Restoring Mood Balance in Depression: Ketamine Reverses Deficit in Dopamine-Dependent Synaptic Plasticity

Pauline Belujon and Anthony A. Grace

Background: One of the most novel and exciting findings in major depressive disorder research over the last decade is the discovery of the fast-acting and long-lasting antidepressant effects of ketamine. Indeed, the therapeutic effects of classic antidepressants, such as selective serotonin reuptake inhibitors, require a month or longer to be expressed, with about a third of major depressive disorder patients resistant to treatment. Clinical studies have shown that a low dose of ketamine exhibits fast-acting relatively sustained antidepressant action, even in treatment-resistant patients. However, the mechanisms of ketamine action at a systems level remain unclear.

Methods: Wistar-Kyoto rats were exposed to inescapable, uncontrollable footshocks. To evaluate learned helplessness behavior, we used an active avoidance task in a shuttle box equipped with an electrical grid floor. After helplessness assessment, we performed in vivo electrophysiological recordings first from ventral tegmental area dopaminergic (DA) neurons and second from accumbens neurons responsive to fimbria stimulation. Ketamine was injected and tested on helpless behavior and electrophysiological recordings.

Results: We show that ketamine is able to restore the integrity of a network by acting on the DA system and restoring synaptic dysfunction observed in stress-induced depression. We show that part of the antidepressant effect of ketamine is via the DA system. Indeed, injection of ketamine restores a decreased dopamine neuron population activity, as well as synaptic plasticity (long-term potentiation) in the hippocampus-accumbens pathway, via, in part, activation of D1 receptors.

Conclusions: This work provides a unique systems perspective on the mechanisms of ketamine on a disrupted limbic system.

Key Words: Dopamine, ketamine, learned helplessness, nucleus accumbens, synaptic plasticity, ventral tegmental area

Major depressive disorder (MDD) is the most common mental disorder in the United States (1). A recent advance shows that a single low dose of ketamine, a functional noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist, relieves symptoms in treatment-resistant depression within hours and its effects can last for up to 10 days (2), and repeated injections induce sustained antidepressant action with mild side effects (3).

Cellular mechanisms of ketamine involve the rapid induction of synaptic proteins in the prefrontal cortex and the hippocampus of rats (4,5). Ketamine rapidly reverses the stress-induced deficit in spine density (6) by activation of the mammalian target of rapamycin signaling pathway (4,6,7). However, mechanisms at a systems level remain unclear.

We focused on two systems in the learned helplessness (LH) model of stress-induced depression, the first being the dopaminergic (DA) reward system of the ventral tegmental area (VTA), in which dysfunctions (8) are thought to lead to the core MDD symptom of anhedonia that is also found in the LH model (9). Second, the ventral subiculum of the hippocampus (vSub), which is involved in context-dependent regulation of behavior and stress integration (10), was examined due to its potential involvement in ruminative behavior, a condition associated with an abnormal focus on internal states (11,12). Therefore, stress-induced disruptions of vSub-nucleus accumbens (NAc) contextual focus could drive an organism to a ruminative state (13). Mental rumination itself is not measurable in rats; however, LH can be maintained over time by processes that may be similar to those occurring in rumination (14). We thus proposed to investigate the impact of LH on DA neuron activity and synaptic transmission in the vSub-NAc pathway and how this system is influenced by ketamine administration.

Methods and Materials

Animals

Adult male Wistar-Kyoto rats (300–350 g; Charles River Laboratories, Wilmington, Massachusetts) were used for their susceptibility to LH (15). Rats were housed singly on a reversed 12-hour dark/light cycle (lights on: 7:00 P.M.) with food and water ad libitum. All experiments were performed in accordance with the guidelines outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Behavioral and electrophysiological experiments are detailed in the Supplemental Methods & Materials in Supplement 1.

Learned Helplessness Paradigm

In the LH paradigm (16,17), inescapable stress occurred on day 1 in one chamber of a two-chamber shuttle box (Med Associates, St. Albans, Vermont). Control animals (no-shock) were placed in the shocking chamber in parallel without shocking.

Helpless behavior and the effect of repeated injections of ketamine were assessed using an active avoidance task on days 2, 3, and 4. Failure was recorded if no crossing was made during the shock. The criterion of 40% failures to escape and 8-second latency to escape (17) was used to discriminate between nonhelpless and helpless rats.

From the Departments of Neuroscience, Psychiatry, and Psychology, University of Pittsburgh, Pittsburgh, Pennsylvania.

Address correspondence to Pauline Belujon, Ph.D., University of Pittsburgh, Department of Neuroscience, A210 Langley Hall, Pittsburgh, PA 15260; E-mail: belujon@pitt.edu.

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A subanesthetic and subanalgesic (18) dose of ketamine (dissolved in saline, 5 mg/kg, intraperitoneal [IP]) or saline (1 ml/kg, IP) was injected 20 minutes or 2 hours before the beginning of the active avoidance task. Ketamine was injected either repeatedly (three times, on days 3, 4, and 5) or acutely after testing for active avoidance on day 2. For acute injection, on day 3, one group of rats was tested for behavior and another was used for electrophysiological recordings.

Extracellular Recordings

Recordings were performed in chloral hydrate anesthetized rats (400 mg/kg, IP) 24 hours after the last active avoidance task and as previously described (19–23).

VTA Recordings. Microelectrodes were lowered through the VTA (anteroposterior [AP] −5.5 to −5.9 mm, mediolateral [ML] +.6 to +1.0 mm from bregma and dorsoventral [DV] −6.5 to −9.5 mm from dura) (24–26). Three parameters of activity were measured: 1) population activity (Figure S1 in Supplement 1); 2) basal firing rate; and 3) the proportion of action potentials occurring in bursts (24). Electrophysiological identification of dopaminergic neurons in the VTA is shown in Figure S2 in Supplement 1.

Ketamine (5 mg/kg, IP) or saline (1 ml/kg, IP) was injected 20 minutes, 2 hours, or 24 hours before the beginning of the first track.

NAc Recordings. Microelectrodes were lowered through the NAc (AP +1.5 mm from bregma; ML +1.1 mm from midline; DV −5 to −7.5 mm from the dura). Single-pulse (intensity 1 mA; pulse-width .25 msec) and high-frequency stimulation (HFS) (50 Hz; 2 seconds at suprathreshold) were applied to the fimbria [AP −1.6 mm from bregma; ML +1.3 mm from the midline; DV −4.5 mm from the dura (20)]. The D1 antagonist SCH23390 (.5 μg/.5 μL) or Dulbecco's phosphate buffered saline was infused locally into the NAc at a rate of .5 μL per minute via a 33-gauge cannula. Ketamine was injected IP (5 mg/kg, in saline).

Histology

Recording electrode placement was verified via electrophoretic ejection of Chicago Sky Blue dye (Sigma-Aldrich, St. Louis, Missouri) into the recording site and stimulation electrode placement was verified by delivering a 10-second pulse at 200 μA. Rats were euthanized with a lethal dose of chloral hydrate (additional 400 mg/kg) and brains were removed following decapitation. The tissue was fixed in 8% paraformaldehyde for at least 48 hours and then transferred to a 25% sucrose solution for cryoprotection. Once saturated, brains were frozen and sliced coronally at 60 μm thick using a cryostat (Leica Frigocut 2800; Leica, Bannockburn, Illinois) and mounted onto gelatin-chromalum coated slides. Tissue was stained with a combination of neutral red and cresyl violet.

Analysis

For behavior, results were expressed as mean number of failures (± SEM) and mean latency to escape (± SEM) recorded over 25 trials each day. Two-way analysis of variance (ANOVA) followed by a Dunnett's *t* test was performed with treatment as the between-subject factor and session as the within-subject factor.

Electrophysiological data were analyzed using a one-way ANOVA (DA recordings) and a one-way ANOVA with repeated measures (NAc recordings) followed by the Holm–Sidak test, with time as the within-subject factor. When the normality test failed, a one-way ANOVA on ranks (Kruskal-Wallis H-test) was performed. Multiple comparisons were analyzed using a two-way ANOVA followed by the Holm–Sidak test, with treatment as the between-subject factor and time as the within-subject factor.

Results

Repeated Injections of Ketamine Restore Escape Behavior in Helpless Rats

Rats received inescapable shocks on day 1 and were tested for escape behavior on 3 consecutive days before electrophysiological recordings (Figure 1A, B). As previously reported (17,27), inescapable

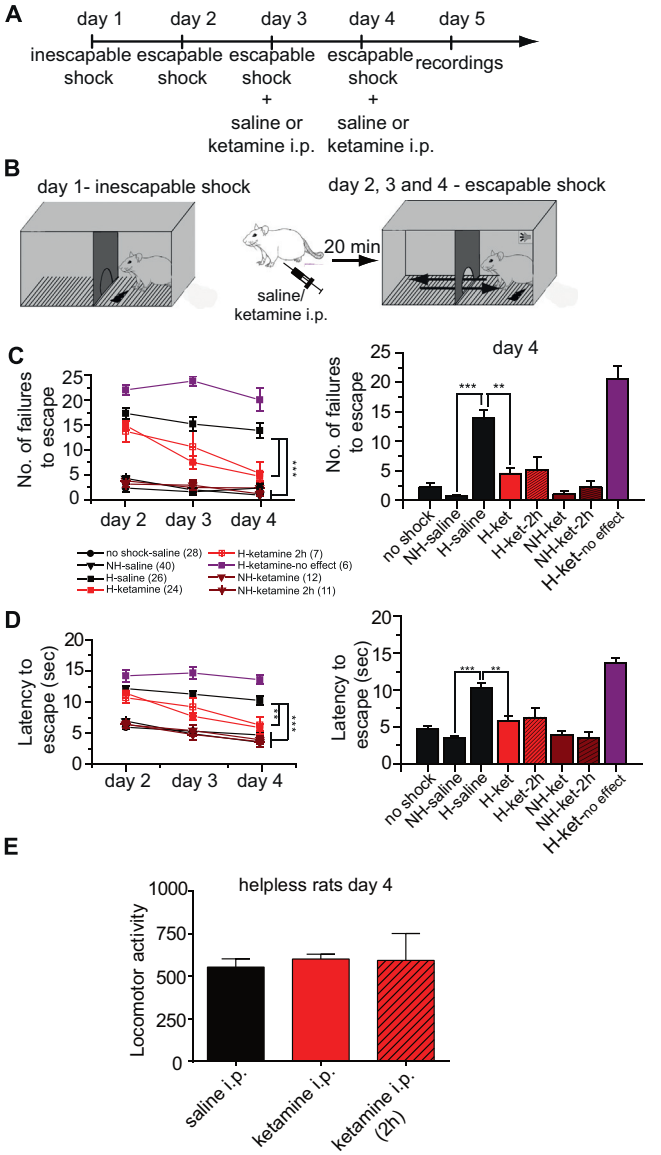

Figure 1. Learned helplessness is reversed by repeated injections of ketamine. **(A)** Experimental timeline. **(B)** Helplessness paradigm. **(C)** Number of failures to escape across 3 consecutive days of escapable shock sessions (left). Data for the escapable session on day 4 are summarized in bar graphs (right). Rats fall into two groups: those showing escape (triangles, nonhelpless; circles, no-shock) and those failing to escape (squares, helpless). Ketamine (red) causes helpless rats to show escape behavior. **(D)** Latency to escape across 3 consecutive days of escapable shock sessions, showing results consistent with escape failures (left). Data for the escapable session on day 4 are summarized in bar graphs (right). Red, purple, and brown represent data for ketamine. **(E)** There was no difference in locomotor activity measured in both sides of the shuttle box during the escapable shock session on day 4 in helpless rats following saline or ketamine 20 minutes or 2 hours after (striped bar) the injection. $^{**}p < .01$; $^{***}p < .001$. Error bars represent SEM. H, helpless rats; i.p., intraperitoneal; ket, ketamine; NH, nonhelpless rats.