Manuscript Details

Manuscript number	BBR_2019_976_R2
Title	The metaplastic effects of ketamine on sucrose renewal and contextual memory reconsolidation in rats
Article type	Research Paper

Abstract

Metaplastic effects of the NMDARs blocker ketamine at the neural and behavioural levels have been described as potential mechanisms underlying the beneficial effects in treatment-resistant depression. However, ketamine effects on addictive behaviours are still unexplored. In the present study, we investigated the effects of ketamine given under a “metaplasticity-inducing dose regimen” on sucrose-related renewal and contextual memory reconsolidation in rats. After a molecular analysis of ketamine modulation of GluN2B, GluA1 and mGluR5 receptors levels in nucleus accumbens, hippocampus and amygdala, two behavioural models were used to investigate ketamine effects: i) context-induced renewal of sucrose-seeking, and ii) sucrose memory reconsolidation. Ketamine was administrated 24h before the renewal test or the retrieval. At the molecular level, ketamine i) decreased GluN2B, GluA1 and mGluR5 receptors in hippocampus, ii) decreased GluA1 and mGluR5 but increased GluN2B in nucleus accumbens and iii) increased GluN2B and mGluR5 in amygdala. At the behavioural level, ketamine given prior to renewal significantly inhibited responding compared to vehicle, while no significant effects were observed on reconsolidation of contextual memory. In conclusion, the molecular analysis of ketamine metaplastic effects in key brain areas suggest a possible involvement of glutamatergic receptors in the inhibition of sucrose renewal but not of contextual memory reconsolidation. The inhibition of renewal could be correlated to hippocampal and accumbal decreased levels of GluA1 and mGluR5, whereas, the lack of effect on contextual memory reconsolidation could be correlated to decreased GluN2B expression in hippocampus, landmark of destabilization-insensitive state.

Keywords	Renewal, memory reconsolidation; metaplasticity; ketamine.

Corresponding Author	Alessandro Piva
Order of Authors	Alessandro Piva, Lucia Caffino, Laura Padovani, Nicholas Pintori, Francesca Mottarlini, Giuseppe Sferrazza, Giovanna Paolone, Fabio Fumagalli, Cristiano Chiamulera
Suggested reviewers	Wickliffe Abraham, Rita Fuchs, Hans Crombag

Submission Files Included in this PDF

File Name [File Type]

BBR_2019_976V2 - Response Letter V2.docx [Response to Reviewers]

BBR_2019_976V2 - Highlights V2.docx [Highlights]

BBR_2019_976V2 - Revised manuscript V2.docx [Manuscript File]

Figure 1 metaKet HD.pptx [Figure]

Figure 2 metaKet HD.pptx [Figure]

Figure 3 metaKet HD.pptx [Figure]

BBR_2019_976V2 - Revised manuscript - marked V2.docx [Supporting File]

BBR_2019_976V2 - Revised manuscript (supplementary material) V2.docx [Supporting File]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Verona, November 06, 2019

To: Keith Trujillo,
Guest Editors, *Behavioural Brain Research*, Ketamine Special Issue

Dear Dr Trujillo,

It is a pleasure to submit a revised version of the manuscript “The metaplastic effects of ketamine on sucrose renewal and contextual memory reconsolidation in rats” by Piva et al. for publication in *Behavioural Brain Research* Journal Special Issue on “Ketamine”. We appended at the end of this letter our responses to reviewers’ comments, on a point-by-point basis with references to pages/sections of the revised version of the manuscript.

The work described has not been previously published (except in the form of an abstract or as part of a published lecture), and it is not under consideration for publication elsewhere.
The publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out.
If accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.
We hope that this manuscript could be of interest for the readers of *Behavioural Brain Research* and considered for publication.

We thank you for the attention.
Looking forward to hearing from you.

Sincerely,
Alessandro Piva

Dept. of Diagnostics and Public Health, Sect. Pharmacology
University of Verona
Piazzale L.A. Scuro, 10 - 37134 Verona (Italy)
Email: alessandro.piva@univr.it
Phone: +39 045 802 7223

Responses to Reviewers' Comments

Reviewer #2

This revised manuscript addressed all of my queries. I have only a few further minor comments.

Reviewer #2 Point 1: In the Highlights, the authors should refrain from using Metaplastic as an adjective for ketamine. Better would be to say in the first one: Ketamine metaplastically inhibits… and for the second one to delete "Metaplastic" altogether as there was no effect in this condition, or else say, "has no metaplastic effect.."
Response: We thank the Reviewer for the comment. We revised the "Highlights" according to Reviewer's suggestion.

Reviewer #2 Point 2: In a similar vein, in the second abstract sentence, I would recommend saying "metaplasticity dose regimen" or "metaplasticity-inducing dose regimen", as the dose regimen is not itself metaplastic. See also first paragraph of the discussion.
Response: The correction has been done both in the abstract and in the discussion.

Reviewer #2 Point 3: Introduction, para 3, line 10, perhaps say "affecting" instead of "contrasting"
And on line 12, delete "however" as the sentence starts with Although.
Response: Done.

Reviewer #2 Point 4: Western blot images. The bands for beta-actin are wider than the bands for the glutamate receptors. Thus it would seem that they have come from different blots. This should be double-checked to ensure that the images all come from the same blot where they are compared in a figure.
Response: The bands, selected as representative, indeed come from the same blot. The difference between each β-actin band and the corresponding proteins should be due to technical innate feature of WB (i.e., polyacrylamide gel percentage and different molecular weight of the protein). However, in order to avoid this doubt, we made a different, hopefully better, selection of β-actin bands of extra-synaptic fraction in figure 1 panel a and b.

Reviewer #3

The authors have modified the manuscript to address a number of considerations. I find that it will be a useful resource as a paper, which could be made more impactful by future potential experiments. However, in its present form I believe there are still some considerations to be addressed.
Reviewer #3 Point 1: The authors responded to the concerns regarding over interpretation of the relationship of the effects of an acute injection of ketamine on GlutR subunit expression to the behaviour in their letter and added some clarification at the end of the discussion. However this inference of correlation hasn't been removed from the article 'highlights'. Likewise, this is not addressed in the abstract which I find less clear now. The way in which the abstract is written infers that the molecular study was after behaviour. For clarity, 'in naïve animals' could be added when describing the effects, just so it is clear that the changes seen were not due to any behavioural testing. For example, now the final sentences following "The inhibition of renewal is correlated to hippocampal and accumbal decreased levels of GluA1" are misleading.
Response: We thank the Reviewer for comments and suggestions. We removed the inference of correlation from the "Highlights", and the Abstract has been modified to avoid misunderstanding about the order of molecular and behavioural experiments. The final sentence of the abstract has been modified to avoid misleading interpretation.

Reviewer #3 Point 2: The authors have more clearly indicated the number of animals used in the different experiments. Two rats were excluded from the reconsolidation experiment as they did not

meet a criteria for sucrose seeking renewal, I am a little confused as to whether by renewal they are referring to the Retrieval session?. However as this renewal is a measure of interest I have concerns as to whether these rats *should* be excluded on this basis? They may represent some natural variation in the levels of relapse to seeking that is seen in a control (vehicle here) group. Would the interpretation of the data change if these rats were included?
Response: First of all, for *renewal* we are referring to the Renewal experiment and not to the Reconsolidation experiment.
We actually did not detail correctly the criteria for animal exclusion from the anaysis. The current sentence ("*Two rats of the vehicle group in the renewal experiment were excluded from the analyses because they did not meet the criterion of relapse to sucrose-seeking (ALP performed during test were greater than ALP performed during the last extinction session*) " must be more correctly rephrased as, "*Two rats of the vehicle group in the renewal experiment were excluded from the analysis because they did not meet the actual criterion of extinction of lever pressing for sucrose, in spite of showing a number of ALP lower than 50% of responding on the first extinction session. In fact, their responding on the last three days of extinction were similar to the ALP values during the last three self-administration sessions*". We now add this sentence in the new version of the manuscript.

Reviewer #3 Point 3: I appreciate that the authors have reanalysed the data and stats. Still for the reconsolidation experiment, the Retrieval session (equivalent to Renewal session only shorter) shows enhanced lever presses relative to the last extinction session (ie. Renewal) in both groups. So in this experiment Ketamine does not prevent renewal, even with different statistical analysis this finding stands. Perhaps the data of responding during the first time bins during Ext 2 can speak to this (albeit there is a context change)?
The authors include discussion of whether the 'metaplastic' effect could enhance extinction rather than alter destabilisation of the original memory in their response to Reviewer 2. These are worthy points of discussion, particularly as the authors mentioned that there was no difference in response rates of the time bins in the long renewal test (60mins) nor in the short renewal test (ie. Retrieval 15mins), but perhaps comparing responses across these experiments might reveal why there is not an effect on renewal in Experiment 2.
It does look like the vehicle rats conditioned slightly less strongly in experiment 2, perhaps it is worth looking at whether the strength of training has a relationship to the extent of renewal in the vehicle animals, because if so perhaps they had less renewal in experiment 2 due to training differences. This is speculative of course, but without due discussion the main conclusion of the paper is weakened and at present the discussion still talks more about the effects of an acute ketamine injection and less so about the behavioural results.
Response: The Retrieval session took place in the conditioning Context A, whereas the last extinction session took place in the Context B, thus the latter cannot be defined as Renewal. Similarly, responding during Ext 2 occurred in Context B, so that responding cannot be compared to responding in conditioning Context A. Nevertheless, we analysed the time-bin data from the Retrieval and the Reinstatement session (now called Test), but we did not find significant differences between the two groups.
We did not find a statistically significant difference in conditioning between the vehicle and the ketamine rats, so we could exclude a different training (i.e., memory) strength between the two treatment groups.
We appreciate the interest of Reviewer #3 to have more information and discussion about the behavioural data of the reconsolidation experiment. We agree that in the orginal version of the Discussion section there is only a limited space for behavioural data. We therefore expanded this part in the Discussion paragraph in this second revision version.

Reviewer #3 Point 4: In reference to the name given to the second experiment 'Test' it still says reinstatement in the Figure and I wouldn't necessarily agree that is how the term is used in literature. Reinstatement is traditionally considered the term for when the US is presented again after extinction, which is not the case here, so in effect it is a (second) test of renewal.
Response: We changed the nomenclature in the Figure 3 panel a) from reinstatement to simply "Test". As far as concerns the nomenclature, there is an historical difference between the fear

conditioning and the drug addiction literature (for a recent review about these differences see Goode & Maren, 2019), where the latter used to define ‘reinstatement’ as the induction of responding for previous reinforced lever by exposure to cues and/or context (i.e., conditioned stimuli and/or conditioning context), mild footshock (i.e., stress) or a single non-contingent drug administration (i.e., priming) before the start of the test session. For this reason we used the term ‘reinstatement’.

- Ketamine metaplastically inhibits sucrose context-induced renewal
- Ketamine has no metaplastic effect on contextual memory reconsolidation
- Ketamine metaplastically induces glutamate receptors changes

The metaplastic effects of ketamine on sucrose renewal and contextual memory reconsolidation in rats

Piva Alessandro[a] §, Caffino Lucia[b] §, Padovani Laura[a], Pintori Nicholas[a], Mottarlini Francesca[b], Sferrazza Giuseppe[a], Paolone Giovanna[a], Fumagalli Fabio[b] *, Chiamulera Cristiano[a] *

[a] Neuropsychopharmacology Lab, Section Pharmacology, Department Diagnostic & Public Health, University of Verona, P.le Scuro 10, 37134, Verona, Italy;

[b] Department of Pharmacological and Biomolecular Sciences, University of Milano Via Balzaretti 9, 20133, Milano, Italy;

§ share the authorship

* share the seniorship

Corresponding author:

Alessandro Piva, Ph.D.

Sezione Farmacologia, Policlinico GB Rossi, P.le Scuro 10, 37134 Verona, Italy.

E-mail: alessandro.piva@univr.it

Phone: +39 0458027223

Author disclosure:

Declarations of interest: none

All authors have approved the final version of the article.

Abstract

Metaplastic effects of the NMDARs blocker ketamine at the neural and behavioural levels have been described as potential mechanisms underlying the beneficial effects in treatment-resistant depression. However, ketamine effects on addictive behaviours are still unexplored. In the present study, we investigated the effects of ketamine given under a “metaplasticity-inducing dose regimen” on sucrose-related renewal and contextual memory reconsolidation in rats.

After a molecular analysis of ketamine modulation of GluN2B, GluA1 and mGluR5 receptors levels in nucleus accumbens, hippocampus and amygdala, two behavioural models were used to investigate ketamine effects: i) context-induced renewal of sucrose-seeking, and ii) sucrose memory reconsolidation. Ketamine was administrated 24h before the renewal test or the retrieval.

At the molecular level, ketamine i) decreased GluN2B, GluA1 and mGluR5 receptors in hippocampus, ii) decreased GluA1 and mGluR5 but increased GluN2B in nucleus accumbens and iii) increased GluN2B and mGluR5 in amygdala. At the behavioural level, ketamine given prior to renewal significantly inhibited responding compared to vehicle, while no significant effects were observed on reconsolidation of contextual memory.

In conclusion, the molecular analysis of ketamine metaplastic effects in key brain areas suggest a possible involvement of glutamatergic receptors in the inhibition of sucrose renewal but not of contextual memory reconsolidation. The inhibition of renewal could be correlated to hippocampal and accumbal decreased levels of GluA1 and mGluR5, whereas, the lack of effect on contextual memory reconsolidation could be correlated to decreased GluN2B expression in hippocampus, landmark of destabilization-insensitive state.

Keywords

Renewal, memory reconsolidation; metaplasticity; ketamine

1. Introduction

Context may be an important determinant factor of relapse to natural and drug rewards [1] and for the definition of intervention strategies [2]. Preclinical research has extensively investigated the conditions under which drug-associated context affect drug seeking behaviour [3-5]. For instance, conditioned response is reinstated (renewal effect) when subjects are re-exposed to a conditioning context (A) after extinction of responding in a different context (B). These studies have also identified the brain areas and the molecular mechanisms involved in context effects on conditioned response for drugs of abuse and natural rewards [6-11].

Context is also important in the reconsolidation of appetitive memories. The reactivation of a consolidated memory through conditioned or unconditioned stimuli presentation during a retrieval session may induce the destabilization and labilization of the memory trace. In order to restabilize the original trace, a process of restabilization/reconsolidation is needed [12]. The memory reactivation and reconsolidation processes have been largely investigated and demonstrated for Pavlovian memories, and only recently they have been demonstrated also for instrumental learning [13-18].

Several studies have shown that drugs of abuse may induce permissive changes that subsequently affect synaptic plasticity events, through a mechanism that has been defined as '*plasticity of synaptic plasticity*', also called *metaplasticity* [19,20]. Metaplasticity has been observed at different cellular and behavioural levels and can be induced by modification of synaptic plasticity or neural assembly and connectivity [21-23] or by the exposure to environmental enrichment, stress or drugs of abuse [22,24,25]. In drug addiction, metaplasticity has been characterized as a process that renders neural circuits more *crystallized* and less susceptible to remodelling [26]. On the other hand, little is known about metaplasticity as a phenomenon affecting relapse (e.g. acting against the effect of determinant factors of drug-seeking relapse), or by facilitating extinction. Although recent evidence indicates that metaplasticity may modulate extinction expression in conditioned fear experiments [27], no studies investigated whether metaplastic stimulations could affect the extinction of conditioned responses to appetitive reinforcers. Finnie and Nader [28] proposed that the molecular events able to affect memory reactivation and reconsolidation act as metaplastic mechanisms, changing "*the types of behavioural experience*" necessary to modulate memory. However, very limited reports are available on the role of metaplasticity on the reconsolidation of appetitive memories: for example, we showed that NMDA receptor antagonist MK-801–induced metaplasticity was able to inhibit the reconsolidation of instrumental memory for sucrose [29]. NMDA receptor antagonists such as MK-801 and ketamine have been shown to induce metaplasticity changes of long-term potentiation (LTP),

i.e. facilitating tetanus-induced LTP in ex-vivo hippocampal slices 24 hours after treatment ([30,31]; for a review see [32]). Single ketamine dosing has been shown to induce a cascade of molecular events leading to enhanced synaptic activity in brain areas involved in mood, motivation and emotional memory (e.g., [33,34]). For instance, ketamine altered glutamate receptors expression and CaMKII autophosphorylation when given 24 hours earlier [35,36]. Nowadays ketamine and ketamine-like antidepressant effects are commonly defined in literature as *metaplastic* effects [31,37,38].

Based on these hypotheses, we aimed to explore whether ketamine was able to induce metaplastic changes affecting context-induced renewal or contextual memory reconsolidation in rats. After a molecular validation of the metaplastic effects of ketamine on different brain areas, we investigated the behavioural effects of ketamine on sucrose-seeking behaviour. For the molecular assay, rats were treated with i.v. ketamine 24 hours before assessment of relevant molecular markers such as the NMDA receptor subunit GluN2B [39,40], the AMPA receptor subunit GluA1 [41] and the metabotropic receptor mGluR5 [27,42,43], in hippocampus, nucleus accumbens and amygdala. The dose of ketamine used here was shown to facilitate LTP in ex-vivo hippocampal slices 24 hours after acute treatment [31]. For the behavioural assessment, ketamine was administered to rats 24 hours (-24h) before i) testing the sucrose-seeking behaviour in a renewal protocol or ii) contextual memory retrieval in a memory reconsolidation protocol. In the former, rats were initially trained to sucrose self-administration (S/A) in a conditioning context, followed by instrumental extinction training in a different context; twenty-four hours after the last extinction session, rats were treated with i.v. ketamine and, 24 hours later, tested for sucrose-seeking behaviour in the conditioning context (renewal test). For the contextual memory reconsolidation experiment, after training, extinction and ketamine injection as in the renewal protocol, rats were briefly exposed to the conditioning context for instrumental memory retrieval [44] and, after a second extinction phase, tested for sucrose-seeking behaviour in the conditioning context (reconsolidation test).

2. Material and methods

2.1 Animals

Fifty male Sprague-Dawley rats (Charles River, Italy) were housed in pairs in temperature and humidity-controlled environment (19-23°C, 60 ± 20 %) on a 12-h light/dark cycle, with light ON at 7:30 pm. Rats were food restricted to maintain their body weight in the range of 250 ± 10 g (daily checked) throughout the experiments, and food (two to three pellets, 10-15 g/day) was made available after each experimental session. Water was available *ad libitum*,

except during experimental sessions. Animals were trained or tested once daily during the dark phase of the light/dark cycle, and all the experimental procedure were carried out in accordance with the U.K. Animals (Scientific Procedures) Act of 1986 and associated guidelines, and with EU Directive 2010/63/EU for animal experiments. All efforts were made to minimize animal suffering and to keep the lowest number of animals used.

2.2 Apparatus

Rats were trained and tested in operant chambers encased in sound-insulated cubicles equipped with ventilation fans (Med Associates Inc., Georgia Regional Industrial Park, Fairfax, VT, USA). Each chamber was equipped with two levers, an active (right) and an inactive (left) lever symmetrically oriented laterally to the food magazine, on the frontal panel. Levers were located 2 cm and food magazine 1 cm above the grid floor. A 2-W white house light was located 26 cm above the grid floor on the back panel of the operant chambers and provided ambient illumination during the entire session duration of all the experimental phases, except for time-out (TO) periods during training and extinction phases. During training, right lever presses produced the delivery of a 45-mg sucrose food pellet (Bilaney Consultants Ltd, UK) with a fixed-ratio 1 (FR1) schedule of reinforcement. During extinction, right lever presses did not correspond to pellet delivery. Left lever presses did not have programmed consequences during the entire experimental protocol. Lever presses and pellet deliveries were recorded, as well schedule parameters and data acquisition were controlled, by Med-PC IV software (Med Associates Inc., Georgia Regional Industrial Park, Fairfax, VT, USA).

2.3 Pharmacological effects and Western Blot Assays

To elucidate the metaplastic effects of ketamine on glutamate receptors level, two groups of rats (5 each group) were treated with ketamine 10 mg/kg/mL i.v. or vehicle 1 mL/kg i.v. and 24h later were anesthetized with 350 mg/kg/2 mL i.p. chloral hydrate (Fluka, Italy) before sacrifice. Then, brains were removed and 1-mm fresh tissue slices containing nuclei accumbens (+1.70 mm), hippocampi and amygdalae (bregma -3.00 mm) were dissected by using a 1-mm Coronal Brain Matrix (SouthPointe Surgical Supply, Florida, USA).

After dissection of brain areas, proteins of post-synaptic density and extra-synaptic fraction were analyzed as previously described [45] with minor modifications. Briefly, nuclei accumbens, hippocampi and amygdalae were homogenized in a teflon-glass potter in cold 0.32 M sucrose buffer pH 7.4 containing 1 mM HEPES, 1 mM $MgCl_2$, 1 mM $NaHCO_3$ and 0.1 mM PMSF, in presence of commercial cocktails of protease (Roche, Monza, Italy) and phosphatase (Sigma-Aldrich, Milan, Italy). Each homogenate was centrifuged at 800 g for 5 min; the obtained supernatant was then centrifuged at 13000 g for 15 min, obtaining a pellet.

This pellet was re-suspended in a buffer containing 75 mM KCl and 1% Triton X-100 and centrifuged at 100000 g for 1 h. The resulting supernatant, referred as Triton X-100 soluble fraction (TSF, extra-synaptic fraction), was stored at -20°C; the pellet, referred as PSD or Triton X-100 insoluble fraction (TIF, post-synaptic density), was homogenized in a glass–glass potter in 20 mM HEPES, protease and phosphatase inhibitors and stored at -20°C in presence of glycerol 30%. Total proteins have been measured in the TIF and TSF fractions according to the Bradford Protein Assay procedure (Bio-Rad, Milan, Italy), using bovine serum albumin as calibration standard.

Equal amounts of proteins of the TIF fraction (8 µg) and of TSF fraction (15 µg) 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 h at room temperature with 10% non-fat dry milk in TBS + 0,1% Tween-20 buffer and then incubated with antibodies against the proteins of interest. The conditions of the primary antibodies were the following: anti-GluN2B (1:1000, Cell signalling technology, USA), anti-GluA1 (1:2000, Cell signalling technology, USA), anti-mGluR5 (1:1000, Millipore, Italy) and anti-β-Actin (1:10000, Sigma-Aldrich, Italy). Results were standardized using β-actin as the 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).

2.4 General Procedure

Schematic diagrams of the behavioural protocols are shown in Figures 2a and 3a. Protocols were designed according to the procedure used in Fuchs's lab to study contextual memory reconsolidation [46] and context-induced renewal [8]. Briefly, for the renewal protocol, following training to sucrose pellets S/A in the conditioning context and instrumental memory extinction in the extinction context, rats were injected with ketamine i.v. and, 24h later, sucrose-seeking behaviour was tested in the training context. For memory reconsolidation protocol, after training to sucrose pellets S/A, extinction and ketamine injection phases as in the renewal protocol, rats were exposed to a retrieval (Ret) session in the conditioning context and, after a second extinction phase in the extinction context, tested for sucrose-seeking behaviour in the conditioning context. Contextual bias was controlled counterbalancing contexts A and B for the experiments, with half of the rats of each experimental group conditioned in context A and the other half conditioned in context B. Context B was a modified version of the operant chamber, with 5-cm blank striped sheets on all the walls and a 1cm-side grid on the floor [47].

2.5 Lever press shaping and training to sucrose self-administration

Forty rats were initially trained to associate right lever presses with sucrose pellets as reinforcement in the conditioning context. The schedule was FR1: 45-mg sucrose food pellet, no TO, session duration up to 100 reinforcements or 120 min. Once the criterion of 100 reinforcements/session was reached, rats started training in the conditioning context. During training, right lever presses corresponded to the delivery of sucrose reinforcement with the schedule: FR1:45-mg sucrose pellet, 60-s TO, session duration up to 12 reinforcements or 60 min. During TO period, right lever presses had no programmed consequences. Light was on throughout shaping and training sessions, except for TO periods during which it switched off. Left lever presses were never associated with programmed consequences. Training lasted for 10 continuous days, and all lever presses during shaping and training were recorded.

2.6 Renewal experiment

Twenty-four hours after the last training session, 24 rats started extinction training, receiving 1h daily session of instrumental extinction in the extinction context. Extinction session schedule was maintained identical to training schedule, except for a fixed duration (1h) and for the absence of any delivery of sucrose pellets. For the renewal protocol, extinction training lasted until rats performed, for three consecutive sessions, less than 50% of lever presses on sucrose-paired lever performed during the first extinction session [47,48]. Noteworthy, increased responding to the active lever on the first day of extinction has been often reported in drug and food S/A studies (e.g., [49]). Generally, extinction criterion was reached between the fourth and the seventh day of extinction training. Twenty-four hours after the last extinction session, 17 rats were treated with 1 mL/kg i.v. vehicle and 7 with 10 mg/kg/mL i.v. ketamine [31]. Following injection, rats remained in the home cage for 24 hours before renewal test. Test session lasted for 60 minutes, with house light on throughout the session and no TO. Both levers were presented but not associated with programmed consequences. All lever presses were recorded during extinction training and sucrose-seeking test.

2.7 Contextual memory reconsolidation experiment

Twenty-four hours after the last training session, 16 rats started extinction training, receiving 1h daily session of instrumental extinction in the extinction context. Extinction session schedule was maintained identical to training schedule, except for a fixed duration (1h) and for the absence of any delivery of sucrose pellets. For the memory reconsolidation protocol, extinction training lasted for seven consecutive days, regardless of the number of active or inactive lever presses performed during the sessions. This criterion was applied following the

protocol described in Arguello et al. [44] to obtain a similar strengthening of the extinction memory in all rats before ketamine treatment and retrieval. In fact, memory age, which determine memory strength, has been listed as one of the boundary conditions that can regulate retrieval ability to reactivate and destabilize memory trace [16]. Twenty-four hours after the last extinction session, 8 rats were treated with 1 mL/kg i.v. vehicle and 8 rats with 10 mg/kg/mL i.v. ketamine [31]. Following injection, rats remained in the home cage until, 24h later, rats were exposed to a retrieval session. Retrieval session was performed in the conditioning context and lasted for 15 minutes, without any TO period. During retrieval, both levers were presented, but not associated with programmed consequences. Twenty-four hours after retrieval, a second extinction training phase in the extinction context was performed to re-extinguish instrumental response before the final test. Here, extinction training lasted until rats performed, for three consecutive sessions, less than 50% of lever presses on sucrose-paired lever performed during the first extinction session of the first extinction training. The day after the last extinction session, sucrose-seeking behaviour was tested in the conditioning context. Test session lasted for 60 minutes, with house light on throughout the session and no TO. Both levers were presented but not associated with programmed consequences. All lever presses were recorded during the extinction training phases, retrieval and the final sucrose-seeking test.

2.8 Data and Statistical Analysis

For the western blot assays, data were analysed by an unpaired two-tailed Student's t-test.

For the renewal experiment, the number of active (ALPs) and inactive (ILPs) lever presses were separately analysed for possible pre-existing group differences with a repeated measures (RM) two-way analyses of variance (ANOVAs) followed by Sidak's multiple comparisons post-hoc test with Session (mean of the last three S/A sessions, first extinction session, mean of the last three extinction sessions) as the within-subject factor and Treatment (vehicle, ketamine) as the between-subject factor. The number of ALPs and ILPs during the renewal test and previous last three extinction sessions were analysed with a RM two-way ANOVA followed by Sidak's multiple comparisons post-hoc test with factors Session (mean of the last three extinction sessions, renewal) and Treatment (vehicle, ketamine) to assess the effect of -24h ketamine.

For the contextual memory reconsolidation experiment, the ALPs and ILPs were separately analysed for possible pre-existing group differences with a RM two-way ANOVA followed by Sidak's multiple comparisons post-hoc test with factors Session (mean of the last three S/A sessions, first extinction session, mean of the last three sessions of extinction-1) and Treatment (vehicle, ketamine). The number of ALPs and ILPs were separately analysed with a RM one-way ANOVA followed by Sidak's multiple comparisons post-hoc test with factors

Session (mean of the last three sessions of extinction-1, retrieval, mean of the last three sessions of extinction-2) and Treatment (vehicle, ketamine) to assess the effects of conditioning context to induce retrieval of contextual memory. The number of ALPs and ILPs were separately analysed with a RM one-way ANOVA followed by Sidak's multiple comparisons post-hoc test with factors Session (mean of the last three sessions of extinction-2, test) and Treatment (vehicle, ketamine) to assess the effect of -24h ketamine on lever pressing. Two rats of the vehicle group in the renewal experiment were excluded from the analysis because they did not meet the actual criterion of extinction of lever pressing for sucrose, in spite of showing a number of ALP lower than 50% of responding on the first extinction session. In fact, their responding on the last three days of extinction were similar to the ALP values during the last three self-administration sessions. All analyses were performed using the GraphPad software package (Prism, version 4; GraphPad, San Diego, California, USA). Alpha was set at 0.05.

3. Results

3.1 Western Blot Assays

Western blot assays revealed that the post-synaptic level of GluN2B in the nucleus accumbens, 24h after ketamine, was significantly increased (+20.22 % ± 6.49, $t_{(8)}$=3.118, p < 0.05), while the post-synaptic levels of GluA1 and the extra-synaptic level of mGluR5 were significantly decreased (respectively -15.43 % ± 5.31, $t_{(8)}$=2.905; -21.80 % ± 8.30, $t_{(8)}$=2.627; p < 0.05) compared to vehicle (Fig. 1a). In the hippocampus, GluN2B, GluA1 and mGluR5 levels were significantly decreased compared to vehicle (respectively -12.05 % ± 5.19, $t_{(8)}$=2.320; -21.54 % ± 8.51, $t_{(8)}$=2.532; -26.77 % ± 8.30, $t_{(8)}$=3.227; p < 0.05) (Fig. 1b). In the amygdala, the level of GluN2B and mGluR5 were significantly increased (respectively +13.07 % ± 4.15, $t_{(8)}$=3.149, p < 0.05; +45.53 % ± 13.32, $t_{(8)}$=3.417, p < 0.01), while GluA1 level did not show any significant difference compared to vehicle (+11.64 % ± 10.39, $t_{(8)}$=1.119, p = 0.30) (Fig. 1c). The whole blot image is reported in the Supplementary material, section 1.

3.2 Renewal experiment

The protocol design of the renewal experiment is reported in Figure 2a. Prior to pharmacological treatment, experimental groups did not show statistical differences in lever responding. The ANOVA of ALPs during the last three S/A sessions, the first extinction session and the last three extinction sessions showed a significant main effect of Session [$F(2,40) = 66.76$; $p < 0.0001$] but no effects of Treatment [$F(1,20) = 0.003$; $p = 0.96$] nor of interaction Session x Treatment [$F(2,40) = 0.38$; $p = 0.68$]. The Sidak's tests between

treatments (prospective vehicle vs. ketamine) showed no significant differences between the last three S/A sessions (91.47 ± 8.38 and 81.81 ± 7.21, $p = 0.92$), the first extinction session (144.50 ± 14.78 and 151.00 ± 19.15, $p = 0.97$) and last three extinction sessions (27.33 ± 2.60 and 32.29 ± 4.39, $p = 0.99$). The Sidak's tests of ALPs between sessions and the two-way ANOVA with post-hoc test of ILPs is reported in the Supplementary material, section 2 .

The treatment with ketamine impaired the responding on the active lever during renewal test compared to vehicle. The ANOVA of ALPs during the last three extinction sessions and the renewal test showed a significant main effect of Session [$F(1,20) = 10.81$; $p < 0.01$] and of interaction Session x Treatment [$F(1,20) = 6.19$; $p < 0.05$], but not of Treatment [$F(1,20) = 1.16$; $p = 0.29$]. The Sidak's tests between treatments showed a significant decrease of ALPs at the renewal test for ketamine compared to vehicle (54.20 ± 6.16 and 36.00 ± 5.20; $p < 0.05$), but no differences between the last three extinction sessions (27.33 ± 2.60 and 32.29 ± 4.39; $p = 0.77$). The Sidak's tests between sessions showed a significant difference between the last three extinction sessions and the renewal test for vehicle (27.33 ± 2.60 and 54.20 ± 6.16; $p < 0.001$) but not for ketamine group (32.29 ± 4.39 and 36.00 ± 5.20; $p = 0.87$) (Fig. 2b). The two-way ANOVA with post-hoc test of ILPs is reported in the Supplementary material, section 3.

3.3 Contextual memory reconsolidation experiment

The protocol design of contextual memory reconsolidation experiment is reported in Figure 3a. Prior to pharmacological treatment, experimental groups did not show statistical differences in lever responding. The ANOVA of ALPs during the last three S/A sessions, the first extinction session and the last three sessions of extinction-1 showed a significant main effect of Session [$F(2,28) = 31.66$; $p < 0.0001$] but no effect of Treatment $F(1,14) = 1.73$; $p = 0.21$] nor of interaction Session x Treatment [$F(2,28) = 1.70$; $p = 0.20$].

The Sidak's tests between treatments (prospective vehicle vs. ketamine) showed no significant differences between the last three S/A sessions (73.46 ± 9.54 and 91.13 ± 10.19, $p = 0.93$), the first extinction session (141.60 ± 29.24 and 214.80 ± 47.15, $p = 0.10$) and the last three sessions of extinction-1 (19.21 ± 4.85 and 23.04 ± 3.71, $p > 0.99$). The Sidak's tests of ALPs between sessions and the two-way ANOVA with post-hoc test of ILPs is reported in the Supplementary material, section 4.

Re-exposure to conditioning context during the retrieval session significantly increased responding on the active lever compared to extinction-1 and extinction-2 for both experimental groups, with no differences between ketamine and vehicle group. The ANOVA of ALPs during the last three sessions of extinction-1, the retrieval session and the last three sessions of extinction-2 showed a significant main effect of Session [$F(2,28) = 26.57$; $p <$

0.0001] but no effects of Treatment F(1,14) = 0.34; p = 0.57] nor of interaction Session x Treatment [F(2,28) = 0.03; p = 0.98].

The Sidak's tests between treatments showed no significant differences for last three sessions of extinction-1 (19.21 ± 4.85 and 23.04 ± 3.71, p = 0.95), for the retrieval sessions (40.88 ± 9.27 and 45.38 ± 5.85, p = 0.92) and for the last three sessions of extinction-2 (11.96 ± 3.76 and 14.58 ± 4.46, p = 0.98). The Sidak's tests of ALPs between sessions and the two-way ANOVA with post-hoc test of ILPs is reported in the Supplementary material, section 5.

The treatment with ketamine before retrieval session did not impair the responding on the active lever during test, compared to vehicle. The ANOVA of ALPs during the last three sessions of extinction-2 and the test showed a significant main effect of Session [F(1,14) = 30.90; p < 0.0001] but no effects of Treatment F(1,14) = 2.04; p = 0.18] nor of interaction Session x Treatment [F(1,14) = 2.18; p = 0.16].

The Sidak's tests between treatments showed no significant differences for the last three sessions of extinction-2 (11.96 ± 3.76 and 14.58 ± 4.46, p = 0.89) and for the test (25.25 ± 4.09 and 37.50 ± 4.98, p = 0.11). The Sidak's tests between sessions showed a significant difference between the last three sessions of extinction-2 and the test both for vehicle (11.96 ± 3.76 and 25.25 ± 4.09, p < 0.05) and for ketamine group (14.58 ± 4.46 and 37.50 ± 4.98, p < 0.001) (Fig. 3b). The two-way ANOVA with post-hoc test of ILPs is reported in the Supplementary material, section 6.

Fig. 1

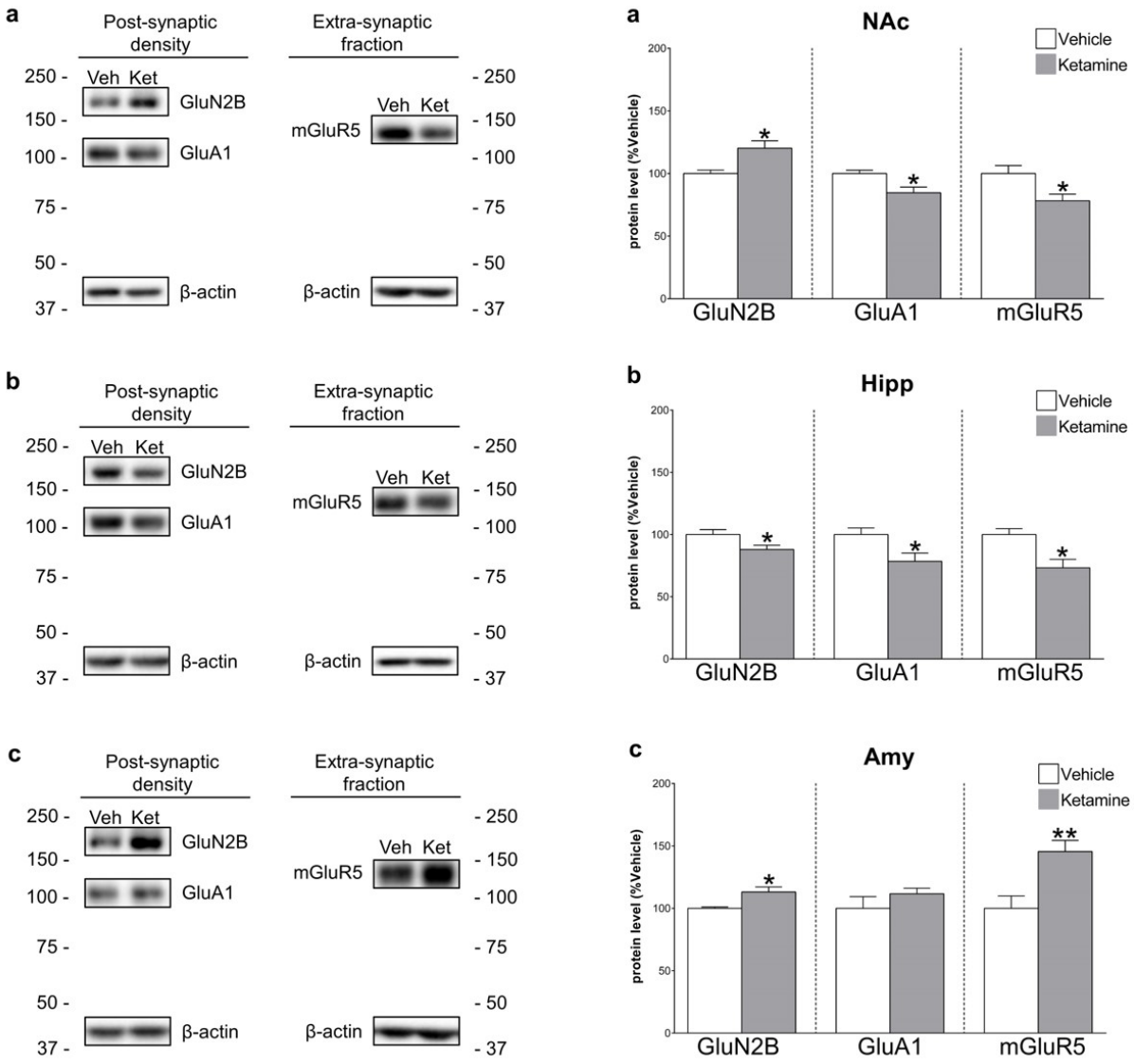


Figure 1. Effect of vehicle or ketamine on GluN2B-containing NMDARs and GluA1-containing AMPARs in the post-synaptic density and on mGluR5 in the extra-synaptic fraction of nucleus accumbens, hippocampus and amygdala. (**Left**) representative images of western blot bands with GluN2B (180 kDa, left) GluA1 (108 kDa, left) and mGluR5 (130 kDa, right) compared to β-actin (43 kDa) as control for nucleus accumbens (**a**), hippocampus (**b**) and amygdala (**c**). (**Right**) quantification of GluN2B-NMDARs and GluA1-AMPARs level in the post-synaptic density and of mGluR5 in the extra-synaptic fraction 24h after vehicle or ketamine treatment in nucleus accumbens (NAc, **a**), hippocampus (Hipp, **b**) and amygdala (Amy, **c**). Data are shown as the mean + SEM and are expressed as percentage of the vehicle. N=5 rats/group. *$p < 0.05$, **$p < 0.01$ vs vehicle; unpaired two-tailed Student's t-test.

Fig. 2

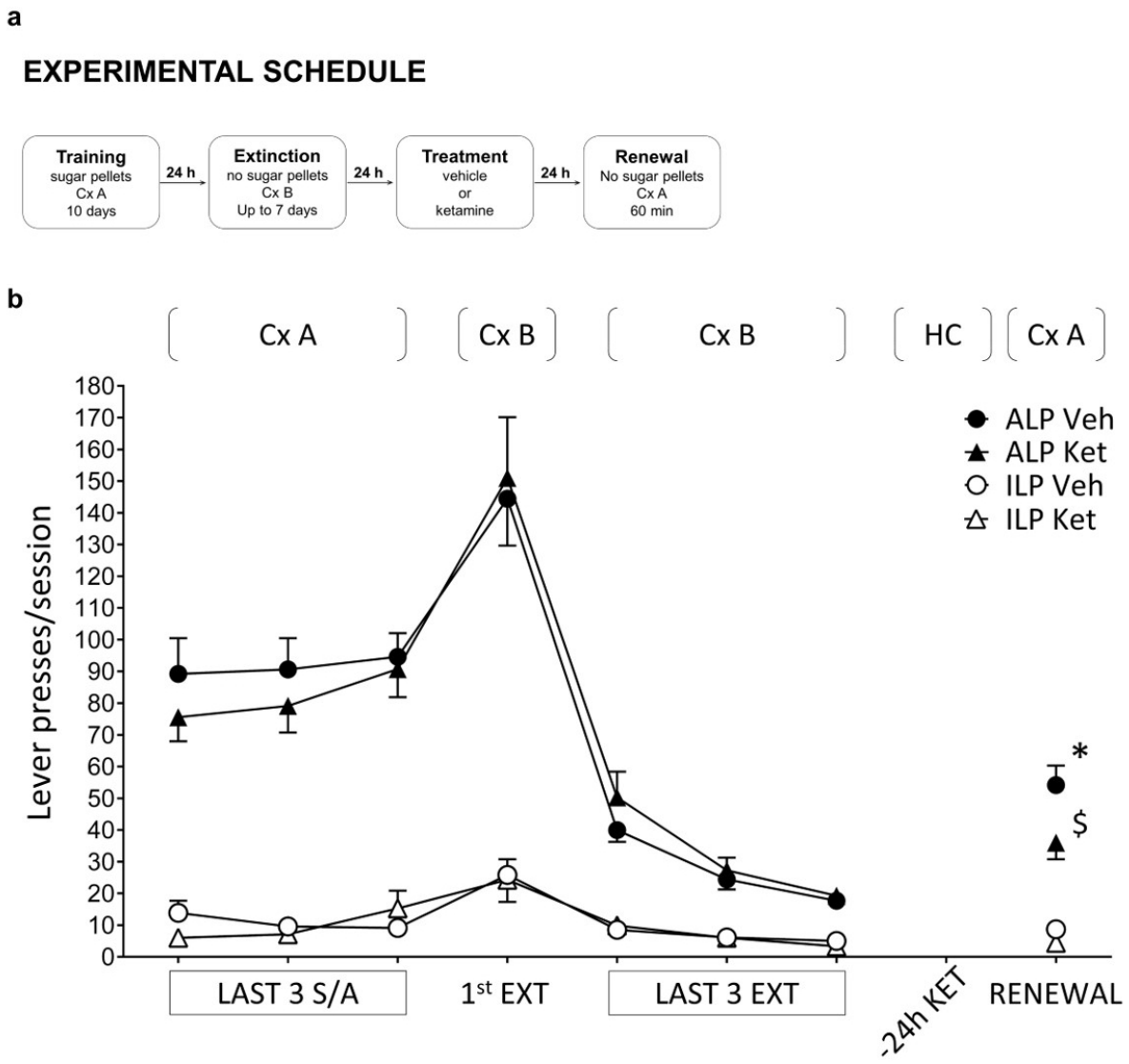


Figure 2. (**a**) Schematic diagram of the experimental protocol and groups. Boxes represent the different procedures used at the different phases of the study. Arrow represents time progression between consecutive phases. Cx A = sucrose self-administration (S/A) training (conditioning) context, Cx B = extinction context. (**b**) Rate of responding on active (solid circle/triangle) or inactive (open circle/triangle) lever per sessions during the different phases of the renewal protocol for rats treated with vehicle (Veh; circle) or ketamine (Ket; triangle). Rate of responding is indicated for the last 3 sessions of S/A (LAST 3 S/A), the first extinction session (1st EXT), the last 3 extinction sessions (LAST 3 EXT) and sucrose-seeking behaviour test (RENEWAL). "-24h KET" indicates the day of treatment, without behavioural session. On top, Cx A indicates the conditioning context, Cx B the extinction context and HC the home cage. Data are expressed as the mean ± SEM. N = 15 rats/vehicle group, N = 7 rats/ketamine group. *$p < 0.001$ vehicle RENEWAL vs LAST 3 EXT, $^{\$}p < 0.05$ ketamine vs

vehicle RENEWAL, repeated measures two-way ANOVA with post-hoc Sidak's multiple comparisons test.

Fig. 3

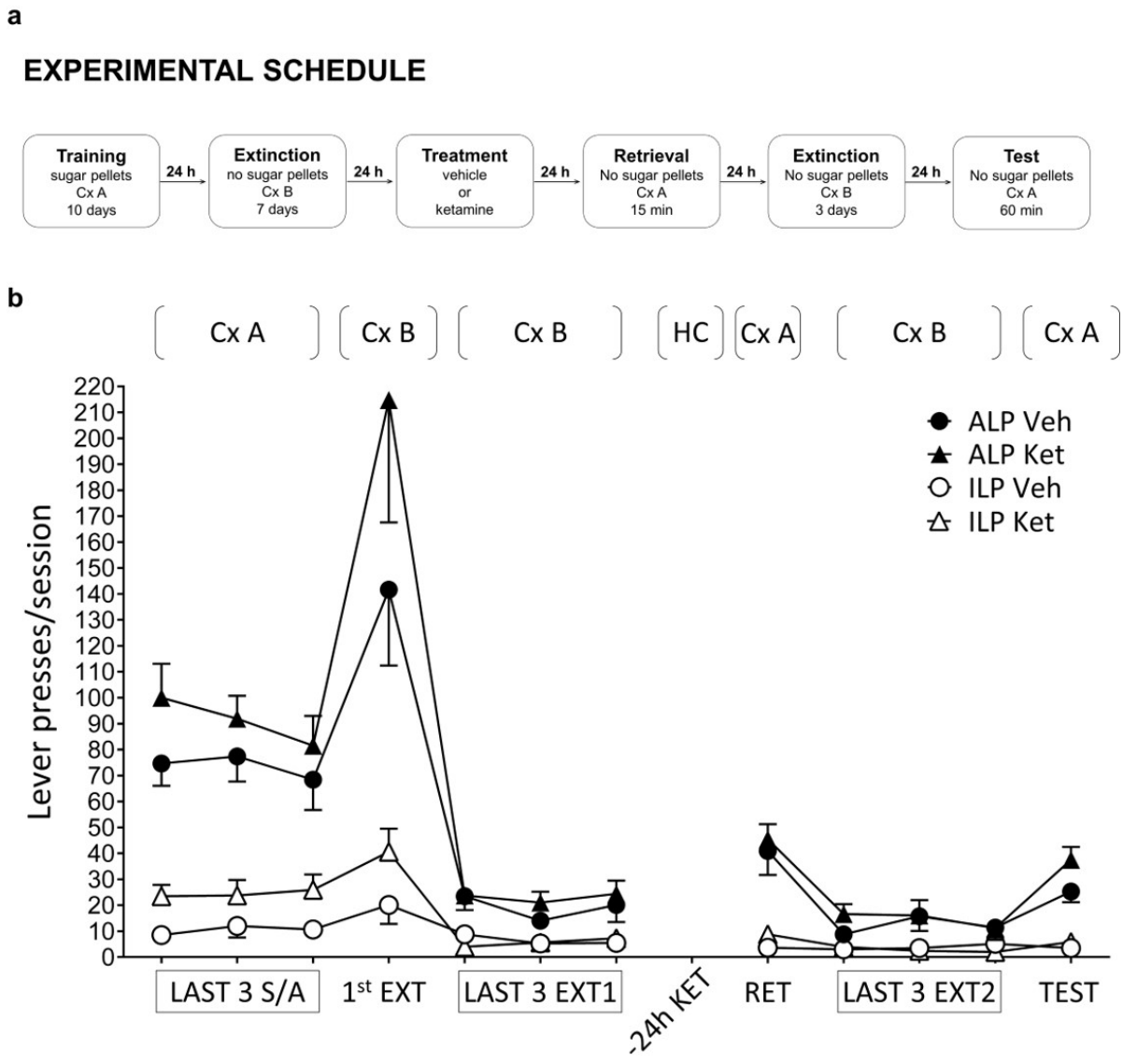


Figure 3. (**a**) Schematic diagram of the experimental protocol and groups. Boxes represent the different procedures used at the different phases of the study. Arrow represents time progression between consecutive phases. Cx A = sucrose S/A training (conditioning) context, Cx B = extinction context. (**b**) Rate of responding on active (solid circle/triangle) or inactive (open circle/triangle) lever per sessions during the different phases of the memory reconsolidation protocol for rats treated with vehicle (Veh; circle) or ketamine (Ket; triangle).

Rate of responding is indicated for the last 3 sessions of S/A (LAST 3 S/A), the first extinction training session (1st EXT), the last 3 extinction sessions of the first extinction phase (LAST 3 EXT1), the retrieval session (RET), the last 3 extinction sessions of the second extinction phase (LAST 3 EXT2) and the sucrose-seeking behaviour test (TEST). "-24h KET" indicates the day of treatment, without behavioural session. On top, Cx A indicates the conditioning context, Cx B the extinction context and HC the home cage. Data are expressed as the mean ± SEM. N = 8 rats/vehicle group, N = 8 rats/ketamine group.

4. Discussion

Ketamine given 24 hours prior to renewal of sucrose-seeking significantly inhibited conditioned responding on the previously sucrose-associated lever compared to vehicle. On the other hand, no significant effect was observed when given 24 hours prior to reactivation of contextual memory. Therefore, ketamine given to rats under a "metaplasticity-inducing dose regimen" was able to affect renewal but not sucrose memory reconsolidation. The behavioural effects of ketamine were observed at doses able to induce expression changes of glutamate receptors relevant for drug-seeking and appetitive memory reconsolidation in amygdala, hippocampus and nucleus accumbens. We therefore suggest that these molecular changes might be causally related to renewal inhibition and, on the other hand, to reconsolidation resistance to disruption.

The behavioural experiments described in this paper were based on protocol designs reported in literature for assessing the effects of context on drug-seeking and drug-memory reconsolidation: the renewal protocol is widely reported in literature (for a review see [3]), whereas the contextual memory reconsolidation protocol has been extensively validated by the Fuchs's group [44,46], with some analogies to instrumental learning protocols developed by our and other groups [16-18,29]. The protocol included an extinction phase where lever pressing was extinguished in a context (CxB) different from the conditioning one (CxA). The re-exposure to CxA induced an increased responding (renewal) that is assumed to be solely due to the conditioning context CxA, since extinction of instrumental responding took place in a different context (CxB). Thus, we exclude the occurrence of an a-priori partial extinction of behaviour in CxA. The reduced responding in the ketamine group during the renewal session, could be due to a faster learning of extinction rather than an inhibition of the renewal. The analysis of responding pattern during session at 15min time bins did not show any difference over the renewal session time period between ketamine and vehicle groups. We therefore hypothesize that the ketamine effect was an impairment of renewal rather than

a potentiation of extinction – even though the latter explanation cannot be excluded. Similarly, we did not find significant differences between the two groups during the early time bin of the retrieval session in the reconsolidation experiments. Furthermore, we analysed the time-bin data from the retrieval and the test (reinstatement) session, but we did not find significant differences between the two groups.

Similar to MK-801 [29,30,32], ketamine induced metaplastic changes assessed on the day after its administration, such as facilitation of tetanus-induced LTP in *ex-vivo* hippocampal slices 24 hours after treatment [31]. Thus, we defined the ketamine dose regimen used in our studies as “metaplastic” in accordance to similar definition given for explaining ketamine effects assessed at time points longer than its half-life (intravenous ketamine half-life in rats is approximately 1 hour; [50]). Indeed, several reports on ketamine antidepressant effects defined the molecular and behavioural changes assessed 24 hours after treatment as “metaplasticity effects” [31,37]. In our study, ketamine induced significant expression changes of glutamate receptors in brain areas relevant for renewal and contextual memory reconsolidation. In fact, glutamate receptors GluN2B, GluA1 and mGluR5 have been reported to be involved in different forms of metaplasticity ([42,51,52]; for reviews see [19,43]).

In hippocampus - an important brain area for processing of spatial context information, drug-seeking [7,8,53] and memory deficits in ketamine users [54] - ketamine significantly decreased synaptic expression levels of GluN2B, GluA1, mGluR5 (Fig. 1, panel b). This general ‘hypo-glutamatergic’ effect might be the compensatory consequence of an increased glutamate release induced by high dose ketamine treatment given to rats 24 hours earlier [35]. Considering that increased GluN2B phosphorylation has been shown in drug-seeking [55-57], its reduction by ketamine in hippocampus may be associated with renewal inhibition. Caffino et al. [36] suggested that the decrease of AMPA receptors induced by ketamine in hippocampus may in turn reduce NMDA receptors activation. The concomitant effect of ketamine, and of its metabolite (2S,6S)-hydroxynorketamine acting as a GluA1 agonist [37,58,59], might have induced AMPA receptors endocytosis and decrease of GluA1 receptors (event though in contrast to what described by Zanos et al. [37]). Interestingly, we showed that metabotropic mGluR5 receptor expression was decreased [36], as also reported by Esterlis et al. [60]. This receptor has been reported to play an important role in hippocampal plasticity [61], so that its down-regulation might explain the reduced effect of conditioning context re-exposure–induced renewal. It is paradoxical that ketamine effect would induce a mGluR5 reduction, which is actually a counter-plasticity expression change [19].

In the nucleus accumbens, mGluR5 antagonism or deletion (removal) has also been shown to reduce relapse to drug-seeking behaviour [62]. Ketamine reduction of mGluR5 in nucleus accumbens could be a further mechanism underlying ketamine inhibitory effect on renewal. The decrease of mGluR5 in this brain area – which is associated with decrease of GluA1 – may be explained as an increased glutamatergic release induced by ketamine 24 hours earlier, lately resulting at renewal in a reduced glutamatergic control over accumbal activation.

We hypothesize that the increased expression of GluN2B in nucleus accumbens and amygdala might be due to a compensatory increase of membrane translocation of these subunits as a consequence of ketamine GluN2B antagonism. It is however unclear which role GluN2B changes might have played in sucrose renewal: although in Pavlovian drug-seeking GluN2B receptor expression was reduced [63,64], it is however not known its role in instrumental contextual learning [65]. It might be the case that increased expression of amygdalar GluN2B and mGluR5 are not relevant for the specific contextual renewal effect observed in our rats well-trained to sucrose S/A for which, on the other hand, hippocampal activity played a major role.

In our study we were not able to observe any significant effect of ketamine on the reconsolidation of contextual sucrose memory. As reviewed in the Introduction section, the retrieval of a consolidated memory may induce destabilization and labilization of the memory trace, followed by a process of restabilization/reconsolidation involving specific brain circuitries, also demonstrated for instrumental learning [13,14,16-18].

Metaplasticity changes in hippocampus have been shown to shift LTD to LTP, which in turn promote memory reconsolidation [66]. Ketamine induced an overall decrease of GluN2B, GluA1 and mGluR5 in hippocampus, perhaps suggesting a general stabilization of the appetitive memory and leading to a condition of contextual memory resistant to retrieval [67,68] as also shown in other paradigms [69,70]. Noteworthy, Wells et al. [65] showed a lack of involvement for hippocampal GluN2B in cocaine-induced memory reconsolidation.

In amygdala, ketamine induced expression changes of GluN2B similar to changes induced by MK-801 in our previous report [29]. GluN2B activation destabilizes memory, making the retrieved memory vulnerable to manipulation [39,71,72]. In Piva et al. [29], we have seen that this effect allowed for sucrose memory inhibition; here, otherwise, it appears that in amygdala it is not relevant at the behavioural level. Similarly, we cannot speculate how the increased level of mGluR5 in amygdala by ketamine is correlated to its lack of effects, considering the limited literature on Group I metabotropic glutamate receptors and memory reconsolidation [73,74].

In our previous report, the instrumental memory reconsolidation inhibition induced by MK-801 was associated with a pattern of glutamatergic facilitation of memory destabilization in nucleus accumbens [29]. Here, 24 hours after ketamine, we observed a similar effect on GluN2B but opposite on GluA1 and mGluR5 expression. Considering that nucleus accumbens plays an important role for Pavlovian memories [63,64] but not for instrumental contextual memories, such ketamine–induced metaplasticity in nucleus accumbens could be irrelevant for our contextual memory reconsolidation (e.g., [75,76]).

The high "metaplastic dose regimen" herein used is an experimental tool that allowed us to understand how NMDARs blockade might induce late effects at the level of behavioural relapse – in this case induced by re-exposure to sucrose-conditioned context or reactivation of contextual sucrose memory. Ketamine effects described in the present study are different from those described in our previous report [29]. In that study, we used a different protocol of instrumental memory reconsolidation, where the conditioning context component effect was not controlled (see also [18]). The two NMDAR blockers should therefore be investigated and compared with similar protocols.

In conclusion, our findings show that ketamine, given under a "metaplastic dose regimen", inhibits sucrose-seeking renewal but not sucrose contextual memory reconsolidation. These metaplastic effects of ketamine suggest a possible involvement of glutamatergic receptors in the inhibition of sucrose renewal but not of contextual memory reconsolidation. For renewal, the inhibition of instrumental responding could be explained as a consequence of hippocampal and accumbal decreased levels of GluA1 and mGluR5; for contextual memory reconsolidation, the lack of effect could be due to resistance of the memory trace to destabilization as a consequence of a general 'hypoglutamatergia' in hippocampus, in particular for GluN2B. The major limitation of our study is the lack of control conditions investigating the molecular ketamine effects in experimental subjects exposed to the entire behavioural protocol. Further studies are therefore needed to study the combined effects of the behavioural history (i.e., sucrose S/A and extinction) and the "metaplastic dose regimen" on glutamatergic receptors.

Acknowledgements

AP and GS performed the behavioural experiments. AP, LP and NP analysed the behavioural data. LC and FM performed and analysed the data of western blot assays. CC, FF, AP, LC and GP planned and designed the whole study and each experiment. CC, FF,

AP and LC wrote the manuscript, and all authors have approved the final version of the article.

The research performed at the University of Verona did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The research performed at the University of Milano was supported by grants from MIUR Progetto Eccellenza.

References

[1] M.E. Bouton, Extinction of instrumental (operant) learning: interference, varieties of context, and mechanisms of contextual control., Psychopharmacology (Berl). 236 (2019) 7–19. doi:10.1007/s00213-018-5076-4.

[2] C.A. Conklin, Environments as cues to smoke: Implications for human extinction-based research and treatment., Exp Clin Psychopharmacol. 14 (2006) 12–19. doi:10.1037/1064-1297.14.1.12.

[3] H.S. Crombag, J.M. Bossert, E. Koya, Y. Shaham, Context-induced relapse to drug seeking: a review., Philos Trans R Soc Lond B Biol Sci. 363 (2008) 3233–43. doi:10.1098/rstb.2008.0090.

[4] A. Badiani, D. Belin, D. Epstein, D. Calu, Y. Shaham, Opiate versus psychostimulant addiction: the differences do matter., Nat Rev Neurosci. 12 (2011) 685–700. doi:10.1038/nrn3104.

[5] S.Y.-S. Khoo, G.D. Gibson, A.A. Prasad, G.P. McNally, How contexts promote and prevent relapse to drug seeking., Genes Brain Behav. 16 (2017) 185–204. doi:10.1111/gbb.12328.

[6] H.S. Crombag, Y. Shaham, Renewal of drug seeking by contextual cues after prolonged extinction in rats., Behav Neurosci. 116 (2002) 169–73. doi:10.1037/0735-7044.116.1.169.

[7] P.W. Marinelli, D. Funk, W. Juzytsch, Z. Li, A.D. Lê, Effects of opioid receptor blockade on the renewal of alcohol seeking induced by context: relationship to c-fos mRNA expression., Eur J Neurosci. 26 (2007) 2815–23. doi:10.1111/j.1460-9568.2007.05898.x.

[8] R.A. Fuchs, K.A. Evans, C.C. Ledford, M.P. Parker, J.M. Case, R.H. Mehta, R.E. See, The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats., Neuropsychopharmacology. 30 (2005) 296–309. doi:10.1038/sj.npp.1300579.

[9] L. Diergaarde, W. de Vries, H. Raasø, A.N.M. Schoffelmeer, T.J. De Vries, Contextual renewal of nicotine seeking in rats and its suppression by the cannabinoid-1 receptor

antagonist Rimonabant (SR141716A)., Neuropharmacology. 55 (2008) 712–6. doi:10.1016/j.neuropharm.2008.06.003.

[10] S. Nakajima, S. Tanaka, K. Urushihara, H. Imada, Renewal of extinguished lever-press responses upon return to the training context., Learn Motiv. 31 (2000) 416–31. doi:10.1006/LMOT.2000.1064.

[11] A.S. Hamlin, K.E. Blatchford, G.P. McNally, Renewal of an extinguished instrumental response: Neural correlates and the role of D1 dopamine receptors., Neuroscience. 143 (2006) 25–38. doi:10.1016/j.neuroscience.2006.07.035.

[12] K. Nader, G.E. Schafe, J.E. Le Doux, Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval., Nature. 406 (2000) 722–26. doi:10.1038/35021052.

[13] A.C. Reichelt, J.L.C. Lee, Memory reconsolidation in aversive and appetitive settings., Front Behav Neurosci. 7 (2013) 118. doi:10.3389/fnbeh.2013.00118.

[14] V. Tedesco, A. Mutti, A. Auber, C. Chiamulera, Nicotine-seeking reinstatement is reduced by inhibition of instrumental memory reconsolidation., Behav Pharmacol. 25 (2014) 725–31. doi:10.1097/FBP.0000000000000088.

[15] M.T.J. Exton-McGuinness, R.C. Patton, L.B. Sacco, J.L.C. Lee, Reconsolidation of a well-learned instrumental memory., Learn Mem. 21 (2014) 468–77. doi:10.1101/lm.035543.114.

[16] M.T.J. Exton-McGuinness, J.L.C. Lee, A.C. Reichelt, Updating memories-the role of prediction errors in memory reconsolidation., Behav Brain Res. 278 (2015) 375–84. doi:10.1016/j.bbr.2014.10.011.

[17] G.H. Vousden, A.L. Milton, The chains of habits: too strong to be broken by reconsolidation blockade?, Curr Opin Behav Sci. 13 (2017) 158–63. doi:10.1016/J.COBEHA.2016.11.010.

[18] A. Piva, E. Gerace, M. Di Chio, L. Padovani, G. Paolone, D.E. Pellegrini-Giampietro, C. Chiamulera, Reconsolidation of sucrose instrumental memory in rats: the role of retrieval context., Brain Res. 1714 (2019) 193–201. doi:10.1016/j.brainres.2019.03.006.

[19] W.C. Abraham, Metaplasticity: tuning synapses and networks for plasticity., Nat Rev Neurosci. 9 (2008) 387–387. doi:10.1038/nrn2356.

[20] W.C. Abraham, M.F. Bear, Metaplasticity: the plasticity of synaptic plasticity., Trends Neurosci. 19 (1996) 126–30. doi: 10.1016/S0166-2236(96)80018-X.

[21] S.R. Hulme, O.D. Jones, W.C. Abraham, Emerging roles of metaplasticity in behaviour and disease., Trends Neurosci. 36 (2013) 353–62. doi:10.1016/j.tins.2013.03.007.

[22] G. Richter-Levin, M. Maroun, Stress and amygdala suppression of metaplasticity in the medial prefrontal cortex., Cereb Cortex. 20 (2010) 2433–41.

doi:10.1093/cercor/bhp311.

[23] M.V. Schmidt, W.C. Abraham, M. Maroun, O. Stork, G. Richter-Levin, Stress-induced metaplasticity: from synapses to behavior., Neuroscience. 250 (2013) 112–20. doi:10.1016/j.neuroscience.2013.06.059.

[24] M.J. Eckert, W.C. Abraham, Effects of environmental enrichment exposure on synaptic transmission and plasticity in the hippocampus., Curr Top Behav Neurosci. 15 (2013) 165–87. doi:10.1007/7854_2012_215.

[25] M.C. Creed, C. Lüscher, Drug-evoked synaptic plasticity: beyond metaplasticity., Curr Opin Neurobiol. 23 (2013) 553–58. doi:10.1016/j.conb.2013.03.005.

[26] D. Neuhofer, P. Kalivas, Metaplasticity at the addicted tetrapartite synapse: a common denominator of drug induced adaptations and potential treatment target for addiction., Neurobiol Learn Mem. 154 (2018) 97–111. doi:10.1016/j.nlm.2018.02.007.

[27] B.J. Stansley, N.M. Fisher, R.G. Gogliotti, C.W. Lindsley, P.J. Conn, C.M. Niswender, Contextual fear extinction induces hippocampal metaplasticity mediated by metabotropic glutamate receptor 5., Cereb Cortex. 28 (2018) 4291–304. doi:10.1093/cercor/bhx282.

[28] P.S.B. Finnie, K. Nader, The role of metaplasticity mechanisms in regulating memory destabilization and reconsolidation., Neurosci Biobehav Rev. 36 (2012) 1667–707. doi:10.1016/j.neubiorev.2012.03.008.

[29] A. Piva, E. Gerace, M. Di Chio, L. Osanni, L. Padovani, L. Caffino, F. Fumagalli, D.E. Pellegrini-Giampietro, C. Chiamulera, The metaplastic effects of NMDA receptors blockade on reactivation of instrumental memories in rats., Neurobiol Learn Mem. 154 (2018) 87–96. doi:10.1016/j.nlm.2018.01.007.

[30] N. Buck, S. Cali, J. Behr, Enhancement of long-term potentiation at CA1-subiculum synapses in MK-801-treated rats., Neurosci Lett. 392 (2006) 5–9. doi:10.1016/j.neulet.2005.08.054.

[31] J. Burgdorf, X. Zhang, K.L. Nicholson, R.L. Balster, J. David Leander, P.K. Stanton, A.L. Gross, R.A. Kroes, J.R. Moskal, GLYX-13, a NMDA receptor glycine-site functional partial agonist, induces antidepressant-like effects without ketamine-like side effects., Neuropsychopharmacology. 38 (2013) 729–42. doi:10.1038/npp.2012.246.

[32] C.F. Zorumski, Y. Izumi, NMDA receptors and metaplasticity: mechanisms and possible roles in neuropsychiatric disorders., Neurosci Biobehav Rev. 36 (2012) 989–1000. doi:10.1016/j.neubiorev.2011.12.011.

[33] R.S. Duman, N. Li, R.-J. Liu, V. Duric, G. Aghajanian, Signaling pathways underlying the rapid antidepressant actions of ketamine., Neuropharmacology. 62 (2012) 35–41. doi:10.1016/j.neuropharm.2011.08.044.

[34] L. Caffino, M. Di Chio, G. Giannotti, M. Venniro, A. Mutti, L. Padovani, D. Cheung, G.F. Fumagalli, D.T. Yew, F. Fumagalli, C. Chiamulera, The modulation of BDNF expression and signalling dissects the antidepressant from the reinforcing properties of ketamine: effects of single infusion vs. chronic self-administration in rats., Pharmacol Res. 104 (2016) 22–30. doi:10.1016/j.phrs.2015.12.014.

[35] L. Caffino, A. Piva, G. Giannotti, M. Di Chio, F. Mottarlini, M. Venniro, D.T. Yew, C. Chiamulera, F. Fumagalli, Ketamine self-administration reduces the homeostasis of the glutamate synapse in the rat brain., Mol Neurobiol. 54 (2017) 7186–93. doi:10.1007/s12035-016-0231-6.

[36] L. Caffino, A. Piva, F. Mottarlini, M. Di Chio, G. Giannotti, C. Chiamulera, F. Fumagalli, Ketamine self-administration elevates αCaMKII autophosphorylation in mood and reward-related brain regions in rats., Mol Neurobiol. 55 (2018) 5453–61. doi:10.1007/s12035-017-0772-3.

[37] P. Zanos, R. Moaddel, P.J. Morris, P. Georgiou, J. Fischell, G.I. Elmer, M. Alkondon, P. Yuan, H.J. Pribut, N.S. Singh, K.S.S. Dossou, Y. Fang, X.-P. Huang, C.L. Mayo, I.W. Wainer, E.X. Albuquerque, S.M. Thompson, C.J. Thomas, C.A. Zarate Jr, T.D. Gould, NMDAR inhibition-independent antidepressant actions of ketamine metabolites., Nature. 533 (2016) 481–86. doi:10.1038/nature17998.

[38] L.R. Vose, P.K. Stanton, Synaptic plasticity, metaplasticity and depression., Curr Neuropharmacol. 15 (2017) 71–86. doi: 10.2174/1570159X14666160202121111.

[39] S.H. Wang, L. de Oliveira Alvares, K. Nader, Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation., Nat Neurosci. 12 (2009) 905–12. doi:10.1038/nn.2350.

[40] C.D. Gipson, K.J. Reissner, Y.M. Kupchik, A.C.W. Smith, N. Stankeviciute, M.E. Hensley-Simon, P.W. Kalivas, Reinstatement of nicotine seeking is mediated by glutamatergic plasticity., Proc Natl Acad Sci USA. 110 (2013) 9124–9. doi:10.1073/pnas.1220591110.

[41] M.H. Monfils, K.K. Cowansage, E. Klann, J.E. LeDoux, Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories., Science. 324 (2009) 951–55. doi:10.1126/science.1167975.

[42] Z.A. Bortolotto, V.J. Collett, F. Conquet, Z. Jia, H. van der Putten, G.L. Collingridge, The regulation of hippocampal LTP by the molecular switch, a form of metaplasticity, requires mGlu5 receptors., Neuropharmacology. 49 (2005) 13–25. doi:10.1016/j.neuropharm.2005.05.020.

[43] T.M. Marton, M.G. Hussain Shuler, P.F. Worley, Homer 1a and mGluR5 phosphorylation in reward-sensitive metaplasticity: a hypothesis of neuronal selection and bidirectional synaptic plasticity., Brain Res. 1628 (2015) 17–28.

doi:10.1016/j.brainres.2015.06.037.

[44] A.A. Arguello, R. Wang, C.M. Lyons, J.A. Higginbotham, M.A. Hodges, R.A. Fuchs, Role of the agranular insular cortex in contextual control over cocaine-seeking behavior in rats., Psychopharmacology (Berl). 234 (2017) 2431–41. doi:10.1007/s00213-017-4632-7.

[45] L. Caffino, G. Giannotti, F. Mottarlini, G. Racagni, F. Fumagalli, Developmental exposure to cocaine dynamically dysregulates cortical Arc/Arg3.1 modulation in response to a challenge., Neurotox Res. 31 (2017) 289–97. doi:10.1007/s12640-016-9683-8.

[46] R.A. Fuchs, G.H. Bell, D.R. Ramirez, J.L. Eaddy, Z. Su, Basolateral amygdala involvement in memory reconsolidation processes that facilitate drug context-induced cocaine seeking., Eur J Neurosci. 30 (2009) 889–900. doi:10.1111/j.1460-9568.2009.06888.x.

[47] A. Auber, N.S. Muthu Karuppasamy, M. Pedercini, D. Bertoglio, V. Tedesco, C. Chiamulera, The effect of postretrieval extinction of nicotine pavlovian memories in rats trained to self-administer nicotine., Nicotine Tob Res. 16 (2014) 1599–605. doi:10.1093/ntr/ntu110.

[48] C. Chiamulera, V. Tedesco, L. Zangrandi, C. Giuliano, G. Fumagalli, Propranolol transiently inhibits reinstatement of nicotine-seeking behaviour in rats., J Psychopharmacol. 24 (2010) 389–95. doi:10.1177/0269881108097718.

[49] A. Pushparaj, Y. Pryslawsky, B. Forget, Y. Yan, B. Le Foll, Extinction bursts in rats trained to self-administer nicotine or food in 1-h daily sessions., Am J Transl Res. 4 (2012) 422–31. PMID: 23145210.

[50] C.L. Shaffer, S.M. Osgood, D.L. Smith, J. Liu, P.E. Trapa, Enhancing ketamine translational pharmacology via receptor occupancy normalization., Neuropharmacology. 86 (2014) 174–80. doi:10.1016/j.neuropharm.2014.07.008.

[51] G. Tenorio, S.A. Connor, D. Guévremont, W.C. Abraham, J. Williams, T.J. O'Dell, P. V Nguyen, "Silent" priming of translation-dependent LTP by ß-adrenergic receptors involves phosphorylation and recruitment of AMPA receptors., Learn Mem. 17 (2010) 627–38. doi:10.1101/lm.1974510.

[52] K. Yang, C. Trepanier, B. Sidhu, Y.-F. Xie, H. Li, G. Lei, M.W. Salter, B.A. Orser, T. Nakazawa, T. Yamamoto, M.F. Jackson, J.F. MacDonald, Metaplasticity gated through differential regulation of GluN2A versus GluN2B receptors by Src family kinases., EMBO J. 31 (2012) 805–16. doi:10.1038/emboj.2011.453.

[53] R.A. Fuchs, J.L. Eaddy, Z.-I. Su, G.H. Bell, Interactions of the basolateral amygdala with the dorsal hippocampus and dorsomedial prefrontal cortex regulate drug context-induced reinstatement of cocaine-seeking in rats., Eur J Neurosci. 26 (2007) 487–98.

doi:10.1111/j.1460-9568.2007.05674.x.

[54] C.J.A. Morgan, C.M. Dodds, H. Furby, F. Pepper, J. Fam, T.P. Freeman, E. Hughes, C. Doeller, J. King, O. Howes, J.M. Stone, Long-term heavy ketamine use is associated with spatial memory impairment and altered hippocampal activation., Front. Psychiatry. 5 (2014) 149. doi:10.3389/fpsyt.2014.00149.

[55] J. Wang, S. Carnicella, K. Phamluong, J. Jeanblanc, J.A. Ronesi, N. Chaudhri, P.H. Janak, D.M. Lovinger, D. Ron, Ethanol induces long-term facilitation of NR2B-NMDA receptor activity in the dorsal striatum: implications for alcohol drinking behavior., J Neurosci. 27 (2007) 3593–602. doi:10.1523/JNEUROSCI.4749-06.2007.

[56] J. Wang, M.F. Lanfranco, S.L. Gibb, Q. V. Yowell, S. Carnicella, D. Ron, Long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum play a crucial role in alcohol consumption and relapse., J Neurosci. 30 (2010) 10187–98. doi:10.1523/JNEUROSCI.2268-10.2010.

[57] X. Xie, A.A. Arguello, A.M. Wells, A.M. Reittinger, R.A. Fuchs, Role of a hippocampal Src-family kinase-mediated glutamatergic mechanism in drug context-induced cocaine seeking., Neuropsychopharmacology. 38 (2013) 2657–65. doi:10.1038/npp.2013.175.

[58] N. Yao, O. Skiteva, X. Zhang, P. Svenningsson, K. Chergui, Ketamine and its metabolite (2R,6R)-hydroxynorketamine induce lasting alterations in glutamatergic synaptic plasticity in the mesolimbic circuit., Mol Psychiatry. 23 (2018) 2066–77. doi:10.1038/mp.2017.239.

[59] G. Collo, L. Cavalleri, C. Chiamulera, E. Merlo Pich, (2R,6R)-Hydroxynorketamine promotes dendrite outgrowth in human inducible pluripotent stem cell-derived neurons through AMPA receptor with timing and exposure compatible with ketamine infusion pharmacokinetics in humans., Neuroreport. 29 (2018) 1425–30. doi:10.1097/WNR.0000000000001131.

[60] I. Esterlis, N. DellaGioia, R.H. Pietrzak, D. Matuskey, N. Nabulsi, C.G. Abdallah, J. Yang, C. Pittenger, G. Sanacora, J.H. Krystal, R. V Parsey, R.E. Carson, C. DeLorenzo, Ketamine-induced reduction in mGluR5 availability is associated with an antidepressant response: an [11C]ABP688 and PET imaging study in depression., Mol Psychiatry. 23 (2018) 824–32. doi:10.1038/mp.2017.58.

[61] K. Naie, D. Manahan-Vaughan, Regulation by metabotropic glutamate receptor 5 of LTP in the dentate gyrus of freely moving rats: relevance for learning and memory formation., Cereb Cortex. 14 (2004) 189–98. doi:10.1093/cercor/bhg118.

[62] C. Chiamulera, C.M. Marzo, D.J.K. Balfour, Metabotropic glutamate receptor 5 as a potential target for smoking cessation., Psychopharmacology (Berl). 234 (2017) 1357–70. doi:10.1007/s00213-016-4487-3.

[63] C.A. Miller, J.F. Marshall, Molecular substrates for retrieval and reconsolidation of

cocaine-associated contextual memory., Neuron. 47 (2005) 873–84. doi:10.1016/j.neuron.2005.08.006.

[64] F.R.M. Theberge, A.L. Milton, D. Belin, J.L.C. Lee, B.J. Everitt, The basolateral amygdala and nucleus accumbens core mediate dissociable aspects of drug memory reconsolidation., Learn Mem. 17 (2010) 444–53. doi:10.1101/lm.1757410.

[65] A.M. Wells, X. Xie, J.A. Higginbotham, A.A. Arguello, K.L. Healey, M. Blanton, R.A. Fuchs, Contribution of an SFK-mediated signaling pathway in the dorsal hippocampus to cocaine-memory reconsolidation in rats., Neuropsychopharmacology. 41 (2016) 675–85. doi:10.1038/npp.2015.217.

[66] W.C. Abraham, J.M. Williams, LTP maintenance and its protein synthesis-dependence., Neurobiol Learn Mem. 89 (2008) 260–8. doi:10.1016/j.nlm.2007.10.001.

[67] P.J. Hernandez, A.E. Kelley, Long-term memory for instrumental responses does not undergo protein synthesis-dependent reconsolidation upon retrieval., Learn Mem. 11 (2004) 748–54. doi:10.1101/lm.84904.

[68] D.R. Ramirez, G.H. Bell, H.C. Lasseter, X. Xie, S.A. Traina, R.A. Fuchs, Dorsal hippocampal regulation of memory reconsolidation processes that facilitate drug context-induced cocaine-seeking behavior in rats., Eur J Neurosci. 30 (2009) 901–12. doi:10.1111/j.1460-9568.2009.06889.x.

[69] M.C. Inda, E. V Muravieva, C.M. Alberini, Memory retrieval and the passage of time: from reconsolidation and strengthening to extinction., J Neurosci. 31 (2011) 1635–43. doi:10.1523/JNEUROSCI.4736-10.2011.

[70] M.J. Honsberger, J.R. Taylor, P.R. Corlett, Memories reactivated under ketamine are subsequently stronger: a potential pre-clinical behavioral model of psychosis., Schizophr Res. 164 (2015) 227–33. doi:10.1016/j.schres.2015.02.009.

[71] C. Ben Mamou, K. Gamache, K. Nader, NMDA receptors are critical for unleashing consolidated auditory fear memories., Nat Neurosci. 9 (2006) 1237–9. doi:10.1038/nn1778.

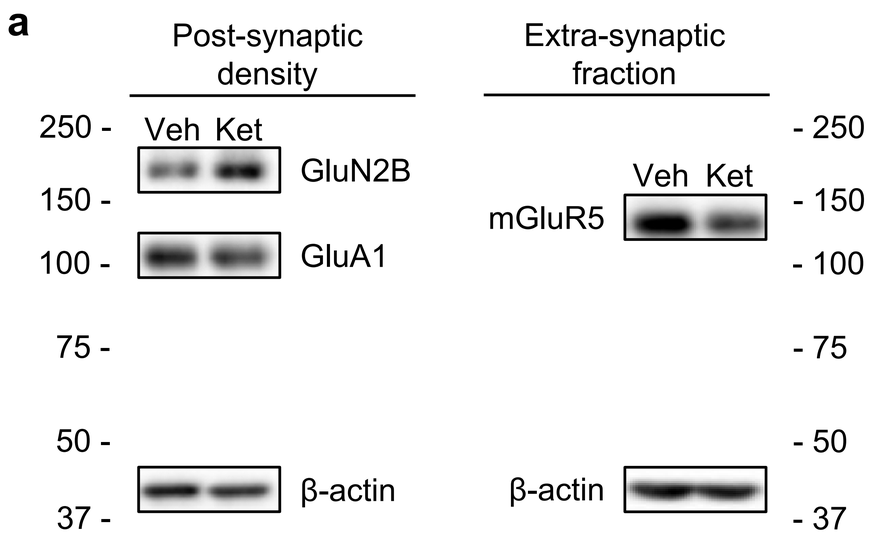
[72] A.L. Milton, E. Merlo, P. Ratano, B.L. Gregory, J.K. Dumbreck, B.J. Everitt, Double dissociation of the requirement for GluN2B- and GluN2A-containing NMDA receptors in the destabilization and restabilization of a reconsolidating memory., J Neurosci. 33 (2013) 1109–15. doi:10.1523/JNEUROSCI.3273-12.2013.

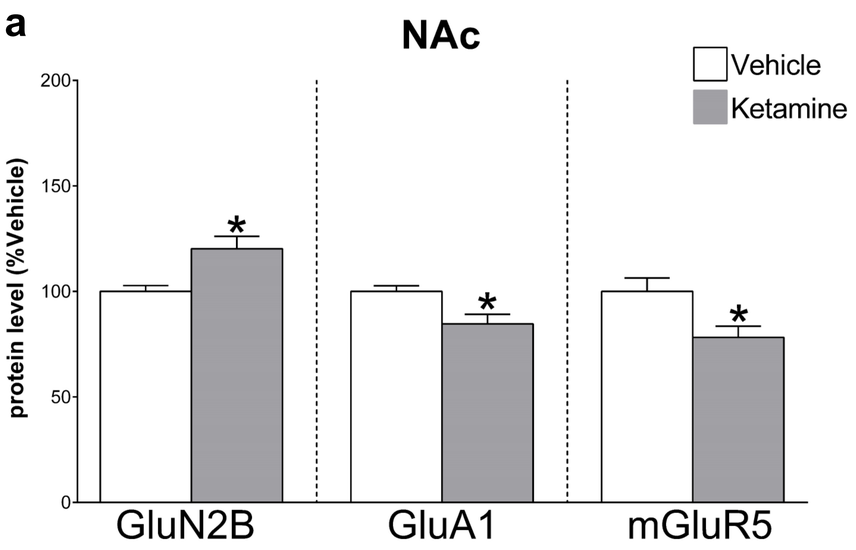
[73] K. Gieros, A. Sobczuk, E. Salinska, Differential involvement of mGluR1 and mGluR5 in memory reconsolidation and retrieval in a passive avoidance task in 1-day old chicks., Neurobiol Learn Mem. 97 (2012) 165–72. doi:10.1016/j.nlm.2011.11.004.

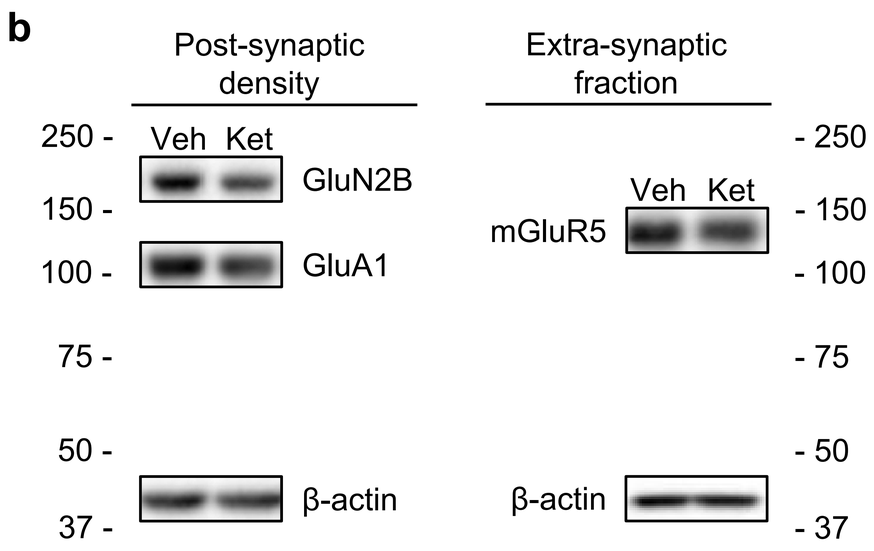
[74] E. Salinska, The role of group I metabotropic glutamate receptors in memory consolidation and reconsolidation in the passive avoidance task in 1-day-old chicks., Neurochem Int. 48 (2006) 447–52. doi:10.1016/j.neuint.2005.11.015.

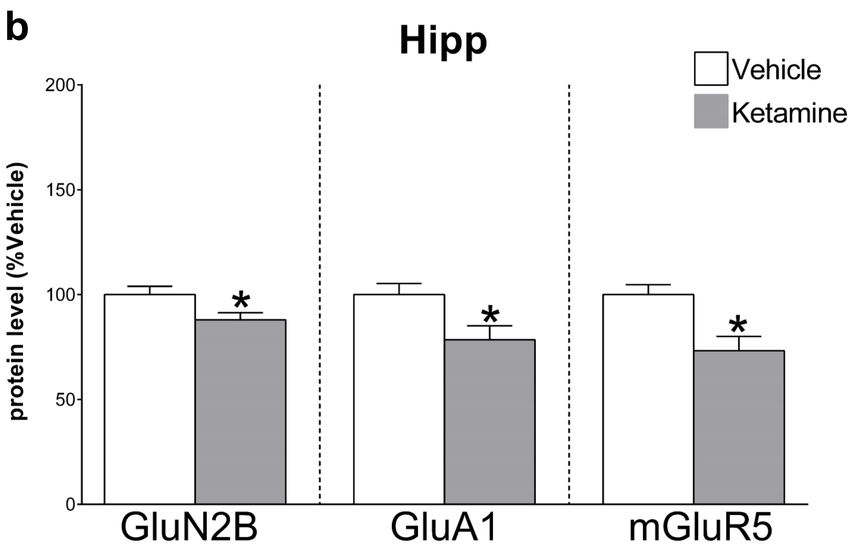
[75] M.W. Shiflett, R.P. Martini, J.C. Mauna, R.L. Foster, E. Peet, E. Thiels, Cue-elicited reward-seeking requires extracellular signal-regulated kinase activation in the nucleus accumbens., J Neurosci. 28 (2008) 1434–43. doi:10.1523/JNEUROSCI.2383-07.2008.

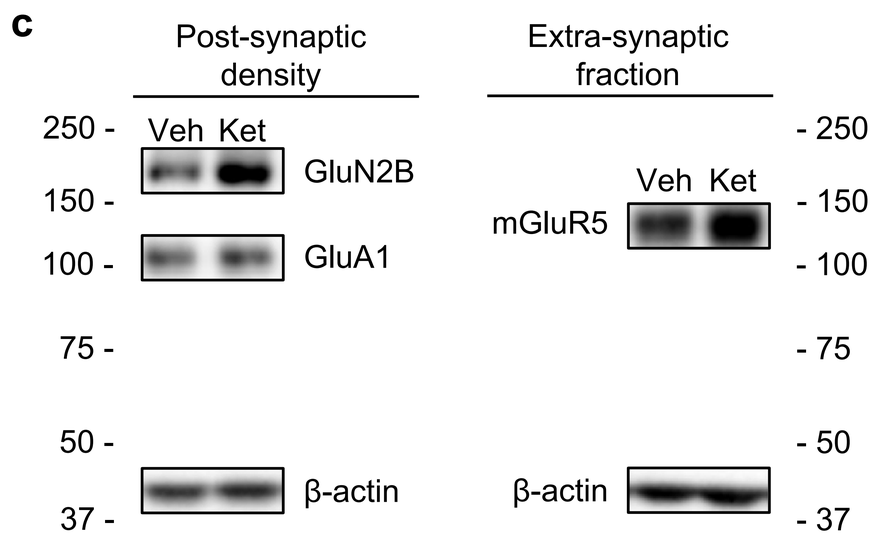
[76] A.M. Wells, A.A. Arguello, X. Xie, M.A. Blanton, H.C. Lasseter, A.M. Reittinger, R.A. Fuchs, Extracellular signal-regulated kinase in the basolateral amygdala, but not the nucleus accumbens core, is critical for context-response-cocaine memory reconsolidation in rats., Neuropsychopharmacology. 38 (2013) 753–62. doi:10.1038/npp.2012.238.

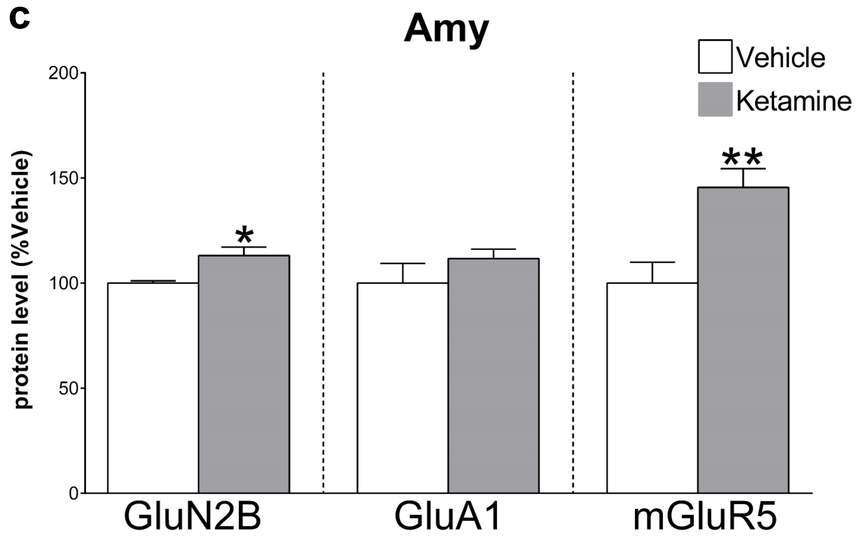

a
Post-synaptic density
Extra-synaptic fraction
Veh Ket
GluN2B
GluA1
β-actin
mGluR5
250 -
150 -
100 -
75 -
50 -
37 -


a
NAc
Vehicle
Ketamine
protein level (%Vehicle)
GluN2B
GluA1
mGluR5


b
Post-synaptic density
Extra-synaptic fraction
Veh Ket
GluN2B
GluA1
β-actin
mGluR5
250 -
150 -
100 -
75 -
50 -
37 -

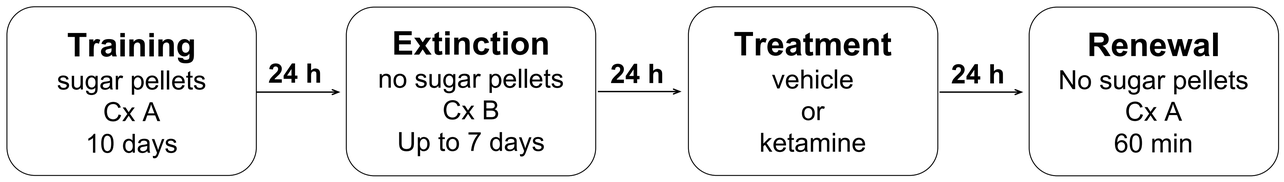

b
Hipp
Vehicle
Ketamine
protein level (%Vehicle)
GluN2B
GluA1
mGluR5


c
Post-synaptic density
Extra-synaptic fraction
Veh Ket
GluN2B
GluA1
β-actin
mGluR5
250 -
150 -
100 -
75 -
50 -
37 -

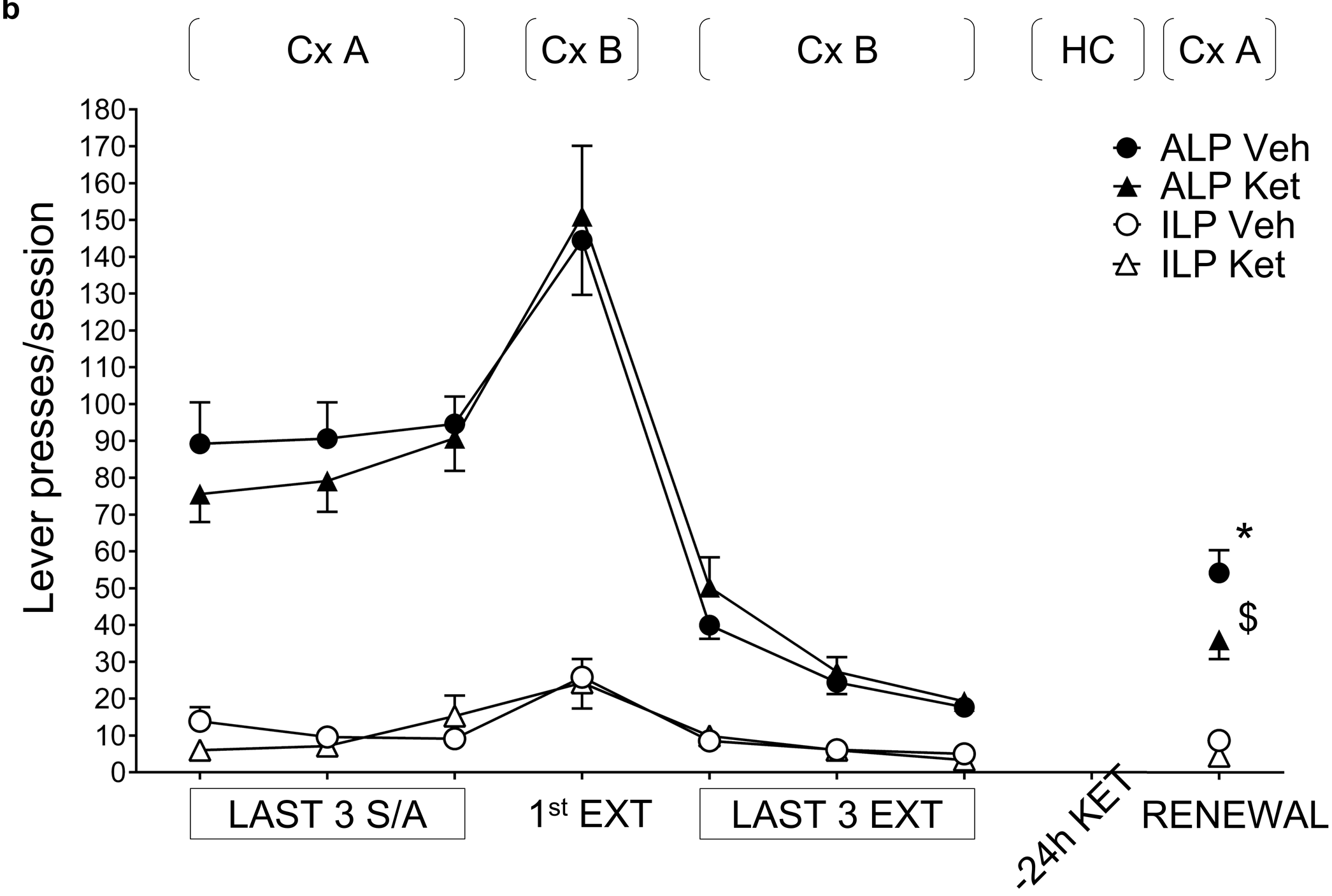

c
Amy
Vehicle
Ketamine
protein level (%Vehicle)
GluN2B
GluA1
mGluR5

a

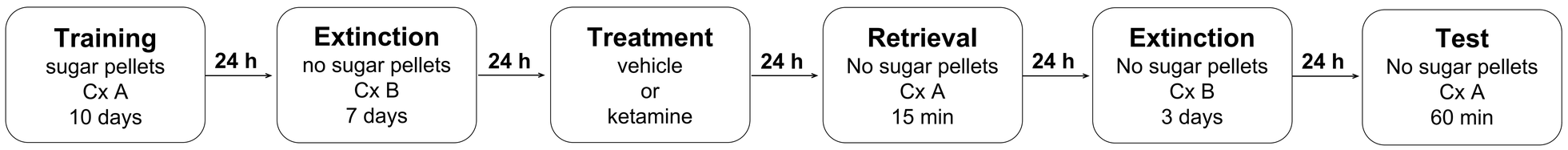
EXPERIMENTAL SCHEDULE



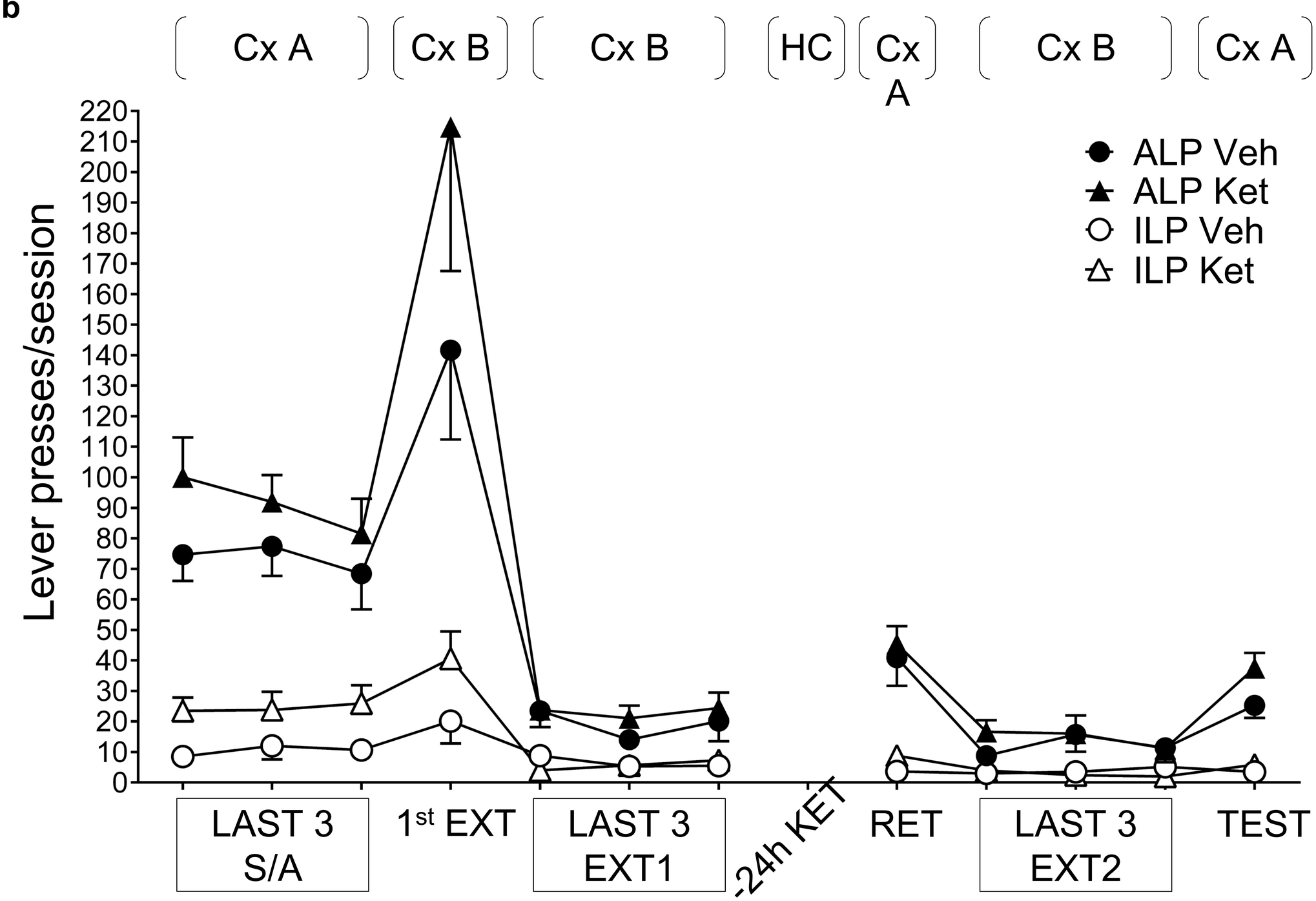
b



a


EXPERIMENTAL SCHEDULE
Training
sugar pellets
Cx A
10 days
24 h
Extinction
no sugar pellets
Cx B
7 days
24 h
Treatment
vehicle
or
ketamine
24 h
Retrieval
No sugar pellets
Cx A
15 min
24 h
Extinction
No sugar pellets
Cx B
3 days
24 h
Test
No sugar pellets
Cx A
60 min

b


Cx A
Cx B
Cx B
HC
Cx A
Cx B
Cx A
Lever presses/session
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ALP Veh
ALP Ket
ILP Veh
ILP Ket
LAST 3 S/A
1st EXT
LAST 3 EXT1
-24h KET
RET
LAST 3 EXT2
TEST

The metaplastic effects of ketamine on sucrose renewal and contextual memory reconsolidation in rats

Piva Alessandro[a] §, Caffino Lucia[b] §, Padovani Laura[a], Pintori Nicholas[a], Mottarlini Francesca[b], Sferrazza Giuseppe[a], Paolone Giovanna[a], Fumagalli Fabio[b] *, Chiamulera Cristiano[a] *

[a] Neuropsychopharmacology Lab, Section Pharmacology, Department Diagnostic & Public Health, University of Verona, P.le Scuro 10, 37134, Verona, Italy;

[b] Department of Pharmacological and Biomolecular Sciences, University of Milano Via Balzaretti 9, 20133, Milano, Italy;

§ share the authorship

* share the seniorship

Corresponding author:

Alessandro Piva, Ph.D.

Sezione Farmacologia, Policlinico GB Rossi, P.le Scuro 10, 37134 Verona, Italy.

E-mail: alessandro.piva@univr.it

Phone: +39 0458027223

Author disclosure:

Declarations of interest: none

All authors have approved the final version of the article.

Abstract

Metaplastic effects of the NMDARs blocker ketamine at the neural and behavioural levels have been described as potential mechanisms underlying the beneficial effects in treatment-resistant depression. However, ketamine effects on addictive behaviours are still unexplored. In the present study, we investigated the effects of ketamine given under a "metaplasticity-inducing dose regimen" on sucrose-related renewal and contextual memory reconsolidation in rats.

After a molecular analysis of ketamine modulation of GluN2B, GluA1 and mGluR5 receptors levels in nucleus accumbens, hippocampus and amygdala, two behavioural models were used to investigate ketamine effects: i) context-induced renewal of sucrose-seeking, and ii) sucrose memory reconsolidation. Ketamine was administrated 24h before the renewal test or the retrieval.

At the molecular level, ketamine i) decreased GluN2B, GluA1 and mGluR5 receptors in hippocampus, ii) decreased GluA1 and mGluR5 but increased GluN2B in nucleus accumbens and iii) increased GluN2B and mGluR5 in amygdala. At the behavioural level, ketamine given prior to renewal significantly inhibited responding compared to vehicle, while no significant effects were observed on reconsolidation of contextual memory.

In conclusion, the molecular analysis of ketamine metaplastic effects in key brain areas suggest a possible involvement of glutamatergic receptors in the inhibition of sucrose renewal but not of contextual memory reconsolidation. The inhibition of renewal could be correlated to hippocampal and accumbal decreased levels of GluA1 and mGluR5, whereas, the lack of effect on contextual memory reconsolidation could be correlated to decreased GluN2B expression in hippocampus, landmark of destabilization-insensitive state.

Keywords

Renewal, memory reconsolidation; metaplasticity; ketamine

1. Introduction

Context may be an important determinant factor of relapse to natural and drug rewards [1] and for the definition of intervention strategies [2]. Preclinical research has extensively investigated the conditions under which drug-associated context affect drug seeking behaviour [3-5]. For instance, conditioned response is reinstated (renewal effect) when subjects are re-exposed to a conditioning context (A) after extinction of responding in a different context (B). These studies have also identified the brain areas and the molecular mechanisms involved in context effects on conditioned response for drugs of abuse and natural rewards [6-11].

Context is also important in the reconsolidation of appetitive memories. The reactivation of a consolidated memory through conditioned or unconditioned stimuli presentation during a retrieval session may induce the destabilization and labilization of the memory trace. In order to restabilize the original trace, a process of restabilization/reconsolidation is needed [12]. The memory reactivation and reconsolidation processes have been largely investigated and demonstrated for Pavlovian memories, and only recently they have been demonstrated also for instrumental learning [13-18].

Several studies have shown that drugs of abuse may induce permissive changes that subsequently affect synaptic plasticity events, through a mechanism that has been defined as '*plasticity of synaptic plasticity*', also called *metaplasticity* [19,20]. Metaplasticity has been observed at different cellular and behavioural levels and can be induced by modification of synaptic plasticity or neural assembly and connectivity [21-23] or by the exposure to environmental enrichment, stress or drugs of abuse [22,24,25]. In drug addiction, metaplasticity has been characterized as a process that renders neural circuits more *crystallized* and less susceptible to remodelling [26]. On the other hand, little is known about metaplasticity as a phenomenon affecting relapse (e.g. acting against the effect of determinant factors of drug-seeking relapse), or by facilitating extinction. Although recent evidence indicates that metaplasticity may modulate extinction expression in conditioned fear experiments [27], no studies investigated whether metaplastic stimulations could affect the extinction of conditioned responses to appetitive reinforcers. Finnie and Nader [28] proposed that the molecular events able to affect memory reactivation and reconsolidation act as metaplastic mechanisms, changing "*the types of behavioural experience*" necessary to modulate memory. However, very limited reports are available on the role of metaplasticity on the reconsolidation of appetitive memories: for example, we showed that NMDA receptor antagonist MK-801–induced metaplasticity was able to inhibit the reconsolidation of instrumental memory for sucrose [29]. NMDA receptor antagonists such as MK-801 and ketamine have been shown to induce metaplasticity changes of long-term potentiation (LTP),

i.e. facilitating tetanus-induced LTP in ex-vivo hippocampal slices 24 hours after treatment ([30,31]; for a review see [32]). Single ketamine dosing has been shown to induce a cascade of molecular events leading to enhanced synaptic activity in brain areas involved in mood, motivation and emotional memory (e.g., [33,34]). For instance, ketamine altered glutamate receptors expression and CaMKII autophosphorylation when given 24 hours earlier [35,36]. Nowadays ketamine and ketamine-like antidepressant effects are commonly defined in literature as *metaplastic* effects [31,37,38].

Based on these hypotheses, we aimed to explore whether ketamine was able to induce metaplastic changes affecting context-induced renewal or contextual memory reconsolidation in rats. After a molecular validation of the metaplastic effects of ketamine on different brain areas, we investigated the behavioural effects of ketamine on sucrose-seeking behaviour. For the molecular assay, rats were treated with i.v. ketamine 24 hours before assessment of relevant molecular markers such as the NMDA receptor subunit GluN2B [39,40], the AMPA receptor subunit GluA1 [41] and the metabotropic receptor mGluR5 [27,42,43], in hippocampus, nucleus accumbens and amygdala. The dose of ketamine used here was shown to facilitate LTP in ex-vivo hippocampal slices 24 hours after acute treatment [31]. For the behavioural assessment, ketamine was administered to rats 24 hours (-24h) before i) testing the sucrose-seeking behaviour in a renewal protocol or ii) contextual memory retrieval in a memory reconsolidation protocol. In the former, rats were initially trained to sucrose self-administration (S/A) in a conditioning context, followed by instrumental extinction training in a different context; twenty-four hours after the last extinction session, rats were treated with i.v. ketamine and, 24 hours later, tested for sucrose-seeking behaviour in the conditioning context (renewal test). For the contextual memory reconsolidation experiment, after training, extinction and ketamine injection as in the renewal protocol, rats were briefly exposed to the conditioning context for instrumental memory retrieval [44] and, after a second extinction phase, tested for sucrose-seeking behaviour in the conditioning context (reconsolidation test).

2. Material and methods

2.1 Animals

Fifty male Sprague-Dawley rats (Charles River, Italy) were housed in pairs in temperature and humidity-controlled environment (19-23°C, 60 ± 20 %) on a 12-h light/dark cycle, with light ON at 7:30 pm. Rats were food restricted to maintain their body weight in the range of 250 ± 10 g (daily checked) throughout the experiments, and food (two to three pellets, 10-15 g/day) was made available after each experimental session. Water was available *ad libitum*,

except during experimental sessions. Animals were trained or tested once daily during the dark phase of the light/dark cycle, and all the experimental procedure were carried out in accordance with the U.K. Animals (Scientific Procedures) Act of 1986 and associated guidelines, and with EU Directive 2010/63/EU for animal experiments. All efforts were made to minimize animal suffering and to keep the lowest number of animals used.

2.2 Apparatus

Rats were trained and tested in operant chambers encased in sound-insulated cubicles equipped with ventilation fans (Med Associates Inc., Georgia Regional Industrial Park, Fairfax, VT, USA). Each chamber was equipped with two levers, an active (right) and an inactive (left) lever symmetrically oriented laterally to the food magazine, on the frontal panel. Levers were located 2 cm and food magazine 1 cm above the grid floor. A 2-W white house light was located 26 cm above the grid floor on the back panel of the operant chambers and provided ambient illumination during the entire session duration of all the experimental phases, except for time-out (TO) periods during training and extinction phases. During training, right lever presses produced the delivery of a 45-mg sucrose food pellet (Bilaney Consultants Ltd, UK) with a fixed-ratio 1 (FR1) schedule of reinforcement. During extinction, right lever presses did not correspond to pellet delivery. Left lever presses did not have programmed consequences during the entire experimental protocol. Lever presses and pellet deliveries were recorded, as well schedule parameters and data acquisition were controlled, by Med-PC IV software (Med Associates Inc., Georgia Regional Industrial Park, Fairfax, VT, USA).

2.3 Pharmacological effects and Western Blot Assays

To elucidate the metaplastic effects of ketamine on glutamate receptors level, two groups of rats (5 each group) were treated with ketamine 10 mg/kg/mL i.v. or vehicle 1 mL/kg i.v. and 24h later were anesthetized with 350 mg/kg/2 mL i.p. chloral hydrate (Fluka, Italy) before sacrifice. Then, brains were removed and 1-mm fresh tissue slices containing nuclei accumbens (+1.70 mm), hippocampi and amygdalae (bregma -3.00 mm) were dissected by using a 1-mm Coronal Brain Matrix (SouthPointe Surgical Supply, Florida, USA).

After dissection of brain areas, proteins of post-synaptic density and extra-synaptic fraction were analyzed as previously described [45] with minor modifications. Briefly, nuclei accumbens, hippocampi and amygdalae were homogenized in a teflon-glass potter in cold 0.32 M sucrose buffer pH 7.4 containing 1 mM HEPES, 1 mM $MgCl_2$, 1 mM $NaHCO_3$ and 0.1 mM PMSF, in presence of commercial cocktails of protease (Roche, Monza, Italy) and phosphatase (Sigma-Aldrich, Milan, Italy). Each homogenate was centrifuged at 800 g for 5 min; the obtained supernatant was then centrifuged at 13000 g for 15 min, obtaining a pellet.

This pellet was re-suspended in a buffer containing 75 mM KCl and 1% Triton X-100 and centrifuged at 100000 g for 1 h. The resulting supernatant, referred as Triton X-100 soluble fraction (TSF, extra-synaptic fraction), was stored at -20°C; the pellet, referred as PSD or Triton X-100 insoluble fraction (TIF, post-synaptic density), was homogenized in a glass–glass potter in 20 mM HEPES, protease and phosphatase inhibitors and stored at -20°C in presence of glycerol 30%. Total proteins have been measured in the TIF and TSF fractions according to the Bradford Protein Assay procedure (Bio-Rad, Milan, Italy), using bovine serum albumin as calibration standard.

Equal amounts of proteins of the TIF fraction (8 µg) and of TSF fraction (15 µg) 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 h at room temperature with 10% non-fat dry milk in TBS + 0,1% Tween-20 buffer and then incubated with antibodies against the proteins of interest. The conditions of the primary antibodies were the following: anti-GluN2B (1:1000, Cell signalling technology, USA), anti-GluA1 (1:2000, Cell signalling technology, USA), anti-mGluR5 (1:1000, Millipore, Italy) and anti-β-Actin (1:10000, Sigma-Aldrich, Italy). Results were standardized using β-actin as the 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).

2.4 General Procedure

Schematic diagrams of the behavioural protocols are shown in Figures 2a and 3a. Protocols were designed according to the procedure used in Fuchs's lab to study contextual memory reconsolidation [46] and context-induced renewal [8]. Briefly, for the renewal protocol, following training to sucrose pellets S/A in the conditioning context and instrumental memory extinction in the extinction context, rats were injected with ketamine i.v. and, 24h later, sucrose-seeking behaviour was tested in the training context. For memory reconsolidation protocol, after training to sucrose pellets S/A, extinction and ketamine injection phases as in the renewal protocol, rats were exposed to a retrieval (Ret) session in the conditioning context and, after a second extinction phase in the extinction context, tested for sucrose-seeking behaviour in the conditioning context. Contextual bias was controlled counterbalancing contexts A and B for the experiments, with half of the rats of each experimental group conditioned in context A and the other half conditioned in context B. Context B was a modified version of the operant chamber, with 5-cm blank striped sheets on all the walls and a 1cm-side grid on the floor [47].

2.5 Lever press shaping and training to sucrose self-administration

Forty rats were initially trained to associate right lever presses with sucrose pellets as reinforcement in the conditioning context. The schedule was FR1: 45-mg sucrose food pellet, no TO, session duration up to 100 reinforcements or 120 min. Once the criterion of 100 reinforcements/session was reached, rats started training in the conditioning context. During training, right lever presses corresponded to the delivery of sucrose reinforcement with the schedule: FR1:45-mg sucrose pellet, 60-s TO, session duration up to 12 reinforcements or 60 min. During TO period, right lever presses had no programmed consequences. Light was on throughout shaping and training sessions, except for TO periods during which it switched off. Left lever presses were never associated with programmed consequences. Training lasted for 10 continuous days, and all lever presses during shaping and training were recorded.

2.6 Renewal experiment

Twenty-four hours after the last training session, 24 rats started extinction training, receiving 1h daily session of instrumental extinction in the extinction context. Extinction session schedule was maintained identical to training schedule, except for a fixed duration (1h) and for the absence of any delivery of sucrose pellets. For the renewal protocol, extinction training lasted until rats performed, for three consecutive sessions, less than 50% of lever presses on sucrose-paired lever performed during the first extinction session [47,48]. Noteworthy, increased responding to the active lever on the first day of extinction has been often reported in drug and food S/A studies (e.g., [49]). Generally, extinction criterion was reached between the fourth and the seventh day of extinction training. Twenty-four hours after the last extinction session, 17 rats were treated with 1 mL/kg i.v. vehicle and 7 with 10 mg/kg/mL i.v. ketamine [31]. Following injection, rats remained in the home cage for 24 hours before renewal test. Test session lasted for 60 minutes, with house light on throughout the session and no TO. Both levers were presented but not associated with programmed consequences. All lever presses were recorded during extinction training and sucrose-seeking test.

2.7 Contextual memory reconsolidation experiment

Twenty-four hours after the last training session, 16 rats started extinction training, receiving 1h daily session of instrumental extinction in the extinction context. Extinction session schedule was maintained identical to training schedule, except for a fixed duration (1h) and for the absence of any delivery of sucrose pellets. For the memory reconsolidation protocol, extinction training lasted for seven consecutive days, regardless of the number of active or inactive lever presses performed during the sessions. This criterion was applied following the

protocol described in Arguello et al. [44] to obtain a similar strengthening of the extinction memory in all rats before ketamine treatment and retrieval. In fact, memory age, which determine memory strength, has been listed as one of the boundary conditions that can regulate retrieval ability to reactivate and destabilize memory trace [16]. Twenty-four hours after the last extinction session, 8 rats were treated with 1 mL/kg i.v. vehicle and 8 rats with 10 mg/kg/mL i.v. ketamine [31]. Following injection, rats remained in the home cage until, 24h later, rats were exposed to a retrieval session. Retrieval session was performed in the conditioning context and lasted for 15 minutes, without any TO period. During retrieval, both levers were presented, but not associated with programmed consequences. Twenty-four hours after retrieval, a second extinction training phase in the extinction context was performed to re-extinguish instrumental response before the final test. Here, extinction training lasted until rats performed, for three consecutive sessions, less than 50% of lever presses on sucrose-paired lever performed during the first extinction session of the first extinction training. The day after the last extinction session, sucrose-seeking behaviour was tested in the conditioning context. Test session lasted for 60 minutes, with house light on throughout the session and no TO. Both levers were presented but not associated with programmed consequences. All lever presses were recorded during the extinction training phases, retrieval and the final sucrose-seeking test.

2.8 Data and Statistical Analysis

For the western blot assays, data were analysed by an unpaired two-tailed Student's t-test.

For the renewal experiment, the number of active (ALPs) and inactive (ILPs) lever presses were separately analysed for possible pre-existing group differences with a repeated measures (RM) two-way analyses of variance (ANOVAs) followed by Sidak's multiple comparisons post-hoc test with Session (mean of the last three S/A sessions, first extinction session, mean of the last three extinction sessions) as the within-subject factor and Treatment (vehicle, ketamine) as the between-subject factor. The number of ALPs and ILPs during the renewal test and previous last three extinction sessions were analysed with a RM two-way ANOVA followed by Sidak's multiple comparisons post-hoc test with factors Session (mean of the last three extinction sessions, renewal) and Treatment (vehicle, ketamine) to assess the effect of -24h ketamine.

For the contextual memory reconsolidation experiment, the ALPs and ILPs were separately analysed for possible pre-existing group differences with a RM two-way ANOVA followed by Sidak's multiple comparisons post-hoc test with factors Session (mean of the last three S/A sessions, first extinction session, mean of the last three sessions of extinction-1) and Treatment (vehicle, ketamine). The number of ALPs and ILPs were separately analysed with a RM one-way ANOVA followed by Sidak's multiple comparisons post-hoc test with factors

Session (mean of the last three sessions of extinction-1, retrieval, mean of the last three sessions of extinction-2) and Treatment (vehicle, ketamine) to assess the effects of conditioning context to induce retrieval of contextual memory. The number of ALPs and ILPs were separately analysed with a RM one-way ANOVA followed by Sidak's multiple comparisons post-hoc test with factors Session (mean of the last three sessions of extinction-2, test) and Treatment (vehicle, ketamine) to assess the effect of -24h ketamine on lever pressing. Two rats of the vehicle group in the renewal experiment were excluded from the analysis because they did not meet the actual criterion of extinction of lever pressing for sucrose, in spite of showing a number of ALP lower than 50% of responding on the first extinction session. In fact, their responding on the last three days of extinction were similar to the ALP values during the last three self-administration sessions. All analyses were performed using the GraphPad software package (Prism, version 4; GraphPad, San Diego, California, USA). Alpha was set at 0.05.

3. Results

3.1 Western Blot Assays

Western blot assays revealed that the post-synaptic level of GluN2B in the nucleus accumbens, 24h after ketamine, was significantly increased (+20.22 % ± 6.49, $t_{(8)}$=3.118, p < 0.05), while the post-synaptic levels of GluA1 and the extra-synaptic level of mGluR5 were significantly decreased (respectively -15.43 % ± 5.31, $t_{(8)}$=2.905; -21.80 % ± 8.30, $t_{(8)}$=2.627; p < 0.05) compared to vehicle (Fig. 1a). In the hippocampus, GluN2B, GluA1 and mGluR5 levels were significantly decreased compared to vehicle (respectively -12.05 % ± 5.19, $t_{(8)}$=2.320; -21.54 % ± 8.51, $t_{(8)}$=2.532; -26.77 % ± 8.30, $t_{(8)}$=3.227; p < 0.05) (Fig. 1b). In the amygdala, the level of GluN2B and mGluR5 were significantly increased (respectively +13.07 % ± 4.15, $t_{(8)}$=3.149, p < 0.05; +45.53 % ± 13.32, $t_{(8)}$=3.417, p < 0.01), while GluA1 level did not show any significant difference compared to vehicle (+11.64 % ± 10.39, $t_{(8)}$=1.119, p = 0.30) (Fig. 1c). The whole blot image is reported in the Supplementary material, section 1.

3.2 Renewal experiment

The protocol design of the renewal experiment is reported in Figure 2a. Prior to pharmacological treatment, experimental groups did not show statistical differences in lever responding. The ANOVA of ALPs during the last three S/A sessions, the first extinction session and the last three extinction sessions showed a significant main effect of Session [F(2,40) = 66.76; p < 0.0001] but no effects of Treatment [F(1,20) = 0.003; p = 0.96] nor of interaction Session x Treatment [F(2,40) = 0.38; p = 0.68]. The Sidak's tests between

treatments (prospective vehicle vs. ketamine) showed no significant differences between the last three S/A sessions (91.47 ± 8.38 and 81.81 ± 7.21, $p = 0.92$), the first extinction session (144.50 ± 14.78 and 151.00 ± 19.15, $p = 0.97$) and last three extinction sessions (27.33 ± 2.60 and 32.29 ± 4.39, $p = 0.99$). The Sidak's tests of ALPs between sessions and the two-way ANOVA with post-hoc test of ILPs is reported in the Supplementary material, section 2 .

The treatment with ketamine impaired the responding on the active lever during renewal test compared to vehicle. The ANOVA of ALPs during the last three extinction sessions and the renewal test showed a significant main effect of Session [$F(1,20) = 10.81$; $p < 0.01$] and of interaction Session x Treatment [$F(1,20) = 6.19$; $p < 0.05$], but not of Treatment [$F(1,20) = 1.16$; $p = 0.29$]. The Sidak's tests between treatments showed a significant decrease of ALPs at the renewal test for ketamine compared to vehicle (54.20 ± 6.16 and 36.00 ± 5.20; $p < 0.05$), but no differences between the last three extinction sessions (27.33 ± 2.60 and 32.29 ± 4.39; $p = 0.77$). The Sidak's tests between sessions showed a significant difference between the last three extinction sessions and the renewal test for vehicle (27.33 ± 2.60 and 54.20 ± 6.16; $p < 0.001$) but not for ketamine group (32.29 ± 4.39 and 36.00 ± 5.20; $p = 0.87$) (Fig. 2b). The two-way ANOVA with post-hoc test of ILPs is reported in the Supplementary material, section 3.

3.3 Contextual memory reconsolidation experiment

The protocol design of contextual memory reconsolidation experiment is reported in Figure 3a. Prior to pharmacological treatment, experimental groups did not show statistical differences in lever responding. The ANOVA of ALPs during the last three S/A sessions, the first extinction session and the last three sessions of extinction-1 showed a significant main effect of Session [$F(2,28) = 31.66$; $p < 0.0001$] but no effect of Treatment $F(1,14) = 1.73$; $p = 0.21$] nor of interaction Session x Treatment [$F(2,28) = 1.70$; $p = 0.20$].

The Sidak's tests between treatments (prospective vehicle vs. ketamine) showed no significant differences between the last three S/A sessions (73.46 ± 9.54 and 91.13 ± 10.19, $p = 0.93$), the first extinction session (141.60 ± 29.24 and 214.80 ± 47.15, $p = 0.10$) and the last three sessions of extinction-1 (19.21 ± 4.85 and 23.04 ± 3.71, $p > 0.99$). The Sidak's tests of ALPs between sessions and the two-way ANOVA with post-hoc test of ILPs is reported in the Supplementary material, section 4.

Re-exposure to conditioning context during the retrieval session significantly increased responding on the active lever compared to extinction-1 and extinction-2 for both experimental groups, with no differences between ketamine and vehicle group. The ANOVA of ALPs during the last three sessions of extinction-1, the retrieval session and the last three sessions of extinction-2 showed a significant main effect of Session [$F(2,28) = 26.57$; $p <$

0.0001] but no effects of Treatment F(1,14) = 0.34; p = 0.57] nor of interaction Session x Treatment [F(2,28) = 0.03; p = 0.98].

The Sidak's tests between treatments showed no significant differences for last three sessions of extinction-1 (19.21 ± 4.85 and 23.04 ± 3.71, p = 0.95), for the retrieval sessions (40.88 ± 9.27 and 45.38 ± 5.85, p = 0.92) and for the last three sessions of extinction-2 (11.96 ± 3.76 and 14.58 ± 4.46, p = 0.98). The Sidak's tests of ALPs between sessions and the two-way ANOVA with post-hoc test of ILPs is reported in the Supplementary material, section 5.

The treatment with ketamine before retrieval session did not impair the responding on the active lever during test, compared to vehicle. The ANOVA of ALPs during the last three sessions of extinction-2 and the test showed a significant main effect of Session [F(1,14) = 30.90; p < 0.0001] but no effects of Treatment F(1,14) = 2.04; p = 0.18] nor of interaction Session x Treatment [F(1,14) = 2.18; p = 0.16].

The Sidak's tests between treatments showed no significant differences for the last three sessions of extinction-2 (11.96 ± 3.76 and 14.58 ± 4.46, p = 0.89) and for the test (25.25 ± 4.09 and 37.50 ± 4.98, p = 0.11). The Sidak's tests between sessions showed a significant difference between the last three sessions of extinction-2 and the test both for vehicle (11.96 ± 3.76 and 25.25 ± 4.09, p < 0.05) and for ketamine group (14.58 ± 4.46 and 37.50 ± 4.98, p < 0.001) (Fig. 3b). The two-way ANOVA with post-hoc test of ILPs is reported in the Supplementary material, section 6.

Fig. 1

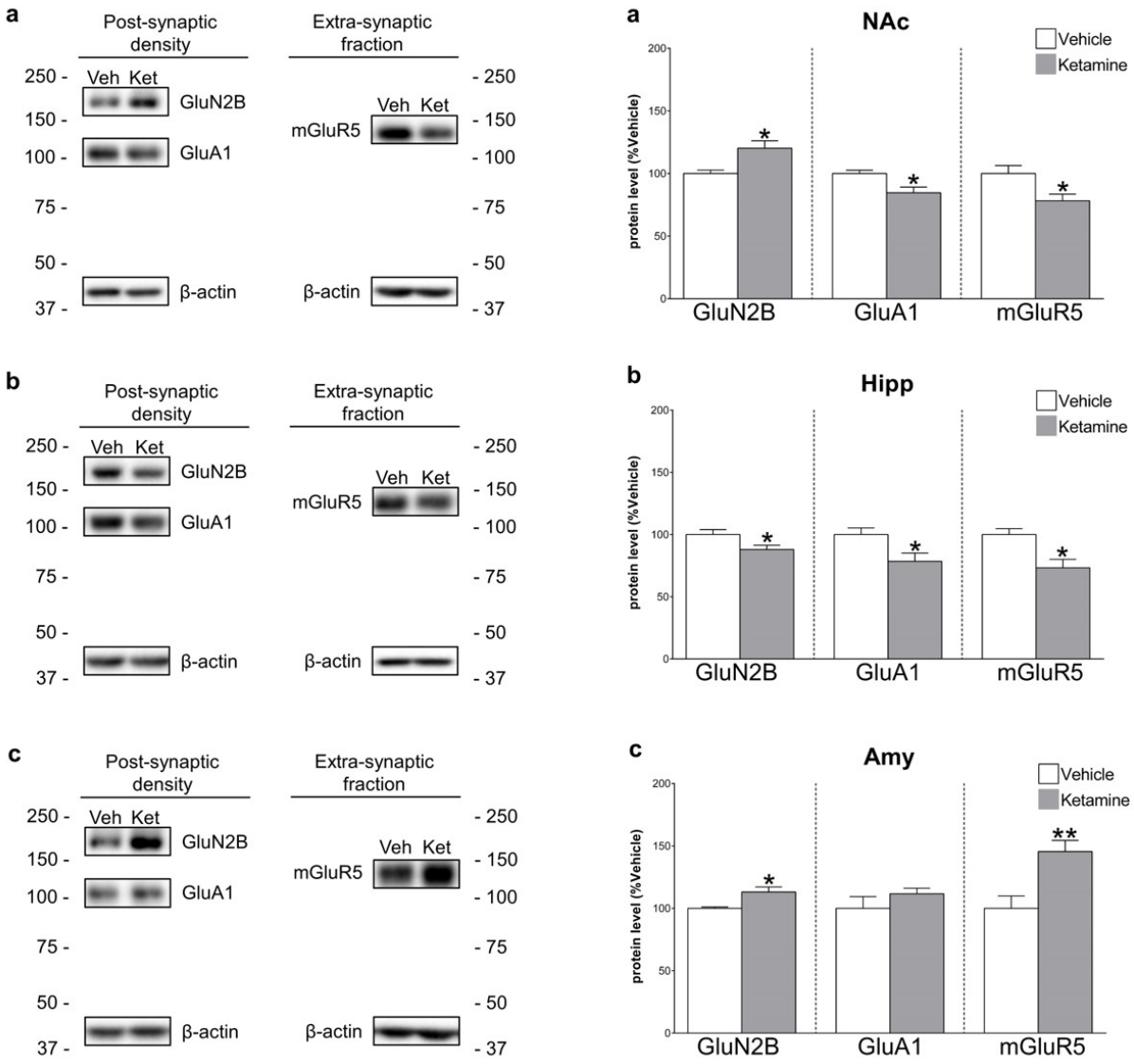


Figure 1. Effect of vehicle or ketamine on GluN2B-containing NMDARs and GluA1-containing AMPARs in the post-synaptic density and on mGluR5 in the extra-synaptic fraction of nucleus accumbens, hippocampus and amygdala. (**Left**) representative images of western blot bands with GluN2B (180 kDa, left) GluA1 (108 kDa, left) and mGluR5 (130 kDa, right) compared to β-actin (43 kDa) as control for nucleus accumbens (**a**), hippocampus (**b**) and amygdala (**c**). (**Right**) quantification of GluN2B-NMDARs and GluA1-AMPARs level in the post-synaptic density and of mGluR5 in the extra-synaptic fraction 24h after vehicle or ketamine treatment in nucleus accumbens (NAc, **a**), hippocampus (Hipp, **b**) and amygdala (Amy, **c**). Data are shown as the mean + SEM and are expressed as percentage of the vehicle. N=5 rats/group. *$p < 0.05$, **$p < 0.01$ vs vehicle; unpaired two-tailed Student's t-test.

Fig. 2

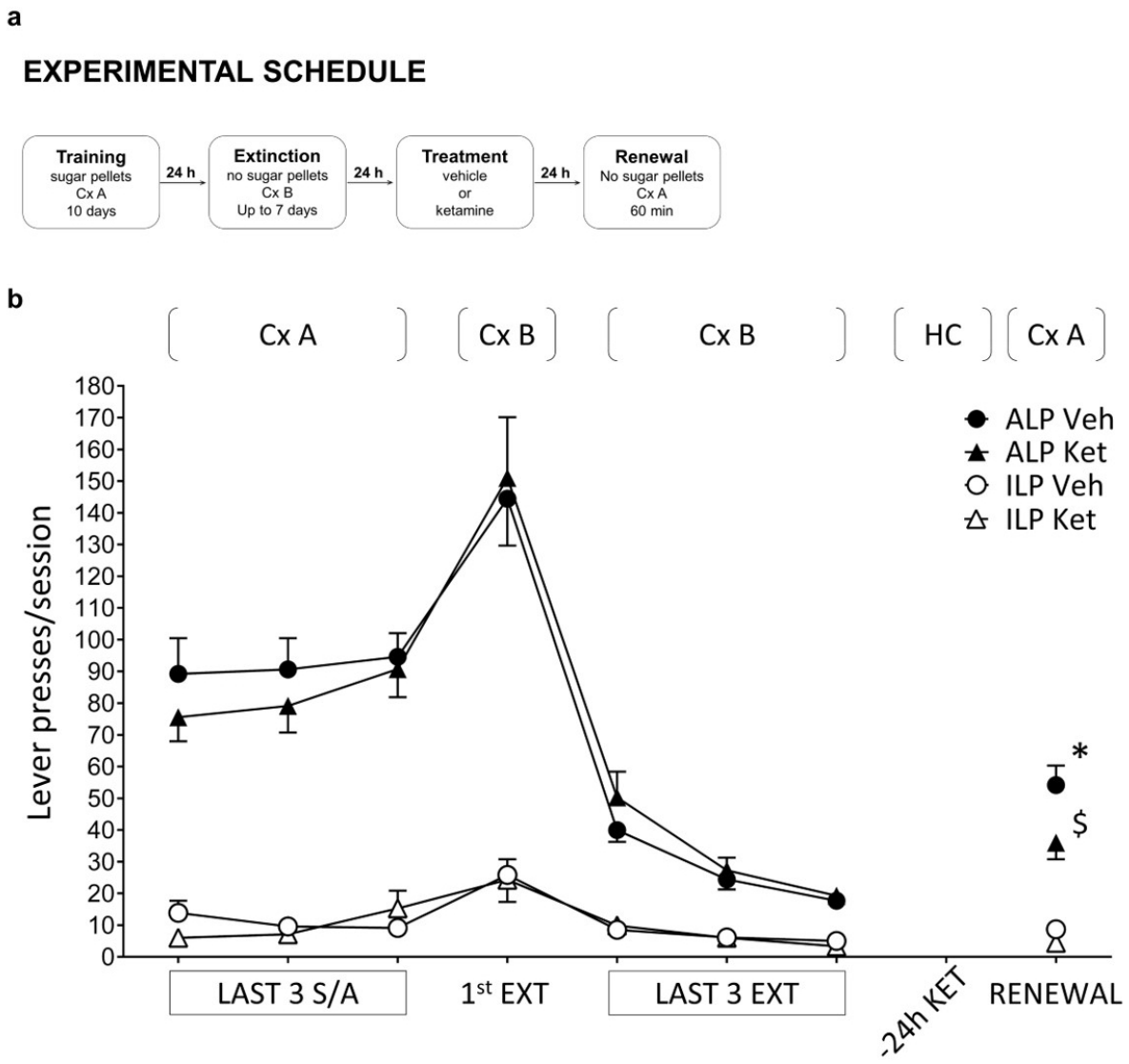


Figure 2. (**a**) Schematic diagram of the experimental protocol and groups. Boxes represent the different procedures used at the different phases of the study. Arrow represents time progression between consecutive phases. Cx A = sucrose self-administration (S/A) training (conditioning) context, Cx B = extinction context. (**b**) Rate of responding on active (solid circle/triangle) or inactive (open circle/triangle) lever per sessions during the different phases of the renewal protocol for rats treated with vehicle (Veh; circle) or ketamine (Ket; triangle). Rate of responding is indicated for the last 3 sessions of S/A (LAST 3 S/A), the first extinction session (1st EXT), the last 3 extinction sessions (LAST 3 EXT) and sucrose-seeking behaviour test (RENEWAL). "-24h KET" indicates the day of treatment, without behavioural session. On top, Cx A indicates the conditioning context, Cx B the extinction context and HC the home cage. Data are expressed as the mean ± SEM. N = 15 rats/vehicle group, N = 7 rats/ketamine group. *$p < 0.001$ vehicle RENEWAL vs LAST 3 EXT, $^{\$}p < 0.05$ ketamine vs

vehicle RENEWAL, repeated measures two-way ANOVA with post-hoc Sidak's multiple comparisons test.

Fig. 3

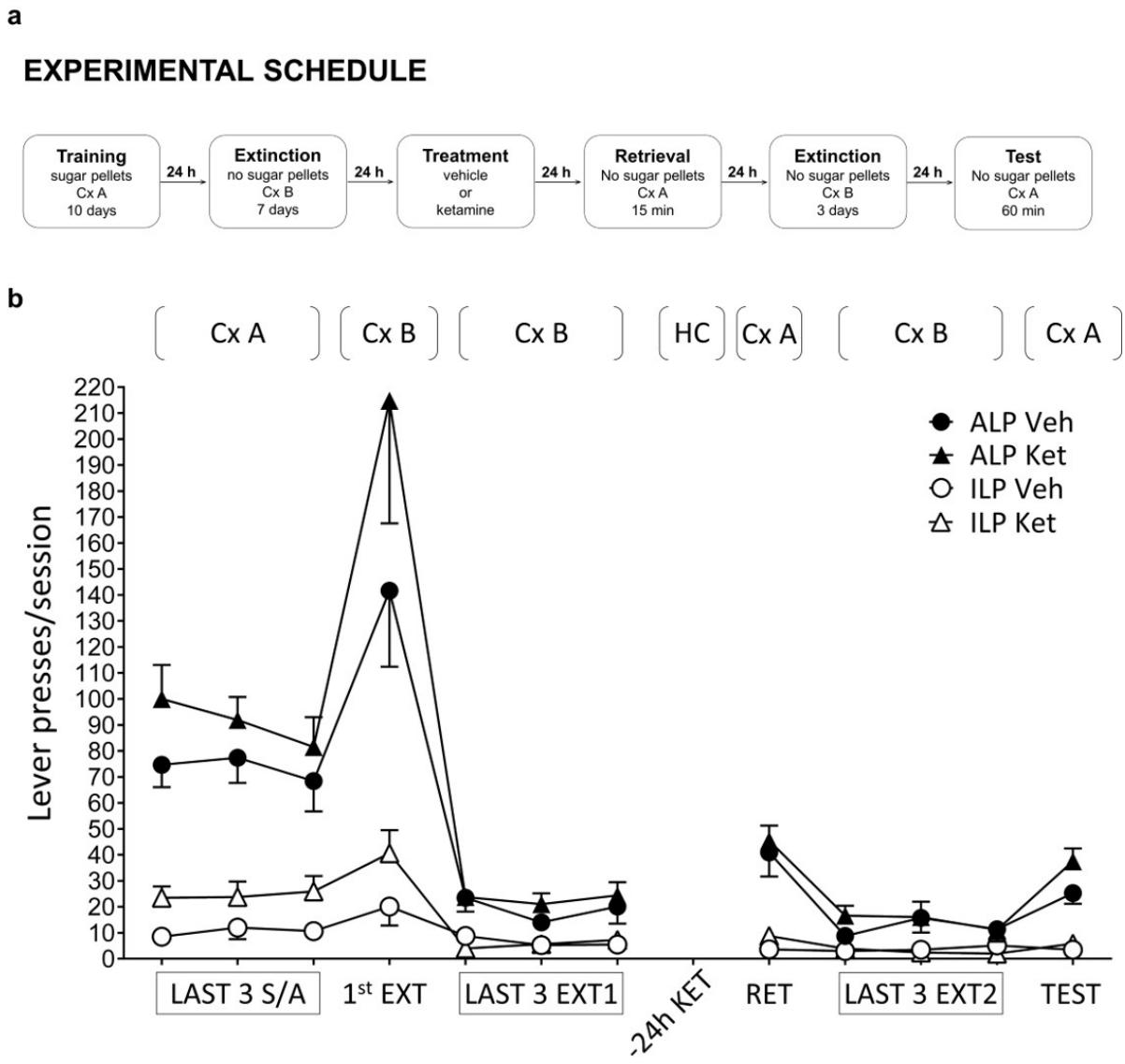


Figure 3. (**a**) Schematic diagram of the experimental protocol and groups. Boxes represent the different procedures used at the different phases of the study. Arrow represents time progression between consecutive phases. Cx A = sucrose S/A training (conditioning) context, Cx B = extinction context. (**b**) Rate of responding on active (solid circle/triangle) or inactive (open circle/triangle) lever per sessions during the different phases of the memory reconsolidation protocol for rats treated with vehicle (Veh; circle) or ketamine (Ket; triangle).

Rate of responding is indicated for the last 3 sessions of S/A (LAST 3 S/A), the first extinction training session (1st EXT), the last 3 extinction sessions of the first extinction phase (LAST 3 EXT1), the retrieval session (RET), the last 3 extinction sessions of the second extinction phase (LAST 3 EXT2) and the sucrose-seeking behaviour test (TEST). "-24h KET" indicates the day of treatment, without behavioural session. On top, Cx A indicates the conditioning context, Cx B the extinction context and HC the home cage. Data are expressed as the mean ± SEM. N = 8 rats/vehicle group, N = 8 rats/ketamine group.

4. Discussion

Ketamine given 24 hours prior to renewal of sucrose-seeking significantly inhibited conditioned responding on the previously sucrose-associated lever compared to vehicle. On the other hand, no significant effect was observed when given 24 hours prior to reactivation of contextual memory. Therefore, ketamine given to rats under a "metaplasticity-inducing dose regimen" was able to affect renewal but not sucrose memory reconsolidation. The behavioural effects of ketamine were observed at doses able to induce expression changes of glutamate receptors relevant for drug-seeking and appetitive memory reconsolidation in amygdala, hippocampus and nucleus accumbens. We therefore suggest that these molecular changes might be causally related to renewal inhibition and, on the other hand, to reconsolidation resistance to disruption.

The behavioural experiments described in this paper were based on protocol designs reported in literature for assessing the effects of context on drug-seeking and drug-memory reconsolidation: the renewal protocol is widely reported in literature (for a review see [3]), whereas the contextual memory reconsolidation protocol has been extensively validated by the Fuchs's group [44,46], with some analogies to instrumental learning protocols developed by our and other groups [16-18,29]. The protocol included an extinction phase where lever pressing was extinguished in a context (CxB) different from the conditioning one (CxA). The re-exposure to CxA induced an increased responding (renewal) that is assumed to be solely due to the conditioning context CxA, since extinction of instrumental responding took place in a different context (CxB). Thus, we exclude the occurrence of an a-priori partial extinction of behaviour in CxA. The reduced responding in the ketamine group during the renewal session, could be due to a faster learning of extinction rather than an inhibition of the renewal. The analysis of responding pattern during session at 15min time bins did not show any difference over the renewal session time period between ketamine and vehicle groups. We therefore hypothesize that the ketamine effect was an impairment of renewal rather than

a potentiation of extinction – even though the latter explanation cannot be excluded. Similarly, we did not find significant differences between the two groups during the early time bin of the retrieval session in the reconsolidation experiments. Furthermore, we analysed the time-bin data from the retrieval and the test (reinstatement) session, but we did not find significant differences between the two groups.

Similar to MK-801 [29,30,32], ketamine induced metaplastic changes assessed on the day after its administration, such as facilitation of tetanus-induced LTP in *ex-vivo* hippocampal slices 24 hours after treatment [31]. Thus, we defined the ketamine dose regimen used in our studies as “metaplastic” in accordance to similar definition given for explaining ketamine effects assessed at time points longer than its half-life (intravenous ketamine half-life in rats is approximately 1 hour; [50]). Indeed, several reports on ketamine antidepressant effects defined the molecular and behavioural changes assessed 24 hours after treatment as “metaplasticity effects” [31,37]. In our study, ketamine induced significant expression changes of glutamate receptors in brain areas relevant for renewal and contextual memory reconsolidation. In fact, glutamate receptors GluN2B, GluA1 and mGluR5 have been reported to be involved in different forms of metaplasticity ([42,51,52]; for reviews see [19,43]).

In hippocampus - an important brain area for processing of spatial context information, drug-seeking [7,8,53] and memory deficits in ketamine users [54] - ketamine significantly decreased synaptic expression levels of GluN2B, GluA1, mGluR5 (Fig. 1, panel b). This general ‘hypo-glutamatergic’ effect might be the compensatory consequence of an increased glutamate release induced by high dose ketamine treatment given to rats 24 hours earlier [35]. Considering that increased GluN2B phosphorylation has been shown in drug-seeking [55-57], its reduction by ketamine in hippocampus may be associated with renewal inhibition. Caffino et al. [36] suggested that the decrease of AMPA receptors induced by ketamine in hippocampus may in turn reduce NMDA receptors activation. The concomitant effect of ketamine, and of its metabolite (2S,6S)-hydroxynorketamine acting as a GluA1 agonist [37,58,59], might have induced AMPA receptors endocytosis and decrease of GluA1 receptors (event though in contrast to what described by Zanos et al. [37]). Interestingly, we showed that metabotropic mGluR5 receptor expression was decreased [36], as also reported by Esterlis et al. [60]. This receptor has been reported to play an important role in hippocampal plasticity [61], so that its down-regulation might explain the reduced effect of conditioning context re-exposure–induced renewal. It is paradoxical that ketamine effect would induce a mGluR5 reduction, which is actually a counter-plasticity expression change [19].

In the nucleus accumbens, mGluR5 antagonism or deletion (removal) has also been shown to reduce relapse to drug-seeking behaviour [62]. Ketamine reduction of mGluR5 in nucleus accumbens could be a further mechanism underlying ketamine inhibitory effect on renewal. The decrease of mGluR5 in this brain area – which is associated with decrease of GluA1 – may be explained as an increased glutamatergic release induced by ketamine 24 hours earlier, lately resulting at renewal in a reduced glutamatergic control over accumbal activation.

We hypothesize that the increased expression of GluN2B in nucleus accumbens and amygdala might be due to a compensatory increase of membrane translocation of these subunits as a consequence of ketamine GluN2B antagonism. It is however unclear which role GluN2B changes might have played in sucrose renewal: although in Pavlovian drug-seeking GluN2B receptor expression was reduced [63,64], it is however not known its role in instrumental contextual learning [65]. It might be the case that increased expression of amygdalar GluN2B and mGluR5 are not relevant for the specific contextual renewal effect observed in our rats well-trained to sucrose S/A for which, on the other hand, hippocampal activity played a major role.

In our study we were not able to observe any significant effect of ketamine on the reconsolidation of contextual sucrose memory. As reviewed in the Introduction section, the retrieval of a consolidated memory may induce destabilization and labilization of the memory trace, followed by a process of restabilization/reconsolidation involving specific brain circuitries, also demonstrated for instrumental learning [13,14,16-18].

Metaplasticity changes in hippocampus have been shown to shift LTD to LTP, which in turn promote memory reconsolidation [66]. Ketamine induced an overall decrease of GluN2B, GluA1 and mGluR5 in hippocampus, perhaps suggesting a general stabilization of the appetitive memory and leading to a condition of contextual memory resistant to retrieval [67,68] as also shown in other paradigms [69,70]. Noteworthy, Wells et al. [65] showed a lack of involvement for hippocampal GluN2B in cocaine-induced memory reconsolidation.

In amygdala, ketamine induced expression changes of GluN2B similar to changes induced by MK-801 in our previous report [29]. GluN2B activation destabilizes memory, making the retrieved memory vulnerable to manipulation [39,71,72]. In Piva et al. [29], we have seen that this effect allowed for sucrose memory inhibition; here, otherwise, it appears that in amygdala it is not relevant at the behavioural level. Similarly, we cannot speculate how the increased level of mGluR5 in amygdala by ketamine is correlated to its lack of effects, considering the limited literature on Group I metabotropic glutamate receptors and memory reconsolidation [73,74].

In our previous report, the instrumental memory reconsolidation inhibition induced by MK-801 was associated with a pattern of glutamatergic facilitation of memory destabilization in nucleus accumbens [29]. Here, 24 hours after ketamine, we observed a similar effect on GluN2B but opposite on GluA1 and mGluR5 expression. Considering that nucleus accumbens plays an important role for Pavlovian memories [63,64] but not for instrumental contextual memories, such ketamine–induced metaplasticity in nucleus accumbens could be irrelevant for our contextual memory reconsolidation (e.g., [75,76]).

The high "metaplastic dose regimen" herein used is an experimental tool that allowed us to understand how NMDARs blockade might induce late effects at the level of behavioural relapse – in this case induced by re-exposure to sucrose-conditioned context or reactivation of contextual sucrose memory. Ketamine effects described in the present study are different from those described in our previous report [29]. In that study, we used a different protocol of instrumental memory reconsolidation, where the conditioning context component effect was not controlled (see also [18]). The two NMDAR blockers should therefore be investigated and compared with similar protocols.

In conclusion, our findings show that ketamine, given under a "metaplastic dose regimen", inhibits sucrose-seeking renewal but not sucrose contextual memory reconsolidation. These metaplastic effects of ketamine suggest a possible involvement of glutamatergic receptors in the inhibition of sucrose renewal but not of contextual memory reconsolidation. For renewal, the inhibition of instrumental responding could be explained as a consequence of hippocampal and accumbal decreased levels of GluA1 and mGluR5; for contextual memory reconsolidation, the lack of effect could be due to resistance of the memory trace to destabilization as a consequence of a general 'hypoglutamatergia' in hippocampus, in particular for GluN2B. The major limitation of our study is the lack of control conditions investigating the molecular ketamine effects in experimental subjects exposed to the entire behavioural protocol. Further studies are therefore needed to study the combined effects of the behavioural history (i.e., sucrose S/A and extinction) and the "metaplastic dose regimen" on glutamatergic receptors.

Acknowledgements

AP and GS performed the behavioural experiments. AP, LP and NP analysed the behavioural data. LC and FM performed and analysed the data of western blot assays. CC, FF, AP, LC and GP planned and designed the whole study and each experiment. CC, FF,

AP and LC wrote the manuscript, and all authors have approved the final version of the article.

The research performed at the University of Verona did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The research performed at the University of Milano was supported by grants from MIUR Progetto Eccellenza.

References

[1] M.E. Bouton, Extinction of instrumental (operant) learning: interference, varieties of context, and mechanisms of contextual control., Psychopharmacology (Berl). 236 (2019) 7–19. doi:10.1007/s00213-018-5076-4.

[2] C.A. Conklin, Environments as cues to smoke: Implications for human extinction-based research and treatment., Exp Clin Psychopharmacol. 14 (2006) 12–19. doi:10.1037/1064-1297.14.1.12.

[3] H.S. Crombag, J.M. Bossert, E. Koya, Y. Shaham, Context-induced relapse to drug seeking: a review., Philos Trans R Soc Lond B Biol Sci. 363 (2008) 3233–43. doi:10.1098/rstb.2008.0090.

[4] A. Badiani, D. Belin, D. Epstein, D. Calu, Y. Shaham, Opiate versus psychostimulant addiction: the differences do matter., Nat Rev Neurosci. 12 (2011) 685–700. doi:10.1038/nrn3104.

[5] S.Y.-S. Khoo, G.D. Gibson, A.A. Prasad, G.P. McNally, How contexts promote and prevent relapse to drug seeking., Genes Brain Behav. 16 (2017) 185–204. doi:10.1111/gbb.12328.

[6] H.S. Crombag, Y. Shaham, Renewal of drug seeking by contextual cues after prolonged extinction in rats., Behav Neurosci. 116 (2002) 169–73. doi:10.1037/0735-7044.116.1.169.

[7] P.W. Marinelli, D. Funk, W. Juzytsch, Z. Li, A.D. Lê, Effects of opioid receptor blockade on the renewal of alcohol seeking induced by context: relationship to c-fos mRNA expression., Eur J Neurosci. 26 (2007) 2815–23. doi:10.1111/j.1460-9568.2007.05898.x.

[8] R.A. Fuchs, K.A. Evans, C.C. Ledford, M.P. Parker, J.M. Case, R.H. Mehta, R.E. See, The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats., Neuropsychopharmacology. 30 (2005) 296–309. doi:10.1038/sj.npp.1300579.

[9] L. Diergaarde, W. de Vries, H. Raasø, A.N.M. Schoffelmeer, T.J. De Vries, Contextual renewal of nicotine seeking in rats and its suppression by the cannabinoid-1 receptor antagonist Rimonabant (SR141716A)., Neuropharmacology. 55 (2008) 712–6.

doi:10.1016/j.neuropharm.2008.06.003.

[10] S. Nakajima, S. Tanaka, K. Urushihara, H. Imada, Renewal of extinguished lever-press responses upon return to the training context., Learn Motiv. 31 (2000) 416–31. doi:10.1006/LMOT.2000.1064.

[11] A.S. Hamlin, K.E. Blatchford, G.P. McNally, Renewal of an extinguished instrumental response: Neural correlates and the role of D1 dopamine receptors., Neuroscience. 143 (2006) 25–38. doi:10.1016/j.neuroscience.2006.07.035.

[12] K. Nader, G.E. Schafe, J.E. Le Doux, Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval., Nature. 406 (2000) 722–26. doi:10.1038/35021052.

[13] A.C. Reichelt, J.L.C. Lee, Memory reconsolidation in aversive and appetitive settings., Front Behav Neurosci. 7 (2013) 118. doi:10.3389/fnbeh.2013.00118.

[14] V. Tedesco, A. Mutti, A. Auber, C. Chiamulera, Nicotine-seeking reinstatement is reduced by inhibition of instrumental memory reconsolidation., Behav Pharmacol. 25 (2014) 725–31. doi:10.1097/FBP.0000000000000088.

[15] M.T.J. Exton-McGuinness, R.C. Patton, L.B. Sacco, J.L.C. Lee, Reconsolidation of a well-learned instrumental memory., Learn Mem. 21 (2014) 468–77. doi:10.1101/lm.035543.114.

[16] M.T.J. Exton-McGuinness, J.L.C. Lee, A.C. Reichelt, Updating memories-the role of prediction errors in memory reconsolidation., Behav Brain Res. 278 (2015) 375–84. doi:10.1016/j.bbr.2014.10.011.

[17] G.H. Vousden, A.L. Milton, The chains of habits: too strong to be broken by reconsolidation blockade?, Curr Opin Behav Sci. 13 (2017) 158–63. doi:10.1016/J.COBEHA.2016.11.010.

[18] A. Piva, E. Gerace, M. Di Chio, L. Padovani, G. Paolone, D.E. Pellegrini-Giampietro, C. Chiamulera, Reconsolidation of sucrose instrumental memory in rats: the role of retrieval context., Brain Res. 1714 (2019) 193–201. doi:10.1016/j.brainres.2019.03.006.

[19] W.C. Abraham, Metaplasticity: tuning synapses and networks for plasticity., Nat Rev Neurosci. 9 (2008) 387–387. doi:10.1038/nrn2356.

[20] W.C. Abraham, M.F. Bear, Metaplasticity: the plasticity of synaptic plasticity., Trends Neurosci. 19 (1996) 126–30. doi: 10.1016/S0166-2236(96)80018-X.

[21] S.R. Hulme, O.D. Jones, W.C. Abraham, Emerging roles of metaplasticity in behaviour and disease., Trends Neurosci. 36 (2013) 353–62. doi:10.1016/j.tins.2013.03.007.

[22] G. Richter-Levin, M. Maroun, Stress and amygdala suppression of metaplasticity in the medial prefrontal cortex., Cereb Cortex. 20 (2010) 2433–41. doi:10.1093/cercor/bhp311.

[23] M.V. Schmidt, W.C. Abraham, M. Maroun, O. Stork, G. Richter-Levin, Stress-induced metaplasticity: from synapses to behavior., Neuroscience. 250 (2013) 112–20. doi:10.1016/j.neuroscience.2013.06.059.

[24] M.J. Eckert, W.C. Abraham, Effects of environmental enrichment exposure on synaptic transmission and plasticity in the hippocampus., Curr Top Behav Neurosci. 15 (2013) 165–87. doi:10.1007/7854_2012_215.

[25] M.C. Creed, C. Lüscher, Drug-evoked synaptic plasticity: beyond metaplasticity., Curr Opin Neurobiol. 23 (2013) 553–58. doi:10.1016/j.conb.2013.03.005.

[26] D. Neuhofer, P. Kalivas, Metaplasticity at the addicted tetrapartite synapse: a common denominator of drug induced adaptations and potential treatment target for addiction., Neurobiol Learn Mem. 154 (2018) 97–111. doi:10.1016/j.nlm.2018.02.007.

[27] B.J. Stansley, N.M. Fisher, R.G. Gogliotti, C.W. Lindsley, P.J. Conn, C.M. Niswender, Contextual fear extinction induces hippocampal metaplasticity mediated by metabotropic glutamate receptor 5., Cereb Cortex. 28 (2018) 4291–304. doi:10.1093/cercor/bhx282.

[28] P.S.B. Finnie, K. Nader, The role of metaplasticity mechanisms in regulating memory destabilization and reconsolidation., Neurosci Biobehav Rev. 36 (2012) 1667–707. doi:10.1016/j.neubiorev.2012.03.008.

[29] A. Piva, E. Gerace, M. Di Chio, L. Osanni, L. Padovani, L. Caffino, F. Fumagalli, D.E. Pellegrini-Giampietro, C. Chiamulera, The metaplastic effects of NMDA receptors blockade on reactivation of instrumental memories in rats., Neurobiol Learn Mem. 154 (2018) 87–96. doi:10.1016/j.nlm.2018.01.007.

[30] N. Buck, S. Cali, J. Behr, Enhancement of long-term potentiation at CA1-subiculum synapses in MK-801-treated rats., Neurosci Lett. 392 (2006) 5–9. doi:10.1016/j.neulet.2005.08.054.

[31] J. Burgdorf, X. Zhang, K.L. Nicholson, R.L. Balster, J. David Leander, P.K. Stanton, A.L. Gross, R.A. Kroes, J.R. Moskal, GLYX-13, a NMDA receptor glycine-site functional partial agonist, induces antidepressant-like effects without ketamine-like side effects., Neuropsychopharmacology. 38 (2013) 729–42. doi:10.1038/npp.2012.246.

[32] C.F. Zorumski, Y. Izumi, NMDA receptors and metaplasticity: mechanisms and possible roles in neuropsychiatric disorders., Neurosci Biobehav Rev. 36 (2012) 989–1000. doi:10.1016/j.neubiorev.2011.12.011.

[33] R.S. Duman, N. Li, R.-J. Liu, V. Duric, G. Aghajanian, Signaling pathways underlying the rapid antidepressant actions of ketamine., Neuropharmacology. 62 (2012) 35–41. doi:10.1016/j.neuropharm.2011.08.044.

[34] L. Caffino, M. Di Chio, G. Giannotti, M. Venniro, A. Mutti, L. Padovani, D. Cheung,

G.F. Fumagalli, D.T. Yew, F. Fumagalli, C. Chiamulera, The modulation of BDNF expression and signalling dissects the antidepressant from the reinforcing properties of ketamine: effects of single infusion vs. chronic self-administration in rats., Pharmacol Res. 104 (2016) 22–30. doi:10.1016/j.phrs.2015.12.014.

[35] L. Caffino, A. Piva, G. Giannotti, M. Di Chio, F. Mottarlini, M. Venniro, D.T. Yew, C. Chiamulera, F. Fumagalli, Ketamine self-administration reduces the homeostasis of the glutamate synapse in the rat brain., Mol Neurobiol. 54 (2017) 7186–93. doi:10.1007/s12035-016-0231-6.

[36] L. Caffino, A. Piva, F. Mottarlini, M. Di Chio, G. Giannotti, C. Chiamulera, F. Fumagalli, Ketamine self-administration elevates αCaMKII autophosphorylation in mood and reward-related brain regions in rats., Mol Neurobiol. 55 (2018) 5453–61. doi:10.1007/s12035-017-0772-3.

[37] P. Zanos, R. Moaddel, P.J. Morris, P. Georgiou, J. Fischell, G.I. Elmer, M. Alkondon, P. Yuan, H.J. Pribut, N.S. Singh, K.S.S. Dossou, Y. Fang, X.-P. Huang, C.L. Mayo, I.W. Wainer, E.X. Albuquerque, S.M. Thompson, C.J. Thomas, C.A. Zarate Jr, T.D. Gould, NMDAR inhibition-independent antidepressant actions of ketamine metabolites., Nature. 533 (2016) 481–86. doi:10.1038/nature17998.

[38] L.R. Vose, P.K. Stanton, Synaptic plasticity, metaplasticity and depression., Curr Neuropharmacol. 15 (2017) 71–86. doi: 10.2174/1570159X14666160202121111.

[39] S.H. Wang, L. de Oliveira Alvares, K. Nader, Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation., Nat Neurosci. 12 (2009) 905–12. doi:10.1038/nn.2350.

[40] C.D. Gipson, K.J. Reissner, Y.M. Kupchik, A.C.W. Smith, N. Stankeviciute, M.E. Hensley-Simon, P.W. Kalivas, Reinstatement of nicotine seeking is mediated by glutamatergic plasticity., Proc Natl Acad Sci USA. 110 (2013) 9124–9. doi:10.1073/pnas.1220591110.

[41] M.H. Monfils, K.K. Cowansage, E. Klann, J.E. LeDoux, Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories., Science. 324 (2009) 951–55. doi:10.1126/science.1167975.

[42] Z.A. Bortolotto, V.J. Collett, F. Conquet, Z. Jia, H. van der Putten, G.L. Collingridge, The regulation of hippocampal LTP by the molecular switch, a form of metaplasticity, requires mGlu5 receptors., Neuropharmacology. 49 (2005) 13–25. doi:10.1016/j.neuropharm.2005.05.020.

[43] T.M. Marton, M.G. Hussain Shuler, P.F. Worley, Homer 1a and mGluR5 phosphorylation in reward-sensitive metaplasticity: a hypothesis of neuronal selection and bidirectional synaptic plasticity., Brain Res. 1628 (2015) 17–28. doi:10.1016/j.brainres.2015.06.037.

[44] A.A. Arguello, R. Wang, C.M. Lyons, J.A. Higginbotham, M.A. Hodges, R.A. Fuchs, Role of the agranular insular cortex in contextual control over cocaine-seeking behavior in rats., Psychopharmacology (Berl). 234 (2017) 2431–41. doi:10.1007/s00213-017-4632-7.

[45] L. Caffino, G. Giannotti, F. Mottarlini, G. Racagni, F. Fumagalli, Developmental exposure to cocaine dynamically dysregulates cortical Arc/Arg3.1 modulation in response to a challenge., Neurotox Res. 31 (2017) 289–97. doi:10.1007/s12640-016-9683-8.

[46] R.A. Fuchs, G.H. Bell, D.R. Ramirez, J.L. Eaddy, Z. Su, Basolateral amygdala involvement in memory reconsolidation processes that facilitate drug context-induced cocaine seeking., Eur J Neurosci. 30 (2009) 889–900. doi:10.1111/j.1460-9568.2009.06888.x.

[47] A. Auber, N.S. Muthu Karuppasamy, M. Pedercini, D. Bertoglio, V. Tedesco, C. Chiamulera, The effect of postretrieval extinction of nicotine pavlovian memories in rats trained to self-administer nicotine., Nicotine Tob Res. 16 (2014) 1599–605. doi:10.1093/ntr/ntu110.

[48] C. Chiamulera, V. Tedesco, L. Zangrandi, C. Giuliano, G. Fumagalli, Propranolol transiently inhibits reinstatement of nicotine-seeking behaviour in rats., J Psychopharmacol. 24 (2010) 389–95. doi:10.1177/0269881108097718.

[49] A. Pushparaj, Y. Pryslawsky, B. Forget, Y. Yan, B. Le Foll, Extinction bursts in rats trained to self-administer nicotine or food in 1-h daily sessions., Am J Transl Res. 4 (2012) 422–31. PMID: 23145210.

[50] C.L. Shaffer, S.M. Osgood, D.L. Smith, J. Liu, P.E. Trapa, Enhancing ketamine translational pharmacology via receptor occupancy normalization., Neuropharmacology. 86 (2014) 174–80. doi:10.1016/j.neuropharm.2014.07.008.

[51] G. Tenorio, S.A. Connor, D. Guévremont, W.C. Abraham, J. Williams, T.J. O'Dell, P. V Nguyen, "Silent" priming of translation-dependent LTP by ß-adrenergic receptors involves phosphorylation and recruitment of AMPA receptors., Learn Mem. 17 (2010) 627–38. doi:10.1101/lm.1974510.

[52] K. Yang, C. Trepanier, B. Sidhu, Y.-F. Xie, H. Li, G. Lei, M.W. Salter, B.A. Orser, T. Nakazawa, T. Yamamoto, M.F. Jackson, J.F. MacDonald, Metaplasticity gated through differential regulation of GluN2A versus GluN2B receptors by Src family kinases., EMBO J. 31 (2012) 805–16. doi:10.1038/emboj.2011.453.

[53] R.A. Fuchs, J.L. Eaddy, Z.-I. Su, G.H. Bell, Interactions of the basolateral amygdala with the dorsal hippocampus and dorsomedial prefrontal cortex regulate drug context-induced reinstatement of cocaine-seeking in rats., Eur J Neurosci. 26 (2007) 487–98. doi:10.1111/j.1460-9568.2007.05674.x.

[54] C.J.A. Morgan, C.M. Dodds, H. Furby, F. Pepper, J. Fam, T.P. Freeman, E. Hughes, C. Doeller, J. King, O. Howes, J.M. Stone, Long-term heavy ketamine use is associated with spatial memory impairment and altered hippocampal activation., Front. Psychiatry. 5 (2014) 149. doi:10.3389/fpsyt.2014.00149.

[55] J. Wang, S. Carnicella, K. Phamluong, J. Jeanblanc, J.A. Ronesi, N. Chaudhri, P.H. Janak, D.M. Lovinger, D. Ron, Ethanol induces long-term facilitation of NR2B-NMDA receptor activity in the dorsal striatum: implications for alcohol drinking behavior., J Neurosci. 27 (2007) 3593–602. doi:10.1523/JNEUROSCI.4749-06.2007.

[56] J. Wang, M.F. Lanfranco, S.L. Gibb, Q. V. Yowell, S. Carnicella, D. Ron, Long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum play a crucial role in alcohol consumption and relapse., J Neurosci. 30 (2010) 10187–98. doi:10.1523/JNEUROSCI.2268-10.2010.

[57] X. Xie, A.A. Arguello, A.M. Wells, A.M. Reittinger, R.A. Fuchs, Role of a hippocampal Src-family kinase-mediated glutamatergic mechanism in drug context-induced cocaine seeking., Neuropsychopharmacology. 38 (2013) 2657–65. doi:10.1038/npp.2013.175.

[58] N. Yao, O. Skiteva, X. Zhang, P. Svenningsson, K. Chergui, Ketamine and its metabolite (2R,6R)-hydroxynorketamine induce lasting alterations in glutamatergic synaptic plasticity in the mesolimbic circuit., Mol Psychiatry. 23 (2018) 2066–77. doi:10.1038/mp.2017.239.

[59] G. Collo, L. Cavalleri, C. Chiamulera, E. Merlo Pich, (2R,6R)-Hydroxynorketamine promotes dendrite outgrowth in human inducible pluripotent stem cell-derived neurons through AMPA receptor with timing and exposure compatible with ketamine infusion pharmacokinetics in humans., Neuroreport. 29 (2018) 1425–30. doi:10.1097/WNR.0000000000001131.

[60] I. Esterlis, N. DellaGioia, R.H. Pietrzak, D. Matuskey, N. Nabulsi, C.G. Abdallah, J. Yang, C. Pittenger, G. Sanacora, J.H. Krystal, R. V Parsey, R.E. Carson, C. DeLorenzo, Ketamine-induced reduction in mGluR5 availability is associated with an antidepressant response: an [11C]ABP688 and PET imaging study in depression., Mol Psychiatry. 23 (2018) 824–32. doi:10.1038/mp.2017.58.

[61] K. Naie, D. Manahan-Vaughan, Regulation by metabotropic glutamate receptor 5 of LTP in the dentate gyrus of freely moving rats: relevance for learning and memory formation., Cereb Cortex. 14 (2004) 189–98. doi:10.1093/cercor/bhg118.

[62] C. Chiamulera, C.M. Marzo, D.J.K. Balfour, Metabotropic glutamate receptor 5 as a potential target for smoking cessation., Psychopharmacology (Berl). 234 (2017) 1357–70. doi:10.1007/s00213-016-4487-3.

[63] C.A. Miller, J.F. Marshall, Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory., Neuron. 47 (2005) 873–84.

doi:10.1016/j.neuron.2005.08.006.

[64] F.R.M. Theberge, A.L. Milton, D. Belin, J.L.C. Lee, B.J. Everitt, The basolateral amygdala and nucleus accumbens core mediate dissociable aspects of drug memory reconsolidation., Learn Mem. 17 (2010) 444–53. doi:10.1101/lm.1757410.

[65] A.M. Wells, X. Xie, J.A. Higginbotham, A.A. Arguello, K.L. Healey, M. Blanton, R.A. Fuchs, Contribution of an SFK-mediated signaling pathway in the dorsal hippocampus to cocaine-memory reconsolidation in rats., Neuropsychopharmacology. 41 (2016) 675–85. doi:10.1038/npp.2015.217.

[66] W.C. Abraham, J.M. Williams, LTP maintenance and its protein synthesis-dependence., Neurobiol Learn Mem. 89 (2008) 260–8. doi:10.1016/j.nlm.2007.10.001.

[67] P.J. Hernandez, A.E. Kelley, Long-term memory for instrumental responses does not undergo protein synthesis-dependent reconsolidation upon retrieval., Learn Mem. 11 (2004) 748–54. doi:10.1101/lm.84904.

[68] D.R. Ramirez, G.H. Bell, H.C. Lasseter, X. Xie, S.A. Traina, R.A. Fuchs, Dorsal hippocampal regulation of memory reconsolidation processes that facilitate drug context-induced cocaine-seeking behavior in rats., Eur J Neurosci. 30 (2009) 901–12. doi:10.1111/j.1460-9568.2009.06889.x.

[69] M.C. Inda, E. V Muravieva, C.M. Alberini, Memory retrieval and the passage of time: from reconsolidation and strengthening to extinction., J Neurosci. 31 (2011) 1635–43. doi:10.1523/JNEUROSCI.4736-10.2011.

[70] M.J. Honsberger, J.R. Taylor, P.R. Corlett, Memories reactivated under ketamine are subsequently stronger: a potential pre-clinical behavioral model of psychosis., Schizophr Res. 164 (2015) 227–33. doi:10.1016/j.schres.2015.02.009.

[71] C. Ben Mamou, K. Gamache, K. Nader, NMDA receptors are critical for unleashing consolidated auditory fear memories., Nat Neurosci. 9 (2006) 1237–9. doi:10.1038/nn1778.

[72] A.L. Milton, E. Merlo, P. Ratano, B.L. Gregory, J.K. Dumbreck, B.J. Everitt, Double dissociation of the requirement for GluN2B- and GluN2A-containing NMDA receptors in the destabilization and restabilization of a reconsolidating memory., J Neurosci. 33 (2013) 1109–15. doi:10.1523/JNEUROSCI.3273-12.2013.

[73] K. Gieros, A. Sobczuk, E. Salinska, Differential involvement of mGluR1 and mGluR5 in memory reconsolidation and retrieval in a passive avoidance task in 1-day old chicks., Neurobiol Learn Mem. 97 (2012) 165–72. doi:10.1016/j.nlm.2011.11.004.

[74] E. Salinska, The role of group I metabotropic glutamate receptors in memory consolidation and reconsolidation in the passive avoidance task in 1-day-old chicks., Neurochem Int. 48 (2006) 447–52. doi:10.1016/j.neuint.2005.11.015.

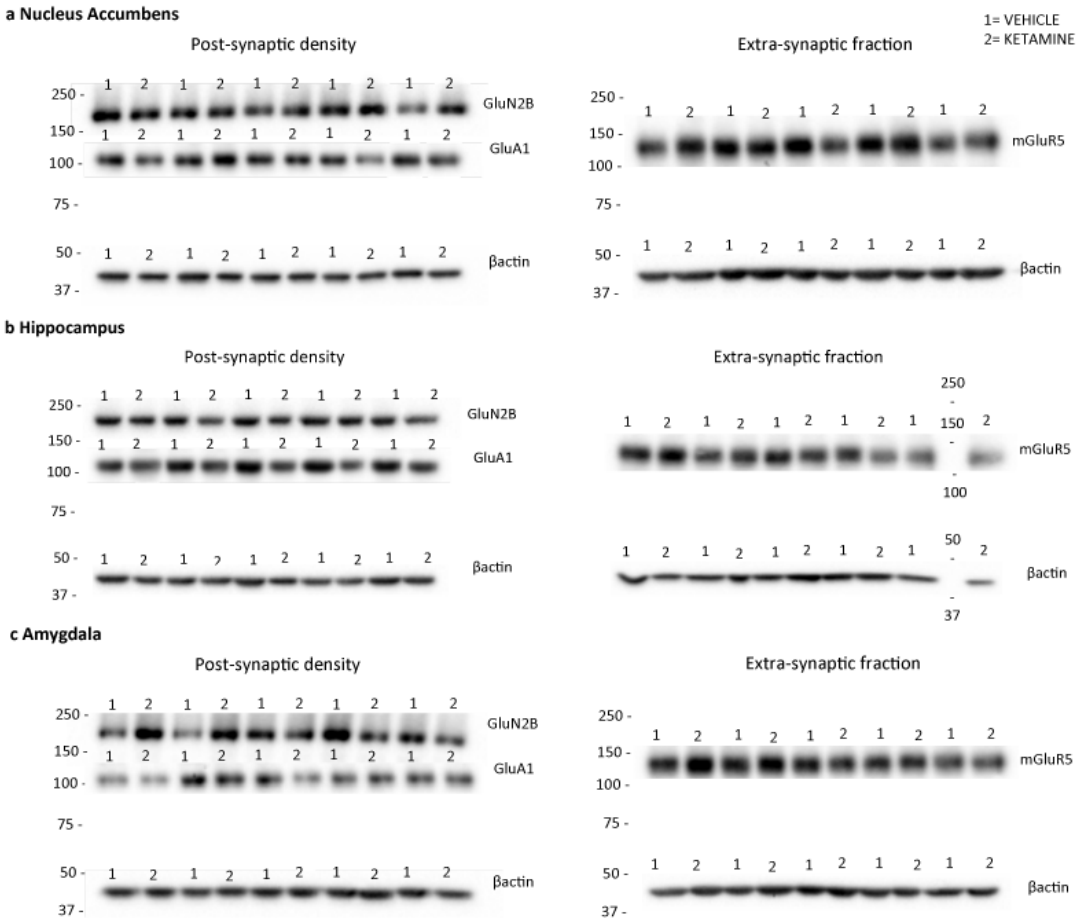
[75] M.W. Shiflett, R.P. Martini, J.C. Mauna, R.L. Foster, E. Peet, E. Thiels, Cue-elicited

reward-seeking requires extracellular signal-regulated kinase activation in the nucleus accumbens., J Neurosci. 28 (2008) 1434–43. doi:10.1523/JNEUROSCI.2383-07.2008.

[76] A.M. Wells, A.A. Arguello, X. Xie, M.A. Blanton, H.C. Lasseter, A.M. Reittinger, R.A. Fuchs, Extracellular signal-regulated kinase in the basolateral amygdala, but not the nucleus accumbens core, is critical for context-response-cocaine memory reconsolidation in rats., Neuropsychopharmacology. 38 (2013) 753–62. doi:10.1038/npp.2012.238.

Supplementary material 1

Uncropped immunoblot related to the western blot data presented in Fig. 1



Supplementary material 2

The Sidak's tests of ALPs between sessions of prospective vehicle group showed significant differences between the last three S/A sessions and the first extinction session (91.47 ± 8.38 and 144.50 ± 14.78, $p < 0.001$), the first extinction session and the last three extinction sessions (144.50 ± 14.78 and 27.33 ± 2.60, $p < 0.0001$) and the last 3 S/A and the last three extinction sessions (91.47 ± 8.38 and 27.33 ± 2.60, $p < 0.0001$). The Sidak's tests of ALPs between sessions of prospective ketamine group showed significant differences between the last three S/A sessions and the first extinction session (81.81 ± 7.21 and 151.00 ± 19.15, $p < 0.001$), the first extinction session and the last three extinction sessions (151.00 ± 19.15 and 32.29 ± 4.39, $p < 0.0001$) and the last three S/A and the last three extinction sessions (81.81 ± 7.21 and 32.29 ± 4.39, $p < 0.05$).
The ANOVA of ILPs during the last three S/A sessions, the first extinction session and the last three extinction sessions showed a significant main effect of Session [$F(2,40) = 18.49$; $p < 0.0001$] but no effects of Treatment [$F(1,20) = 0.07$; $p = 0.80$] nor of interaction Session x Treatment

[F(2,40) = 0.03; p = 0.97]. The Sidak's tests of ILPs between treatments (prospective vehicle vs. ketamine) showed no significant differences for the mean of the last three S/A sessions (10.87 ± 1.95 and 9.48 ± 2.72, p = 0.99), for the first extinction session (25.80 ± 4.98 and 24.29 ± 6.98, p = 0.99) and for the mean of the last three extinction sessions (6.56 ± 0.89 and 6.38 ± 1.24, p > 0.99). The Sidak's tests of ILPs between sessions of prospective vehicle group showed significant differences between the last three S/A sessions and the first extinction session (10.87 ± 1.95 and 25.80 ± 4.98, p < 0.001) and between the first extinction session and the last three extinction sessions (25.80 ± 4.98 and 6.56 ± 0.89, p < 0.0001) but not between the last three S/A sessions and the last three extinction sessions (10.87 ± 1.95 and 6.56 ± 0.89, p = 0.57). The Sidak's tests of ILPs between sessions of prospective ketamine group showed significant differences between the last three S/A sessions and the first extinction session (9.48 ± 2.72 and 24.29, p < 0.05) and between the first extinction session and the last three extinction sessions (24.29 ± 6.98 and 6.38 ± 1.24, p < 0.01) but not between the last three S/A sessions and the last three extinction sessions (9.48 ± 2.72 and 6.68 ± 1.24, p = 0.92).

Supplementary material 3

The ANOVA of ILPs during the last three extinction sessions and the renewal test showed no significant main effect of factor Session [F(1,20) = 0.01; p = 0.93], of factor Treatment [F(1,20) = 1.12; p = 0.30] and of interaction Session x Treatment [F(1,20) = 2.39; p = 0.14]. The Sidak's tests between treatments showed no significant differences neither for the renewal test (8.73 ± 2.05 and 4.43 ± 1.43; p = 0.18) nor for last three extinction sessions (6.56 ± 0.89 and 6.38 ±1.24; p > 0.99). The Sidak's tests between sessions showed no significant difference between the last three extinction sessions and the renewal test for vehicle (6.56 ± 0.89 and 8.73 ± 2.05; p = 0.30) nor for ketamine group (6.38 ±1.24 and 4.43 ± 1.43; p = 0.62).

Supplementary material 4

The Sidak's tests of ALPs between sessions of prospective vehicle group showed no significant differences between the last three S/A sessions and the first extinction session (73.46 ± 9.54 and 141.60 ± 29.24, p = 0.07) and the last three S/A sessions and the last three sessions of extinction-1 (73.46 ± 9.54 and 19.21 ± 4.85, p = 0.18), and a significant difference between the first extinction session and the last three sessions of extinction-1 (141.60 ± 29.24 and 19.21 ± 4.85, p < 0.001).
The Sidak's tests of ALPs between sessions of prospective ketamine group showed significant differences between the mean of the last three S/A sessions and the first extinction session (91.13

± 10.19 and 214.80 ± 47.15, $p < 0.001$) and the first extinction session and the last three sessions of extinction-1 (214.80 ± 47.15 and 23.04 ± 3.71, $p < 0.0001$), but no significant differences between the last three S/A sessions and the last three sessions of extinction-1 (91.13 ± 10.19 and 23.04 ± 3.71, $p = 0.07$).

The ANOVA of ILPs during the last three S/A sessions, the first extinction session and the last three sessions of extinction-1 showed a significant main effect of Session [$F(2,28) = 13.62$; $p < 0.0001$], but no effects of Treatment [$F(1,14) = 4.38$; $p = 0.06$] nor of interaction Session x Treatment [$F(2,28) = 2.76$; $p = 0.08$]. The Sidak's tests of ILPs between treatments showed no significant differences for the last three S/A sessions (10.38 ± 3.10 and 24.38 ± 4.94, $p = 0.20$) and for the last three sessions of extinction-1 (6.50 ± 1.85 and 5.63 ± 1.72, $p > 0.99$), but a significant difference between the first extinction session (20.13 ± 7.34 and 40.63 ± 8.87, $p < 0.05$). The Sidak's tests of ILPs between sessions of prospective vehicle group showed no significant differences between the last three S/A sessions and the first extinction session (10.38 ± 3.10 and 20.13 ± 7.34, $p = 0.39$), the last three S/A sessions and the last three sessions of extinction-1 (10.38 ± 3.10 and 6.50 ± 1.85, $p = 0.92$), and the first extinction session and the last three sessions of extinction-1 (20.13 ± 7.34 and 6.50 ± 1.85, $p = 0.14$). The Sidak's tests of ILPs between sessions of prospective ketamine group showed no significant difference between the last three S/A sessions and the first extinction session (24.38 ± 4.94 and 40.63 ± 8.87 , $p = 0.06$) but significant differences between the last three S/A sessions and the last three sessions of extinction-1 (24.38 ± 4.94 and 5.63 ± 1.72, $p < 0.05$), and the first extinction session and the last three sessions of extinction-1 (40.63 ± 8.87 and 5.63 ± 1.72, $p < 0.0001$).

Supplementary material 5

The Sidak's tests of ALPs between sessions of vehicle group showed significant differences between the last three sessions of extinction-1 and the retrieval session (19.21 ± 4.85 and 40.88 ± 9.27, $p < 0.01$) and between the retrieval and the last three sessions of extinction-2 (40.88 ± 9.27 and 11.96 ± 3.76, $p < 0.001$) but not between the last three sessions of extinction-1 and last three sessions of extinction-2 (19.21 ± 4.85 and 11.96 ± 3.76, $p = 0.56$). The Sidak's tests of ALPs between sessions of ketamine group showed significant differences between the last three sessions of extinction-1 and the retrieval session (23.04 ± 3.71 and 45.38 ± 5.85, $p < 0.01$) and between the retrieval and the last three sessions of extinction-2 (45.38 ± 5.85 and 14.58 ± 4.46, $p < 0.0001$) but not between the last three sessions of extinction-1 and the last three sessions of extinction-2 (23.04 ± 3.71 and 14.58 ± 4.46, $p = 0.43$).

The ANOVA of ILPs during the last three sessions of extinction-1, the retrieval session and the last three sessions of extinction-2 showed a significant main effect of Session [$F(2,28) = 3.46$; $p < 0.05$]

and of interaction Session x Treatment [$F(2,28) = 4.10$; $p < 0.05$], but not of factor Treatment $F(1,14) = 0.26$; $p = 0.62$]. The Sidak's tests of ILPs between treatments showed no significant differences for the last three sessions of extinction-1 (6.50 ± 1.85 and 5.63 ± 1.72, $p = 0.98$), for the retrieval session (3.63 ± 1.43 and 8.75 ± 2.89, $p = 0.13$) and for the last three sessions of extinction-2 (3.88 ± 1.25 and 2.75 ± 0.69, $p = 0.96$).

The Sidak's tests of ILPs between sessions of prospective vehicle group showed no significant differences between the last three sessions of extinction-1 and the retrieval session (6.50 ± 1.85 and 3.63 ± 1.43, $p = 0.30$), the last three sessions of extinction-1 and the last three sessions of extinction-2 (6.50 ± 1.85 and 3.88 ± 1.25, $p = 0.37$), and the retrieval session and the last three sessions of extinction-2 (3.63 ± 1.43 and 3.88 ± 1.25, $p > 0.99$). The Sidak's tests of ILPs between sessions of prospective ketamine group showed no significant difference between the last three sessions of extinction-1 and retrieval session (5.63 ± 1.72 and 8.75 ± 2.89 , $p = 0.23$), the last three sessions of extinction-1 and the last three sessions of extinction-2 (5.63 ± 1.72 and 2.75 ± 0.69, $p = 0.30$), but a significant difference between the retrieval session and the last three sessions of extinction-2 (8.75 ± 2.89 and 2.75 ± 0.69, $p < 0.01$).

Supplementary material 6

The treatment with ketamine before retrieval session did not impair the responding on the active lever during test compared to vehicle. The ANOVA of ILPs during the last three sessions of extinction-2 and the test showed no significant effect of factor Session [$F(1,14) = 1.67$; $p = 0.22$], nor of Treatment $F(1,14) = 0.24$; $p = 0.63$] and nor of interaction Session x Treatment [$F(1,14) = 2.70$; $p = 0.12$]. The Sidak's tests between treatments showed no significant differences for the last three sessions of extinction-2 (3.88 ± 1.25 and 2.75 ± 0.69, $p = 0.76$) and for the test (3.50 ± 0.80 and 5.88 ± 1.70, $p = 0.30$). The Sidak's tests between sessions showed no significant differences between the last three sessions of extinction-2 and the test for vehicle (3.88 ± 1.25 and 3.50 ± 0.80; $p = 0.96$) nor for ketamine group (2.75 ± 0.69 and 5.88 ± 1.70; $p = 0.11$).