Ketamine self-administration reduces the homeostasis of the glutamate synapse in the rat brain

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Abstract

Ketamine is a non-competitive antagonist of the NMDA glutamate receptor with psychotomimetic and reinforcing properties, although recent work has pointed out its antidepressant action following acute exposure.

Our aim was to investigate the expression of crucial components of the glutamate synapse following chronic ketamine self-administration (S/A), focusing our attention on medial prefrontal cortex (mPFC) and hippocampus (Hip), two brain regions involved in compulsive drug-seeking and drug-related cognitive disorders.

Rats self-administered ketamine at a sub-anesthetic dose for 5-6 weeks and were sacrificed 24 hours after the last drug exposure.

We found a general down-regulation of glutamate receptor expression that was brain region-dependent. In fact, in the mPFC, we found reduced expression of NMDA receptor subunits, whereas AMPA receptor protein levels were reduced in Hip; of note, specific scaffolding proteins of NMDA and AMPA receptors were also reduced in mPFC and Hip, respectively. Moreover, the metabotropic mGluR5 receptor was similarly down-regulated in these brain regions.

These findings reveal a dynamic impairment of glutamate homeostasis in the mPFC and Hip that may represent a signature of long-term exposure to ketamine S/A. Further, this decrement, similarly observed in humans and animal models of schizophrenia may represent a specific feature of the human disease endophenotype.

Introduction

Ketamine is a recreational drug with psychotomimetic properties causing great social and economic burden worldwide [1]. The reinforcing properties of ketamine, i.e. its ability to initiate and maintain self-administration [2], can be attributable to inhibition of NMDA receptors on GABAergic neurons which, through a disinhibitory mechanism, can cause excessive glutamate and dopamine release in prefrontal cortex and limbic striatal regions [3]. Adding complexity to the issue of ketamine abuse, several lines of evidence have recently shown that a single infusion of ketamine induces a rapid antidepressant response in subjects with major depression [4-6] thus causing a dramatic upsurge on this drug. For this reason, most of the research has focused on its rapid effects since, up to date, antidepressant drugs require chronic administration to produce their therapeutic effects; conversely, research addressing the effects of its long-term exposure is so far sparse, even though several lines of evidence have shown that repeated exposure to ketamine causes cognitive dysfunction and behavioral effects that closely mimic symptoms of schizophrenia [7,8]. Taken together, these considerations highlight the crucial importance of unveiling the mechanism(s) through which ketamine brings about its multiple actions, which include drug-seeking, psychotomimetic effects and antidepressant actions. To this end, we have recently contributed to this issue by disentangling the role of the neurotrophin BDNF in the antidepressant or reinforcing properties of this drug [9]. However, much has still to be done, primarily with respect to the long-term action of ketamine.

Drug addiction is a chronic relapsing disorder hypothesized to be due to altered neuroplasticity that renders individuals vulnerable to craving-inducing drug –related stimuli. While medial prefrontal cortex (mPFC) is a key site for compulsive drug-seeking, we know very little about ketamine-induced plasticity in this brain area, especially in terms of changes of excitatory signaling. Glutamate plays a crucial role in cognitive processes and compulsive behaviors [10] and dysregulation of glutamate signaling in cortical region has been widely demonstrated in animal models of schizophrenia [11], a disorder characterized by cognitive deficit and comorbid with drugs of abuse [12]. Moreover, studies in animals and humans also implicate hippocampus (Hip) as pivotal for changes in learning and memory due to drugs of abuse [13].

For these reasons, the major aim of our study was to investigate the expression of critical determinants of the glutamate synapse following chronic ketamine self-administration (S/A) at sub-anesthetic dosage, focusing our attention on mPFC and Hip. To this end, animals self-administered ketamine for 5-6 weeks and were sacrificed 24 hours after the last drug exposure immediately prior the daily ketamine S/A session. Molecular assessment was thus performed at a time point that was after a period of chronic ketamine (i.e., 5-6 weeks), at a ketamine-free state (i.e., 24 hours after last ketamine S/A) and before a potential ketamine-seeking condition (i.e., the expected next daily S/A session).

Material and Methods

Subjects

Adult male Sprague Dawley rats (Harlan, Italy) were individually housed in a temperature-controlled environment (19-23 °C) on a 12 hours light–dark cycle with light ON at 07.00 p.m. All the experimental procedures were conducted within the dark phase of the light-dark cycle.

Animals were food restricted to maintain their body weight range between 240 and 260 g (daily checked). Food diet (two to three pellets, for a total of 10–15 g/d) was made available during the entire experimental period. Animals had ad libitum access to water except during experimental sessions. All animal procedures were carried out in accordance with the Principles of laboratory animal care (NIH publication No. 85-23, revised 1985), the European Communities Council Directive (2010/63/UE). All efforts were made to minimize animal suffering and to keep the lowest number of animals used.

Surgery

After one week of acclimatization and one week of handling, chronic indwelling jugular catheters were implanted in all rats. Rats were anaesthetized with 0.5 mg/kg/0.5 mL medetomidine (Domitor®, Pfizer, Italy), 10 mg/kg tiletamine + 10 mg/kg zolazepam (Zoletil 100®, Virbac, Italy; 0.2 mL/kg intramuscular), and then implanted with a silastic catheter (inner diameter 0.30 mm, outer diameter 0.63 mm, Cam Caths, Cambridgeshire, UK) in the right jugular vein. Immediately after surgery, animals were medicated with 5 mg/kg/mL subcutaneous carprophene (Rimadyl®, Pfizer, Italy) and 25,000,000 IU benzyl-penicillin + 1 g/kg dihydrostreptomycin (Rubrocillina Forte®, Intervet, Italy; 1 mL/kg subcutaneous), 0.5 mg/kg/0.1 mL intramuscular atipamezole (Antisedan®, Pfizer, Italy). Each day after recovery, animals received an intravenous injection of 0.1 mL of heparin solution (30 IU/mL heparin sodium, Sigma, Italy) before and after the experimental session. Rats with catheter not patented or leaking were removed from the study.

Apparatus

Ketamine or vehicle infusions were delivered in operant chambers encased in sound-insulated cubicles, equipped with ventilation fans (Med Associates Inc., St Albans, Vermont, USA). Each chamber contained two levers. In the S/A group, right lever (ketamine-paired lever) presses corresponding to Fixed-Ratio (FR) value of 1 produced the activation of the infusion pump (Med Associates Inc.). Lever pressing during the Time-Out (TO) period was recorded, although it did not have any consequence. Left lever presses did not have any consequence. All types of lever presses were recorded for all the groups. A 2 W house light located on the back panel near the chamber ceiling provides ambient illumination during the entire session duration, except during infusions and TO periods. The house light

indicated the availability of the drug. Data acquisition and schedule parameters were controlled by Med-PC software (Med Associates Inc.).

Drugs

Ketamine hydrochloride (LGC Standards s.r.l., Italy) solutions were freshly prepared immediately before the self-administration session. Ketamine was diluted in heparinized bacteriostatic saline (0.9% NaCl plus 0.9% benzyl alcohol plus 1 IU/mL heparin), and pH was adjusted to 7.4 with NaOH. Ketamine unit doses were expressed as mg/kg of body weight/infusion. Adjustment of ketamine concentration to changes in rat body weight was not needed since rat body weight was kept stable at 250 g (± 10 g). Ketamine solution was administered via the infusion pump at a volume of 0.186 mL during a 4-s period, associated with a 4-s turn-on of stimulus light placed above the ketamine-paired lever (acting as a conditioned stimulus for the S/A group; CS).

Treatment groups and procedures

After the period of recovery from surgery, rats were divided into three groups: one group receiving a single 0.186 mL vehicle infusion during a 4-s period, and then placed into the operant chamber for 1 hour without consequences upon responding on the levers, or self-administrating vehicle (n = 9 rats in total; Vehicle or control group) for only one session, and one group trained to self-administer ketamine 0.5 mg/kg/infusion (n = 10 rats in total; S/A group). Rats in the S/A group were kept on daily S/A session for a period ranging between 35 to 43 days from the start of the experiment.

Since the two Vehicle sub-groups (one vehicle infusion with no consequences upon responding, and the chronic vehicle self-administration; respectively n = 6 and n = 3) did not show significant differences in protein expressions (data not shown), the values from the two sub-groups were pooled. In the S/A group, animals were trained to intravenously self-administer ketamine on a daily basis with following schedule of reinforcement FR1: ketamine 0.5 mg/kg/infusion, 4-s infusion duration contingently to 4-s CS, followed by TO 40 s. Session duration lasted 3 h for the first two sessions, and then 1 h for the other sessions. A priming injection of ketamine 0.5 mg/kg/infusion was administered at the start of each S/A session. Training and priming ketamine unit dose was chosen according to Venniro et al. [2] and to our unpublished dose-response experiments. The 0.5 mg/kg dose corresponds to the maximal level of responding for ketamine infusion within the range 0.125-1.0 mg/kg.

Rats were anesthetized with intraperitoneally 350 mg/kg/2 ml chloralium hydrate (Fluka, Italy). Sacrifice was performed 24 hours after vehicle infusion for the two vehicle sub-groups, or 24 hours after the end of the last 1-h session for the S/A group. Following the sacrifice, the medial prefrontal cortex (defined as Cg1, Cg3, and IL

subregions) corresponding to plates 5–9 of the atlas of Paxinos and Watson [14] has been immediately dissected from 2-mm thick slices and hippocampus was grossly dissected from the whole brain. Tissues were immediately frozen on dry ice and stored at 80°C.

Preparation of Protein Extracts and Western Blot Analyses

Hippocampi and medial prefrontal cortices were homogenized in a glass-glass potter using a cold buffer containing 0.32 M sucrose, 1mM Hepes solution, 0.1 mM EGTA, 0.1 mM PMSF, pH=7.4, in presence of a complete set of protease inhibitors and a phosphatase inhibitor cocktail. Crude synaptosomal fraction was prepared as previously described [15]. The homogenized tissues were centrifuged at 1000 g for 10 minutes; the resulting supernatant was centrifuged at 9000 g for 15 minutes to obtain the pellet corresponding to the crude synaptosomal fraction, which was resuspended in a buffer containing 20 mM HEPES, 0.1 mM dithiothreitol, 0.1 mM EGTA, in presence of a complete set of protease inhibitors and a phosphatase inhibitor cocktail. Total proteins have been measured in the crude synaptosomal fraction by the Bio-Rad Protein Assay (Bio-Rad Laboratories). Ten micrograms of proteins for each sample were run on an sodium dodecyl sulfate-8% polyacrylamide gel under reducing conditions and then electrophoretically transferred onto nitrocellulose membranes (GE Healthcare, Milan, Italy). Blots were blocked 1 hour at room temperature with 10% non-fat dry milk in tris buffered saline + 0.1% Tween-20 buffer and then incubated with antibodies against the total proteins of interest.

The conditions of the primary antibodies were the following: anti total GluN1 (1:1000, Invitrogen, Carlsbad, CA, USA), anti total GluN2B (1:1000, Santa Cruz Biotechonology, Santa Cruz, CA, USA), anti total GluN2A (1:1000, Invitrogen), anti total GluA1 (1:2000, Santa Cruz Biotechonology), anti total GluA2 (1:2000, Cell Signaling Technology Inc., Beverly, MA, USA), anti total PSD-95 (1:4000, Cell Signaling Technology Inc.), anti total SAP102 (1:1000, Cell Signaling Technology Inc.), anti- SAP97 (1:1000, Neuromab, Davis, CA, USA), anti- mGluR5 (1:4000, Chemicon, Temecula, CA, USA) and anti β-actin (1:10000, Sigma-Aldrich). Immunocomplexes were visualized by chemiluminescence using the Chemidoc MP Imaging System (Bio-Rad Laboratories). Results were standardized to β-actin control protein.

Data analysis and statistics

Data were collected in individual animals (independent determinations). Molecular data, as mean ± SEM percentages of the Vehicle/control rats, were analyzed by an unpaired two-tailed Student's t test. Subjects were eliminated from the final molecular dataset if their data deviated from the mean by 2 standard deviations.

Significance for all tests was $p<0.05$. All analyses were performed using the GraphPad software package (Prism, version 4; GraphPad, San Diego, California, USA).

Results:

In this study, rats were trained to self-administer ketamine (1-hour session). Ketamine self-administration was acquired and maintained for 35 to 43 days. Values of ketamine intake during S/A are reported in Table 1 (n=10 rats) and final rates of responding at Figure 1. Although rats met acquisition criteria (active vs. inactive lever discrimination, stable rate of responding and number of reinforcements) at different sessions, we however found that these differences did not affect the variability of final values for active lever presses and number of reinforcement, respectively, 26.7±4.6 and 14.4±2.6 (mean±SEM of the last three self-administration sessions). For Vehicle-treated group (n = 9 rats), 6 rats did not emit any specific response since the IV infusion was received non-contingently upon responding, before the start of the session in the Skinner box, and without any consequence upon responding on both levers during the 1-hour session, while 3 rats received IV infusion of vehicle with the same schedule of ketamine S/A group.

Figure 2 shows the effect of ketamine S/A on the expression of the main NMDA subunits in rat mPFC and Hip. In the mPFC, we found a significant decrease for all the subunits examined in the crude synaptosomal fraction of ketamine S/A rats. In fact, the obligatory subunit GluN1 was reduced (-14%, $t_{(17)}$=2.228, p=0.04) and so were the accessory subunits GluN2A (-28%, $t_{(17)}$=2.174, p=0.044) and GluN2B (-19%, $t_{(15)}$=2.435, p=0.028) (Fig. 2a). Interestingly, in the Hip, we observed only a significant increase in GluN2B levels in ketamine-exposed rats (GluN2B: +28%, $t_{(17)}$=2.653, p= 0.017; GluN1: +10%, $t_{(17)}$=0.7304, p=0.475; GluN2A: -1%, $t_{(17)}$=0.1367, p=0.8929) (Fig. 2b).

Figure 3 shows the effect of ketamine S/A on the expression of the main AMPA receptor subunits in rat crude synaptosomal fraction of mPFC and Hip. No significant changes were observed for AMPA receptor subunits in mPFC (GluA1: -17%, $t_{(10)}$=0.9442, p=0.3673; GluA2: -6%, $t_{(10)}$=0.5829, p=0.5729) (Fig. 3a). Conversely, we found reduced expression of GluA1 (-30%, $t_{(15)}$=2.295, p=0.037) and GluA2 (-36%, $t_{(16)}$=2.664, p=0.017) (Fig. 3b) in the Hip of ketamine S/A rats.

Figure 4 shows a significant ketamine S/A -induced reduction of mGluR5 in both mPFC (-25%, $t_{(16)}$=3.258, p=0.0049, panel A) and Hip (- 27%, $t_{(17)}$=2.350, p=0.031, panel B), whereas Figure 5 shows the effect on the expression of the main NMDA and AMPA receptor scaffolding proteins in rat mPFC and Hip. In the mPFC, ketamine reduced the expression of PSD-95 (-23%, $t_{(17)}$=3.273, p=0.0045) and SAP102 (-24%, $t_{(17)}$=2.417, p=0.027) while no changes were detected for SAP97 (0%, $t_{(16)}$=0.012, p=0.99) (Fig. 5a). A different picture was drawn for scaffolding protein expression in rat hippocampus. In fact, no changes were detected for PSD-95 (+ 8%, $t_{(16)}$=0.9034, p=0.3797) and SAP102 (+7%, $t_{(17)}$=0.5952, p=0.5596) whereas SAP97 expression was reduced by repeated exposure to ketamine by 32% ($t_{(16)}$=2.587, p=0.02) (Fig. 5b).

Discussion:

Our findings show that repeated ketamine S/A markedly reduces the expression of specific glutamate receptor subunits and their scaffolding proteins in mPFC and Hip. These changes, indicative of post-synaptic glutamate alteration, may impair the glutamate synapse homeostasis.

Our results show that ketamine-treated rats acquire and maintain the self-administration behavior suggesting that glutamate hypofunction is correlated to long-term ketamine S/A. Such cortical hypofunction may be relevant and cause a maladaptive response when the system is challenged: in fact, we have recently shown that acute stress dysregulates the glutamate synapse in the mPFC of cocaine-treated rats [17], which were characterized by reduced expression of PSD-95 in the mPFC [18]. The reduced cortical NMDA-mediated tone is strengthened by the reduced expression of metabotropic receptor mGluR5, contributing to delineate an overall picture of cortical hypoglutamatergia as a critical component of long-term ketamine S/A: notably, reduced cortical expression of mGluR5 may be suggestive of relapse liability [19] as well as of reduced ability to extinguish cocaine-seeking [20].

While ketamine-induced changes in translation might alter the composition of NMDA and AMPA receptor complexes, an altered expression of the scaffolding protein may impair glutamate receptors cycling, a dynamic process that is pivotal for glutamate neurotransmission. Of note, these results represent the first evidence of a selective and region-dependent reduction of NMDA- and AMPA-specific scaffolding proteins following ketamine S/A, which contributes to highlight a dynamic impairment of glutamate homeostasis. To this end, it is important to note that, besides acting as scaffolds, these proteins are also necessary for the trafficking of newly synthesized receptor towards the dendritic spines [21]. Therefore, repeated self-administration of ketamine might have compromised the glutamate signaling in mPFC and hippocampus not only via the reduced expression of specific glutamate receptors but, also, through the reduced ability to transport, target and anchor these receptor subunits to the synaptic membrane of the dendritic spine, as shown by the reduced expression of the scaffolding proteins PSD-95, SAP97 and SAP102. To sum up, we can conclude that the ketamine-induced reduction in the expression of specific scaffolding proteins has reduced the synaptic stability of both NMDA and AMPA receptors in a brain region-dependent fashion, perhaps resulting in an impaired ability to properly transmit the glutamate input.

Of note, disorders related to substance abuse share glutamatergic mechanisms with schizophrenia [22]. To this end, a relevant, functional implication of our results stems from the evidence that an hypoglutamatergic state in the mPFC has been closely associated with a schizophrenia-like phenotype [23,24] and deletions of either NMDA or AMPA receptor subunits cause schizophrenia-like symptoms [25,26]: these data point to S/A of sub-anesthetic doses of ketamine as a risk factor for the development of schizophrenic symptoms.

Interestingly, our data unveil a different picture in mPFC and Hip of chronically ketamine-treated rats. In fact, long-term abuse of ketamine leads to a region selective reduction of glutamate transmission that involves NMDA receptors and their scaffolding protein in the mPFC whereas AMPA receptors and their specific anchoring proteins are selectively reduced in the Hip. Such peculiar regulation of repeated ketamine S/A highlights structural changes in the mPFC whereas, in the Hip, mechanisms related to receptor depolarization are more likely to come into play. This hypothesis is corroborated by the evidence that the expression of PSD-95, an index of integrity and stability of excitatory synapses [27], is reduced in the mPFC but not Hip, in line with previous reports in humans [28]. Given that the reduced expression of PSD-95 might be suggestive of a reduced number of spines after repeated exposure to drugs of abuse [18], we hypothesize that ketamine has caused structural changes in this brain region that, together with reduced NMDA-mediated transmission, may contribute to the hypoglutamatergia observed in the post-mortem brains of schizophrenic patients [29,30]. This possibility is reinforced by the evidence that mGluR5 receptors, whose expression is reduced in the mPFC of ketamine-treated rats, regulate NMDA mediated cognitive functions [31] presumably via reduced activation of NMDA currents [32]. Further, dysregulation of mGluR5 causes behavioral phenotypes associated with schizophrenia [33,34].

Conversely, in the Hip, the lack of changes in NMDA receptor subunit expression is in line with previous reports in humans [35,36] and so are the reductions of AMPA receptors that have been previously reported in humans [37,38] and in animal models characterized by cognitive deficit [39]. Notably, recent data show hippocampal-dependent memory deficit in ketamine users [40]. The similarity between human and animal data indicates that long-term ketamine S/A reflects specific hippocampal features of the human disease endophenotype. Intriguingly, the reduced expression of AMPA receptors in the Hip may be functionally relevant and, albeit indirectly, contribute to the ketamine-induced schizophrenia-like phenotype. In fact, it is well established that the desensitization promoted by AMPA receptors is crucial for the NMDA channel to open [41] and, therefore, it could be hypothesized that, in the Hip, the reduction of the GluA1 and GluA2 subunits may impede or, at least, slow down the activation of NMDA receptors, thus contributing to the overall down-regulation and functional impairment on NMDA-mediated transmission typical of schizophrenia. Interestingly, the very recent data about the effects of ketamine metabolite (*R*)(*2R,6R*) isoform of hydroxynorketamine (*2R,6R*-HNK), which is active as antidepressant in mice through a mechanism involving AMPA receptor agonism but not NMDA receptor antagonism showed that a single treatment with *2R,6R*-HNK, as well as with ketamine, induced an increase of GluA1 and GluA2 receptor expression in Hip but not PFC synaptoneurosomes which lasted 24 hours [42]. Our data, showing an opposite regional profile of AMPA receptors modulation after chronic exposure to ketamine, support a discrimination between the effects of an antidepressant dosing regimen of ketamine on AMPA receptors (up-regulation) (as in [40]) and those induced by chronic reinforcing dosing (down-regulation) in Hip

in our study, in accordance to a similar discrimination that we previously reported for BDNF [9]. This possibility is strengthened by the evidence that mGluR5, that are also decreased in the Hip of ketamine-treated rats, are pivotal for hippocampal-mediated long-term potentiation and learning [43].

In conclusion, we here provide evidence indicating that long-term self-administration of sub-anesthetic doses of ketamine reduces glutamate transmission in mPFC and Hip through receptor-specific alterations. Such defect is reinforced by reduced expression of specific scaffolding proteins depicting a dynamic deficit of glutamate-mediated neuroplasticity in ketamine-treated rats. These results have broad implications that range between drug abuse and schizophrenia and may contribute to explain, at least partially, the comorbidity between these two disorders.

Figure legends

Fig. 1: Ketamine self-administration (S/A) rate of responding on last S/A sessions [mean± standard error (SEM) of the last three self-administration sessions]. Ordinate represents number of lever presses (solid circle = active; solid square = inactive) at the three last sessions (respectively, -3, -2 and -1, with the latter as the last S/A session).

Fig. 2: Effects of chronic ketamine self-administration (S/A) on the NMDA GluN1, GluN2A and GluN2B protein levels in the crude synaptosomal fraction from rat medial prefrontal cortex (mPFC) (panel A) and hippocampus (Hip) (panel B). Animals were sacrificed 24 hours after the last S/A session. In the upper part of the figure, representative immunoblots are shown for GluN2A (180 kDa), GluN2B (180 kDa) and GluN1 (120 kDa) proteins in the mPFC and Hip.

Results are expressed as percentages of vehicle-treated rats (Control). Histograms represent the mean+SEM of 8-10 rats per group. * $p< 0.05$ vs. control rats (unpaired Student's t-test).

Fig. 3: Effects of chronic ketamine self-administration (S/A) on the AMPA receptor subunits GluA1 and GluA2 protein levels in the crude synaptosomal fraction from rat mPFC (panel A) and Hip (Panel B). Animals were sacrificed 24 hours after the last S/A session. In the upper part of the figure, representative immunoblots are shown for GluA1 (108 kDa) and GluA2 (108 kDa) proteins in the mPFC and Hip.

Results are expressed as percentages of vehicle-treated rats (Control). Histograms represent the mean+SEM of 6-10 rats per group. * $p< 0.05$ vs. control rats (unpaired Student's t-test).

Fig. 4: Effects of chronic ketamine self-administration (S/A) on mGluR5 receptor expression in the crude synaptosomal fraction of mPFC (panel A) and HIP (panel B). Animals were sacrificed 24 hours after the last S/A session. Below the graphs, representative immunoblots are shown for mGluR5 (132 kDa) protein in the mPFC and Hip.

Results are expressed as percentages of vehicle-treated rats (Control). Histograms represent the mean+SEM of 8-10 rats per group. * $p< 0.05$, ** $p<0.01$ vs. control rats (unpaired Student's t-test).

Fig. 5: Effects of chronic ketamine self-administration (S/A) on scaffolding protein expression in the crude synaptosomal fraction of mPFC (panel A) and Hip (panel B). Animals were sacrificed 24 hours after the last S/A session. In the upper part of the figure, representative immunoblots are shown for PSD-95 (95 kDa), SAP102 (102 kDa) and SAP97 (97 kDa) proteins in the mPFC and Hip.

Results are expressed as percentages of vehicle-treated rats (Control). Histograms represent the mean+SEM of 8-10 rats per group. * $p< 0.05$, ** $p<0.01$ vs. control rats (Student's t-test).

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The authors report no biomedical financial interests or potential conflicts of interest.

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Table 1

Values of ketamine intake during self-administration (S/A) training

Total ketamine intake (mg/kg)	Daily ketamine intake (mg/kg)	3 last session ketamine intake (mg/kg)
267.6 ± 143.5	8.9 ± 4.8	7.2 ± 1.3

Data represent the mean ± standard deviation (SD) for total and daily ketamine intake during the whole S/A period, and the mean ± standard error (S.E.M.) for ketamine intake during the last three S/A sessions.