Ketamine self-administration elevates αCaMKII autophosphorylation in mood and reward-related brain regions in rats

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Abstract

Modulation of αCaMKII expression and phosphorylation is a feature shared by drugs of abuse with different mechanisms of action. Accordingly, we investigated whether αCaMKII expression and activation could be altered by self-administration of ketamine, a non-competitive antagonist of the NMDA glutamate receptor, with antidepressant, psychotomimetic as well as reinforcing properties.

Rats self-administered ketamine at a sub-anesthetic dose for 43 days and were sacrificed 24 hours after the last drug exposure; reward-related brain regions, such as medial prefrontal cortex (PFC), ventral striatum (vS) and hippocampus (Hip), were used for the measurement of αCaMKII-mediated signaling.

αCaMKII phosphorylation was increased in these brain regions suggesting that ketamine, similarly to other reinforcers, activates this kinase. We next measured the two main targets of αCaMKII, i.e. GluN2B(S1303) and GluA1(S831), and found increased activation of GluN2B(S1303) together with reduced phosphorylation of GluA1(S831). Since GluN2B, via inhibition of ERK, regulates the membrane expression of GluA1, we measured ERK2 phosphorylation in the crude synaptosomal fraction of these brain regions, which was significantly reduced suggesting that ketamine-induced phosphorylation of αCaMKII promotes GluN2B(S1303) phosphorylation that, in turn, inhibits ERK 2 signaling, an effect that results in reduced membrane expression and phosphorylation of GluA1.

Taken together, our findings point to αCaMKII autophosphorylation as a critical signature of ketamine self-administration providing an intracellular mechanism to explain the different effects caused by αCaMKII autophosphorylation on the post-synaptic GluN2B- and GluA1-mediated functions. These data add ketamine to the list of drugs of abuse converging on αCaMKII to sustain their addictive properties.

Introduction

The Ca^{2+}/calmodulin dependent protein kinase II (αCaMKII) is a multifunctional kinase that co-localizes with, and regulates, glutamate receptors, primarily NMDA and AMPA receptors [1], contributing to neuronal excitability and playing a pivotal role in various forms of synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD). Its phosphorylation in the threonine residue in position 286 generates autonomous kinase activity, which locks αCaMKII in a Ca^{++}-independent state [2] , thus rendering the system less flexible and unable to adjust the response to calcium fluctuations.

Besides its physiological role, accumulating evidence has shown that αCaMKII is crucially involved in the long-term molecular and structural changes that characterize addiction [3]. Most of these studies involved the psychostimulant cocaine. Easton and colleagues have shown that αCaMKII controls the establishment of cocaine reinforcing effects in mice and humans [4]. Enhanced αCaMKII gene expression and phosphorylation in the ventral striatum (vS) seem to be pivotal for the motivation to self-administer cocaine [5] as well as the reinstatement of cocaine seeking [6]. We have recently contributed to this topic by showing that short-term abstinence from cocaine self-administration enhances αCaMKII autophosphorylation in the vS and medial prefrontal cortex (PFC) [7], suggesting that this kinase is regulated not only by cocaine-taking but also by its cessation, implying a role of this kinase in cocaine withdrawal syndrome. In addition, by employing a yoked control paradigm, we demonstrated that cocaine-induced elevation of αCaMKII autophosphorylation occurs only in the animals that self-administered cocaine, further strengthening its role in the motivation to self-administer the drug [7]. Notably, the potential role of αCaMKII in the action of cocaine has been recently expanded including a novel mechanism of αCaMKII regulation such as the phosphorylation at serine 331 [8].

Although different drugs of abuse act through distinct mechanisms, activation of αCaMKII appears to be a common feature of many of them. An elegant manuscript has recently summarized the state of art by exploring the role of αCaMKII primarily in the action of opiates and cocaine [9]. Interestingly, these authors have shown that αCaMKII, although it does play a role in the action of both classes of drugs, was however primarily implicated in opiate action as critical determinant of dependence/withdrawal whereas its role in the action of the psychostimulant cocaine was tightly dependent on the paradigm employed, i.e. duration of treatment, modality of drug administration and previous exposure, or not, to self-administration [9]. Adding to these lines of evidence, recent data have indicated that αCaMKII is critical also for the positive reinforcing effects of alcohol [10-13]. Taken together, the available data suggest that changes in αCaMKII autophosphorylation are among the molecular neuroadaptations set in motion by different classes of drugs of abuse in association to their addictive effects.

To this end, we extended our analysis to ketamine, a widely abused recreational drug that causes dissociative and hallucinatory symptoms together with severe medical problems [14,15]. Ketamine is a non-competitive NMDA antagonist that, via a disinhibitory mechanism, increases dopamine release in striatum and prefrontal cortex [16], an effect thought to be responsible of the psychotic-like symptoms exerted by ketamine; of note, we have also shown that repeated ketamine self-administration impairs glutamate receptors expression in PFC and hippocampus (Hip) [17], suggesting a perturbation of glutamate homeostasis following long-term exposure to ketamine. In line with ketamine-induced impairment of glutamate functions, repeated exposure to phencyclidine, another non-competitive NMDA antagonist, has been shown to cause a decline in αCaMKII autophosphorylation in rat prefrontal cortex [18]. However, these authors used a dosing regimen and a route of administration that allows the investigation of psychotomimetic, but not reinforcing, properties of ketamine. Conversely, based on the above-mentioned considerations on the effects of several drugs of abuse on αCaMKII, we hypothesized that it might play a role in the reinforcing properties of ketamine and therefore we incorporated a chronic self-administration paradigm with subanaesthetic dosage of ketamine. Accordingly, animals self-administered ketamine for 43 days and were sacrificed 24 hours after the last self-administration session, to investigate the long-term effect of the drug and avoid effects related to abstinence. We focused our attention on reward-related brain regions (PFC and vS) but also on Hip that is known to be important for the development and maintenance of addiction, via the modulation of specific behaviors, such as instrumental learning [19], drug-reinforced instrumental behaviors [20] and memory systems [21-23], as well as via the regulation of specific mechanisms such as adult hippocampal neurogenesis [24]. In order to evaluate whether ketamine self-administration might influence further glutamate homeostasis, we focused our attention on critical determinants of glutamate neurotransmission such as the NMDA receptor subunit GluN2B and the AMPA receptor subunit GluA1 that are critical for synaptic plasticity and memory formation [25-27]. To this end we measured the expression and phosphorylation of GluN2B and GluA1, whose serine residues in 1303 [28] and 831 [29,30], respectively, are known targets of αCaMKII and whose regulation has been shown to play a role in animal models of psychiatric disorders [31,32]. We also examined the expression and phosphorylation of the MAP kinase ERK2 [33], which may link together αCaMKII with these post-synaptic excitatory receptors, in an attempt to provide a cohesive picture of the ketamine-induced alteration of the glutamate synapse.

Material and Methods

Subjects

For this manuscript, we took advantage of a previous experiment [17] and examined αCaMKII expression and phosphorylation, together with the activation of its major intracellular targets, in PFC, vS and Hip for two reasons: first, to further explore ex-vivo changes in rats whose addiction-like behaviour was previously established [17] and, secondly, to comply with animal care and welfare European and National legislation on reduction of number of animals, whenever possible. 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.

After one week of acclimatization and one week of handling, chronic indwelling jugular catheters were implanted in all rats as described in [17]. 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.

Drugs

Ketamine hydrochloride was a commercial solution "LOBOTOR100 mg/mL" (ACME, Italy). Ketamine solutions were freshly prepared immediately before the infusion 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 self-administration group; CS).

Treatment groups and procedures

After the period of recovery from surgery, rats were divided into groups receiving ketamine or vehicle. Rats allocated to receive ketamine through self-administration (S/A group) were kept on daily ketamine 0.5 mg/kg/infusion

S/A session (n = 10 rats in total) for a period ranging between 35 to 43 days from the start of the experiment. The Vehicle group included i), rats injected with a single 0.186 mL vehicle infusion during a 4-s period, and then placed into the operant chamber (Apparatus described in details in [17]; Med Associates Inc., St Albans, Vermont, USA) for 1 hour without consequences upon responding on the levers, and ii), rats self-administrating vehicle for only one session. Since these two vehicle sub-groups (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 a Time-Out (TO) period of 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. [34] 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 chloral 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 [35] 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

Ventral striata, 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 [17]. 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 a 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: pαCaMKII T286 (1:2000, Thermoscientific, Italy); αCaMKII (1:5000, Millipore, Italy); pGluN2B S1303 (1:1000, Upstate); anti total GluN2B (1:1000, Santa Cruz Biotechonology, Santa Cruz, CA, USA), pGluA1 S831 (1:500, Thermoscientific); anti total GluA1 (1:2000, Santa Cruz Biotechonology), anti phospho-ERK2 T185/187 (1:1000, Cell Signaling Technology, USA); anti total ERK2 (1:5000, Cell Signaling Technology), and anti β-actin (1:10000, Sigma-Aldrich). Results were standardized to β-actin control protein, which was detected by evaluating the band density at 43 kDa. Immunocomplexes were visualized by chemiluminescence using the Chemidoc MP Imaging System (Bio-Rad Laboratories).

Statistical analysis

Data were collected in individual animals (independent determinations) and are presented as means and standard errors. The effects produced by repeated ketamine treatment were analyzed by an unpaired Student's *t* test. Statistical significance was assumed at $p<0.05$.

Results

The following analyses were undertaken in the crude synaptosomal fraction, enriched in cell membranes. Figure 1 shows the effect of ketamine S/A on the expression and autophosphorylation of αCaMKII in rat nucleus accumbens (vS) (panel A), medial prefrontal cortex (PFC) (panel B) and hippocampus (Hip) (panel C). In all the brain areas analyzed, repeated exposure to ketamine increased αCaMKII autophosphorylation in threonine 286 (vS: +48%, $t_{(16)}$= 2.36, p= 0.031; PFC: +48%, $t_{(15)}$= 2.97, p= 0.01; Hip: +32%, $t_{(17)}$= 3.36, p= 0.004). At variance from PFC and Hip, in the vS, we found a significant increase of αCaMKII expression (vS: +54%, $t_{(16)}$= 2.37, p= 0.031; PFC: -4%, $t_{(13)}$= 0.46, p= 0.65; Hip: +8%, $t_{(17)}$= 1.12, p= 0.28).

Figure 2 shows the effect of ketamine S/A in the vS on the expression and phosphorylation of the accessory NMDA subunit GluN2B and the main AMPA subunit GluA1, whose serine residues in 1303 and 831, respectively, are well established targets of αCaMKII. We found a significantly reduced expression of GluN2B with increased phosphorylation of GluN2B(S1303) (GluN2B: -27%, $t_{(15)}$= 2.23, p= 0.042; pGluN2B: +68%, $t_{(16)}$= 2.57, p= 0.02; panel A) whereas both GluA1 levels and GluA1(S831) phosphorylation were significantly reduced (GluA1: -32%, $t_{(15)}$= 2.20, p= 0.044; pGluA1: -38%, $t_{(14)}$= 2.67, p= 0.018; panel B). We next measured the expression and phosphorylation of ERK2, since it has been shown that its GluN2B-induced modulation may regulate GluA1 surface delivery [33]. We found a significant reduction in pERK2(T186/Y187) (-54%, $t_{(10)}$= 3.40, p= 0.007) with no changes of ERK2 levels (+22%, $t_{(10)}$= 0.89, p= 0.397; panel C).

Figure 3 shows the effect of ketamine S/A on the expression and phosphorylation of the same targets in PFC. In line with the vS, we found a significant reduction of GluN2B expression with increased phosphorylation of GluN2B(S1303) (GluN2B: -25%, $t_{(14)}$= 3.42, p= 0.004; pGluN2B: +42%, $t_{(13)}$= 2.49, p= 0.027; panel A) and a reduced GluA1(S831) phosphorylation with no changes in GluA1 protein levels (GluA1: -26%, $t_{(10)}$= 1.60, p= 0.141; pGluA1: -31%, $t_{(10)}$= 2.65, p= 0.024; panel B). The analysis of the expression and phosphorylation levels of ERK2 revealed a significant reduction in pERK2(T186/Y187) with no changes of ERK2 levels (pERK2: -50%, $t_{(17)}$= 3.45, p= 0.003; ERK2: +16%, $t_{(17)}$= 1.15, p= 0.266; panel C).

Figure 4 shows the effect of ketamine S/A on the expression and phosphorylation of the same targets in Hip. We found a significant increase in both expression of GluN2B and phosphorylation of GluN2B(S1303) (GluN2B: +30%, $t_{(17)}$= 2.50, p= 0.023; pGluN2B: +37%, $t_{(14)}$= 2.58, p= 0.022; panel A) whereas both GluA1 levels and GluA1(S831) phosphorylation were significantly reduced (GluA1: -33%, $t_{(17)}$= 2.70, p= 0.015; pGluA1: -26%, $t_{(17)}$= 2.29, p= 0.035; panel B). As previously shown in the vS and PFC, also in the Hip the analysis of the expression and phosphorylation levels of ERK2 revealed a significant reduction in pERK2(T186/Y187) with no changes of ERK2 levels (pERK2: -36%, $t_{(17)}$= 2.25, p= 0.038; ERK2: +31%, $t_{(17)}$= 1.57, p= 0.134; panel C).

Discussion

We here show that ketamine self-administration increased αCaMKII autophosphorylation in brain regions involved in both potential antidepressant and addictive properties of ketamine such as vS, PFC and Hip. These findings add ketamine to the list of addictive drugs (alcohol, cocaine, opioids) promoting enhanced autophosphorylation of αCaMKII, and further support the hypothesis that αCaMKII activation is a convergent mechanism in the action of different drugs of abuse.

Of note, we here show that αCaMKII autophosphorylation does not lead to a general activation of its main targets: in fact, while the phosphorylation of the NMDA subunit GluN2B(S1303) is increased, the phosphorylation of the AMPA subunit GluA1(S831) is reduced, suggesting the targeting of specific processes rather than a global interference with glutamate homeostasis. The GluN2B subunit of the NMDA receptor is phosphorylated by αCaMKII at Ser1303 [28]; although the functional 'in vivo' relevance of GluN2B(Ser1303) phosphorylation is still largely elusive, such αCaMKII-mediated activation has been however shown to increase NMDA-mediated currents and modulate NMDA signaling via changes in receptor trafficking as well as interactions with scaffolding proteins [36-39]. It is conceivable to hypothesize that αCaMKII-induced enhancement of pGluN2B(Ser1303) may increase calcium influx into spines [40,41] resulting in abnormal strengthening of synapses. This effect may cause synaptic saturation, thus contributing to enduring drug-seeking behaviour, presumably usurping networks implicated in physiological learning [42]. αCaMKII phosphorylates also the GluA1 AMPA receptor subunit at Ser831 causing an increase in channel conductance, an effect that also contributes to hippocampal LTP [29,30]. Unexpectedly, we observed a reduced GluA1(Ser831) phosphorylation suggesting that ketamine may have uncoupled the kinase from this receptor. Notably, it appears that the profile of αCaMKII expression differs between brain regions: in fact, in the vS, cocaine increases protein levels of αCaMKII, presumably leading to increased phosphorylation whereas, in the PFC and Hip, cocaine directly activates αCaMKII autophsopshorylation. This evidence indicates different mechanisms underlying the activation of the kinase.

In an attempt to investigate the mechanism responsible of the dichotomy of GluN2B and GluA1 phosphorylation, we focused our attention on ERK signaling, whose phosphorylation has been shown to play a pivotal role in glutamate receptor trafficking (Kim et al., 2005). We found that, in the crude synaptosomal fraction of these brain regions, ketamine caused a marked decrease of ERK2 phosphorylation, in line with the previously shown effect in the whole homogenate [43]. Given that GluN2B activation is coupled to inhibition of ERK signaling, an effect that leads to reduced GluA1 expression in cell membranes (Kim et al., 2005), we propose a cohesive picture suggesting that ketamine self-administration enhances αCaMKII autophosphorylation which, in turn, stimulates the phosphorylation of

GluN2B in the Ser1303 residue; such activation inhibits ERK signaling, as previously shown [33], resulting in reduced membrane expression and phosphorylation of GluA1 (Fig 5).

Of note, based on evidence from the literature showing that the non-contingent treatment reduced αCaMKII autophosphorylation [44,45], it is possible to speculate that increased αCaMKII autophosphorylation may be critical for the motivation to self-administer ketamine.

Elevated αCaMKII autophosphorylation levels are tightly linked with alterations of synaptic plasticity and cognition. Transgenic mice with constitutively active αCaMKII autophosphorylation show reduced excitatory postsynaptic currents in the hippocampus [46] while mice with α-thalassemia X-linked mental retardation show elevated CaMKII activity in the PFC associated with learning deficit [47]; in addition, animal models of ADHD show cognitive deficit due to aberrant CaMKII activity in the PFC [48]. Based on these results, we suggest that increased αCaMKII autophosphorylation, caused by exposure to drugs of abuse, represents a molecular signature that may lead to defective cognition, perhaps contributing to the addictive process. Also, αCaMKII is emerging as a critical target of neuropsychiatric disorders suggesting that changes in its expression and/or activation may represent a mechanism to explain, at least partially, the comorbidity with, for instance, depression [49].

In conclusion, our results show that ketamine self-administration enhanced αCaMKII autophosphorylation in brain regions known to play a critical role in drug addiction, suggesting that up-regulation of αCaMKII autophosphorylation may represent a contributing factor for the reinforcing properties of ketamine. We propose a molecular mechanism through which ketamine self-administration reorganizes the glutamate synapse, influencing its homeostasis in addiction-associated brain regions via specific changes that are likely to cause opposite effects on the post-synaptic functions mediated by the glutamate receptors GluN2B and GluA1, which represent the main targets of αCaMKII.

Financial Disclosures/Conflicts of Interest

The authors report no conflicts of interest.

Figure legends:

Fig. 1: Effect of ketamine self-administration on p-αCaMKII(Thr286) and total αCaMKII levels in the crude synaptosomal fraction of ventral striatum (vS, panel A), medial prefrontal cortex (PFC, panel B) and hippocampus (Hip, panel C). Rats [ketamine self-administration (S/A) and vehicle-exposed (control)] were killed 24 h after the last ketamine session. Below the graphs, representative immunoblots are shown for p-αCaMKII(Thr286) (50 KDa) and αCaMKII (50 KDa) proteins in the crude synaptosomal fraction of vS, PFC and Hip of rats exposed to ketamine. Results are expressed as percentages of control rats. Histograms show the mean±SEM of at least 7–10 rats per group. *p<0.05, **p<0.01 vs. control rats (unpaired Student's *t* test).

Fig. 2: Effect of ketamine self-administration on the main targets of αCaMKII in the crude synaptosomal fraction of nucleus accumbens.

Panel A shows the phosphorylation and expression levels of GluN2B; Panel B shows the phosphorylation and expression levels of GluA1 and panel C shows the phosphorylation and expression levels of ERK2. Representative immunoblots are shown in panel D.

Rats [ketamine self-administration (S/A) and vehicle-exposed (control)] were killed 24 h after the last ketamine session. Results are expressed as percentages of control rats. Histograms show the mean±SEM of at least 6–10 rats per group. *p<0.05, **p<0.01 vs. control rats (unpaired Student's *t* test).

Fig. 3: Effect of ketamine self-administration on the main targets of αCaMKII in the crude synaptosomal fraction of medial prefrontal cortex.

Panel A shows the phosphorylation and expression levels of GluN2B; Panel B shows the phosphorylation and expression levels of GluA1 and panel C shows the phosphorylation and expression levels of ERK2. Representative immunoblots are shown in panel D.

Rats [ketamine self-administration (S/A) and vehicle-exposed (control)] were killed 24 h after the last ketamine session. Results are expressed as percentages of control rats. Histograms show the mean±SEM of at least 6–10 rats per group. *p<0.05, **p<0.01 vs. control rats (unpaired Student's *t* test).

Fig. 4: Effect of ketamine self-administration on the main targets of αCaMKII in the crude synaptosomal fraction of hippocampus.

Panel A shows the phosphorylation and expression levels of GluN2B; Panel B shows the phosphorylation and expression levels of GluA1 and panel C shows the phosphorylation and expression levels of ERK2. Representative immunoblots are shown in panel D.

Rats [ketamine self-administration (S/A) and vehicle-exposed (control)] were killed 24 h after the last ketamine session. Results are expressed as percentages of control rats. Histograms show the mean±SEM of at least 8–10 rats per group. *p<0.05 vs. control rats (unpaired Student's *t* test).

Fig. 5: αCaMKII-induced different phosphorylation of pGluN2B and pGluA1: schematic representation of the hypothesized molecular mechanism.

The homeostasis of the glutamate synapse is altered in reward-related brain regions of rats exposed to ketamine. Repeated ketamine self-administration activates αCaMKII autophosphorylation (1). Such activation increases pGluN2B phosphorylation (2) that, in turn, reduces ERK 2 signaling (3) thus reducing membrane expression and phosphorylation of GluA1 (4).

Abbreviations: Ket S/A= ketamine self-administration; P= phosphorylation**;** **α**CaMKII = **α**Ca^{2+}/calmodulin-dependent protein kinase; GluN2B = glutamate NMDA receptor subunit 2B; GluA1 = glutamate AMPA receptor subunit 1; ERK2 = Extracellular Receptor Kinase

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