overflow (Olney and Farber, 1995; Moghaddam et al., 1997; Adams and Moghaddam, 1998; Cochran et al., 2003; Homayoun and Moghaddam, 2007; Hou et al., 2013; Keilhoff et al., 2004; Muller et al., 2013).

4.2. Neurobiological mechanisms of the dosing regimen effects of ketamine on novelty processing

The two dosing regimens of repeated ketamine administration also induced opposite effects on amphetamine-induced locomotor activity, providing evidence for the importance of neural adaptations occurring in the mesocortico-limbic dopamine (DA) pathway in potentially giving rise to the observed alterations in cognition and novelty detection. A locomotor test in response to a challenge dose of amphetamine, a DA (and noradrenaline) releaser, is commonly used as a test of behavioral sensitization in animals that had undergone repeated exposure to the psychostimulant. Substantial data implicate the augmentation of the locomotor response to a psychostimulant challenge (sensitization) to be a reflection of a state of hyperresponsiveness (increased efflux) in dopaminergic neurotransmission in the nucleus accumbens (Kelly et al., 1975; Pijnenburg et al., 1975; Sharp et al., 1987; Vanderschuren et al., 1999), but not noradrenaline neurotransmission (Vanderschuren et al., 2003). The LD ketamine regimen in the present study produced locomotor sensitization in response to an amphetamine challenge, despite evidence of reduced spontaneous locomotor activity. In contrast, the HD ketamine regimen generated an attenuated locomotor response to amphetamine, which could not be attributed to the induction of stereotypy. It has been proposed that acute, as well as subchronic administration of ketamine leads to increased DA outflow in the prefrontal cortex, and that this disruption in mesocortical dopaminergic neurotransmission may underlie cognitive impairments (Moghaddam et al., 1997; Lindefors et al., 1997). Given that the mesoaccumbens DA and mesocortical DA systems may have a pharmacologically and functionally inverse relationship with the latter regulating the former via direct glutamatergic projections, or indirectly through the ventral tegmental area (Le Moal and Simon, 1991; Ventura et al., 2004; Hayen et al., 2014), it is conceivable that the HD ketamine administration regimen in the present study induced neuroadaptations that gave rise to a combination of a hyporesponsive mesoaccumbens DA system and a hyperresponsive mesocortical DA system.

By the same token, the enhanced ability of our LD ketamine pre-treated rats to detect associative mismatches and novel object location may, in part, be linked to a state of hyperresponsivity of the mesoaccumbens DA system, as evidenced by their potentiated locomotor response to amphetamine. This view is consistent with the findings of increased D2R binding in the hippocampus, coupled with an increase in dopamine transporter density in the striatum in rats having undergone the same dosing regimen, which may be indicative of augmented dopamine tone in these areas (Becker et al., 2003). Phasic NAc DA is implicated in signaling novelty, as well as coding for salient events, which generate a prediction error (between expected and actual outcomes) (Schultz, 1998; Redgrave et al., 1999; Horvitz, 2000; Garris and Rebec, 2002; Mirenowicz and Schultz, 1994; Salamone et al., 2005; Roeper, 2013). Thus, we speculate that a state of hyperresponsive NAc dopamine system may have led to the attribution of a disproportionate (increased) level of salience (as coded by DA) to a non-reward-related

prediction error generated by the mismatch trials in our LD ketamine pre-treated rats. Similarly, the placement of familiar objects in novel spatial locations could have generated an exaggerated prediction error signal in our LD ketamine pre-treated rats, leading to their enhanced performance. However, further work is required to establish a *direct* link between alterations in dopamine responsivity in the NAc of subchronic ketamine pre-treated rats, and enhanced function of novelty processing.

It is highly plausible that glutamatergic mechanisms may also have contributed to the present effects of repeated ketamine on novelty processing. In vivo microdialysis studies have consistently reported a rather paradoxical *increase* in glutamate (and dopamine) efflux in the PFC after the administration of subanesthetic doses of ketamine (Lorrain et al., 2003; Moghaddam et al., 1997), as a consequence of a reduction in GABAergic inhibition of glutamatergic neurons (Olney and Farber, 1995). Furthermore, Razoux et al. (2007) observed a potentiation of synaptic transmission between the PFC and NAc and a marked increased in glutamate release in the NAc, immediately after an acute administration of ketamine (25 mg/kg), which was coupled with hyperlocomotion and disrupted latent inhibition. However, of the few studies that have directly investigated the effect of subchronic doses of ketamine on alterations in glutamatergic neurotransmission, one has reported significantly decreased glutamate binding in the frontal cortex, but not the hippocampus or striatum (Becker et al., 2003). It is possible that the neural adaptations that occur in the glutamate system as a result of ketamine administration is time-, and dosing regimen-dependent, with a potentiation of glutamatergic function in the PFC in the acute/early stages of withdrawal, followed by a contrasting depression of glutamate function in later stages of withdrawal following ketamine administration. This progression of ketamine-induced neurochemical adaptations would be highly consistent with studies demonstrating elevated cortical glutamate activity in the early stages of schizophrenia (Théberge et al., 2002), and reduced glutamatergic function once the disease is fully established (Théberge et al., 2003). While this possibility needs to be further investigated, one implication for current therapeutic interventions in development would be that pharmacological agents that attenuate excess glutamate overflow in the PFC (e.g., metabotropic glutamate receptor agonists, see Stone, 2011), would be most efficacious in the early phases of the disease.

4.3. Relevance to schizophrenia

An aberrant prediction error signaling, combined with a dysregulation in the mesolimbic dopamine system has been reported in schizophrenia patients (Roiser et al., 2009; Heinz and Schlagenhauf, 2010; Gradin et al., 2011). In vivo imaging studies have found evidence of increased DA release (Laruelle et al., 1996; Breier et al., 1997; Abi-Dargham et al., 2000) and increased presynaptic DA storage capacity (Kumakura et al., 2007) in the striatum and limbic areas of unmedicated schizophrenia patients. It has also been observed that unmedicated schizophrenics show blunted differences in neural activation of the mPFC to behaviorally significant vs. irrelevant stimuli (Schlagenhauf et al., 2009), indicating that schizophrenia patients may overattribute salience to otherwise irrelevant, or neutral stimuli. However, our results would suggest that an overresponsive mesoaccumbens DA system does not necessarily lead to a chaotic, indiscriminate salience attribution of familiar/irrelevant stimuli. While there was some evidence of our LD ketamine pre-treated animals exhibiting increases in the overall percentage of ORs emitted to both mismatch and match trials in the associative mismatch detection task, and increased total exploration time of all objects in the novel object detection task, we have also demonstrated significant improvements in the ability of these ketamine pre-treated animals to discriminate between novel and familiar stimuli.

4.4. Relevance to depression

While the present study did not specifically aim to investigate the anti-anhedonic effects of repeated ketamine exposure, we observed an enhanced preference for a low concentration of sucrose solution (1%) in the LD ketamine pre-treated animals, which could be an indication of a heightened hedonic state. This view would be consistent with an increasing number of recent clinical studies demonstrating the antidepressant effects of acute and subchronic ketamine administration, and associated changes in glucose metabolism in the PFC (anterior cingulate), orbitofrontal cortex, hippocampus and striatum (putamen) (Aan het Rot et al., 2010; Murrough et al., 2013; Shiroma et al., 2014; Lally et al., 2014, 2015). However, the enhanced preference for the sucrose solution did not persist into the second sucrose preference test, which was conducted two weeks later. The absence of potentiation in sucrose preference in the LD ketamine pre-treated group in the second test may be a reflection of the transient nature of the potentiated hedonic state induced by neural/neurochemical adaptations in the brain. This would have implications for the current use of ketamine in the treatment of depression, especially in terms of the long term efficacy of ketamine action and frequency of administration. Alternatively, the selective potentiation of sucrose preference in the first test may have reflected alterations in novelty processing (reduced neophagia). However, this account is not compatible with our failure to find any significant deficits in our test of neophagia in the ketamine-pretreated rats. Further studies using different behavioral assays of incentive motivation are warranted to differentiate the two possibilities.

4.5. Conclusion

In conclusion, the present study provides novel evidence of the dose-dependent effects of repeated ketamine administration upon novelty processing, cognition and motivation. Our LD repeated ketamine regimen induced marked enhancement in the performance of associative mismatch detection and spatial novelty exploration, and enhanced preference of a low-dose (1%) sucrose solution. In contrast, the HD repeated ketamine regimen induced significant deficits in associative mismatch detection and working memory performance. The starkly contrasting behavioral effects of the two different pre-treatment regimens used in the present study highlight the importance of the careful selection of dosing regimens in the use of subchronic ketamine exposure as a pharmacological model of schizophrenia. Furthermore, the present findings indicate that a dosing regimen that ameliorates depressive symptoms has the potential to lead to a hyper-responsive salience attribution system, and the manifestation of undesirable side effects. The present study, therefore, calls for further work to be conducted to explore the optimal dosing regimen for ketamine in its use as an anti-depressant.

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