Piva et al., The metaplastic effects of NMDA receptors blockade on reactivation of instrumental memories in rats

Highlights (max 85 with spaces)

- MK-801 given 24 h before instrumental memory retrieval affected reconsolidation
- Changes of Zif268 and rpS6P in key brain areas indicated reconsolidation inhibition
- MK-801 given 24 h before also increased glutamate receptors in a metaplastic fashion
- 'Metaplastic" MK-801 might have facilitated extinction than inhibited reconsolidation

The metaplastic effects of NMDA receptors blockade on reactivation of instrumental memories in rats

Piva Alessandro[a], Gerace Elisabetta[b,1], Di Chio Marzia[a], Osanni Lisa[a], Padovani Laura[a], Caffino Lucia[c], Fumagalli Fabio[c], Pellegrini-Giampietro Domenico E[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 Health Sciences, University of Firenze, Piazza di San Marco 4, 50121, Firenze, Italy;
[c] Department of Pharmacological and Biomolecular Sciences, University of Milano Via Balzaretti 9, 20133, Milano, Italy;
[1] present address: Department of Neurosciences, Psychology, Drug Research and Child Health, University of Firenze, Piazza di San Marco 4, 50121, Firenze; Italy

Corresponding author:
Chiamulera Cristiano, PharmD, M.Sc.
Sezione Farmacologia, Policlinico GB Rossi, P.le Scuro 10, 37134 Verona, Italy.
E-mail: cristiano.chiamulera@univr.it
Phone: +39 0458027277

Author disclosure:
Authors declare no conflict of interest. All authors have approved the final version of the article.

Abbreviations

Akt: protein kinase B
ALP: active lever presses
AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
Amy: amygdala
ANOVA: analysis of variance
BLA: basolateral complex of amygdala
CeA: central nucleus of amygdala
Cx: context
DAB: 3,3-diaminobenzidine
ERK1/2: extracellular signal-regulated kinases 1/2
FR1: fixed ratio 1 schedule
HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HS: horse serum
i.p.: intraperitoneal
LTP: long-term potentiation
mTOR: mammalian Target Of Rapamycin
NAc: nucleus accumbens
NAcS: nucleus accumbens shell
NMDAR: N-methyl-D-aspartate receptor
No-Ret: no-retrieval
PBS: phosphate-buffered saline
PFA: paraformaldehyde
PMSF: phenylmethylsulfonyl fluoride
Ret: retrieval
rpS6P: phosphorylated ribosomal protein S6
S/A: self-administration
SEM: standard error of mean
TBS: tris-buffered saline
TIF: Triton X-100 insoluble fraction
TO: time-out
TSF: Triton X-100 soluble fraction
Zif268: zinc finger protein 268

Abstract

Metaplasticity, defined as the plasticity of synaptic plasticity, could affect learning and memory at different neural levels. It was hypothesized that metaplasticity changes on glutamate receptors may affect memory destabilization, promoting or preventing reconsolidation. We investigated the metaplastic effect of NMDA channel blocker MK-801 on sucrose instrumental memory reconsolidation in a behavioural rat model associated to the assessment of molecular markers of metaplasticity, memory retrieval, destabilization and reconsolidation.

Following instrumental conditioning and forced abstinence, rats were intraperitoneally treated with MK-801 or vehicle 24 h before memory retrieval, or not Separate groups were tested for in-vivo extinction of responding (24 h and 7 d after reactivation) or ex-vivo assessment of transcription factor Zif268 and ribosomal protein rpS6 phosphorylation in nucleus accumbens (NAc) and amygdala (Amy).

MK-801 significantly inhibited instrumental responding at extinction test, suggesting reconsolidation blockade of instrumental memory. The decrease of Zif268 and phosphorylated-rpS6 levels in NAc and Amy in MK-801/Retrieval vs. Vehicle/Retrieval group supported the behavioural findings. An increase of GluN2B, GluA1 and mGluR5 in NAc, and GluN2B in Amy, 24 h after MK-801 indicated the trigger of associated metaplastic changes.

Our findings show that metaplastic changes induced by NMDA receptors blockade affected sucrose instrumental memory retrieval as shown by both behavioural and molecular changes. We hypothesize that these findings however suggested a switch to extinction rather than a reconsolidation Inhibition.

Keywords

reconsolidation; extinction; instrumental learning; metaplasticity; MK-801; rat.

1. Introduction

The reactivation of previously consolidated memories may trigger a process of destabilization and lability of the memory trace (Nader, Schafe, & Le Doux, 2000; Przybyslawski & Sara, 1997). The retrieval of the memory is thus followed by a process of restabilization (reconsolidation), which could take place during a temporal period ranging between 1 to 6 hours. Inhibitory manipulations applied within this time window (the 'reconsolidation window') may block the reconsolidation of traumatic and appetitive memories, and the return of memory expression assessed under different conditions (Bouton, 1993). It has been proposed that reconsolidation inhibition may be a potential intervention for maladaptive memory disorders such as post-traumatic stress and substance use disorders (Chiamulera, Hinnenthal, Auber, & Cibin, 2014; Dunbar & Taylor, 2016; Milton & Everitt, 2010).

It was apparent since early studies that reconsolidation takes place only within specific 'boundary conditions' depending on memory features (e.g., age, strength) and on reactivation conditions (e.g., context, schedule and duration of reactivation) (Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013; Lee, 2009; Lee, Nader, & Schiller, 2017; Reichelt and Lee, 2013). It was shown that some mechanisms activated by some reactivation protocols might shift memory to a reconsolidation-resistant state, whereas different protocols may facilitate processes of memory destabilization thus allowing reconsolidation occurrence (Nader & Einarsson, 2010; Nader & Hardt, 2009). Finnie and Nader (2012) proposed that molecular events able to modify the synaptic connection that encode a memory trace may change the process of memory reactivation and the trigger of subsequent reconsolidation via an effect defined as 'metaplasticity', i.e., the plasticity of synaptic plasticity (Abraham & Bear, 1996). Metaplasticity could occur after manipulations at different levels, from synapse to neuronal network, from systems to behaviour (Hulme, Jones, & Abraham, 2013). Behavioural metaplasticity could be expressed as changes in learning and memory mediated by modification of synaptic plasticity, neural assembly connectivity etc. (Richter-Levin & Maroun, 2010; Schmidt, Abraham, Maroun, Stork, & Richter-Levin, 2013). Thus, Finnie and Nader (2012) proposed that metaplasticity events might affect memory trace stabilization by inhibiting or facilitating memory destabilization at reactivation. They suggested that some specific molecular 'metaplastic' events occurring before reactivation might change "*the types of behavioural experience necessary*" for memory reactivation. For instance, down-regulation

of glutamate receptor subtype GluN2B in amygdala (Amy) inhibited memory destabilization and yield to resistance to reconsolidation (Wang, de Oliveira Alvares, & Nader, 2009).

Based on these hypotheses, we aimed to explore whether N-methyl-D-aspartate (NMDA) receptors channel blocker MK-801 was able to induce metaplastic changes affecting subsequent destabilization, reactivation and reconsolidation of appetitive memory in rats. NMDA receptor antagonists such as MK-801 and ketamine have been shown to induce changes in long-term potentiation (LTP) in a metaplastic fashion: both substances were able to enhance tetanus-induced LTP in ex-vivo hippocampal slices 24 hours after treatment (Buck, Cali, & Behr, 2006; Burgdorf et al., 2013; Graef et al., 2015; Izumi & Zorumski, 2014; for a review see Zorumski & Izumi, 2012). Therefore, the aims of the current study were, i), to investigate the behavioural effects of MK-801 on instrumental memory reconsolidation when given 24 hours before memory retrieval, and ii), to correlate these effects with molecular markers of metaplasticity and of memory destabilization, retrieval and reconsolidation. Rats trained to sucrose self-administration for 10 days (Conditioning), and then exposed to a 14-day forced abstinence period, were treated with MK-801 or Vehicle on the last day of abstinence; the dose of MK-801 used in the present study, i.e. 4 mg/kg/mL, is the same dose that Buck and colleagues showed to induce facilitation of LTP in ex-vivo CA1-subiculum synapses of rats hippocampus 24 hours after dosing (Buck et al., 2006). Twenty-four hours after acute treatment, rats were re-exposed to a short instrumental memory reactivation session (Retrieval; Piva et al., under revision; Tedesco, Mutti, Auber, & Chiamulera, 2014). Behavioural test for reinstatement of lever presses was performed twenty-four hours after Retrieval. Different groups of animals were sacrificed two hours after Retrieval (or No-Retrieval control) for the assessment of GluN2B (Wang et al., 2009), GluA1 (Monfils, Cowansage, Klann, & LeDoux, 2009), mGluR5 (Bortolotto et al., 2005), zinc finger protein 268 (Zif268; Lee, Di Ciano, Thomas, & Everitt, 2005; Lee, Milton, & Everitt, 2006; Theberge, Milton, Belin, Lee, & Everitt, 2010; Thomas, Arroyo, & Everitt, 2003), phosphorylated ribosomal protein S6 (rpS6P; Barak et al., 2013; Biever, Valjent, & Puighermanal, 2015; Tedesco, Roquet, DeMis, Chiamulera, & Monfils, 2014) in nucleus accumbens (NAc), central nucleus (CeA) and basolateral complex (BLA) of Amy as molecular correlates of MK-801 effects on metaplasticity and on reconsolidation of appetitive memory.

2. MATERIAL AND METHODS

2.1 Animals

Seventy-seven 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), and food (two to four pellets, 10-20 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 (Coulbourn Instruments, Lehigh Valley, Whitehall, PA, USA) encased in sound-insulated cubicles equipped with ventilation fans (Ugo Basile, Comerio, Italy). Each chamber was equipped with two levers, an active (right) and an inactive lever (left), symmetrically oriented laterally to the food magazine, on the frontal panel. Levels 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 food magazine and provided ambient illumination during the entire session duration of food-shaping, retrieval and test phases, and for the entire session except for time-out (TO) periods during training phase. Right lever press produced the delivery of a 45-mg sucrose food pellet (Bilaney Consultants Ltd, UK) with a Fixed-ratio 1 (FR1) schedule of reinforcement during training. Left lever presses did not have consequences. Lever presses and pellet deliveries were recorded, as well schedule parameters and data acquisition were controlled, by Med-PC software (Med Associates Inc., St Albans, Vermont, USA).

2.3 General Procedure

A schematic diagram of the protocol design is shown in Figure 1, panel A. The experimental protocols were designed according to the procedure used in our laboratory to demonstrated nicotine instrumental memory reconsolidation by Tedesco et al (Tedesco et al., 2014): Phase I, training to sucrose pellets self-administration (S/A); Phase II, forced abstinence in home cage with MK-801 or Vehicle treatment 24 h before Phase III; and Phase III, memory Retrieval (Ret) or No-Retrieval (No-Ret) in the training context. After the three phases, rats were divided in two groups: one group performed an Extinction test 24 h after and a

Spontaneous Recovery test 7 days after Phase III in the training context, while the animals in the second group were sacrificed for immunohistochemical staining 2 h after Phase III.
Two separate groups of rats were treated with MK-801 or Vehicle and sacrificed 24 h later for immunoblotting assays.

2.4 Lever press shaping and training to sucrose self-administration procedures

All rats were initially shaped to associate right lever presses with sucrose pellets as reinforcement. 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, animals started training Phase I. During Phase I, right lever pressing delivered 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 consequences, and the house light switched OFF. Left lever presses had no consequences for all the experimental sessions. After 10 continuous days of sucrose S/A training, rats remained 14 days in home cage for forced abstinence phase.

2.5 Behavioural analysis: Retrieval, Extinction and Spontaneous Recovery tests.

During the last day of forced abstinence, 24 h before Phase III, rats were divided in two groups, one treated with intraperitoneal (i.p.) saline solution (Vehicle) 1 mL/kg and one treated with i.p. MK-801 4 mg/kg/mL (Buck et al., 2006). The day after, both groups were further divided into subgroups exposed to Ret or No-Ret session in the training context. The four resulting subgroups were: Vehicle/Ret, Vehicle/No-Ret, MK-801/Ret and MK-801/No-Ret.
During the Ret session, both levers were presented, and rats were allowed to press right active lever up to 20 times, with house light ON; levers had no consequences during Ret. During the No-Ret session, no levers were presented and house light was OFF. During Ret or No-Ret session, animals spent a similar amount of time in the training context (185 ± 10 s; mean ± SEM). Twenty-four hours after Ret or No-Ret session, all subjects were re-exposed for 60 min to the training context in the presence of levers, house light ON and no TO to test sucrose-seeking behaviour with an Extinction test. The same re-exposure was repeated 7 days after Ret/No-Ret session to test the Spontaneous Recovery of memory. Levers had no consequences during Extinction and Spontaneous Recovery. All lever presses were recorded during Ret, Extinction and Spontaneous Recovery.

2.6 Molecular analysis: Retrieval procedure and brain extraction

For the molecular analysis, four separated groups of rats were treated with Vehicle or MK-801 and exposed to Ret or No-Ret session as described in the previous section. Then, 2 h after the first lever emitted during Ret or 2 h after the beginning of No-Ret session, all rats were sacrificed for Zif268 or rpS6P immunohistochemistry.

2.7 Immunohistochemistry

Rats were anesthetized with 350 mg/kg/2 mL i.p. chloral hydrate (Fluka, Italy), then transcardially perfused with heparin 100 UI/L (Sigma–Aldrich, Milan, Italy) in saline solution and paraformaldehyde (PFA) 4 % in 1X phosphate buffered saline solution (PBS). Brains were removed and post-fixed for 2 h at 4 °C into PFA 4 % in PBS, then washed 3 times with PBS and left in sucrose 30 % in PBS for cryoprotection for 48 h. Free-floating sections (40 µm) containing nucleus accumbens shell (NacS) (corresponding to a bregma +1.70 mm section from Paxinos & Watson, 1998); CeA (bregma -1.88) and BLA (bregma -3.00 mm) were processed for Zif268 or rpS6P immunoreactivity. After washing in PBS, endogenous peroxidase was neutralized with hydrogen peroxide 0.75 % in PBS for 10 min. Sections were blocked with 0.5 % Horse Serum (HS; BioWhittaker-Lonza, Basel, Switzerland) + 0.5 % Triton X-100 (Sigma-Aldrich, Milan, Italy) in PBS wash solution, and then incubated overnight at 4 °C with anti-Zif268 (1:1000, Santa Cruz, rabbit polyclonal) or anti-PSer235/236-rpS6 antibody (1:1000, Cell Signaling, rabbit polyclonal) in wash solution. After washes in wash solution, slices were incubated for 2 h at room temperature with anti-rabbit biotinylated antibody (1:1000, Amersham GE Healthcare Europe, Milan, Italy).

Following washes in wash solution, and finally in PBS, tissue sections were visualized using VectaStain ABC kit (Vector Laboratories, Rome, Italy) and developed in DAB peroxidase substrate (Sigma-Aldrich, Milan, Italy) for 3-4 min. Sections were mounted on gelatin-coated slides, dehydrated and then closed with Entellan (Merck-Millipore, Darmstadt, Germany). The sections were acquired using a light transmission microscope (Axioscope 2 Zeiss, Zeiss). Six images for each region (one for each hemisphere, 3 sections for each rat, that is 2 × 3 = 6 images/region/rat) were acquired by the connected video camera (Optikam B3) using a 10X objective (0.3 mm^2). Counts of the number of neurons positive to Zif268 and rpS6P were carried out using the NIH software 'Image-J' (www.rsbweb.nih.gov) (Caffino et al., 2016).

2.8 Pharmacological effects and Western Blot Assays.

To elucidate the metaplastic effects of MK-801 on the level of glutamate receptors, two separated groups of 5 rats/group were treated with MK-801 4 mg/kg/mL i.p. or Vehicle 1

mL/kg i.p. and 24 h later were anesthetized with 350 mg/kg/2 mL i.p. chloral hydrate (Fluka, Italy) and sacrificed. Then, brains were removed and 1-mm fresh tissue slices containing nuclei accumbens (+1.70 mm) 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 (Caffino, et al., 2017) with minor modifications. Briefly, nuclei accumbens 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 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, Santa Cruz Biotechnology, USA), anti-GluA1 (1:1000, Neuromab, 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).

2.9 Data Analysis

For the behavioural experiment, the number of active lever presses (ALP) after 60-min Extinction test from the four groups Veh/No-Ret, MK-801/No-Ret, Veh/Ret, MK-801/Ret was analysed as dependent variable to assess the effect of drug treatment on retrieval condition.

Two-way analysis of variance (ANOVA) with factors Treatment (Vehicle, MK-801) and Retrieval (Ret, No-Ret) was carried out on the total number of ALP after the Extinction test. After Grubbs' test to identify outliers, one subject for each of Veh/Ret, Veh/No-Ret and MK-801/Ret group were excluded from data analysis.

For the immunohistochemistry experiments, intensity threshold, minimum and maximum cell size values were initially determined in an empirical fashion under blind conditions. The dependent variable for the immunohistochemistry experiments was the positive cell count/mm^2 for Zif268 or rpS6P. Two-way analyses of variance (ANOVAs) with the factors Treatment and Retrieval were carried out on mean ± SEM percentages of Zif268-positive cells/mm^2 and rpS6P-positive cells/mm^2 comparing the four different groups, with Vehicle/No-Ret as control group. For the western blots assays, mean ± SEM percentages of Vehicle group were analysed by an unpaired Student's t-test. All analyses were performed using the GraphPad software package (Prism, version 4; GraphPad, San Diego, California, USA).

3. Results

Two-way ANOVA on extinction test showed a significant main effect of factor Treatment [($F(1, 40) = 5.9$; $p < 0.05$] but not of Retrieval [$F(1, 40) = 0.1$; NS] and of Treatment x Retrieval interaction [$F(1, 40) = 1.6$; NS]. Comparing ALP/60 mins between the different groups, Fisher's LSD post-hoc tests showed no significant difference between Veh/No-Ret and Veh/Ret (94.3 ± 9.3 vs. 103.9 ± 11.1; NS), between MK-801/No-Ret vs MK-801/Ret (82.2 ± 11.8 vs. 64.6 ± 8.3; NS), and between Veh/No-Ret and MK-801/No-Ret (94.3 ± 9.3 vs. 82.2 ± 11.8; NS) but a significant decrease of active lever presses was observed for MK-801/Ret compared to Veh/Ret group (64.6 ± 8.3 vs. 103.9 ± 11.1; $p < 0.05$) (Fig. 1B). Two-way ANOVA on 7 days spontaneous recovery test showed no significant effect of factor Treatment [($F(1, 40) = 0.8$; NS], of Retrieval [$F(1, 40) = 0.3$; NS] and of Treatment x Retrieval interaction [$F(1, 40) = 0.01$; NS]. Comparing ALP/60 mins between the different groups, Fisher's LSD post-hoc tests showed no significant difference between Veh/No-Ret and Veh/Ret (37.9 ± 3.1 vs. 42.9 ± 5.3; NS), between MK-801/No-Ret vs MK-801/Ret (45.1 ± 8.2 vs. 48.5 ± 10.1; NS), between Veh/No-Ret and MK-801/No-Ret (37.9 ± 3.1 vs. 45.1 ± 8.2; NS) and between Veh/Ret and MK-801/Ret (42.9 ± 5.3 vs. 48.5 ± 10.1; NS) (Fig. 1C).

Fig. 1

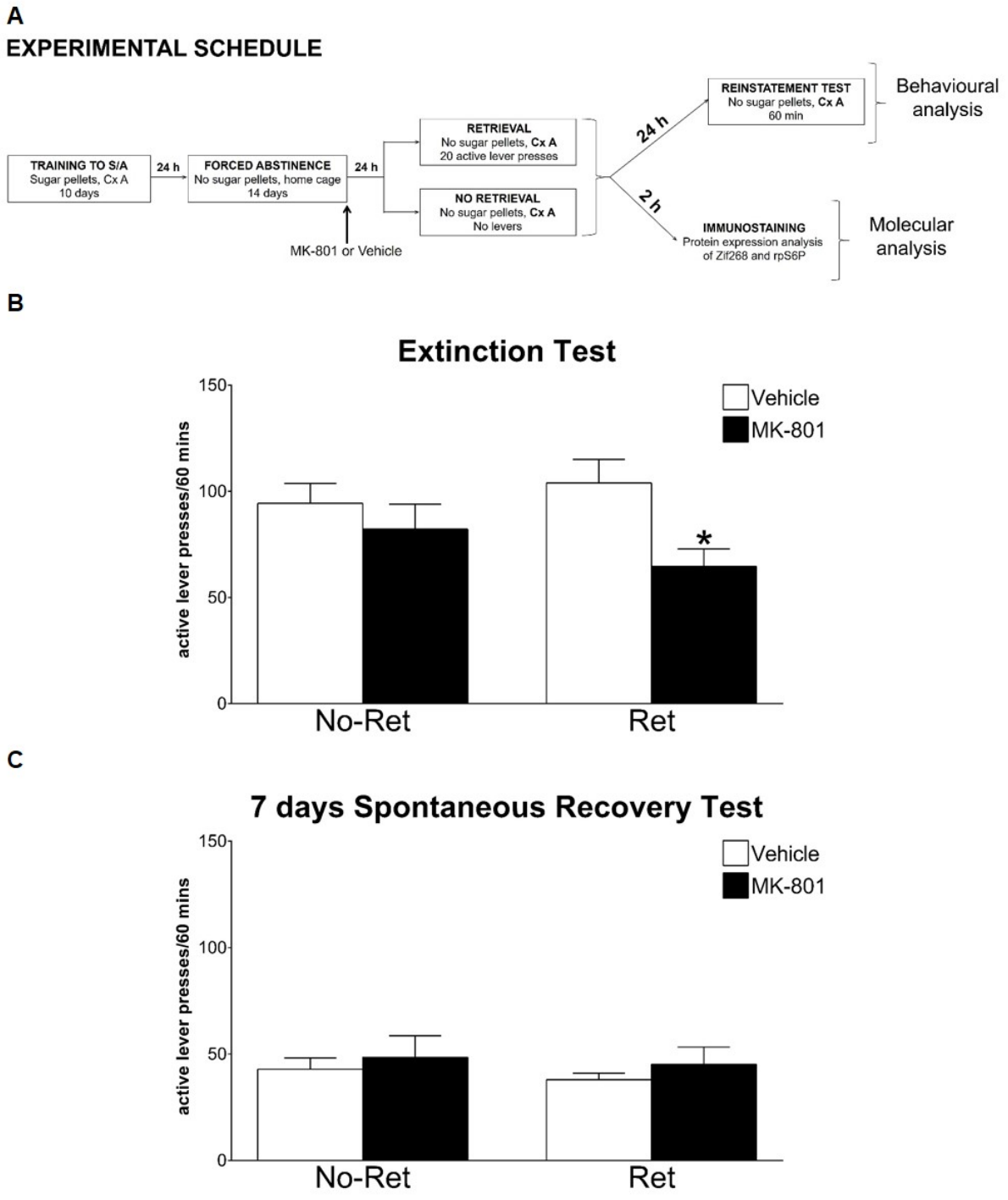

Figure 1. (**A**) Schematic diagram of the experimental protocol and groups. Boxes represent the different procedures used at the different phases of the study. Arrow represents time progression between consecutive phases. Cx A = sucrose self-administration training (conditioning) context. (**B**) Effect of Veh (open columns) or MK-801 (solid columns) treatment on Extinction test performance when given 24 h before Retrieval (Ret) or No-Retrieval (No-

Ret). (C) Effect of Veh (open columns) or MK-801 (solid columns) treatment given 24 h before Ret/No-Ret on Spontaneous Recovery test performed 7 days after Ret/No-Ret. Ordinate represents number of lever presses after behavioural tests. Data are expressed as mean + SEM. N = 9-12 rats/Veh groups, N=10-13 rats/MK-801 groups. *$p < 0.05$, Fisher's LSD post-hoc tests.

The analysis of the expression of Zif268 in the NAcS showed a significant main effect of factor Retrieval [two-way ANOVA, ($F(1, 16) = 14.1$; $p < 0.01$] and of Treatment x Retrieval interaction [$F(1, 16) = 35.7$; $p < 0.0001$] but not of Treatment [$F(1, 16) = 0.6$; NS]. Comparing the different groups, Tukey's post-hoc tests showed a significant percentual increase of Zif268 expression in the MK-801/No-Ret compared to Veh/No-Ret (+37.1 ± 10.0; $p < 0.01$), a significant decrease for MK-801/Ret compared to Veh/Ret (-48.2 ± 10.2; $p < 0.01$) and a significant increase for Veh/Ret compared to Veh/No-Ret (+69.4 ± 10.6; $p < 0.0001$). No significant difference was observed between MK-801/No-Ret and MK-801/Ret (+15.9 ± 9.6; NS) (Fig. 2C).

In the CeA, the analysis of Zif268 expression showed a significant main effect of factor Treatment [$F(1, 16) = 20.3$; $p < 0.001$] and of Treatment x Retrieval interaction [$F(1, 16) = 96.6$; $p < 0.0001$] but not of Retrieval [$F(1, 16) = 0.3$; NS]. Comparing the groups, Tukey's post-hoc tests showed a significant increase of Zif268 in MK-801/No-Ret compared to Veh/No-Ret (+85.4 ± 8.3; $p < 0.0001$) and a significant decrease of MK-801/Ret compared to Veh/Ret (-31.7 ± 8.5; $p < 0.01$). Moreover, Veh/Ret was significantly increased compared to Veh/No-Ret (+61.9 ± 8.8; $p < 0.0001$) and MK-801/Ret was significantly decreased compared to MK-801/No-Ret (-55.1 ± 8.0; $p < 0.0001$) (Fig. 2E). Similarly in the BLA, two-way ANOVA test showed a significant main effect of Treatment x Retrieval interaction [$F(1, 16) = 90.0$; $p < 0.0001$] but not of factor Treatment [$F(1, 16) = 0.8$; NS] nor of Retrieval [$F(1, 16) = 1.6$; NS]. Comparing the groups, Tukey's post-hoc tests showed a significant increase of Zif268 in the MK-801/No-Ret compared to Veh/No-Ret (+90.4 ± 12.9; $p < 0.0001$), and a significant decrease for MK-801/Ret compared to Veh/Ret (-74.9 ± 11.7; $p < 0.0001$). Moreover, Veh/Ret was significantly increased compared to Veh/No-Ret (+71.7 ± 13.0; $p < 0.001$) MK-801/Ret was significantly decreased compared to MK-801/No-Ret (-93.5 ± 11.6; $p < 0.0001$) (Fig.2G).

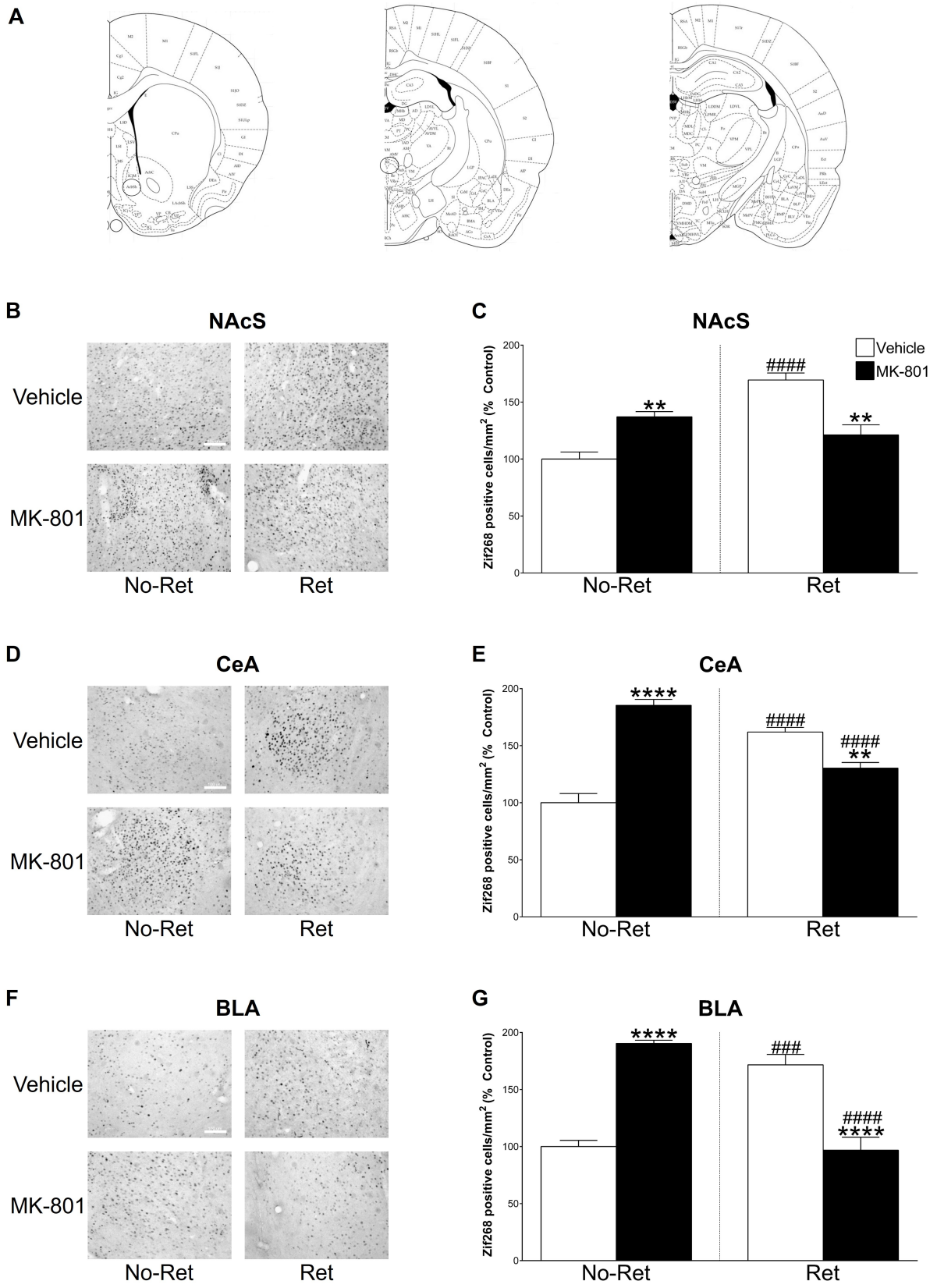

Figure 2. Immunohistochemistry assessment of Zif268 expression 24 h after Vehicle or MK-801 treatment, and 2 h after Ret or No-Ret session. (**A**) Representative images of brain

areas of interest, with circles indicating the microscopic frame of the region under analysis. (**B, D, F)** Representative images of microscope sections of nucleus accumbens shell (NAcS, **B**), central nucleus of the amygdala (CeA, **D**) and basolateral complex of the amygdala (BLA, **F**) 24 h after Vehicle (open columns) or MK-801 (solid columns) and 2 h after No-Retrieval (No-Ret) or Retrieval (Ret) session. Zeiss Axioskop 2, objective 10X. Scale bar, 100 µm. (**C, E, G**) Number of Zif268 positive cells/mm^2 in NAcS, CeA and BLA 24 h after Vehicle or MK-801 and 2 h after No-Ret or Ret. Data are shown as mean + SEM percentual values of Vehicle/No-Ret. Three adjacent sections, both hemispheres, N = 4 - 6 rats/group. **$p < 0.01$; ****$p < 0.0001$ between treatments (same Ret or No-Ret), $^{###}p < 0.001$; $^{####}p < 0.0001$ vs. No-Ret within treatment, two-way ANOVA followed by Tukey's post-hoc tests.

To confirm the occurrence of memory reconsolidation process we assessed the phosphorylation level of protein translation marker rpS6 in the same areas. For the level of rpS6P in the NAcS, two-way ANOVA showed a significant main effect of Treatment x Retrieval interaction [F (1, 16) = 47.9; $p < 0.0001$] but not of factor Treatment [F (1, 16) = 0.01; NS] nor for Retrieval [(F (1, 16) = 1.4; NS]. Comparing the different conditions, Tukey's post-hoc tests showed a significant increase of rpS6P level in the MK-801/No-Ret compared to Veh/No-Ret (+31.3 ± 6.2; $p < 0.001$) and a significant decrease of MK-801/Ret compared to Veh/Ret (-30.3 ± 6.4; $p < 0.01$). Moreover, Veh/Ret was significantly increased compared to Veh/No-Ret (+25.5 ± 6.6; $p < 0.01$) and MK-801/Ret was significantly decreased compared to MK-801/No-Ret (-36.13 ± 6.0; $p < 0.0001$) (Fig. 3C).

In the CeA, two-way ANOVA showed a significant main effect of factor Retrieval [F (1, 16) = 29.1; $p < 0.0001$] and of Treatment x Retrieval interaction [F (1, 16) = 50.0; $p < 0.0001$] but not of Treatment [F (1, 16) = 2.9; NS]. Tukey's post-hoc tests showed a significant increase of rpS6P level in the MK-801/No-Ret compared to Veh/No-Ret (+48.4 ± 7.7; $p < 0.0001$), a significant decrease in the MK-801/Ret compared to Veh/Ret (-29.6 ± 7.9; $p < 0.01$) and a significant increase in the Veh/Ret compared to Veh/No-Ret (+68.7 ± 8.2; $p < 0.0001$). No significant difference was observed between MK-801/No-Ret and MK-801/Ret (+9.2 ± 7.4; NS) (Fig.3E). In BLA, two-way ANOVA showed no significant main effect of Treatment factor [F (1, 16) = 0.5; NS], of Retrieval [F (1, 16) = 0.2; NS] and of Treatment x Retrieval interaction [F (1, 16) = 1.3; NS]. Tukey's post-hoc tests showed no significant differences among the four different experimental groups: MK-801/No-Ret vs. Veh/No-Ret (+16.1 ± 12.2; NS), MK-801/Ret vs. Veh/Ret (-3.7 ± 12.5; NS), Veh/No-Ret vs. Veh/Ret (-13.7 ± 12.9; NS), MK-801/No-Ret vs. MK-801/Ret (+6.0 ± 11.7; NS) (Fig. 3G)

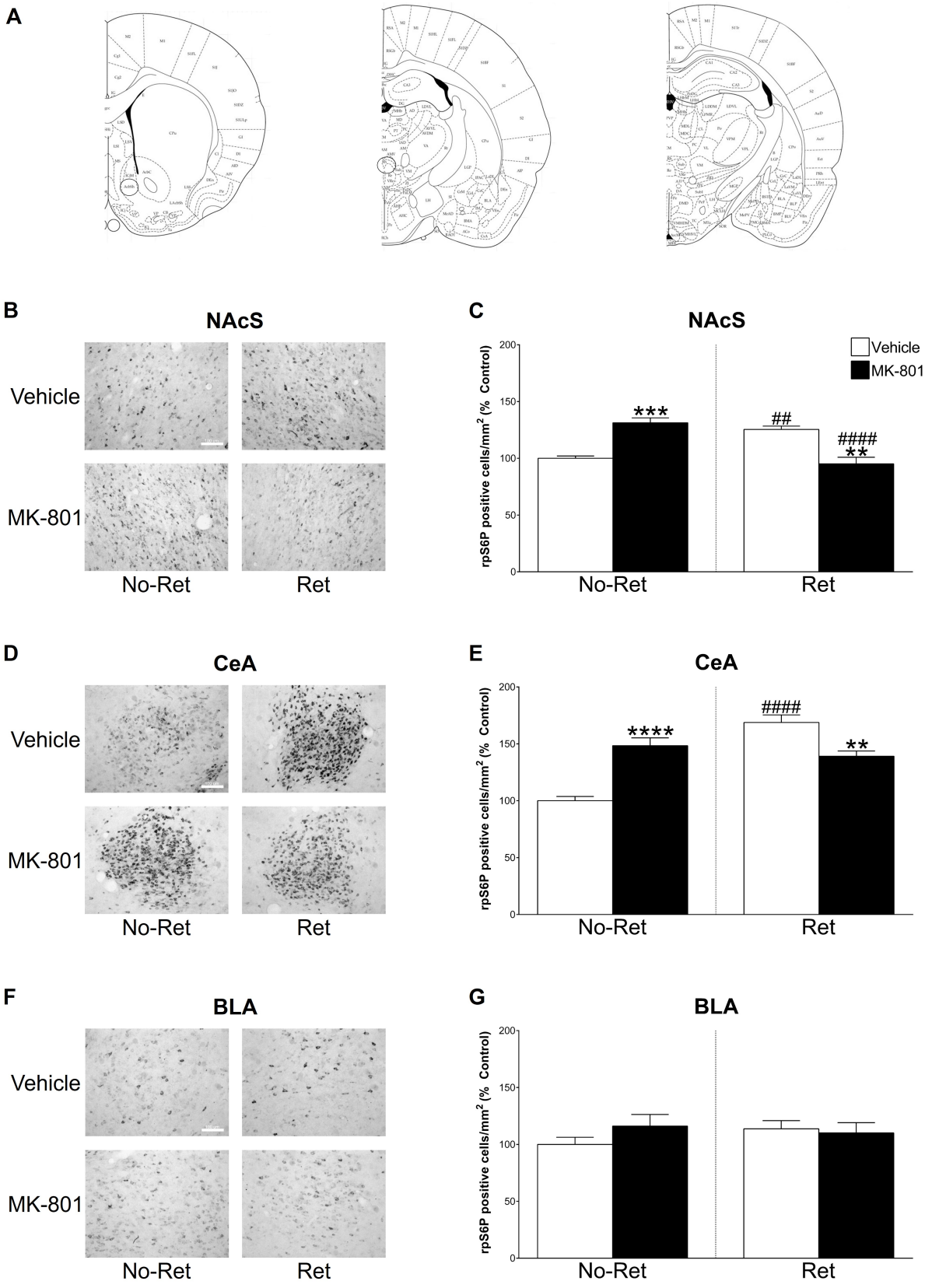

Figure 3. Immunohistochemistry assessment of rpS6P level 24 h after Vehicle or MK-801 treatment and 2 h after Ret or No-Ret session. (**A**) Representative images of brain areas of

interest, with circles indicating the microscopic frame of the region under analysis. (**B, D, F**) Representative images of microscope sections of nucleus accumbens shell (NAcS, **B**), central nucleus of the amygdala (CeA, **D**) and basolateral complex of the amygdala (BLA, **F**) 24 h after Vehicle (open columns) or MK-801 (solid columns) and 2 h after No-Retrieval (No-Ret) or Retrieval (Ret) session. Zeiss Axioskop 2, objective 10X. Scale bar, 100 μm. (**C, E, G**) Number of rpS6P positive cells/mm^2 in NAcS, CeA and BLA 24 h after Vehicle or MK-801 and 2 h after No-Ret or Ret. Data are shown as mean + SEM and are expressed as a percentual values of Vehicle/No-Ret. Three adjacent sections, both hemispheres, N = 4 - 6 rats/group. $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$ between treatments (same Ret or No-Ret), $^{\#\#}p < 0.01$; $^{\#\#\#\#}p < 0.0001$ vs. No-Ret within treatment, two-way ANOVA followed by Tukey's post-hoc tests.

Western blot assays 24 h after MK-801 or Veh treatment in the post-synaptic density of NAc, the level of GluN2B and GluA1 after MK-801 was significantly increased compared to Veh (respectively +31.8 ± 4.9 and +47.4 ± 7.3; $p < 0.001$, Student's t-test). Moreover, the analysis of the extra-synaptic fraction showed that MK-801 significantly increased mGluR5 level compared to Veh (+47.1 ± 7.8; $p < 0.001$, Student's t-test) (Fig.4B).

In the post-synaptic density of Amy, the level of GluN2B after MK-801 was significantly increased compared to Veh (40.2 ± 6.2; $p < 0.001$, Student's t-test), while the levels of GluA1 in the post-synaptic density and of mGluR5 in the extra-synaptic fraction did not show significant difference compared to Vehicle (respectively +3.8 ± 3.3 and -5.3 ± 9.6; NS, Student t-test) (Fig. 4D).

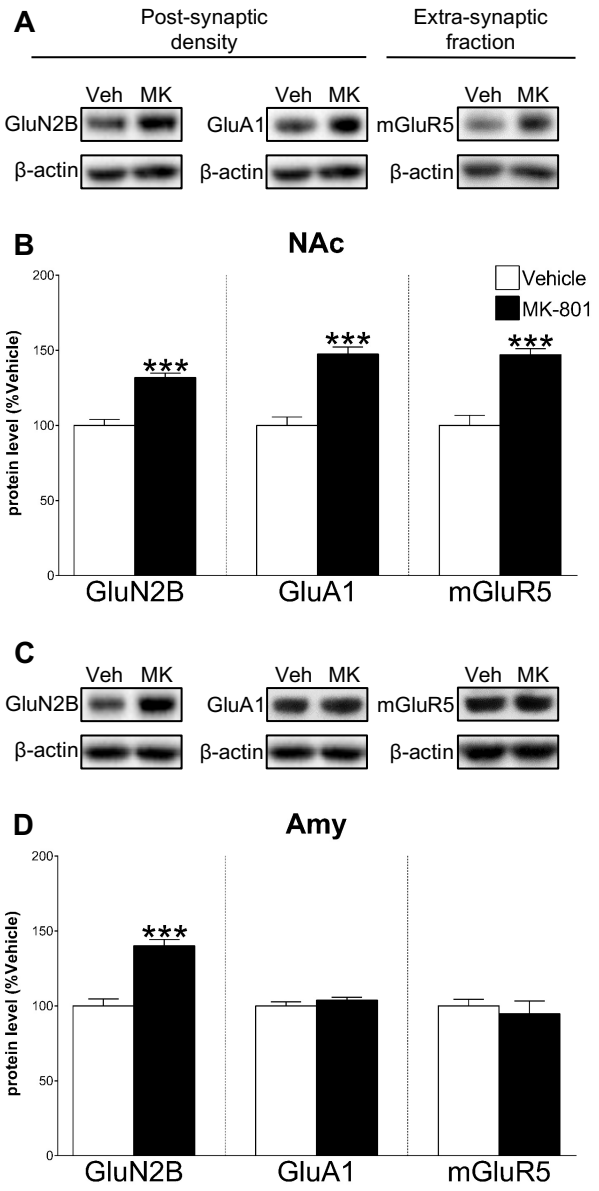

Figure 4. Effect of Vehicle or MK-801 on GluN2B-containing NMDARs and GluA1-containing AMPARs in the post-synaptic density and on mGluR5 in the extra-synaptic fraction of nucleus accumbens and amygdala. (**A**, **C**) representative images of western blot bands with GluN2B (180 kDa, left) GluA1 (108 kDa, middle) and mGluR5 (130 kDa, right) compared to β-actin (43 kDa) as control. (**B**, **D**) quantification of GluN2B-NMDARs and GluA1-AMPARs level in the post-synaptic density and of mGluR5 in the extra-synaptic fraction 24 h after Vehicle or MK-801 treatment in NAc (**B**) and Amy (**D**). Data are shown as the mean + SEM and are expressed as percentage of the Vehicle. N=4-5 rats/group. ***p < 0.001, unpaired Student's t-test.

Table 1. Summary table of the direction of effects of MK-801 or Vehicle treatment on Zif268, rpS6P, GluN2B, GluA1 and mGluR5.

	Nucleus Accumbens Shell		Amygdala	
	Zif268			
	No-Ret	Ret	No-Ret	Ret
Vehicle	0	↑	0	↑
MK-801	↑	↑	↑	↓
	rpS6P			
Vehicle	0	↑	0*	↑*
MK-801	↑	↓	↑*	↑*

	Nucleus Accumbens	Amygdala
	GluN2B	
MK-801 vs Vehicle	↑	↑
	GluA1	
MK-801 vs Vehicle	↑	0
	mGluR5	
MK-801 vs Vehicle	↑	0

Symbols: ↑ = increase; ↓ = decrease; 0 = no change. * = in central nucleus of amygdala only.

4. Discussion

MK-801 given 24 hours prior to the reactivation of instrumental memories (-24h MK-801/Retrieval) significantly inhibited conditioned responding for sucrose at extinction test compared to the Vehicle/Retrieval group. This effect was associated to a decrease of Zif268 in NAcS, CeA and BLA compared to vehicle-induced increase after Retrieval session. However, MK-801 per se increased Zif268 in the same brain areas in No-Retrieval compared to Vehicle/No-Retrieval group, similarly to the effect observed after the Vehicle/Retrieval session. Therefore, the behavioural inhibitory effect observed in the MK-801/Retrieval group could be specifically associated only in amygdala to a significant inhibition of increased Zif268 levels induced by either Vehicle/Retrieval or by MK-801/No-Retrieval. The increased phosphorylation of rpS6P in NAcS and CeA in the Vehicle/Retrieval group was reduced in the MK-801/Retrieval group. However, MK-801 per se also increased phosphorylation of

rpS6 in the same brain areas in the MK-801/No-Retrieval compared to Vehicle/No-Retrieval group. It therefore appears that the MK-801/Retrieval condition (the only one associated to a significant behavioural inhibition at extinction test compared to Vehicle/Retrieval) specifically inhibited increased rpS6 phosphorylation induced by either Vehicle/Retrieval or by MK-801/No-Retrieval only in NAcS.

The behavioural inhibitory effect of -24h MK-801 on instrumental memory responding when given under a dosing protocol known to induce metaplastic effects (Buck et al., 2006; Zorumski & Izumi, 2012) appears to be necessarily associated to the procedure of instrumental memory reactivation. However, even though MK-801/Ret showed lower values vs. MK-801/No-Ret, there is not significant difference between the groups, and it is not possible to confirm a selective MK-801 effect under retrieval. Under our procedure, the No-Ret condition is the same of Ret but for manipulanda availability and lever pressing. We cannot exclude that the convergent effects of MK-801 and Ret (the signal) would have underlying convergent MK-801 and context effects (maybe acting as a noise) involving different mechanisms. For this reason we think that the MK-801/No-Ret vs MK-801/Ret comparison might not be the most appropriate.

The behavioural effect of -24h MK-801 is specifically associated to the inhibition of known markers of retrieval and reconsolidation: Zif268 in BLA and CeA, and rpS6 phosphorylation in NAcS. The former molecular correlation is interestingly linked to -24h MK-801-induced increase of GluN2B synaptic levels in Amy, a proposed metaplasticity marker of memory destabilization (Finnie & Nader, 2012). The effect on rpS6 phosphorylation in NAcS is on the other hand associated to -24h MK-801-induced increase of GluN2B, GluA1 and mGluR5 levels in NAc. These metaplastic molecular changes might have facilitated destabilization, respectively in Amy (GluN2B) and/or in NAc (GluN2B, GluA1, mGluR5), allowed inhibition of memory reconsolidation (but see below an alternative explanation) and then, inhibition of instrumental responding at extinction test.

We hypothesize that the metaplasticity mechanisms triggered by -24h MK-801 might have raised a direction change in the synaptic activation induced by the behavioural manipulation aimed to induce memory reactivation. The question however is: which is the process that after -24h MK-801-primed metaplastic condition and under memory retrieval allowed the inhibition of memory reconsolidation in the MK-801/Retrieval group? Or, as an alternative interpretation, is it possible that a process of extinction took place rather than reconsolidation inhibition? Indeed, it could be speculated that the facilitation of memory destabilization (as supported for instance by the metaplastic increase of GluN2B in Amy) which should allow memory reconsolidation occurrence, it had on the other hand set in turn in the -24h MK-

801/Retrieval group the conditions for a retrieval-contingent process of extinction rather than reconsolidation. The competition between reconsolidation and extinction processes during memory retrieval is a phenomenon that has been previously described for reactivation sessions of intermediate length, that is neither short enough for reconsolidation nor sufficiently long for extinction (Flavell & Lee, 2013; Merlo, Milton, Goozee, Theobald, & Everitt, 2014; see also discussion in Exton-McGuinness & Lee, 2015). The metaplastic synaptic pattern changes induced by -24h MK-801 paired to the Retrieval, but not when paired to the No-Retrieval, condition may have shifted the balance between reconsolidation and extinction by favouring the acquisition of the latter as the predominant process.

The decrease of Zif268 expression levels in Amy in the -24h MK-801/Retrieval group may be interpreted as the possible molecular correlate of such as shift to extinction. The transcription factor Zif268 has been widely used as a marker of memory reactivation and reconsolidation (Besnard, Caboche, & Laroche, 2013; Lee, 2008; Lee, Everitt, & Thomas, 2004). In this study, we have demonstrated that higher expression levels of Zif268 in Amy (Piva et al., under revision; Tedesco, Roquet, et al., 2014), and NAcS (Exton-McGuinness & Lee, 2015; Piva et al., under revision) were correlated to memory reactivation and reconsolidation. Although Zif268 inhibition leads to reconsolidation blockade (Veyrac, Besnard, Caboche, Davis, & Laroche, 2014), however we cannot exclude that decreased Zif268 levels might induced extinction facilitation. Trent et al. (2015) recently showed that the expression of immediate early genes (including Zif268) constrained extinction occurrence during the early phase of contextual memory reactivation; in fact knockdown of Zif268 levels during a short recall favoured extinction occurrence rather than reconsolidation inhibition, whereas during a longer recall, changes in Zif268 had not effect. We could therefore speculate that with our reactivation parameters (similar to a short recall), a decrease of Zif268 levels in Amy in the -24h MK-801/Retrieval group may have induced extinction. Previous studies, including from ours, showed that increased rpS6 phosphorylation is correlated to memory reconsolidation (Barak et al., 2013; Tedesco, Roquet, et al., 2014) and to reinforcing drug effect (Tedesco, Ravagnani, Bertoglio, & Chiamulera, 2013; Zanda et al., 2017). The reduced phosphorylation of rpS6 in NAcS is associated to an increased expression of all three investigated glutamate receptors. Enhanced glutamatergic transmission through glutamate AMPA and NMDA receptors induces rpS6 phosphorylation via different signalling cascades, such as Akt via mTOR, ERK1/2 via p70S6 and p90S6 kinases (Biever, Valjent, Puighermanal, 2015). It could be speculated that a different balancing between signalling cascade predominance brought to reduction of the rpS6 phosphorylation.

An increase of GluN2B has been shown to facilitate destabilization (Ben Mamou, Gamache, & Nader, 2006; Milton et al., 2013; Wang et al., 2009). GluA1 has been shown to be important for memory retrieval (Clem & Huganir, 2010; Monfils et al., 2009) in a way dissociable from its role in destabilization (Milton et al., 2013), suggesting that these two processes may occur in parallel via two separate glutamatergic networks in Amy (Lee & Flavell, 2014; Milton et al., 2013). Although there is a limited literature on Group I metabotropic glutamate receptors and memory reconsolidation (Gieros, Sobczuk, & Salinska, 2012; Salinska, 2006), we have recently proposed that Group I subtype mGluR5 is involved in memory reconsolidation and that mGluR5 antagonism may act as inhibitor based on the role played by mGluR5 in the modulation of glutamatergic transmission (Chiamulera, Marzo, & Balfour, 2017). Moreover, glutamate receptors GluN2B, GluA1 and mGluR5 have been reported to be involved in different forms of metaplasticity (Bortolotto et al., 2005; Tenorio et al., 2010; Yang et al., 2012; for reviews, see Abraham, 2008; Hulme et al., 2013; Marton, Hussain Shuler, & Worley, 2015). In accordance to the suggestions by Finnie & Nader (2012), the increased expression of glutamate receptors after -24h MK-801 may mediate the effects of increased glutamate release that occur when memory is reactivated. GluN2B levels appears to be important both in Amy and NAcS not only for the mediation of metaplasticity and the regulation of memory destabilization, but also as an 'NMDA-dependency' factor that could explain the hypothetical "reconsolidation-to-extinction shift" suggested above, in accordance to the NMDA-dependent modes of transition from reconsolidation to extinction in fear conditioning (Merlo et al., 2014).

The main limitation of our study is the lack of temporal characterization for the molecular mechanisms triggered by MK-801 and occurring during the 24 h temporal period before memory retrieval. Antagonism or signalling knocking-down studies on would help to elucidate the causal relationship between metaplastic changes and molecular correlates of memory reactivation.

In conclusion, our findings showed that NMDA antagonism-induced metaplastic changes by MK-801 affected instrumental responding for sucrose either via reconsolidation inhibition or extinction facilitation. These data suggest that pharmacological modulation of NMDA given under a 'metaplastic dose-regimen' may be relevant for learning and memory at a behavioural level.

Acknowledgements

AP performed all the experiments. AP, MDC, LO and LP performed and analysed the data of immunohistochemical studies. LC and FF performed and analysed the data of western blot assays. CC, DEPG, AP and EG planned and designed the whole study and each experiment. CC and AP wrote the manuscript, and all authors have approved the final version of the article. The project was partially funded by University of Verona Joint Project 2012.

References

Abraham, W. C. (2008). Metaplasticity: tuning synapses and networks for plasticity. *Nature Reviews Neuroscience*, *9*(5), 387–387. https://doi.org/10.1038/nrn2356

Abraham, W. C., & Bear, M. F. (1996). Metaplasticity: The plasticity of synaptic plasticity. *Trends in Neurosciences*. https://doi.org/10.1016/S0166-2236(96)80018-X

Auber, A., Tedesco, V., Jones, C. E., Monfils, M. H., & Chiamulera, C. (2013). Post-retrieval extinction as reconsolidation interference: Methodological issues or boundary conditions? *Psychopharmacology*, *226*(4), 631–647. https://doi.org/10.1007/s00213-013-3004-1

Barak, S., Liu, F., Hamida, S. Ben, Yowell, Q. V, Neasta, J., Kharazia, V., … Ron, D. (2013). Disruption of alcohol-related memories by mTORC1 inhibition prevents relapse. *Nature Neuroscience*, *16*(8), 1111–1117. https://doi.org/10.1038/nn.3439

Ben Mamou, C., Gamache, K., & Nader, K. (2006). NMDA receptors are critical for unleashing consolidated auditory fear memories. *Nat Neurosci*, *9*(10), 1237–1239. https://doi.org/10.1038/nn1778

Besnard, A., Caboche, J., & Laroche, S. (2013). Recall and Reconsolidation of Contextual Fear Memory: Differential Control by ERK and Zif268 Expression Dosage. *PLoS ONE*, *8*(8), 1–8. https://doi.org/10.1371/journal.pone.0072006

Biever, A., Valjent, E., & Puighermanal, E. (2015). Ribosomal Protein S6 Phosphorylation in the Nervous System: From Regulation to Function. *Frontiers in Molecular Neuroscience*, *8*(December), 1–14. https://doi.org/10.3389/fnmol.2015.00075

Bortolotto, Z. A., Collett, V. J., Conquet, F., Jia, Z., Van Der Putten, H., & Collingridge, G. L. (2005). The regulation of hippocampal LTP by the molecular switch, a form of

metaplasticity, requires mGlu5 receptors. *Neuropharmacology*, *49*(SUPPL.), 13–25. https://doi.org/10.1016/j.neuropharm.2005.05.020

Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. *Psychological Bulletin*, *114*(1), 80–99. https://doi.org/10.1037/0033-2909.114.1.80

Buck, N., Cali, S., & Behr, J. (2006). Enhancement of long-term potentiation at CA1-subiculum synapses in MK-801-treated rats. *Neuroscience Letters*, *392*(1–2), 5–9. https://doi.org/10.1016/j.neulet.2005.08.054

Burgdorf, J., Zhang, X., Nicholson, K. L., Balster, R. L., David Leander, J., Stanton, P. K., … Moskal, J. R. (2013). GLYX-13, a NMDA Receptor Glycine-Site Functional Partial Agonist, Induces Antidepressant-Like Effects Without Ketamine-Like Side Effects. *Neuropsychopharmacology*, *38*(5), 729–742. https://doi.org/10.1038/npp.2012.246

Caffino, L., Di Chio, M., Giannotti, G., Venniro, M., Mutti, A., Padovani, L., … Chiamulera, C. (2016). 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. *Pharmacological Research*, *104*, 22–30. https://doi.org/10.1016/j.phrs.2015.12.014

Caffino, L., Giannotti, G., Mottarlini, F., Racagni, G., & Fumagalli, F. (2017). Developmental Exposure to Cocaine Dynamically Dysregulates Cortical Arc/Arg3.1 Modulation in Response to a Challenge. *Neurotoxicity Research*, *31*(2), 289–297. https://doi.org/10.1007/s12640-016-9683-8

Chiamulera, C., Hinnenthal, I., Auber, A., & Cibin, M. (2014). Reconsolidation of maladaptive memories as a therapeutic target: Pre-clinical data and clinical approaches. *Frontiers in Psychiatry*, *5*(AUG), 107. https://doi.org/10.3389/fpsyt.2014.00107

Chiamulera, C., Marzo, C. M., & Balfour, D. J. K. (2017). Metabotropic glutamate receptor 5 as a potential target for smoking cessation. *Psychopharmacology*, *234*(9–10), 1357–1370. https://doi.org/10.1007/s00213-016-4487-3

Clem, R. L., & Huganir, R. L. (2010). Calcium-Permeable AMPA Receptor Dynamics Mediate Fear Memory Erasure. *Science*, *330*(6007), 1108–1112. https://doi.org/10.1126/science.1195298

Dunbar, A. B., & Taylor, J. R. (2016). Reconsolidation and psychopathology: Moving towards reconsolidation-based treatments. *Neurobiology of Learning and Memory*. https://doi.org/10.1016/j.nlm.2016.11.005

Exton-McGuinness, M. T. J., & Lee, J. L. C. (2015). Reduction in Responding for Sucrose and Cocaine Reinforcement by Disruption of Memory Reconsolidation. *eNeuro*, *2*(2). https://doi.org/10.1523/ENEURO.0009-15.2015

Finnie, P. S. B., & Nader, K. (2012). The role of metaplasticity mechanisms in regulating memory destabilization and reconsolidation. *Neuroscience and Biobehavioral Reviews*, *36*(7), 1667–1707. https://doi.org/10.1016/j.neubiorev.2012.03.008

Flavell, C. R., & Lee, J. L. C. (2013). Reconsolidation and extinction of an appetitive pavlovian memory. *Neurobiology of Learning and Memory*, *104*, 25–31. https://doi.org/10.1016/j.nlm.2013.04.009

Gieros, K., Sobczuk, A., & Salinska, E. (2012). Differential involvement of mGluR1 and mGluR5 in memory reconsolidation and retrieval in a passive avoidance task in 1-day old chicks. *Neurobiology of Learning and Memory*, *97*(1), 165–172. https://doi.org/10.1016/j.nlm.2011.11.004

Graef, J. D., Newberry, K., Newton, A., Pieschl, R., Shields, E., Luan, F. N., … Bristow, L. J. (2015). Effect of acute NR2B antagonist treatment on long-term potentiation in the rat hippocampus. *Brain Research*, *1609*(1), 31–39. https://doi.org/10.1016/j.brainres.2015.03.019

Hulme, S. R., Jones, O. D., & Abraham, W. C. (2013). Emerging roles of metaplasticity in behaviour and disease. *Trends in Neurosciences*. Elsevier Ltd. https://doi.org/10.1016/j.tins.2013.03.007

Izumi, Y., & Zorumski, C. F. (2014). Metaplastic effects of subanesthetic ketamine on CA1 hippocampal function. *Neuropharmacology*, *86*, 273–281. https://doi.org/10.1016/j.neuropharm.2014.08.002

Lee, J. L. C. (2008). Memory reconsolidation mediates the strengthening of memories by additional learning. *Nature Neuroscience*, *11*(11), 1264–1266. https://doi.org/10.1038/nn.2205

Lee, J. L. C. (2009). Reconsolidation: maintaining memory relevance. *Trends in Neurosciences*, *32*(8), 413–420. https://doi.org/10.1016/j.tins.2009.05.002

Lee, J. L. C., Di Ciano, P., Thomas, K. L., & Everitt, B. J. (2005). Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior. *Neuron*, *47*(6), 795–801. https://doi.org/10.1016/j.neuron.2005.08.007

Lee, J. L. C., Everitt, B. J., & Thomas, K. L. (2004). Independent Cellular Processes for Hippocampal Memory Consolidation and Reconsolidation, *304*(5672), 839–843.

Lee, J. L. C., & Flavell, C. R. (2014). Inhibition and enhancement of contextual fear memory destabilization. *Frontiers in Behavioral Neuroscience*, *8*(April), 144. https://doi.org/10.3389/fnbeh.2014.00144

Lee, J. L. C., Milton, A. L., & Everitt, B. J. (2006). Cue-Induced Cocaine Seeking and Relapse Are Reduced by Disruption of Drug Memory Reconsolidation. *The Journal of Neuroscience*, *26*(22), 5881–5887. https://doi.org/10.1523/JNEUROSCI.0323-06.2006

Lee, J. L. C., Nader, K., & Schiller, D. (2017). An Update on Memory Reconsolidation Updating. *Trends in Cognitive Sciences*. Elsevier Ltd. https://doi.org/10.1016/j.tics.2017.04.006

Marton, T. M., Hussain Shuler, M. G., & Worley, P. F. (2015). Homer 1a and mGluR5 phosphorylation in reward-sensitive metaplasticity: A hypothesis of neuronal selection and bidirectional synaptic plasticity. *Brain Research*. Elsevier. https://doi.org/10.1016/j.brainres.2015.06.037

Merlo, E., Milton, A. L., Goozee, Z. Y., Theobald, D. E., & Everitt, B. J. (2014). Reconsolidation and extinction are dissociable and mutually exclusive processes: Behavioral and molecular evidence. *The Journal of Neuroscience*, *34*(7), 2422–2431. https://doi.org/10.1523/JNEUROSCI.4001-13.2014

Milton, A. L., & Everitt, B. J. (2010). The psychological and neurochemical mechanisms of drug memory reconsolidation: Implications for the treatment of addiction. *European Journal of Neuroscience*, *31*(12), 2308–2319. https://doi.org/10.1111/j.1460-9568.2010.07249.x

Milton, A. L., Merlo, E., Ratano, P., Gregory, B. L., Dumbreck, J. K., & Everitt, B. J. (2013). Double dissociation of the requirement for GluN2B- and GluN2A-containing NMDA receptors in the destabilization and restabilization of a reconsolidating memory. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(3), 1109–15. https://doi.org/10.1523/JNEUROSCI.3273-12.2013

Monfils, M.-H., Cowansage, K. K., Klann, E., & LeDoux, J. E. (2009). Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories. *Science*, *324*(5929), 951–955. https://doi.org/10.1126/science.1167975

Nader, K., & Einarsson, E. Ö. (2010). Memory reconsolidation: An update. *Annals of the New York Academy of Sciences*. https://doi.org/10.1111/j.1749-6632.2010.05443.x

Nader, K., & Hardt, O. (2009). Historically, learning and memory experiments used a variety of tasks in conjunction with global amnesic treat- ments, *10*(MArCH). https://doi.org/10.1038/nrn2590

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature*, *406*(6797), 722–726. https://doi.org/10.1038/35021052

Piva, A., Gerace, E., Di Chio, M., Padovani, L., Pellegrini-Giampietro, D. E., & Chiamulera, C. (n.d.). Reconsolidation of instrumental memory for sucrose in rats: a molecular assessment. *Neuroscience*.

Przybyslawski, J., & Sara, S. J. (1997). Reconsolidation of memory after its reactivation. *Behavioural Brain Research*, *84*(1–2), 241–246. https://doi.org/10.1016/S0166-4328(96)00153-2

Reichelt and Lee. (2013). Memory reconsolidation in aversive and appetitive settings. *Frontiers in Behavioral Neuroscience*, *7*, 118. https://doi.org/10.3389/fnbeh.2013.00118

Richter-Levin, G., & Maroun, M. (2010). Stress and amygdala suppression of metaplasticity in the medial prefrontal cortex. *Cerebral Cortex*, *20*(10), 2433–2441. https://doi.org/10.1093/cercor/bhp311

Salinska, E. (2006). 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*(6–7), 447–452. https://doi.org/10.1016/j.neuint.2005.11.015

Schmidt, M. V, Abraham, W. C., Maroun, M., Stork, O., & Richter-Levin, G. (2013). Stress-induced metaplasticity: From synapses to behavior. *Neuroscience*. IBRO. https://doi.org/10.1016/j.neuroscience.2013.06.059

Tedesco, V., Mutti, A., Auber, A., & Chiamulera, C. (2014). Nicotine-seeking reinstatement is reduced by inhibition of instrumental memory reconsolidation. *Behavioural Pharmacology*, *25*(8), 725–731. https://doi.org/10.1097/FBP.0000000000000088

Tedesco, V., Ravagnani, C., Bertoglio, D., & Chiamulera, C. (2013). Acute ketamine-induced neuroplasticity: ribosomal protein S6 phosphorylation expression in drug addiction-related rat brain areas. *Neuroreport*, *24*(7), 388–93. https://doi.org/10.1097/WNR.0b013e32836131ad

Tedesco, V., Roquet, R. F., DeMis, J., Chiamulera, C., & Monfils, M. H. (2014). Extinction, applied after retrieval of auditory fear memory, selectively increases zinc-finger protein 268 and phosphorylated ribosomal protein S6 expression in prefrontal cortex and lateral amygdala. *Neurobiology of Learning and Memory*, *115*, 78–85. https://doi.org/10.1016/j.nlm.2014.08.015

Tenorio, G., Connor, S. A., Guévremont, D., Abraham, W. C., Williams, J., O'Dell, T. J., & Nguyen, P. V. (2010). "Silent" priming of translation-dependent LTP by ß-adrenergic receptors involves phosphorylation and recruitment of AMPA receptors. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *17*(12), 627–638. https://doi.org/10.1101/lm.1974510

Theberge, F. R. M., Milton, A. L., Belin, D., Lee, J. L. C., & Everitt, B. J. (2010). The basolateral amygdala and nucleus accumbens core mediate dissociable aspects of drug memory reconsolidation. *Learning & Memory*, *17*(9), 444–453. https://doi.org/10.1101/lm.1757410

Thomas, K. L., Arroyo, M., & Everitt, B. J. (2003). Induction of the learning and plasticity-associated gene Zif268 following exposure to a discrete cocaine-associated stimulus. *European Journal of Neuroscience*, *17*(9), 1964–1972. https://doi.org/10.1046/j.1460-9568.2003.02617.x

Trent, S., Barnes, P., Hall, J., & Thomas, K. L. (2015). Rescue of long-term memory after reconsolidation blockade. *Nature Communications*, *6*, 7897. https://doi.org/10.1038/ncomms8897

Veyrac, A., Besnard, A., Caboche, J., Davis, S., & Laroche, S. (2014). The transcription factor Zif268/Egr1, brain plasticity, and memory. *Progress in Molecular Biology and Translational Science*, *122*, 89–129. https://doi.org/10.1016/B978-0-12-420170-5.00004-0

Wang, S.-H., de Oliveira Alvares, L., & Nader, K. (2009). Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. *Nature Neuroscience*, *12*(7), 905–912. https://doi.org/10.1038/nn.2350

Yang, K., Trepanier, C., Sidhu, B., Xie, Y., Li, H., Lei, G., … MacDonald, J. F. (2012). Metaplasticity gated through differential regulation of GluN2A versus GluN2B receptors by Src family kinases. *The EMBO Journal*, *31*(4), 805–816. https://doi.org/10.1038/emboj.2011.453

Zanda, M. T., Fadda, P., Antinori, S., Di Chio, M., Fratta, W., Chiamulera, C., & Fattore, L. (2017). Methoxetamine affects brain processing involved in emotional response in rats. *British Journal of Pharmacology*. https://doi.org/10.1111/bph.13952

Zorumski, C. F., & Izumi, Y. (2012). NMDA receptors and metaplasticity: Mechanisms and possible roles in neuropsychiatric disorders. *Neuroscience and Biobehavioral Reviews*. Elsevier Ltd. https://doi.org/10.1016/j.neubiorev.2011.12.011