bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

CB1 receptor signaling modulates amygdalar plasticity during context-cocaine memory reconsolidation to promote subsequent cocaine seeking

Running title: Amygdala CB1 receptor signaling and cocaine-memory reconsolidation

Jessica A. Higginbotham[1], Rong Wang[1], Ben D. Richardson[1,2,3], Hiroko Shiina[1], Shi Min Tan[1], Mark A. Presker[4], David J. Rossi[1,5], Rita A. Fuchs[1,5,6*]

[1]Department of Integrative Physiology and Neuroscience, Washington State University College of Veterinary Medicine, Pullman, WA, USA
[2]Department of Biological Engineering, University of Idaho, Moscow, ID, USA
[3]WWAMI Medical Education, University of Idaho, Moscow, ID, USA
[4]Department of Psychology and Neuroscience, University of North Carolina, Chapel Hill, NC, USA
[5]Washington State University Alcohol and Drug Abuse Research Program, Pullman, WA, USA
[6]Translational Addiction Research Center, Washington State University, Pullman, WA, USA

*Corresponding author: Dr. Rita A. Fuchs, rita.fuchs@wsu.edu

Conflict of Interest: The authors have no conflicts of interest to disclose.

Acknowledgements: This research was funded by NIDA R01 DA025646 (RAF), NIDA F31 DA 045430 (JAH), NIAAA R01 AA026078 (DJR), NINDS T32 NS007431 (MAP), and Washington State Initiative 171 (JAH) and 502 (RAF) funds administered through the Washington State University Alcohol and Drug Abuse Research Program.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

ABSTRACT

Contextual drug-associated memories precipitate craving and relapse in cocaine users. Such associative memories can be weakened through interference with memory reconsolidation, a process by which memories are maintained following memory retrieval-induced destabilization. We hypothesized that cocaine-memory reconsolidation requires cannabinoid type 1 receptor (**CB1R**) signaling based on the fundamental role of the endocannabinoid system in synaptic plasticity and emotional memory processing. Using an instrumental rat model of cocaine relapse, we evaluated whether systemic CB1R antagonism (**AM251**; 3 mg/kg, I.P.) during memory reconsolidation alters (a) subsequent drug context-induced cocaine-seeking behavior, as well as (b) cellular adaptations and (c) excitatory synaptic physiology in the basolateral amygdala (**BLA**). Systemic CB1R antagonism – during, but not after, cocaine-memory reconsolidation – reduced drug context-induced cocaine-seeking behavior three days, but not three weeks, later. CB1R antagonism also inhibited memory retrieval-associated increases in BLA zinc finger 268 (**zif268**) and activity regulated cytoskeletal-associated protein (**Arc**) immediate-early gene expression and changes in BLA α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (**AMPAR**) and N-methyl-D-aspartate receptor (**NMDAR**) subunit phosphorylation that likely contribute to increased receptor membrane trafficking and synaptic plasticity during memory reconsolidation. Furthermore, CB1R antagonism increased memory reconsolidation-associated spontaneous excitatory post-synaptic current frequency in BLA principal neurons during memory reconsolidation. Together, these findings suggest that CB1R signaling modulates cellular and synaptic mechanisms in the BLA during cocaine-memory reconsolidation, thereby facilitating cocaine-memory maintenance. These findings identify the CB1R as a potential therapeutic target for relapse prevention.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

SIGNIFICANCE STATEMENT

Drug relapse can be triggered by the retrieval of context-drug memories upon re-exposure to a drug-associated environment. Context-drug associative memories become destabilized upon retrieval and must be reconsolidated into long-term memory stores in order to persist. Hence, targeted interference with memory reconsolidation can weaken maladaptive context-drug memories and reduce the propensity for drug relapse. Our findings indicate that cannabinoid type 1 receptor (**CB1R**) signaling is critical for context-cocaine memory reconsolidation and subsequent drug context-induced reinstatement of cocaine-seeking behavior. Furthermore, cocaine-memory reconsolidation is associated with CB1R-dependent immediate-early gene expression and changes in excitatory synaptic proteins and physiology in the basolateral amygdala. Together, our findings provide initial support for CB1R as a potential therapeutic target for relapse prevention.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

INTRODUCTION

Exposure to drug-associated environmental stimuli triggers the retrieval of maladaptive drug memories that can precipitate drug craving and relapse in cocaine users (Childress et al., 1988, 1999; Crombag et al., 2008). Similar to other associative memories, cocaine-associated memories become labile upon retrieval (Nader et al., 2000). Retention of these memories requires memory reconsolidation processes that involve *de novo* protein synthesis (Nader et al., 2000; Fuchs et al., 2009; Wells et al., 2011a) and glutamatergic synaptic plasticity (Rao-Ruiz et al., 2015; Rich and Torregrossa, 2018). Consequently, targeted interference with memory reconsolidation can weaken contextual cocaine-associated memories and reduce the propensity for drug relapse (Fuchs et al., 2009; Ramirez et al., 2009; Wells et al., 2011, 2013, 2016; Arguello et al., 2014; Stringfield et al., 2017). Accordingly, it is important to investigate the cellular mechanisms of cocaine-memory reconsolidation with a focus on viable therapeutic targets.

Endocannabinoids are retrograde messengers that modulate excitatory and inhibitory synaptic plasticity (Castillo et al., 2012) and some forms of memory reconsolidation through the stimulation of presynaptic cannabinoid type 1 receptors (**CB1Rs**) (see Stern et al., 2018 for review). CB1R antagonism during memory reconsolidation impairs Pavlovian morphine- (De Carvalho et al., 2014), methamphetamine- (Yu et al., 2009), and nicotine- (Fang et al., 2011) conditioned place preference (**CPP**) memories. However, critical gaps remain in our understanding of CB1R involvement in memory reconsolidation. First, it is unclear whether CB1Rs regulate the reconsolidation of *cocaine* memories. Second, it is not known whether CB1Rs play similar roles in the reconsolidation of drug memories forged in instrumental versus Pavlovian paradigms, as extant literature indicates that Pavlovian and instrumental cocaine-associated memories are reconsolidated through partially distinct neural mechanisms (Miller and Marshall, 2005; Theberge et al., 2010; Wells et al., 2013). Finally, the cellular and synaptic physiological mechanisms by which CB1Rs modulate drug-memory reconsolidation have not been explored.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

In the present study, we tested the hypothesis that CB1Rs are critically involved in contextual cocaine-memory reconsolidation in an instrumental model of drug relapse. First, we evaluated whether systemic CB1R antagonism during memory reconsolidation impairs cocaine-memory integrity as indicated by a subsequent, memory retrieval-dependent reduction in cocaine-seeking behavior. Second, we assessed the effects of memory reconsolidation and systemic CB1R antagonism on molecular adaptations and excitatory synaptic physiology in the basolateral amygdala (**BLA**), the critical site for protein synthesis-dependent memory reconsolidation in our model (Fuchs et al., 2009; Wells et al., 2011b). It has been established that auditory fear-memory reconsolidation requires memory retrieval-dependent, transient, NMDAR-dependent synaptic exchange of calcium-impermeable (**CI**, GluA2-containing) AMPARs with calcium-permeable (**CP**, GluA2-lacking) AMPARs in the lateral amygdala (Clem and Huganir, 2010; Hong et al., 2013; Lopez et al., 2015; Yu et al., 2016). While similar research has not explored the molecular mechanisms of contextual appetitive or aversive memory reconsolidation, we have shown that BLA protein kinase A (**PKA**) activation is necessary for cocaine-memory reconsolidation in the drug context-induced reinstatement model (Arguello et al., 2014). Other studies have demonstrated that PKA-mediated GluA1^{S845} phosphorylation enhances GluA1 synaptic recruitment (Clem and Huganir, 2010), whereas Src-family tyrosine kinase (**Src**)-mediated GluA2^{Y876} phosphorylation elicits GluA2 endocytosis (Hayashi and Huganir, 2004). Thus, elevated Ca^{2+} influx through NMDARs activates PKA and Src tyrosine kinases which promote changes in synaptic AMPAR-subunit composition that collectively mediate expression of long-term potentiation (**LTP;** He et al., 2009; Makino and Malinow, 2009). Moreover, Src-mediated GluN2B^{Y1472} phosphorylation is required for proper GluN2B synaptic localization, signaling, and amygdalar synaptic plasticity, raising another kinase control point for plasticity expression and/or initiation (Nakazawa et al., 2006). Accordingly, in the present study, we identified alterations in immediate-early gene (**IEGs**) expression and in glutamate receptor subunit expression and phosphorylation with a focus on GluA1^{S845}, GluA2^{Y876}, and GluN2B^{Y1472} phosphorylation. In an

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

attempt to explore the synaptic physiological significance of these post-translational protein modifications, we determined changes in excitatory postsynaptic currents (**EPSCs**) in glutamatergic pyramidal principal neurons (**PNs**) of the BLA.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (*n* = 108; 275-300 g at the start of the experiment) were individually housed in a temperature- and humidity-controlled vivarium on a reversed light/dark cycle (lights on at 6:00 am). Rats were given *ad libitum* access to water and 20-25 g of standard rat chow per day. The housing and care of animals were conducted in accordance with guidelines defined in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011) and approved by the Washington State University Institutional Animal Care and Use Committee.

Food Training and Surgery

To facilitate the acquisition of drug self-administration, rats were trained to press a lever (active lever) under a continuous food reinforcement schedule in standard operant conditioning chambers (Coulbourn Instruments, Holliston, MA) during a 16-h overnight food training session. Each active-lever response resulted in the unsignaled delivery of one food pellet (45 mg pellets; Bioserv, Flemington, NJ) under a continuous reinforcement schedule. Responses on a second (inactive) lever were recorded but had no programmed consequences. Contextual stimuli used for subsequent cocaine conditioning were not present during the food-training session.

For jugular catheter implantation, rats were fully anesthetized using ketamine hydrochloride and xylazine (100 mg/kg and 5 mg/kg, i.p., respectively; Dechara Veterinary Products, Overland Park, KS and Akorn, Lake Forest, IL) at least 24 h after food training. Jugular

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

141 catheters were constructed in house and surgically implanted into the right jugular vein to facilitate
142 cocaine self-administration. The catheters were maintained and periodically tested for patency as
143 described previously (Fuchs et al., 2007). Rats received the non-steroidal anti-inflammatory
144 analgesic, carprofen (5 g/kg per d, p.o.; ClearH2O, Westbrook, ME), from 24 h before until 48 h
145 after surgery.
146
147 ***Cocaine Self-Administration and Extinction Training***
148 Rats were randomly assigned to one of two different environmental contexts for cocaine
149 self-administration training. The two environmental contexts contained distinct olfactory, auditory,
150 visual, and tactile stimuli, as previously described (Fuchs et al., 2007). Daily 2-h training sessions
151 were conducted in one of the two environmental contexts during the rats' dark cycle. During
152 training sessions, active-lever responses were reinforced under a fixed ratio 1 cocaine
153 reinforcement schedule (0.15 mg cocaine hydrochloride/0.05 mL infusion, i.v.; National Institute
154 on Drug Abuse Drug Supply Program, Research Triangle Park, NC). Cocaine infusions were
155 delivered over 2.25 s followed by a 20-s time-out period, during which active-lever responses had
156 no programmed consequences. Inactive-lever responses had no programmed consequences.
157 Training continued until the rats reached the acquisition criterion (i.e., ≥ 10 cocaine infusions
158 obtained per session on at least 10 training days). Next, rats received daily 2-h extinction training
159 sessions in the alternate environmental context, where lever presses had no programmed
160 consequences. The number of extinction sessions was set at seven to hold memory age constant
161 at the time of the experimental manipulation. Immediately after extinction session 4, the rats
162 received an i.p. injection of saline (1 mL/kg) to acclimate them to the injection procedure.
163
164
165

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

166 ***Experiment 1: Effects of CB1R antagonism immediately after memory retrieval on drug***
167 ***context-induced cocaine seeking three days later***

168 Twenty-four h after the seventh extinction session, rats were re-exposed to the cocaine-
169 paired context for 15 min to trigger memory retrieval and reconsolidation (**Fig. 1A**). During the
170 session, lever presses had no programmed consequences. Cocaine reinforcement was withheld
171 to prevent acute cocaine effects on neurotransmission and endocannabinoid mobilization
172 independent of memory destabilization (Ortinski et al., 2012; Wang et al., 2015). Immediately after
173 the session (i.e., during the putative time of memory reconsolidation), rats received systemic
174 administration of the CB1R antagonist/inverse agonist, *N*- (Piperidin-1-yl)-5- (4-iodophenyl)-1-
175 (2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (**AM251**, 3 mg/kg; Sigma Aldrich, St.
176 Louis, MO), or vehicle (**VEH**; 8% DMSO, 5% Tween80 in saline; 1 mL/kg). AM251 at this dose is
177 sufficient to impair contextual fear learning and memory consolidation (Arenos et al., 2006). On
178 the next day, daily 2-h extinction training sessions resumed in the designated extinction context
179 until the rats reached the extinction criterion (i.e., ≤ 25 active-lever presses per session on two
180 consecutive days; mean number of days to criterion = 2.0 ± 0.0 d). Lever responses in the
181 extinction context were assessed to detect possible off-target effects of experimental
182 manipulations on extinction memories. Twenty-four h after the last extinction session, cocaine-
183 seeking behavior (i.e., non-reinforced lever responses) was assessed in the cocaine-paired
184 context. Rats were euthanized immediately after the 2-h test session, and their brains were flash
185 frozen in isopentane and stored for analysis of BLA IEG and glutamate-receptor subunit
186 expression using western blotting, as described below. Based on the phosphorylation kinetics of
187 glutamate receptor subunits (Clem and Huganir, 2010; Rao-Ruiz et al., 2011), these tissue
188 samples were prepared and analyzed for total-protein levels only.
189
190

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Experiment 2: Effects of CB1R antagonism six hours after memory retrieval on drug context-induced cocaine seeking three days later

Memory reconsolidation impairments require manipulation while the memories are labile (i.e., 2-4 h after memory retrieval; Tronson and Taylor, 2007). Experiment 2 evaluated whether any impairments in cocaine-seeking behavior in Experiment 1 reflected a memory reconsolidation deficit (**Fig. 2A**) as opposed to prolonged impairment in the expression of cocaine-seeking behavior. The procedures in Experiment 2 were identical to those in Experiment 1 except that rats received AM251 (3 mg/kg, i.p.) or VEH *six h* after the 15-min memory retrieval session in the cocaine-paired context, outside of the putative time window of memory reconsolidation. As in Experiment 1, daily 2-h extinction training sessions resumed in the extinction context after the memory retrieval session until the extinction criterion was reached (mean number of days to criterion = 2.00 ± 0.0 d). The extinction sessions were followed by a single 2-h test of cocaine-seeking behavior in the cocaine-paired context. Rats were euthanized immediately after the test session. Their brains were flash frozen in isopentane and stored for analysis of BLA IEG and glutamate receptor subunit expression using western blotting, as in Experiment 1.

Experiment 3: Effects of CB1R antagonism immediately after memory retrieval on drug context-induced cocaine seeking 24 days later

Experiment 3 assessed whether any effects of AM251 on memory integrity persisted over time. The procedures were identical to those in Experiment 1 except that, after memory retrieval and pharmacological manipulation, rats remained in their home cages for 21 days (**Fig. 3A**). Daily 2-h extinction training sessions then resumed until the extinction criterion was reached (mean number of days to criterion = 2.93 ± 0.27 d), and this was followed by a 2-h test of cocaine-seeking behavior (i.e., 24 d post treatment). Rats were euthanized immediately after the test session. Their brains were flash frozen in isopentane and stored for analysis of BLA IEG and glutamate-receptor subunit expression using western blotting, as in Experiment 1.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Experiment 4: Effects of memory reconsolidation and CB1R antagonism on BLA protein expression and phosphorylation

Experiment 4 evaluated memory reconsolidation-related changes in immediate-early gene expression and glutamate-receptor subunit expression and phosphorylation. The procedures in Experiment 4 were identical to those in Experiment 1 except that rats were euthanized 45 min after memory retrieval or no-memory retrieval (home-cage stay) and pharmacological treatment (**Fig. 4A**). This euthanasia time point was selected based on the activation kinetics of Arc and zif268 (Lee, 2004; Li et al., 2005) and associated changes in glutamate-receptor expression and phosphorylation in other models of synaptic plasticity (Clem and Huganir, 2010; Rao-Ruiz et al., 2011). The brains were flash frozen in isopentane and stored for analysis of BLA IEG and glutamate-receptor subunit expression and glutamate-receptor subunit phosphorylation using western blotting.

Western Blotting

Brains were stored at -80 ºC before the collection of BLA tissue punches (o.d. 0.75 mm). Punched tissue was stored at -80 ºC in lysis buffer containing 10 mM HEPES, 1% SDS, and 1x protease and phosphatase inhibitor cocktails (Sigma Aldrich, St. Louis, MO). Samples were thawed, manually homogenized, and total tissue homogenate protein concentrations were determined using the Biorad detergent-compatible protein assay ($R^2 \geq 0.99$). After electrophoresis and transfer, membranes were dried overnight at 4ºC. The next day, membranes were reactivated in methanol and blocked before incubation overnight in Odyssey blocking buffer (Li-Cor Biosciences, Lincoln, NE) with 0.2% Tween 20 and primary antibodies targeting Arc (Cat# sc-17839, RRID:AB_626696), zif268 (Cat# sc-189, RRID: AB_2231020), NMDAR subunit 2B (**GluN2B**; Cat# 06-600, RRID:AB_310193), phospho-Tyr^{1472}-GluN2B (**pGluN2B**; Cat# p1516-1472, RRID:AB_2492182), GluA1 (Cat# sc-13152, RRID:AB_627932), phospho-Ser^{845}-GluA1

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

(**pGluA1**; Cat# AB5849, RRID:AB_92079), GluA2 (Cat# MABN1189, RRID:AB_2737079), phospho-Tyr^{876}-GluA2 (**pGluA2**; Cat# 4027, RRID:AB_1147622), calnexin (**CNX**; Cat# ADI-SPA-860, RRID:AB_10616095), or glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**; Cat# ab8245, RRID:AB_2107448). Membranes were then washed and incubated for 1 h in Odyssey blocking buffer with 0.2% Tween 20 and 0.01% SDS with the following near-infrared fluorescent secondary antibodies: IRDye® 800CW goat anti-mouse (Cat# 926-32210, RRID:AB_621842), IRDye® 800CW goat anti-rabbit (Cat# 926-32211, RRID:AB_621843), IRDye® 680RD donkey anti-mouse (Cat# 926-68072, RRID:AB_10953628), and IRDye® 680LT goat anti-rabbit (Cat# 926-68021, RRID:AB_10706309). For multiplexed targets (based on antibody availability), the 800-nm channel was used to detect the lowest-abundance, phospho-proteins as it provides lower background, maximizing detection sensitivity (Schutz-Geschwender et al., 2004). Total proteins were detected in the 680-nm channel. Membranes were digitally imaged using a Li-Cor Odyssey CLX. Integrated optical intensity values for target protein bands were derived using Li-Cor Image Studio Software (RRID: SCR_015795). Total protein levels were normalized to a loading control and expressed as a percentage of the comparator group (Experiments 1-3: VEH-treated; Experiment 4: VEH-no-memory retrieval). Phospho-specific values were normalized to total protein values. A lane-normalization factor was calculated for the total and house-keeping proteins by dividing the lane's integrated optical intensity value by the highest signal value on the blot. Target-protein integrated intensity values were divided by the respective loading control's lane-normalization factor to normalize signal values across blots (Schutz-Geschwender et al., 2004).

Experiment 5: Effects of post-retrieval CB1R antagonism on excitatory synaptic transmission in BLA PNs at memory reconsolidation

Experiment 5 examined the effects of memory reconsolidation and systemic CB1R antagonism on glutamatergic EPSCs in BLA PNs, the primary output neurons of the BLA

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

(McDonald, 1984). The procedures in Experiment 5 were identical to those in Experiment 1 except that rats were exposed to the cocaine-paired context for 15 min (memory retrieval) or remained in their home cages (no-memory retrieval) before systemic AM251 (3 mg/kg, i.p.) or VEH administration (**Fig. 6A**). Rats were euthanized and brain slices were prepared for whole-cell electrophysiology recordings within an average of 30 min of treatment.

BLA Brain Slice Electrophysiology

All rats used for electrophysiological recordings were deeply anesthetized with isoflurane (3-5%). Rats were transcardially perfused with ice-cold artificial cerebrospinal fluid (**aCSF**), which contained 124 mM NaCl, 26 mM $NaHCO_3$, 2.5 mM KCl, 2.5 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM NaH_2PO_4, 10 mM D-glucose, and 1 mM kynurenic acid, and was bubbled with 95% O_2/5% CO_2 (pH 7.4). After perfusion, the brain was rapidly removed and sliced coronally (225 µm) in ice-cold dissection buffer that contained 220 mM sucrose, 26 mM $NaHCO_3$, 2 mM KCl, 0.5 mM $CaCl_2$, 5 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 2 mM Na-pyruvate, 1 mM ascorbic acid, 10 mM D-glucose, 1 mM kynurenic acid, and was bubbled with 95% O_2/5% CO_2. Tissue slices containing the BLA were incubated in aCSF (with 1 mM kynurenic acid) at 35-37 ºC, after which they were incubated in room temperature aCSF (with 1 mM kynurenic acid) until used. Slices were discarded within 4 h of slice preparation.

Slices were transferred to a recording chamber and continually perfused (~5 mL/min) in aCSF (without kynurenic acid) at a bath temperature of 32-35 °C. All pharmacological agents were dissolved in aCSF and were applied via bath perfusion. BLA pyramidal PNs were visually identified using differential interference contrast imaging through an Olympus 60x (0.9 NA) water-immersion objective. Whole-cell patch-clamp recordings were made using glass pipettes with a resistance of 2-3 MΩ when filled with internal solution that contained 130 mM CsCl, 4 mM NaCl, 0.5 mM $CaCl_2$, 10 mM HEPES, 5 mM EGTA, 4 mM Mg-ATP, 0.5 mM Na_2-GTP, 5 mM QX-314,

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

0.1 mM spermine, and 0.03 mM Alexa568 hydrazide dye with pH adjusted to 7.2–7.3 with CsOH. Cells were voltage-clamped at -60 or +30 mV. Signals were digitized at 20 kHz, low-pass filtered at 10 kHz, and additionally filtered at 2 kHz for presentation. To evoke EPSCs (**eEPSCs**), a concentric bipolar stimulating electrode was placed into the internal capsule (**IC**), and tissue was stimulated by current pulses (20-50 µA, 0.1-ms duration at a frequency of 0.1 Hz). Glutamatergic responses were pharmacologically isolated using 1 µM strychnine and 10 µM gabazine to block glycine and $GABA_A$ receptors, respectively. AMPAR-mediated synaptic currents were isolated by adding 50 µM D-2-amino-5-phosphonovalerate (**AP5**), a broad-spectrum NMDAR antagonist. Ten eEPSCs were averaged in each condition, and their mean amplitudes were analyzed with pClamp software (RRID: SCR_011323). In the absence of AP5, NMDAR-mediated eEPSCs were measured as the average amplitude of the EPSC 50 ms after the onset of the stimulus (V_h = +30 mV), when AMPAR-mediated contributions are negligible (**Fig. 6B**). AMPA/NMDA ratios were calculated as the average inward peak current amplitude (V_h = -60 mV) divided by the outward current (V_h = +30 mV) amplitude at 50 ms after the onset of the stimulus. The rectification index was calculated as the average peak eEPSC amplitude at -60 mV divided by the average peak eEPSC amplitude at +30 mV, both recorded in the presence of AP5 to pharmacologically isolate the AMPAR component of the eEPSC. Spontaneous EPSCs (**sEPSCs**) from at least 300s long whole-cell recordings in each condition were analyzed with MiniAnalysis Program software. sEPSCs were detected automatically with an amplitude detection threshold of 2.5x the amplitude of the peak to peak of the noise. Events were visually confirmed as previously described (Richardson and Rossi, 2017), and the frequency and mean peak amplitude at -60 mV and +30 mV was measured.

Experimental Design and Statistical Analysis

To identify potential pre-existing group differences in behavioral and drug history, active- and inactive-lever presses and cocaine intake during drug self-administration training (last 3

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

sessions) and non-reinforced lever presses during extinction training (first 7 extinction sessions) and during the memory-retrieval session were analyzed using separate mixed-factorial or univariate ANOVAs with subsequent treatment group as the between-subjects factor and time (session) as the within-subject factor, or between-subjects t-tests, where appropriate. Non-reinforced lever presses during the first post-treatment exposure to the extinction context and to the cocaine context were analyzed using mixed-factorial ANOVAs with memory retrieval (retrieval, no-memory retrieval) and treatment (AM251, VEH) as between-subjects factors and context (extinction, cocaine-paired) and time (20-min interval) as within-subjects factors, where appropriate. For Experiment 1-3, normalized total protein levels at test were analyzed using t-tests. For Experiments 4-5, phospho-protein levels, total protein levels, peak and mean eEPSC amplitudes, and sEPSC frequency at memory reconsolidation were analyzed using separate ANOVAs with treatment and memory retrieval as between-subjects factors. Significant interactions and main effects were further analyzed using Sidak's or Tukey's *post hoc* tests. Cumulative probability distributions of sEPSC amplitudes and inter-event intervals across groups (memory retrieval or no-memory retrieval, with AM251 or VEH) were analyzed using non-parametric Kruskal-Wallis tests with Dunn's *post hoc* tests. The relationships between active-lever presses and total protein levels at test were analyzed using Pearson's *r* correlational coefficients. Alpha was set at 0.05 for all analyses.

RESULTS

Behavioral and Drug History

There were no statistically significant differences between the groups in cocaine intake during drug self-administration training or in lever responding during drug self-administration training, extinction training, or memory retrieval in Experiments 1-5 (**Fig. 1-3; Extended Fig. 1-1, 2-1, 3-1, 6-1; Extended Tables 1-1, 2-1, 3-1, 4-1, 6-1**).

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

345 ***Experiment 1: Systemic CB1R antagonism during memory reconsolidation***
346 ***attenuates subsequent drug context-induced cocaine seeking***

347 Systemic AM251 administration immediately after the 15-min cocaine-memory retrieval
348 session (i.e., at the onset of memory reconsolidation) attenuated cocaine-seeking behavior at test
349 in a context-dependent manner (**Fig. 1C**; 2 x 2 ANOVA context x treatment interaction, $F_{(1,13)}$ =
350 10.93, p = 0.006; context main effect, $F_{(1,13)}$ = 111.80, p = 0.0001; treatment main effect, $F_{(1,13)}$ =
351 8.95, p = 0.01). Active-lever responding in the cocaine-paired context was greater than in the
352 extinction context (Sidak's test, p = 0.05). Furthermore, AM251 administered immediately after
353 memory retrieval attenuated active-lever responding in the cocaine-paired (Sidak's test, p = 0.05),
354 but not the extinction, context relative to VEH. Time-course analysis of active-lever presses in the
355 cocaine-paired context revealed that responding declined over time, and AM251 reduced
356 responding during the first 20-min interval relative to VEH (**Fig. 1D**; 2 x 6 ANOVA treatment x time
357 interaction, $F_{(5,65)}$ = 4.11, p = 0.003, Tukey's tests, p < 0.05; time main effect, $F_{(5,65)}$ = 14.59, p =
358 0.0001; treatment main effect $F_{(1,13)}$ = 11.86, p = 0.004). Inactive-lever responding remained low
359 in both contexts independent of treatment (**Fig. 1-1**).
360
361 ***Experiment 2: Systemic CB1R antagonism outside of the memory reconsolidation window***
362 ***does not alter subsequent drug context-induced cocaine seeking***
363 Systemic AM251 administration 6 h after cocaine-memory retrieval (i.e., after
364 reconsolidation into long-term memory stores) did not alter subsequent cocaine-seeking behavior
365 relative to VEH (**Fig. 2C**). Active-lever responding in the cocaine-paired context was higher than
366 in the extinction context regardless of treatment (2 x 2 ANOVA context main effect, $F_{(1,15)}$ = 89.43,
367 p < 0.0001), and delayed AM251 administration did not alter responding in either context relative
368 to VEH (ANOVA treatment main and interaction effects, $F_{(1,15)} \leq 0.21$, $p \geq 0.65$). The time-course
369 analysis of active-lever presses in the cocaine-paired context indicated that responding declined
370 after the first 20-min interval independent of treatment (**Fig. 2D**; 2 x 6 ANOVA time main effect,

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

371 $F_{(5,75)}$ = 20.45, p < 0.0001, interval 1 > intervals 2-6, Tukey's tests, p < 0.05; treatment main and
372 interaction effects, Fs $_{(5,75)}$ ≤ 0.88, p ≥ 0.50). Inactive-lever responding remained low in both
373 contexts independent of treatment (**Fig. 2-1**).
374
375 ***Experiment 3: Systemic CB1R antagonism during memory reconsolidation fails to alter***
376 ***drug context-induced cocaine seeking 24 days later***
377 Systemic AM251 administration immediately after cocaine-memory retrieval (i.e., at the
378 onset of memory reconsolidation) failed to alter cocaine-seeking behavior after a 21-d drug-free
379 period followed by at least two extinction sessions later, relative to VEH (**Fig. 3C**). Active-lever
380 responding in the cocaine-paired context was higher than in the extinction context regardless of
381 treatment (2 x 2 ANOVA context main effect, $F_{(1,15)}$ = 48.56, p < 0.0001; treatment main and
382 interaction effects, $Fs_{(1,15)}$ ≤ 3.32, p ≥ 0.09). Time-course analysis of active-lever presses in the
383 cocaine-paired context indicated that responding declined after the first 20-min interval
384 independent of treatment (**Fig. 3D**; 2 x 6 ANOVA time main effect, F $_{(5,75)}$ = 20.08, p < 0.0001,
385 interval 1 > intervals 2-6, Tukey's tests, p < 0.05; treatment main and interaction effects, Fs $_{(5,75)}$
386 ≤ 0.57, p ≥ 0.46). Inactive-lever responding remained low in both contexts independent of
387 treatment (**Fig. 3-1**).
388
389 ***Experiment 4: Systemic CB1R antagonism inhibits memory retrieval-induced molecular***
390 ***changes in the BLA during memory reconsolidation***
391 In brain tissue collected during memory reconsolidation (**Fig. 4A-B**), BLA IEG expression
392 varied as a function of memory retrieval and AM251 treatment. Memory retrieval increased zif268
393 expression relative to no-memory retrieval (i.e., home cage stay; **Fig. 4C**; 2 x 2 ANOVA, retrieval
394 x treatment interaction, $F_{(1,38)}$ = 18.91, p < 0.0001; retrieval main effect, $F_{(1,38)}$ = 9.16, p = 0.004;
395 treatment main effect, $F_{(1,38)}$ = 9.08, p = 0.005). Systemic AM251 administration after memory
396 retrieval reduced zif268 expression relative to VEH (Sidak's test, p < 0.05), such that zif268

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

expression no longer differed from those in the no-memory retrieval controls. Similar to zif268, memory retrieval increased Arc expression relative to no-memory retrieval (**Fig. 4D**; 2 x 2 ANOVA, retrieval x treatment interaction, $F_{(1,38)} = 5.61$, $p = 0.02$; retrieval main effect, $F_{(1,38)} = 13.03$, $p = 0.0009$; treatment main effect, $F_{(1,38)} = 0.53$, $p = 0.47$). Furthermore, systemic AM251 administration after memory retrieval modestly attenuated Arc expression during memory reconsolidation relative to VEH (Sidak's test, $p = 0.06$), such that Arc expression no longer differed from those in the no-memory retrieval controls.

Similar to IEG expression, glutamate receptor subunit phosphorylation varied as a function of memory retrieval and AM251 treatment. These results are reported in **Fig 4E-G**.

Src-mediated phosphorylation of NMDAR GluN2B^{Y1472} (**pGluN2B**) facilitates proper GluN2B synaptic localization, learning, and amygdalar synaptic plasticity (Nakazawa et al., 2006). BLA pGluN2B levels varied as a function of memory retrieval and treatment (**Fig. 4F**). Memory retrieval increased pGluN2B relative to no-memory retrieval (retrieval x treatment interaction, $F_{(1,38)} = 20.74$, $p < 0.0001$; retrieval main effect, $F_{(1,38)} = 5.60$, $p = 0.02$; treatment main effect, $F_{(1,38)} = 1.43$, $p = 0.24$). Systemic AM251 administration after memory retrieval reduced pGluN2B during memory reconsolidation relative to VEH (Sidak's test, $p < 0.05$), such that pGluN2B levels no longer differed from those in the no-memory retrieval controls. Notably, a trend for an AM251-induced increase in total GluN2B levels (**Fig. 4-1B**; treatment main effect, $F_{(1,38)} = 8.40$, $p = 0.006$; retrieval main and interaction effects, $Fs_{(1,38)} \leq 3.72$, $p \geq 0.06$) could have enhanced this effect by increasing the denominator.

PKA-mediated phosphorylation of AMPAR GluA1^{S845} (**pGluA1**) promotes GluA1 trafficking to the postsynaptic density and fear-memory destabilization after memory retrieval (Clem and Huganir, 2010). BLA pGluA1 levels varied as a function of memory retrieval and AM251 treatment (**Fig. 4F**; retrieval x treatment interaction, $F_{(1,38)} = 8.04$, $p = 0.007$; retrieval main effect, $F_{(1,38)} = 7.15$, $p = 0.01$; treatment main effect, $F_{(1,38)} = 1.70$, $p = 0.20$) with no change in total GluA1 levels (**Fig. 4-1C**; all retrieval and treatment main and interaction effects, $Fs_{(1,38)} \leq 1.83$, $p \geq 0.18$).

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Memory retrieval increased pGluA1 relative to no-memory retrieval (Sidak's test, $p < 0.05$). Moreover, systemic AM251 administration after memory retrieval reduced pGluA1 during memory reconsolidation relative to VEH (Sidak's test, $p < 0.05$), such that pGluA1 levels no longer differed from those in no-memory retrieval controls.

Src-mediated phosphorylation of AMPAR GluA2^{Y876} (**pGluA2**) disrupts GluA2 association with postsynaptic density scaffolding proteins, thereby reducing GluA2 synaptic expression (Hayashi and Huganir, 2004). Memory retrieval reduced BLA pGluA2 during memory reconsolidation relative to no-memory retrieval (**Fig. 4G**; retrieval main effect, $F_{(1,38)} = 6.78$, $p = 0.01$; all treatment main and interaction effects, $Fs_{(1,38)} \leq 0.78$, $p \geq 0.38$), without altering total GluA2 levels (**Fig. 4-1D**; all $Fs_{(1,38)} < 1.59$, $p > 0.22$). In contrast, AM251 failed to alter pGluA2 or total GluA2 levels.

Experiments 1-3: Systemic CB1R antagonism during memory reconsolidation inhibits molecular changes in the BLA during reinstatement three days, but not 24 days, post treatment

To capture potential protracted effects of AM251 on protein expression during the reinstatement test, BLA tissue was collected immediately after the 2-h test session in the cocaine-paired context in Experiments 1-3 (**Fig. 5A**). In Experiment 1, systemic AM251 administration immediately after memory retrieval (i.e., during memory reconsolidation) significantly reduced BLA zif268 ($t_{(13)} = 2.94$, $p = 0.01$), total GluN2B ($t_{(13)} = 3.48$, $p = 0.004$), and total GluA1 ($t_{(13)} = 4.41$, $p = 0.001$) expression relative to VEH three days post treatment. AM251 administration also increased Arc expression ($t_{(13)} = 4.61$, $p = 0.0005$) and failed to alter total GluA2 levels ($t_{(13)} = 1.63$, $p = 0.125$) relative to VEH at the same time point (**Fig. 5B**). Furthermore, there was a positive correlation between zif268 expression and active-lever responding at test three days after VEH treatment (**Fig. 5C**; $r = 0.82$, $p = 0.05$). This correlation was not observed after AM251

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

448 treatment (r = 0.31, p = 0.46) or between active-lever responding and other protein targets (**Table**
449 **5-1,** $r \leq 0.40$, $p \geq 0.12$).

450 In Experiment 2, systemic AM251 administration 6 h after memory retrieval (i.e., outside
451 the memory reconsolidation window) failed to alter BLA zif268, Arc, GluN2B, GluA1, or GluA2
452 levels relative to VEH at test, three days post treatment (**Fig. 5D**; $t_{(13)} \leq 0.51$, $p \geq 0.61$).
453 Furthermore, there was a positive correlation between BLA zif268 expression and active-lever
454 responding at test three days after VEH treatment (r = 0.77, p = 0.02) and a trend for a similar
455 positive correlation after AM251 treatment (**Fig. 5E**; r = 0.69, p = 0.058).

456 In Experiment 3, systemic AM251 administration immediately after memory retrieval did
457 not alter zif268, Arc, GluN2B, GluA1, or GluA2 levels relative to VEH at test, 24 days later (**Fig.**
458 **5F**; all $t_{(15)} \leq 1.18$, $p \geq 0.26$). However, there was a positive correlation between BLA zif268
459 expression and active-lever responding at test after memory retrieval and VEH treatment (**Fig.**
460 **5G**; r = 0.90, p = 0.002) and a trend for a negative correlation following AM251 treatment (r = -
461 0.63, p = 0.07).

462

463 ***Experiment 5: Systemic CB1R antagonism after memory retrieval increases sEPSC***
464 ***frequency in BLA PNs***

465 To determine if AM251-induced memory reconsolidation impairments and associated
466 molecular changes were paralleled by changes in synaptic transmission in the BLA, whole cell
467 patch-clamp recordings were obtained from BLA PNs in slices prepared on average 30 min after
468 memory retrieval or no-memory retrieval and systemic AM251 or VEH treatment (**Fig. 6A**).
469 Application of the NMDAR antagonist, APV (50 µM), was used to pharmacologically isolate the
470 AMPAR component, and subsequent digital subtraction revealed the NMDA component, of the
471 composite eEPSC (**Fig. 6B**). The AMPAR and NMDAR components were operationalized as the
472 peak amplitude and the amplitude at 50 ms of the composite response, respectively. Neither
473 memory retrieval nor AM251 altered the AMPAR-mediated eEPSC amplitude (**Fig. 6C-D**;

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

ANOVA, all retrieval and treatment $Fs_{(1,19)} \leq 0.57$, $p \geq 0.46$), NMDAR-mediated eEPSC amplitude (**Fig. 6E**; ANOVA, all $Fs_{(1,18)} \leq 3.89$, $p \geq 0.06$), or the AMPA/NMDA eEPSC amplitude ratio (**Fig. 6F**; ANOVA, all $Fs_{(1,17)} \leq 2.73$, $p \geq 0.12$).

In contrast to the lack of effects on eEPSCs, the sEPSC frequency varied as a function of memory retrieval and systemic AM251 treatment (**Fig. 6G**). Memory retrieval followed by VEH did not alter the mean sEPSC frequency relative to no-memory retrieval (ANOVA, retrieval x treatment interaction, $F_{(1,18)} = 6.95$, $p = 0.02$; all other $Fs_{(1,18)} \leq 4.17$, $p \geq 0.06$). However, AM251 administration after memory retrieval, but not after no-memory retrieval, increased the mean sEPSC frequency relative to VEH (**Fig. 6H**; Sidak's test, $p < 0.05$) and produced a leftward shift in the cumulative probability distribution of sEPSC inter-event intervals relative to VEH after memory retrieval or AM251 after no-memory retrieval (**Fig. 6I**; Kruskal-Wallis test, $H_{(3)} = 72.14$, $p < 0.0001$, Dunn's test, $p < 0.05$). The effect of memory retrieval on sEPSC amplitude was also dependent on memory retrieval and treatment (**Fig. 6J**; ANOVA, context x treatment interaction, $F_{(1,20)} = 4.737$, $p = 0.04$; all other $Fs_{(1,20)} \leq 0.22$, $p \geq 0.65$), but *post-hoc* comparisons did not indicate group differences. Memory retrieval followed by VEH produced a leftward shift in the cumulative probability distribution of sEPSC amplitudes relative to no-memory retrieval (Kruskal-Wallis test, $H_{(3)} = 11.67$, $p = 0.01$, Dunn's test, $p < 0.05$), and AM251 administration after memory retrieval inhibited this shift relative to VEH (**Fig. 6K**; Dunn's test, $p < 0.05$).

To verify our measurements of non-pharmacologically separated composite EPSCs, we repeated our measurements on pharmacologically isolated AMPAR eEPSCs (**Fig. 7A**). In the presence of the NMDAR antagonist, AP5 (50 µM), neither memory retrieval nor systemic AM251 administration altered synaptic responses to IC stimulation, including peak AMPAR eEPSC amplitude at -60 mV (**Fig. 7B**; ANOVA, all $Fs_{(1,35)} \leq 3.30$, $p \geq 0.08$) and +30 mV (**Fig. 7C**; ANOVA, all $Fs_{(1,35)} \leq 0.68$, $p \geq 0.42$), or the AMPAR rectification index (**Fig. 7D**; ANOVA, all $Fs_{(1,34)} \leq 0.11$, $p \geq 0.75$).

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

In the presence of AP5, the mean AMPAR sEPSC frequency varied as a function of memory retrieval and AM251 treatment (**Fig. 7E**). Memory retrieval followed by VEH did not alter mean sEPSC frequency relative to no-memory retrieval (ANOVA, retrieval x treatment interaction, $F_{(1,41)} = 5.53$, $p = 0.02$; retrieval main effect, $F_{(1,41)} = 6.24$, $p = 0.02$; treatment main effect, $F_{(1,41)} = 1.68$, $p = 0.20$). However, systemic AM251 administration after memory retrieval, but not after no-memory retrieval, increased sEPSC frequency relative to VEH (**Fig. 7F**; Sidak's test, $p < 0.05$) and produced a leftward shift in the cumulative probability distribution of sEPSC inter-event intervals relative to VEH after memory retrieval or AM251 after no-memory retrieval (**Fig. 7G**; Kruskal-Wallis test, $H_{(3)} = 43.18$, $p < 0.0001$; Dunn's test, $p < 0.05$). Neither memory retrieval nor AM251 treatment altered the mean amplitude of sEPSCs (**Fig. 7H**; ANOVA, all $Fs_{(1,43)} \leq 0.73$, $p \geq 0.41$, Fig. figure caption) or the cumulative probability distribution of sEPSC amplitudes (**Fig. 7I**; Kruskal-Wallis test, $H_{(3)} = 0.28$, $p = 0.96$).

DISCUSSION

The main finding of this study is that CB1R signaling critically modulates memory reconsolidation processes necessary for subsequent drug context-induced cocaine-seeking behavior in an instrumental model of drug relapse. Furthermore, memory retrieval induces CB1R-dependent changes in IEG expression, glutamatergic receptor subunit phosphorylation, and excitatory synaptic transmission in the BLA during memory reconsolidation, and some of these changes are predictive of the magnitude of subsequent drug-seeking behavior.

Systemic CB1R antagonism during cocaine-memory reconsolidation (i.e., immediately after memory retrieval) reduced drug context-induced cocaine-seeking behavior three days later, relative to VEH (**Fig. 1**). The CB1R antagonist, AM251 does not alter inhibitory avoidance (Gobira et al., 2013) or grooming behaviors (Hodge et al., 2008) at similar doses, suggesting that AM251 is not acutely aversive. Furthermore, CB1R antagonism alone did not alter the expression of drug-seeking behavior despite its long half-life (i.e., 22-h; McLaughlin et al., 2003) (**Fig. 2**). These

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

observations suggest that CB1R signaling is necessary for cocaine-memory reconsolidation in an instrumental model of drug relapse, thereby expanding on the known involvement of CB1Rs in Pavlovian morphine, nicotine, and methamphetamine memory reconsolidation (Yu et al., 2009; Fang et al., 2011; De Carvalho et al., 2014). CB1R signaling is not required for social reward memory reconsolidation (Achterberg et al., 2014); as such, selective effects of CB1R antagonism on memory reconsolidation across drug classes and paradigms are especially encouraging from a substance use disorder treatment perspective.

Systemic CB1R antagonism inhibited molecular adaptations in the BLA during memory reconsolidation and at the time of the test for drug context-induced cocaine-seeking behavior three days later. Specifically, memory retrieval augmented zif268 and Arc expression during memory reconsolidation, and this effect was blocked by AM251 (**Fig. 4**). CB1R-dependent IEG expression in the BLA is probably required for memory re-stabilization given that intra-BLA zif268 or Arc antisense administration disrupts cocaine-CPP and explicit conditioned stimulus (CS)-cocaine-memory reconsolidation in other paradigms (Lee et al., 2005, 2006; Theberge et al., 2010; Alaghband et al., 2014). At test, there was a CB1R-dependent, positive relationship between the extent of BLA zif268 expression and the magnitude of drug context-induced cocaine-seeking behavior (**Fig. 5C**). This finding is consistent with reports of increased zif268 mRNA expression during reinstatement (Hearing et al., 2010; Ziółkowska et al., 2011) and suggests that BLA zif268 expression tracks drug context-induced motivation for cocaine. However, the *memory retrieval-dependent* AM251 effects indicate that diminished motivation for cocaine at test is due to CB1R antagonist-induced impairment in cocaine-memory strength or retrieval link establishment during memory reconsolidation.

Interestingly, cocaine-memory retrieval triggered CB1R-dependent increases in pGluN2B^{Y1472} and pGluA1^{S845}, consistent with enhanced GluN2B synaptic stability (Nakazawa et al., 2006) and GluA1 synaptic recruitment (Hong et al. 2010), respectively, as well as CB1R-

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

549 independent decreases in pGluA2^{Y876} (**Fig. 4**). Previous research has indicated the necessity of
550 PKA activation (Arguello et al. 2014) and the importance of NMDAR-dependent CI-AMPAR
551 (GluA2-containing) endocytosis followed closely by CP-AMPAR (GluA2-lacking) synaptic
552 trafficking in the amygdala for memory reconsolidation (Clem and Huganir, 2010; Hong et al.,
553 2013; Lopez et al., 2015; Yu et al., 2016). In our study, decreases in pGluA2^{Y876}, consistent with
554 diminished Src-mediated CI-AMPAR endocytosis (Hayashi and Huganir, 2004), likely captured a
555 time point when CI-AMPAR synaptic expression was restored after transient endocytosis, while
556 CP-AMPAR synaptic insertion was still ongoing (Rao-Ruiz et al. 2015). Thus, cocaine-memory
557 reconsolidation may involve CB1R- and NMDAR-dependent CP-AMPAR synaptic insertion and
558 CB1R-independent CI-AMPAR endocytosis.

559 The robust effects of memory retrieval on IEG expression and glutamate-receptor subunit
560 phosphorylation were paralleled by subtle influences on BLA synaptic physiology observed in
561 slices prepared on average 30 min after memory retrieval and systemic AM251 treatment. During
562 reconsolidation, IC stimulation did not reveal changes in AMPA/NMDA ratio, eEPSC peak
563 amplitude, or eEPSC rectification in BLA PNs (**Fig. 6-7**). Similarly, fear-memory reconsolidation
564 is associated with equal-conductance exchange of CI-AMPARs to CP-AMPARs and no change
565 in IC→BLA synaptic strength, with a transient increase in rectification reported in slices prepared
566 5 min, but not one hour, after memory retrieval (Hong et al. 2013; Rao-Ruiz et al. 2015). Since
567 we observed molecular changes in the BLA 45 min after memory retrieval (**Fig. 4**), it is possible
568 that the temporal dynamics of IC→BLA synaptic plasticity during cocaine-memory and fear-
569 memory reconsolidation differ, or the timing of our synaptic physiology protocols did not permit
570 the detection of transient changes in rectification. Finally, the observed molecular adaptations in
571 the BLA might reflect changes in receptor trafficking at *non*-IC→BLA synapses or other cell types
572 (i.e., GABAergic interneurons).

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Importantly, we discovered memory retrieval-dependent increases in sEPSC frequency and reductions in peak sEPSC amplitude in BLA PNs (selected based on their responsiveness to IC stimulation, **Fig. 6-7**). Moreover, sEPSC frequency increases were only observed when CB1Rs were blocked. This suggests that potential cocaine-memory retrieval-associated plasticity occurs at *non*-IC excitatory inputs to BLA PNs (e.g., sensory cortices, prefrontal cortex, or ventral hippocampus; LeDoux, 2007; Sah et al., 2003), and systemic CB1R antagonism enables potentiation at these inputs, and may create "synaptic noise" that impedes plasticity associated with memory strength or reconsolidation. Thus, CB1R signaling may facilitate memory reconsolidation by reducing "synaptic noise." Additionally, memory retrieval-dependent reductions in sEPSC peak amplitude (**Fig. 6J-K)** were observed in a CB1R- and NMDAR-dependent manner **(Fig. 7H-I**). If the rising phase of the NMDAR component contributed to the peak composite sEPSC, then increases in BLA pGluN2B^{Y1472} (**Fig. 4**) may explain the reductions in sEPSC amplitude given the lower open probability of GluN2B-containing NMDARs (Paoletti et al., 2013). The involvement of GluN2B in appetitive memory reconsolidation has not been investigated until now, but earlier studies indicate that GluN2B mediates Pavlovian fear-memory destabilization, but not re-stabilization (Ben Mamou et al., 2006; Milton et al., 2013). Consequently, future research will need to evaluate whether the contributions of GluN2B-containing NMDARs to memory reconsolidation are paradigm specific.

Collectively, cocaine-memory reconsolidation involves at least two forms of plasticity at BLA PN synapses: (a) a memory retrieval-induced increase in excitability or vesicle release probability of glutamatergic afferents that is inhibited by CB1R signaling and (b) an NMDAR- and CB1R-dependent decrease in sEPSC amplitude. The former finding implies that CB1R signaling is necessary to *reduce* glutamatergic synaptic excitation of BLA PNs during memory reconsolidation. Initially, this appears to be contradictory to effects of CB1R antagonism on memory reconsolidation in the present study and to the known role of glutamate in memory

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

598 reconsolidation (Milton et al., 2008; Rao-Ruiz et al., 2015). However, it is consistent with the
599 recognized role of CP-AMPARs in synaptic scaling, a plasticity mechanism important for
600 rebalancing neuronal excitability (Diering and Huganir, 2018). CP-AMPARs preferentially
601 accumulate at synapses after LTP (Turrigiano, 2008; Turrigiano et al., 2014; Diering and Huganir,
602 2018) and facilitate synaptic resistance to down-scaling and ubiquitination (Diering et al., 2014;
603 Sanderson et al., 2016). Future studies will need to investigate whether synaptic scaling is a
604 plasticity mechanism of memory reconsolidation.

605 Contrary to our hypothesis, the effects of AM251 on drug-seeking behavior and BLA
606 protein expression were not present 24 days post treatment (**Fig. 5**). Transient amnesia could
607 result from AM251-induced enhancement in extinction memory consolidation followed by
608 spontaneous recovery. However, this was unlikely because brief re-exposure to the cocaine-
609 paired context did not elicit either appreciable behavioral extinction or synaptic depression in
610 IC→BLA synapses, a phenomenon associated memory extinction (Clem and Huganir, 2010; Rich
611 et al., 2019). Furthermore, testing occurred immediately after two extinction sessions, as opposed
612 to after time away from the testing environment that is required for spontaneous recovery
613 (Rescorla, 2004). It is more likely, instead, that the transient effects of AM251 reflected the
614 delayed availability of alternate memory traces following amnesia due to reconsolidation
615 interference (Nadel and Moscovitch, 1997). Alternatively, AM251 could inhibit neural ensembles
616 that form “retrieval links” (Lewis et al., 1968). Consistent with this idea, reactivation of neural
617 ensembles that are active during encoding is necessary and sufficient for memory expression
618 (Ramirez et al., 2013; Roy et al., 2016; Richards and Frankland, 2017).

619 In conclusion, systemic administration of AM251 in conjunction with re-exposure to the
620 cocaine-paired context, a manipulation analogous to exposure therapy, blocks glutamatergic
621 mechanisms associated with memory reconsolidation and transiently alleviates drug context-
622 induced motivation to seek cocaine in a rodent model of drug relapse. Thus, AM251 may provide

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

623 short-term benefit for individuals with cocaine-use disorders, similar to β-adrenergic receptor
624 antagonism (Saladin et al., 2013). Furthermore, the CB1R may be a suitable therapeutic target
625 for interference with the reconsolidation of maladaptive memories, even though AM251 also has
626 some efficacy as a GPR55 (CB1 orphan receptor) agonist (Kapur et al., 2009) and μ-opioid
627 receptors antagonist (Seely et al., 2012). While additional research is needed to determine the
628 exact mechanisms of action for AM251, the present findings are interesting from a
629 pharmacotherapeutic perspective. *Chronic* daily treatment with the CB1R inverse agonist,
630 rimonabant, is effective for smoking cessation but produces detrimental side effects (Moreira and
631 Crippa, 2009). Therefore, future studies will need to examine whether a less extensive regimen
632 of *repeated* post-retrieval administration of AM251 or other CB1R antagonists can safely produce
633 lasting reductions in cue reactivity and cocaine seeking without detrimental side effects.

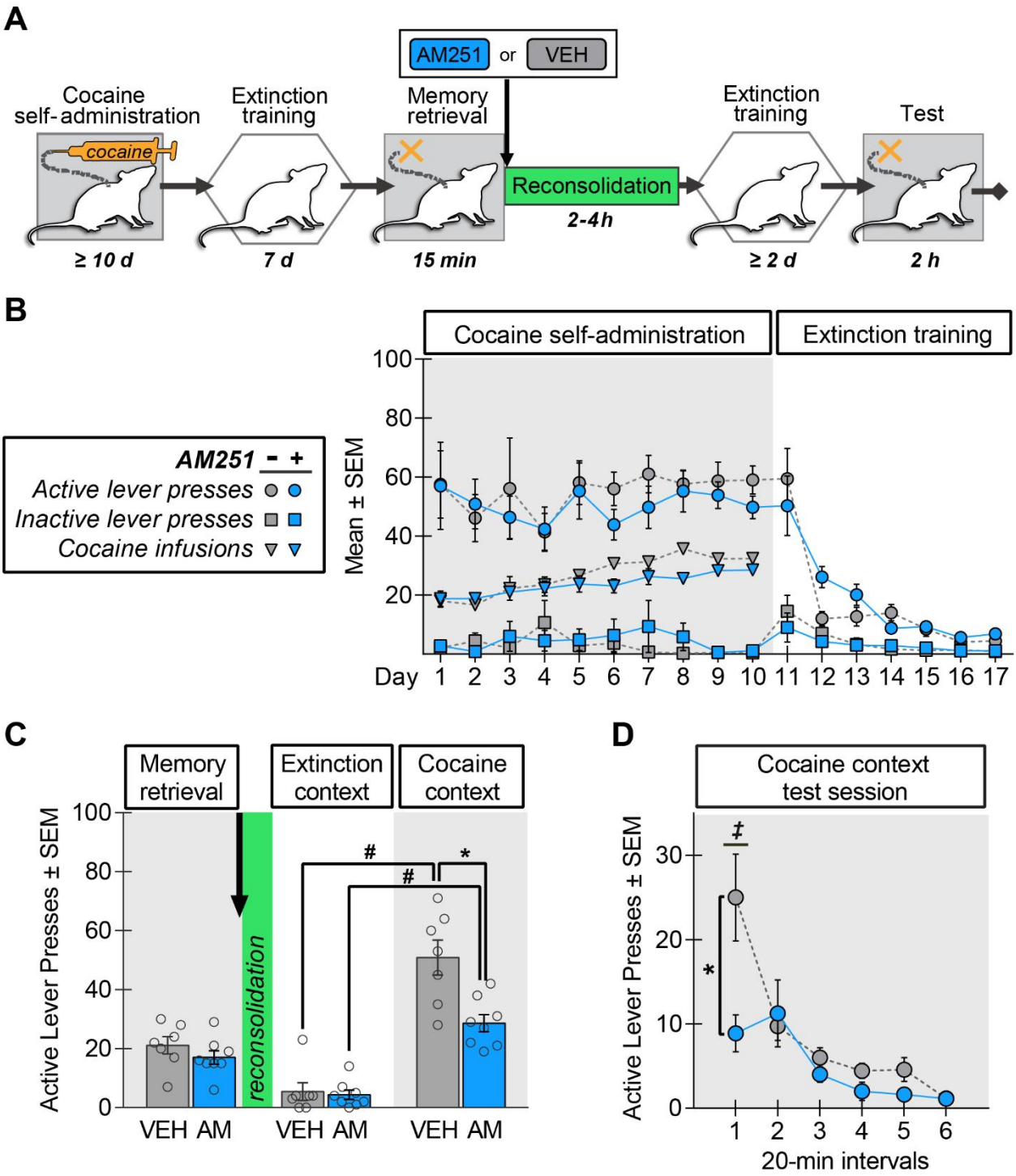
634

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

635 **Figure 1. Systemic AM251 administration *during* memory reconsolidation reduces drug**
636 **context-induced cocaine-seeking behavior three days later. (A)** Experimental timeline. The
637 15-min memory-retrieval session was immediately followed by systemic AM251 (AM; 3 mg/kg,
638 i.p.; *n* = 8) or VEH (*n* = 7) administration. After at least two additional daily extinction sessions,
639 drug-seeking behavior was assessed in the cocaine-paired context. **(B)** Lever responses and
640 cocaine infusions during drug self-administration (last 10 d) and extinction training. **(C)** Active-
641 lever responses during the memory retrieval session in the cocaine-paired context before
642 treatment (*arrow*) and upon first re-exposure to the extinction context and the cocaine-paired
643 context at test. **(D)** Time course of active-lever presses in the cocaine-paired context at test.
644 **Symbols**: ANOVA $^{\#}$context simple main effect, Sidak's test, $p < 0.05$; *****treatment simple main
645 effect, Sidak's test, $p < 0.05$ (Panel C), Tukey's test, $p < 0.05$ (Panel D); ‡time simple main effects
646 (VEH: interval 1 > 2-6; AM251: intervals 1-2 > 4-6), Tukey's tests, ps < 0.05.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

647 **FIGURE 1**



648

649

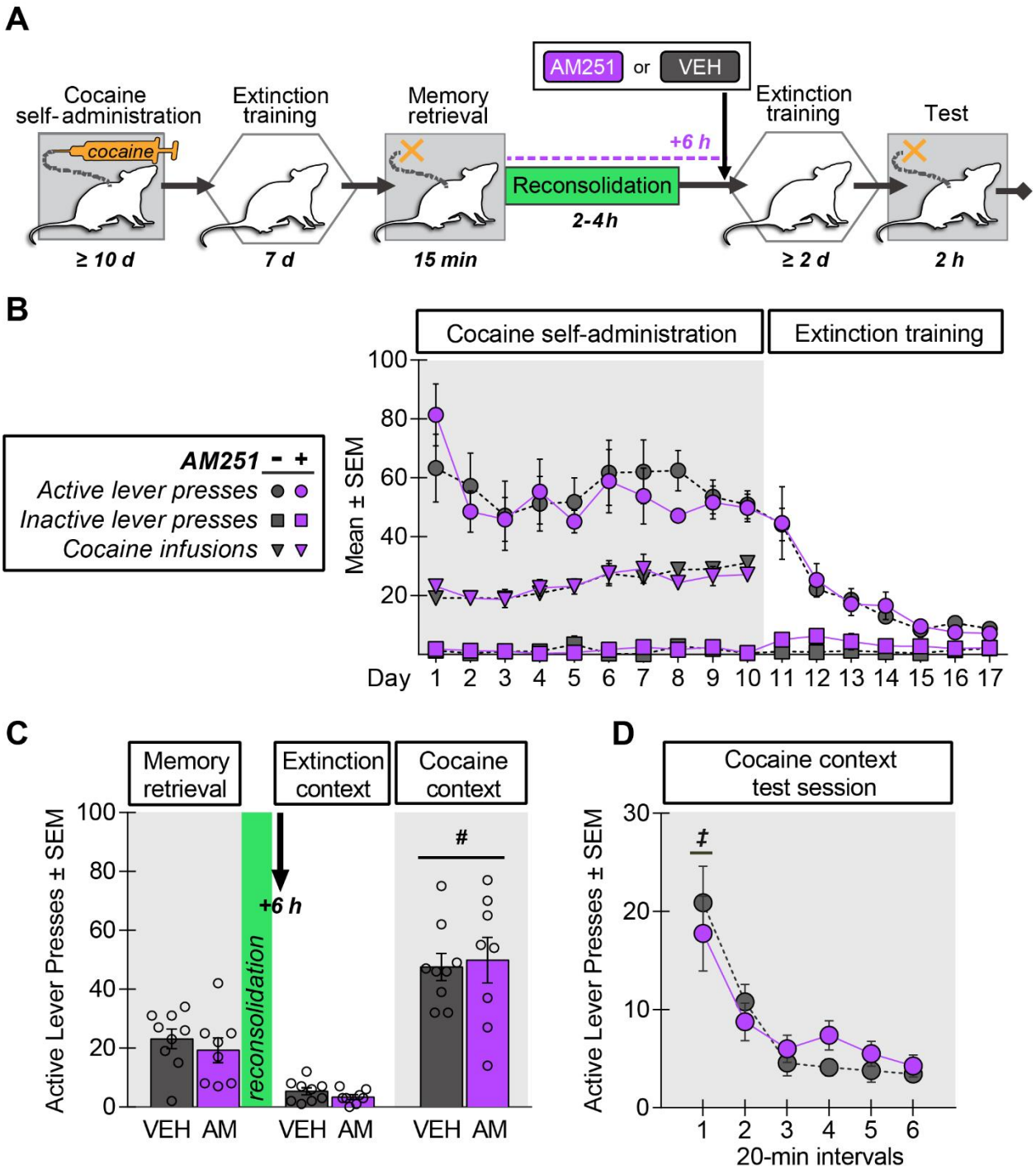
650

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 2. Systemic AM251 administration *after* memory reconsolidation fails to alter context-induced cocaine-seeking behavior three days later. (A) Experimental timeline. The 15-min memory-retrieval session was followed by systemic AM251 (AM; 3 mg/kg, i.p.; *n* = 8) or VEH (*n* = 9) administration 6 hours later (i.e., outside of the memory reconsolidation window). After at least two additional daily extinction sessions with ≤ 25 active-lever responses, drug-seeking behavior was assessed in the cocaine-paired context. **(B)** Lever responses and cocaine infusions during drug self-administration and extinction training. **(C)** Active-lever responses during the memory retrieval session in the cocaine-paired context before treatment (*arrow*) and upon first re-exposure to the extinction context and the cocaine-paired context at test. **(D)** Time course of active-lever responses across in the cocaine-paired context at test. Omnibus ANOVA effects are reported in the Results section. **Symbols**: ANOVA $^{\#}$context main effect, $p < 0.05$; ‡time simple main effects (interval 1 > 2-6), Tukey's tests, ps < 0.05.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

663 **FIGURE 2**



664

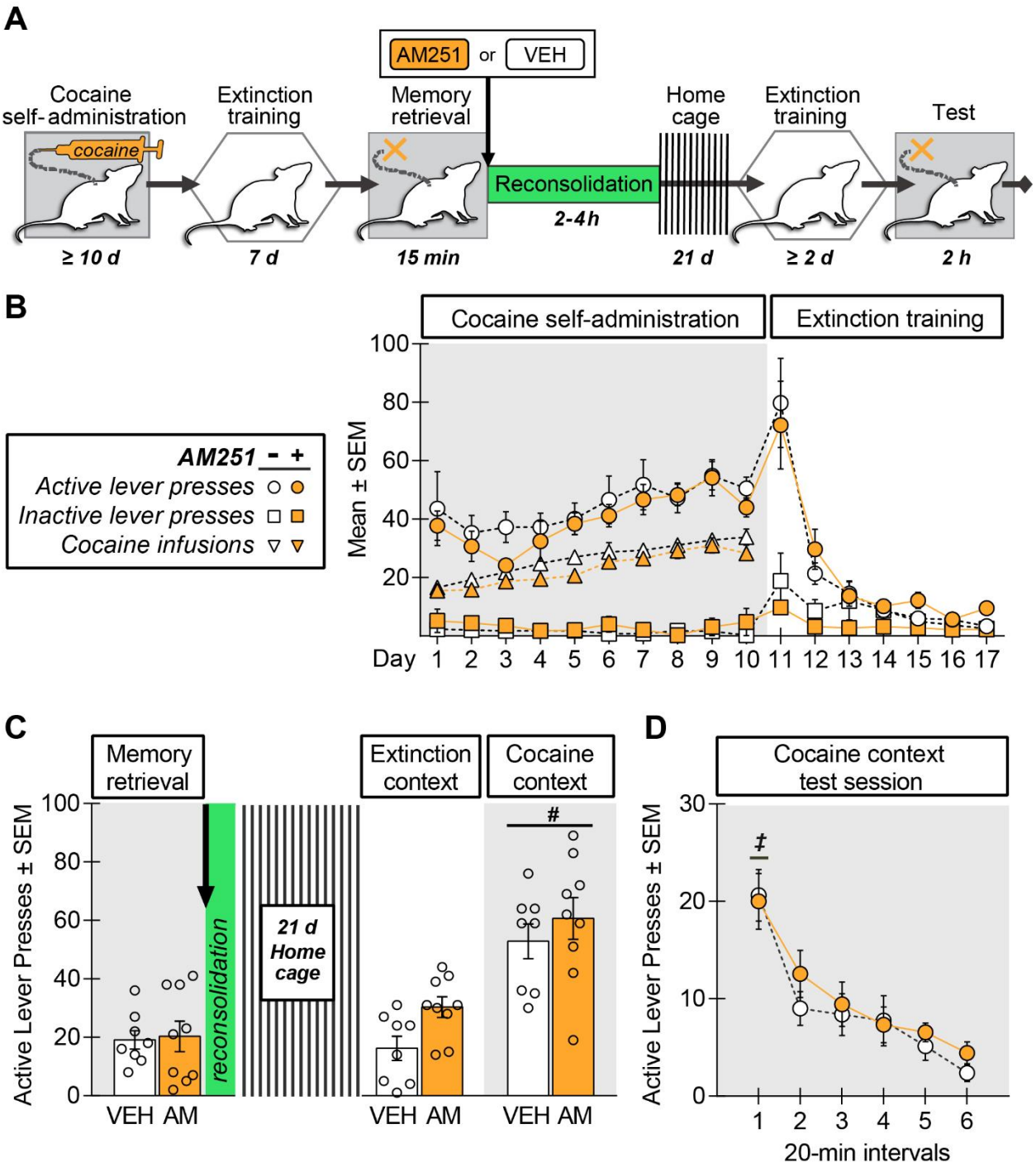
665

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 3. Systemic AM251 administration *during* memory reconsolidation does not alter drug context-induced cocaine-seeking behavior 24 days later. (A) Experimental timeline. The 15-min memory-retrieval session was immediately followed by systemic administration of AM251 (AM; 3 mg/kg, i.p.; *n* = 9) or VEH (*n* = 8). After 21 days of home cage stay, rats received at least two additional daily extinction sessions with ≤ 25 active-lever responses prior to the test of drug-seeking behavior in the cocaine-paired context. **(B)** Lever responses and cocaine infusions during drug self-administration and extinction training. **(C)** Active-lever responses during the memory-retrieval session in the cocaine-paired context before treatment (*arrow*) and after treatment followed by 21 days of home cage stay, upon first re-exposure to the extinction context and the cocaine-paired context at test. **(D)** Time course of active-lever responses in the cocaine-paired context at test. **Symbols**: ANOVA $^{\#}$context main effect, $p < 0.05$; ‡time simple main effects (interval 1 > 2-6), Tukey's tests, ps < 0.05.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

678 **FIGURE 3**



679

680

681

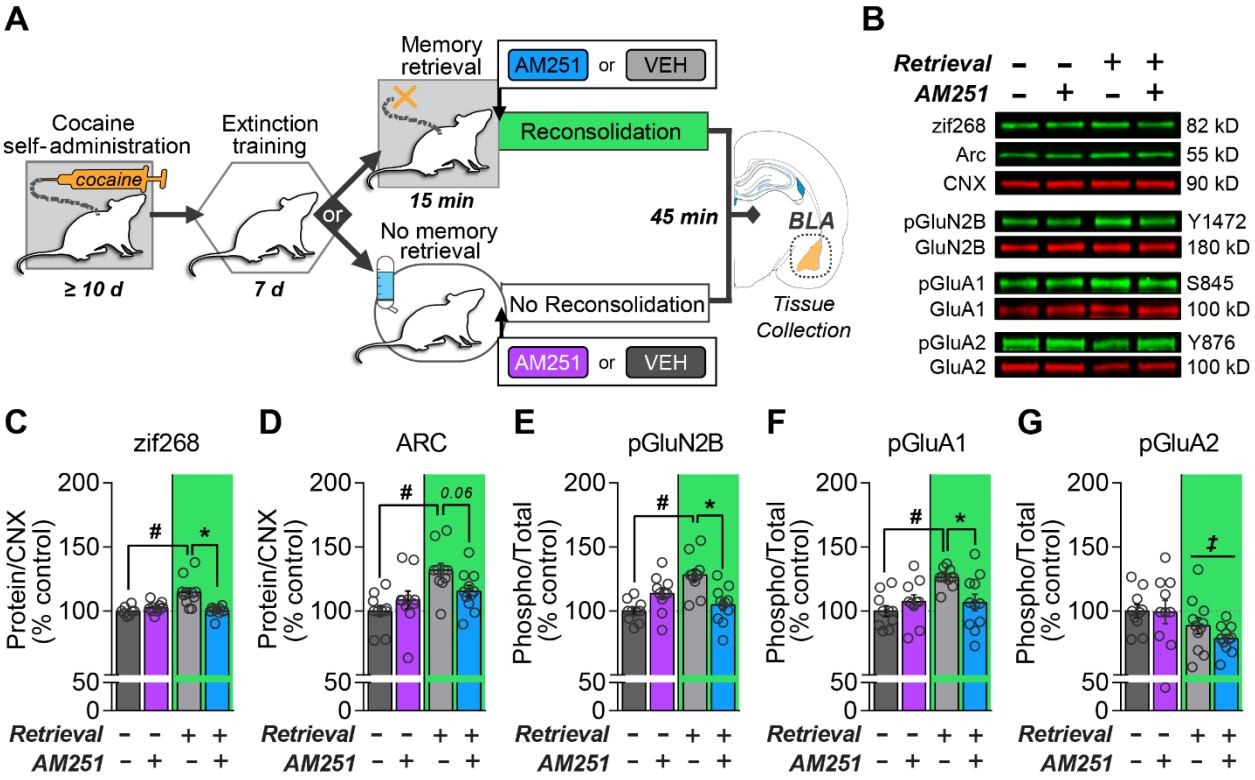
bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 4. Systemic AM251 administration after memory retrieval prevents changes in BLA IEG expression and glutamate receptor subunit phosphorylation *during* memory reconsolidation. (A) Experimental timeline. Systemic AM251 (3 mg/kg, i.p.) or VEH was administered immediately after cocaine-memory retrieval or no-memory retrieval (*n* = 10 to 11 per group). BLA tissue samples were collected 45 min later for analysis of full tissue homogenates. **(B)** Representative western blots. Mean **(C)** zif268 and **(D)** ARC levels normalized to the loading control, calnexin (CNX). Mean levels of **(E)** GluN2B phosphorylation at Y1472 (pGluN2B), **(E)** GluA1 phosphorylation at S845 (pGluA1), and **(F)** GluA2 phosphorylation at Y876 (pGluA2) normalized to total protein levels. See extended **Figure 4-1** for control protein measures. Values are expressed as a percentage of the no-memory retrieval VEH control group. **Symbols**: ANOVA, $^{\#}$retrieval simple main effect, Sidak's test, $p < 0.05$, *****treatment simple main effect, Sidak's test, $p < 0.05$, ‡retrieval main effect, $p < 0.05$.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

694 **FIGURE 4**

695



696

697

698

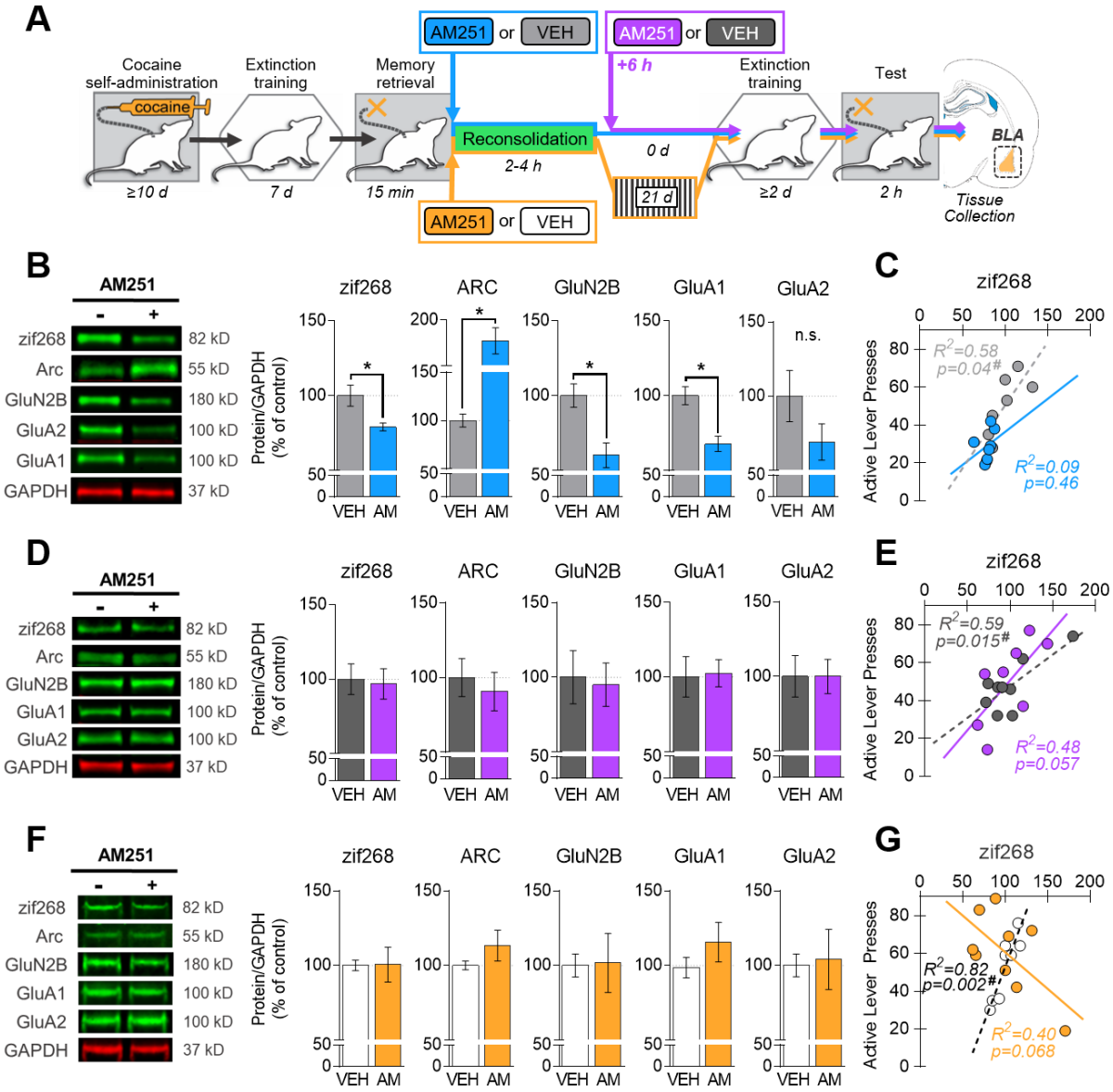
699

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 5. Systemic AM251 administration after memory retrieval elicits time-dependent changes in BLA IEG and total glutamate receptor subunit expression levels at test and in relationship with the magnitude of drug-seeking behavior. (A) Experimental timeline. Systemic AM251 (AM; 3 mg/kg, i.p.) or VEH was administered immediately or six hours after cocaine-memory retrieval. Cocaine-seeking behavior was assessed in the cocaine-paired context three or 24 days later, after at least two extinction sessions in Experiments 1-3. BLA tissue samples were collected immediately after the test session. **(B)** Effects of systemic AM251 administration immediately after memory retrieval on total protein levels (mean ± SEM) in the BLA at test, three days later in Experiment 1. **(C)** Relationship between total zif268 protein levels and active-lever presses at test in Experiment 1. **(D)** Effects of systemic AM251 administration 6 hours after memory retrieval on total protein levels (mean ± SEM) in the BLA at test, three days later in Experiment 2. **(E)** Relationship between total zif268 protein levels and active-lever presses at test in Experiment 2. **(F)** Effects of systemic AM251 administration immediately after memory retrieval on total protein levels (mean ± SEM) in the BLA at test, 24 days later in Experiment 3. **(G)** Relationship between total zif268 protein levels and active-lever presses at test in Experiment 3. Values were normalized to the loading control, GAPDH, and expressed as a percentage of the VEH-treated group. **Symbols**: *t-test, $p < 0.05$; #Pearson's r correlation coefficient, $p < 0.05$ (*n* = 7-9 per group).

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

718 **FIGURE 5**



719

720

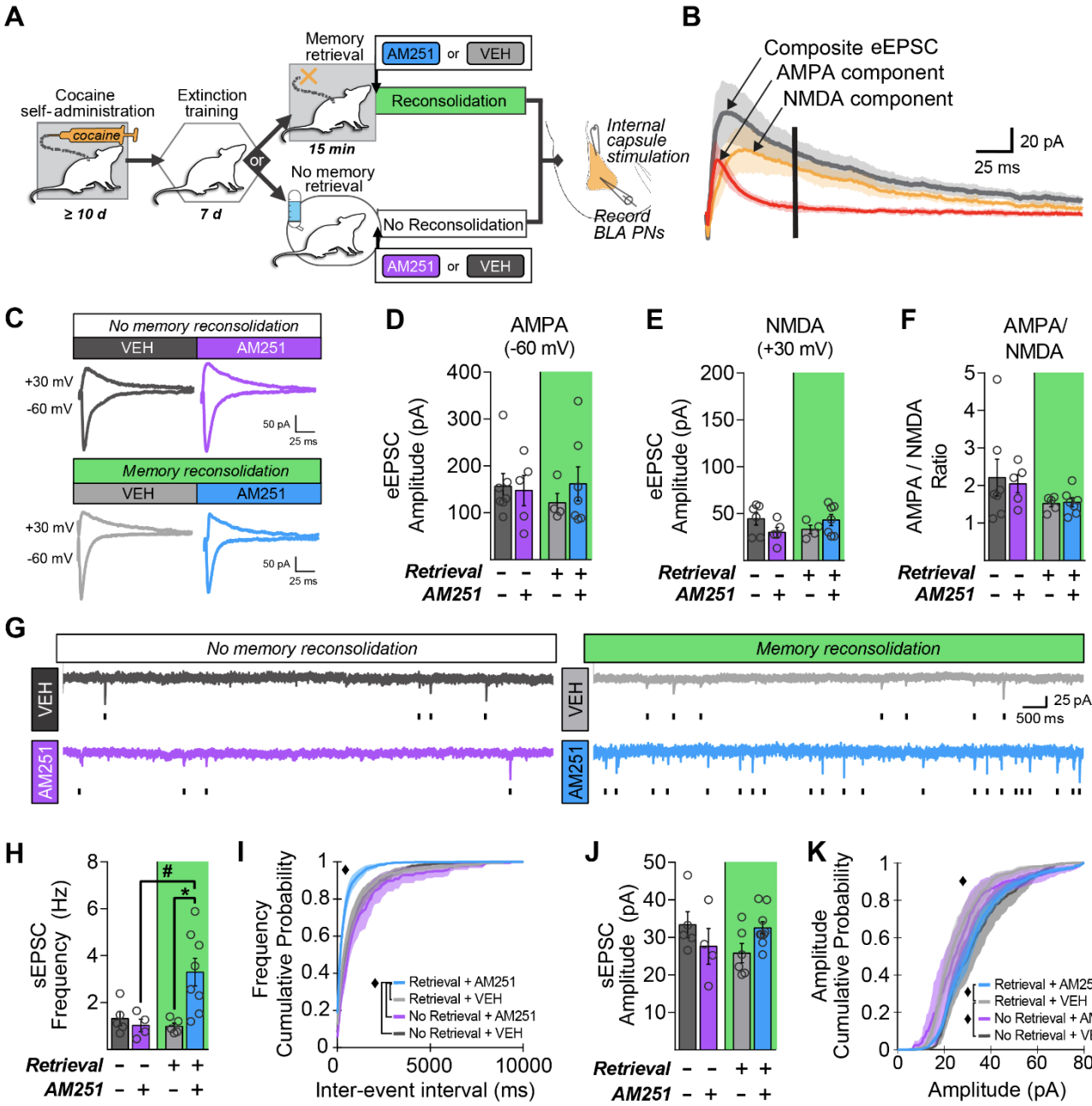
721

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 6. Systemic AM251 administration after memory retrieval increases sEPSC frequency and amplitude in BLA PNs during cocaine-memory reconsolidation. (A) Experimental timeline. AM251 (3 mg/kg, i.p.) or VEH was administered immediately after cocaine-memory retrieval or no-memory retrieval (See **Fig. 6-1** for behavioral history; *n* = 4 to 5 per group). Brain slices were prepared for whole-cell recording within an average of 30 min after treatment. Schematic depicting stimulating electrode placement in the internal capsule and recording electrode placement in the basal nucleus. **(B)** eEPSCs (mean across all cells recorded ± SEM) recorded at +30 mV in aCSF (*gray*) or AP5 (leaving the isolated AMPAR component, *red*), and superimposed digital subtraction (to visualize digitally-derived NMDAR component, *orange*). NMDAR-mediated eEPSC amplitudes were measured 50 ms after stimulus onset to eliminate AMPAR component contribution (*black bar*). **(C)** Representative recordings of eEPSC obtained from BLA PN cells in aCSF, in each group at -60 mV and +30 mV holding potentials. Peak amplitude of AMPA (mean ± SEM, at -60 mV; **D**) and NMDA (mean ± SEM, at +30 mV; **E**) components in aCSF. **(F)** AMPA/NMDA ratio (mean ± SEM). **(G)** Representative sEPSC recordings with ticks indicating individual events. **(H)** sEPSC frequency (mean ± SEM) and **(I)** the cumulative probability distribution of sEPSC inter-event intervals (mean ± SEM). **(J)** sEPSC amplitude (mean ± SEM) and **(K)** the cumulative probability distribution of sEPSC amplitudes (mean ± SEM). **Symbols**: ANOVA, $^{\#}$retrieval simple main effect, Sidak's test, $p < 0.05$; *****treatment simple main effect, Sidak's test, $p < 0.05$; **♦**Kruskal-Wallis test, $p < 0.05$, Dunn's test, $p < 0.05$.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

741 **FIGURE 6**



742

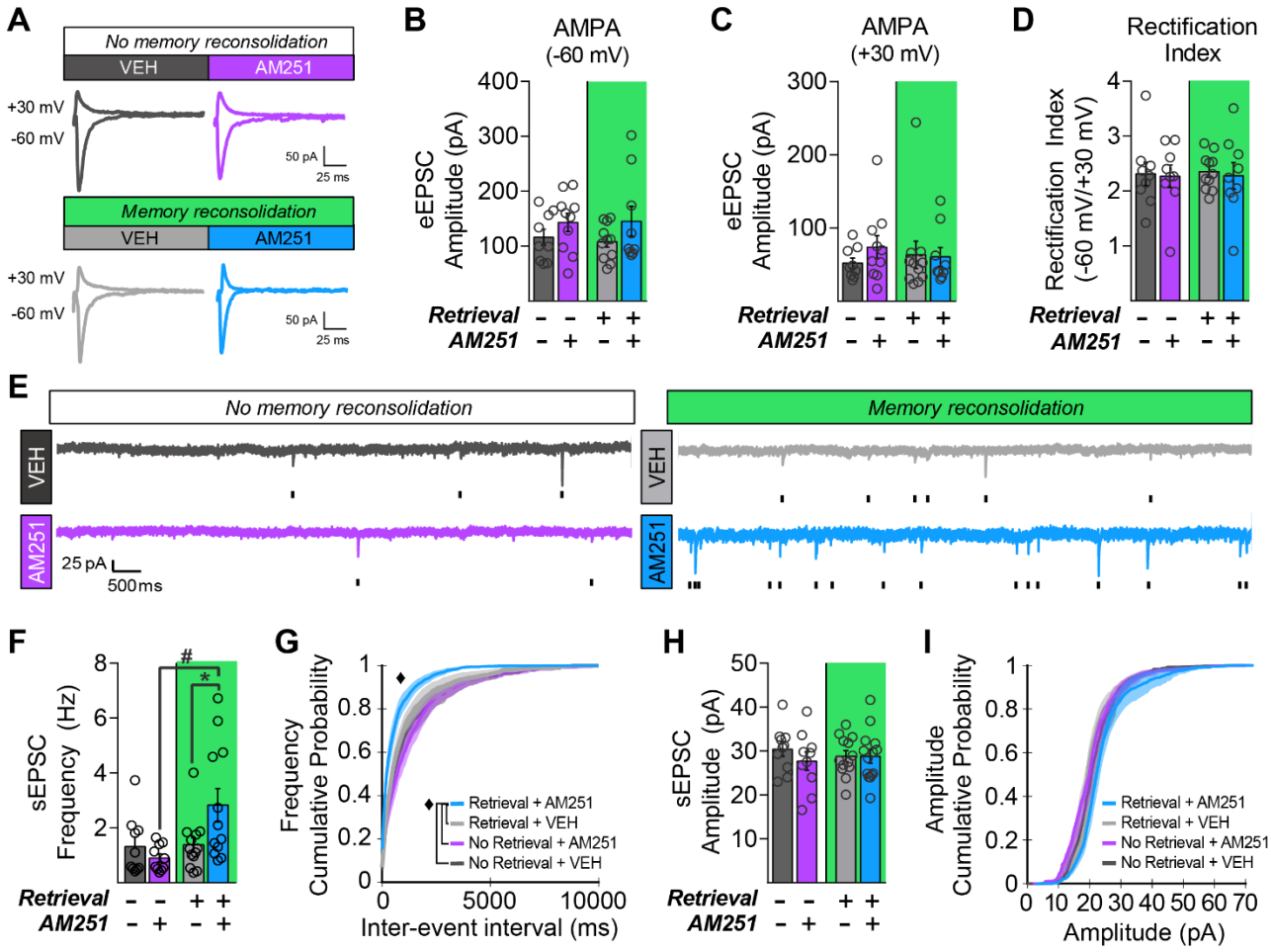
743

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

744 **Figure 7. Systemic AM251 administration after memory retrieval increases AMPAR-**
745 **mediated sEPSC frequency in BLA PNs during cocaine-memory reconsolidation. (A)**
746 Representative eEPSCs recordings in the presence of the NMDAR antagonist, AP5, at +30 mV
747 and -60 mV. Peak AMPAR-mediated eEPSC amplitudes (mean ± SEM) **(B)** at – 60 mV and **(C)**
748 +30 mV. **(D)** Rectification index calculated as the ratio of the peak eEPSC amplitude at -60 mV
749 and +30 mV in the presence of AP5. **(E)** Representative sEPSC recordings with ticks indicating
750 individual events. **(F)** sEPSC frequency (mean ± SEM). **(G)** Cumulative probability distribution of
751 sEPSC inter-event intervals (mean ± SEM). **(H)** sEPSC amplitude (mean ± SEM) and **(I)**
752 cumulative probability distribution of amplitudes (mean ± SEM). **Symbols**: ANOVA, $^{\#}$retrieval
753 simple main effect, Sidak's test, $p < 0.05$; *****ANOVA, treatment simple main effect, Sidak's test, p
754 < 0.05; **♦**Kruskal-Wallis test, $p < 0.05$, Dunn's test, $p < 0.05$.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

FIGURE 7



bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

EXTENDED DATA

FIGURE 1-1

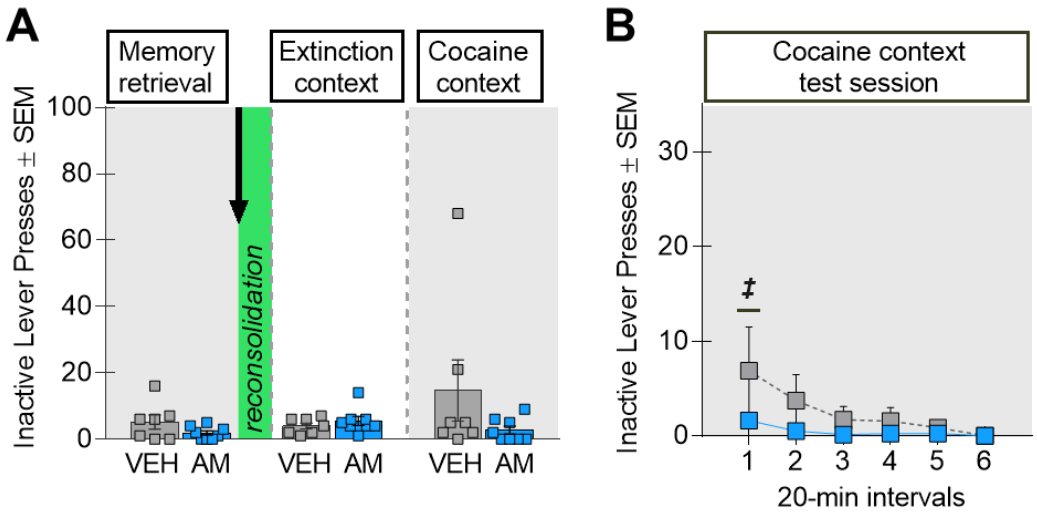


Figure 1-1. Inactive-lever responding in Experiment 1. (A) Inactive-lever responding was not different between the groups during the 15-min memory-retrieval session (*left panel*) before administration of AM251 (AM, $n = 8$) or VEH ($n = 7$) ($t_{(13)} = 1.54$, $p = 0.15$) (*arrow*). After treatment, inactive-lever responding remained low in the extinction and cocaine-paired contexts independent of treatment (ANOVA, all $Fs_{(1,13)} \leq 2.52$, $p \geq 0.14$). **(B)** Inactive-lever responding in the cocaine-paired context at test declined over time independent of treatment (ANOVA, time main effect, $F_{(5,65)} = 2.71$, $p = 0.03$, ‡time simple main effects, Tukey's tests, interval 1 > 2-6, ps < 0.05).

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

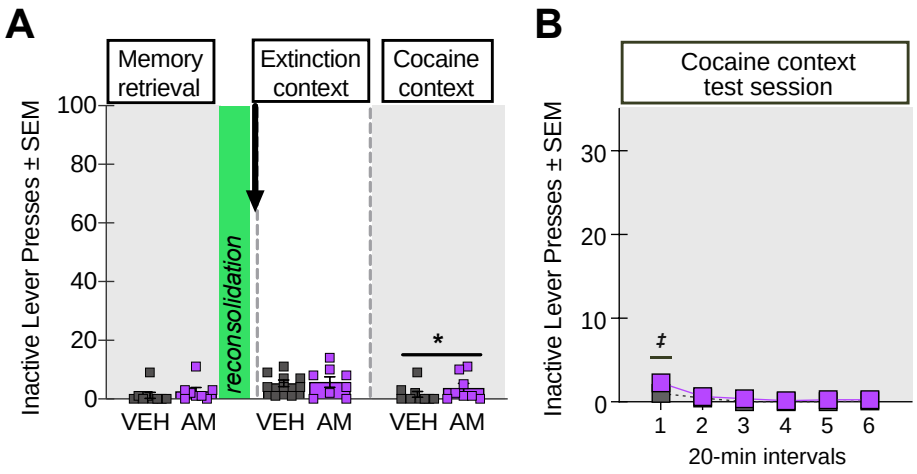
Table 1-1 | Experiment 1 Behavioral History

		Treatment Main Effects				Day Main Effects				Treatment x Day			
Phase	**Measure**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**
Self-Administration	Active Lever	F	1,13	0.60	0.45	F	9,117	0.86	0.57	F	9,117	0.29	0.74
	Inactive Lever	F	1,13	2.29	0.15	F	9,117	1.16	0.33	F	9,117	1.20	0.30
	Cocaine Infusion	F	1,13	0.15	0.71	F	9,117	20.19	<0.0001	F	9,117	2.84	*0.01
Extinction	Active Lever	F	1,13	0.21	0.66	F	6,78	38.91	<0.0001	F	6,78	1.91	0.89
	Inactive Lever	F	1,13	0.22	0.65	F	6,78	8.23	<0.0001	F	6,78	0.81	0.57
Memory Retrieval	Active Lever	t	13	0.20	0.67								
	Inactive Lever	t	13	3.80	0.07								

**VEH > AM251 on day 8*

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

770 **FIGURE 2-1**



771

772 **Figure 2-1. Inactive-lever responding for Experiment 2. (A)** Inactive-lever responding was not
773 different between the groups during the 15-min memory retrieval session (*left panel*) before
774 administration of AM251 (AM, *n* = 8) or VEH (*n* = 9) ($t_{(15)} = 0.89$, p = 0.39) (*arrow*). After treatment,
775 VEH-treated rats exhibited less inactive-lever responding in the cocaine-paired context relative to
776 the extinction context (ANOVA, *****context main effect, $F_{(1,15)} = 6.99$, p = 0.02). **(B)** Inactive-lever
777 responding declined over time during the test in the cocaine-paired context independent of
778 treatment (ANOVA, time main effect, $F_{(5,75)} = 7.35$, p < 0.0001; **‡**time simple main effects, Tukey's
779 tests, interval 1 > 2-6, ps < 0.05).

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

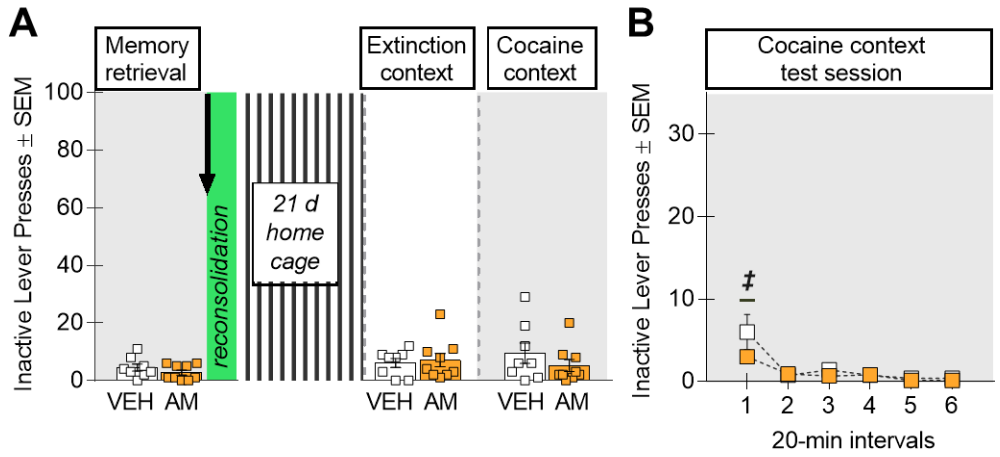
780 **Table 2-1 | Experiment 2 Behavioral History**

		Treatment Main Effects				Day Main Effects				Treatment x Day			
Phase	**Measure**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**
Self-Administration	Active Lever	F	1,15	0.18	0.68	F	9,135	1.57	0.13	F	9,135	0.58	0.81
	Inactive Lever	F	1,15	0.76	0.65	F	9,135	0.62	0.78	F	9,135	0.76	0.65
	Cocaine Infusion	F	1,15	0.02	0.71	F	9,135	7.09	<0.0001	F	9,135	0.90	0.53
Extinction	Active Lever	F	1,15	0.00	0.95	F	6,90	29.05	<0.0001	F	6,90	0.27	0.95
	Inactive Lever	F	1,15	4.48	0.05	F	6,90	1.18	0.32	F	6,90	1.89	0.09
Memory Retrieval	Active Lever	t	15	0.34	0.57								
	Inactive Lever	t	15	0.21	0.65								

781

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

782 **FIGURE 3-1**



783

784 **Figure 3-1. Inactive-lever responding in Experiment 3. (A)** Inactive-lever responding was not
785 different between the groups during the 15-min memory retrieval session (*left panel*) before
786 administration of AM251 (AM, *n* = 9) or VEH (*n* = 8) ($t_{(15)}$ = 1.15, p = 0.27) (*arrow*). After treatment,
787 inactive-lever responding remained low in the extinction and cocaine-paired contexts independent
788 of treatment (ANOVA, all $Fs_{(1,15)} \leq 1.45$, $p \geq 0.25$). **(B)** Inactive-lever responding declined over
789 time in the cocaine-paired context at test independent of treatment (ANOVA, time main effect,
790 $F_{(5,75)} = 13.31$, $p < 0.0001$; ‡ time simple main effects, Tukey's tests, interval 1 > 2-6, ps < 0.05).

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Table 3-1 | Experiment 3 Behavioral History

		Treatment Main Effects				Day Main Effects				Treatment x Day			
Phase	**Measure**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**
Self-Administration	Active Lever	F	1,15	0.93	0.35	F	9,135	4.86	<0.0001	F	9,135	0.30	0.97
	Inactive Lever	F	1,15	2.29	0.15	F	9,117	0.48	0.89	F	9,135	0.56	0.83
	Cocaine Infusion	F	1,15	0.75	0.40	F	9,117	21.33	<0.0001	F	9,135	0.50	0.87
Extinction	Active Lever	F	1,15	0.19	0.67	F	6,90	34.43	<0.0001	F	6,90	0.40	0.88
	Inactive Lever	F	1,15	1.83	0.20	F	6,90	5.15	<0.0001	F	6,90	1.03	0.41
Memory Retrieval	Active Lever	t	15	4.31	0.55								
	Inactive Lever	t	15	0.08	0.78								

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

FIGURE 4-1

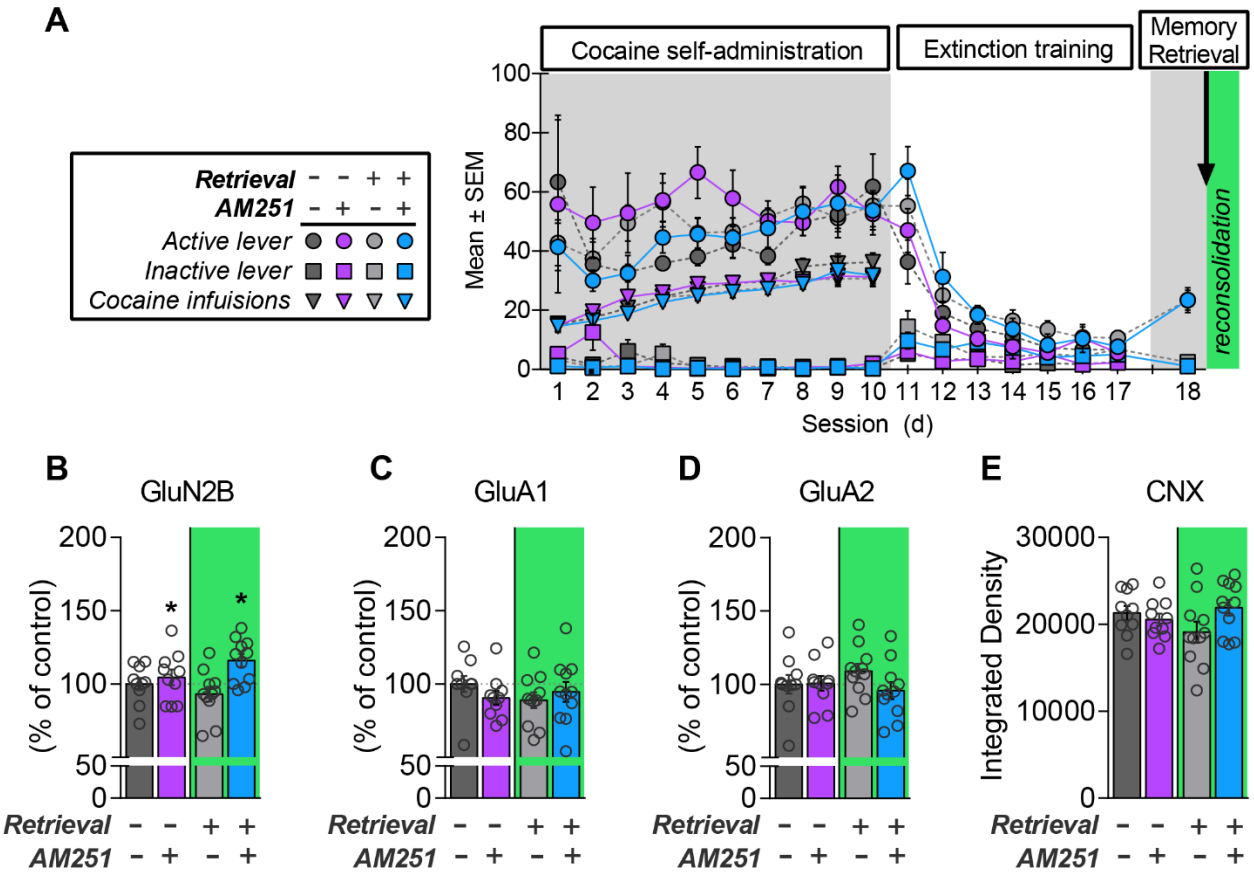


Figure 4-1. Systemic AM251 treatment increases total GluN2B, but not total GluA1 or GluA2 levels in the BLA during memory reconsolidation. (A) Lever responses and cocaine infusions during drug self-administration, extinction training, and the 15-min memory-retrieval session in the cocaine-paired context prior to treatment (*arrow*) in Experiment 4a. **(B)** AM251 increased total GluN2B protein levels independent of memory retrieval (ANOVA, *treatment main effect; p = 0.001). There was no effect of memory retrieval or AM251 treatment on **(C)** total GluA1 or **(D)** total GluA2 protein levels, or on **(E)** the raw integrated density values for the house-keeping protein, calnexin (CNX).

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

803 **Table 4-1 | Experiment 4 Behavioral History**

		Treatment Main Effects				Day Main Effects				Treatment x Day			
Phase	**Measure**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**
Self-Administration	Active Lever	F	3,38	1.19	0.33	F	9,342	1.96	0.43	F	27,342	0.44	0.79
	Inactive Lever	F	3,38	1.97	0.14	F	9,342	3.50	<0.0001	F	27,342	2.47	<0.0001
	Cocaine Infusion	F	3,38	0.65	0.59	F	9,342	51.31	<0.0001	F	27,342	0.77	0.79
Extinction	Active Lever	F	3,38	2.98	0.04	F	6,228	64.27	<0.0001	F	18228	1.56	0.07
	Inactive Lever	F	3,38	1.49	0.23	F	6228	5.47	<0.0001	F	18,228	1.21	0.26
Memory Retrieval	Active Lever	t	20	0.94	0.34								
	Inactive Lever	t	20	1.31	0.27								

804

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

805 **Table 5-1 | Relationship between total protein levels and active-lever presses at test**

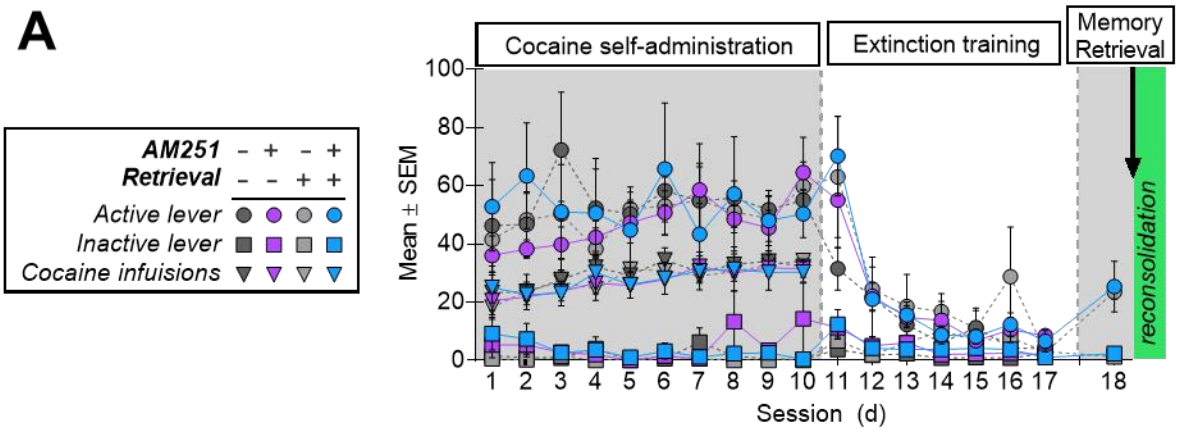
	Experiment 1				Experiment 2				Experiment 3			
	VEH		AM251		VEH		AM251		VEH		AM251	
Target Protein	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*
ARC	0.17	0.36	0.22	0.25	0.12	0.36	0.01	0.86	0.002	0.91	0.26	0.16
GluN2B	0.04	0.68	0.11	0.43	0.01	0.86	0.04	0.63	0.22	0.24	0.04	0.62
GluA1	0.36	0.12	0.15	0.4	0.01	0.77	0.02	0.77	0.001	0.94	0.04	0.62
GluA2	0.37	0.15	<0.001	0.99	<0.001	0.98	0.004	0.88	0.29	0.17	0.01	0.84

806

807

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

808 **FIGURE 6-1**



809

810 **Figure 6-1. Behavioral history in Experiment 5 (A)** Lever responses and cocaine infusions
811 during drug self-administration, extinction training, and the 15-min memory-retrieval session in
812 the cocaine-paired context prior to treatment (*arrow*). Statistics are provided in Table 6-1.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Table 6-1 | Experiment 5 Behavioral History

		Treatment Main Effects				Day Main Effects				Treatment x Day			
Phase	**Measure**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**
Self-Administration	Active Lever	F	3,13	0.29	0.83	F	9,117	0.75	0.66	F	27,117	0.69	0.86
	Inactive Lever	F	3,13	1.13	0.37	F	9,117	0.77	0.65	F	27,117	1.19	0.26
	Cocaine Infusion	F	3,13	0.11	0.95	F	9,117	7.32	<0.0001	F	27,117	0.55	0.96
Extinction	Active Lever	F	3,12	0.10	0.96	F	6,72	22.61	<0.0001	F	6,72	0.21	1.00
	Inactive Lever	F	3,12	1.04	0.41	F	6,72	6.63	<0.0001	F	6,72	1.06	0.41
Memory Retrieval	Active Lever	t	7	5.00	0.06								
	Inactive Lever	t	7	3.42	0.11								

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

817 **Table 6-2 | Outward current frequency and amplitude with aCSF**

		Treatment Main Effects				Day Main Effects				Treatment x Day				Post-hoc effects
Recording Condition	**Measure**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**	**Sidak's or Dunn's test, p < 0.05**
aCSF	sEPSC Frequency (+30 mV)	F	1,20	0.07	0.80	F	1,20	7.54	0.01	F	1,20	3.01	0.10	*NA*
	Frequency Distribution									H	3	259.70	< 0.0001	*Memory retrieval + AM251 > all other groups*
	sEPSC Amplitude (+30 mV)	F	1,20	0.02	0.89	F	1,20	0.16	0.70	F	1,20	1.43	0.25	*NA*
	Amplitude Distribution									H	3	16.36	0.00	*No retrieval + AM251 < all other groups*

818

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

819 **Table 7-1 | Outward current frequency and amplitude with AP5**

		Treatment Main Effects				Day Main Effects				Treatment x Day				Post-hoc effects
Recording Condition	**Measure**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**	**Test**	**df**	**Statistic**	**p**	**Sidak's or Dunn's test, p < 0.05**
AP5	sEPSC Frequency (+30 mV)	F	1,43	2.67	0.11	F	1,43	21.15	<0.0001	F	1,43	10.14	0.00	*Memory retrieval + AM251 > all other groups*
	Frequency Distribution (+30 mV)									H	3	180.70	< 0.0001	*Memory retrieval + AM251 > all other groups*
	sEPSC Amplitude (+30 mV)	F	1,44	0.41	0.53	F	1,44	0.25	0.62	F	1,43	1.67	0.20	*NA*
	Amplitude Distribution (+30 mV)									H	3	0.46	0.93	*NA*

820

821

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

REFERENCES

Achterberg E, Trezza V, Vanderschuren LJ (2014) Glucocorticoid receptor antagonism disrupts the reconsolidation of social reward-related memories in rats. Behav Pharmacol 25:216–225.

Alaghband Y, O'Dell SJ, Azarnia S, Khalaj AJ, Guzowski JF, Marshall JF (2014) Retrieval-induced NMDA receptor-dependent Arc expression in two models of cocaine-cue memory. Neurobiol Learn Mem 116:79–89.

Arenos JD, Musty RE, Bucci DJ (2006) Blockade of cannabinoid CB1 receptors alters contextual learning and memory. Eur J Pharmacol 539:177–183.

Arguello AA, Hodges MA, Wells AM, Lara H, Xie X, Fuchs RA (2014) Involvement of amygdalar protein kinase A, but not calcium/calmodulin- dependent protein kinase II, in the reconsolidation of cocaine-related contextual memories in rats. Psychopharmacology (Berl) 231:55–65.

Ben Mamou C, Gamache K, Nader K (2006) NMDA receptors are critical for unleashing consolidated auditory fear memories. Nat Neurosci 9:1237–1239.

Castillo PE, Younts TJ, Chávez AE, Hashimotodani Y (2012) Endocannabinoid signaling and synaptic function. Neuron 76:70–81.

Childress AR, McLellan TA, Ehrman R, O'Brien CP (1988) Classically conditioned responses in opioid and cocaine dependence: A role in relapse. NIDA Res Monogr 84:25–43.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. Am J Psychiatry 156:11–18.

Clem RL, Huganir RL (2010) Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. Science 330:1108–1112.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

845 Crombag HS, Bossert JM, Koya E, Shaham Y (2008) Review. Context-induced relapse to drug
846 seeking: a review. Philos Trans R Soc Lond B Biol Sci 363:3233–3243.

847 De Carvalho CR, Pamplona FA, Cruz JS, Takahashi RN (2014) Endocannabinoids underlie
848 reconsolidation of hedonic memories in Wistar rats. Psychopharmacology (Berl) 231:1417–
849 1425.

850 Diering GH, Gustina AS, Huganir RL (2014) PKA-GluA1 coupling via AKAP5 controls AMPA
851 receptor phosphorylation and cell-surface targeting during bidirectional homeostatic
852 plasticity. Neuron 84:790–805.

853 Diering GH, Huganir RL (2018) The AMPA receptor code of synaptic plasticity. Neuron
854 100(2):314–329.

855 Fang Q, Li FQ, Li YQ, Xue YX, He YY, Liu JF, Lu L, Wang JS (2011) Cannabinoid CB1 receptor
856 antagonist rimonabant disrupts nicotine reward-associated memory in rats. Pharmacol
857 Biochem Behav 99:738–742.

858 Fuchs RA, Bell GH, Ramirez DR, Eaddy JL, Su Z (2009) Basolateral amygdala involvement in
859 memory reconsolidation processes that facilitate drug context-induced cocaine seeking. Eur
860 J Neurosci 30:889–900.

861 Fuchs RA, Eaddy JL, Su ZI, Bell GH (2007) Interactions of the basolateral amygdala with the
862 dorsal hippocampus and dorsomedial prefrontal cortex regulate drug context-induced
863 reinstatement of cocaine-seeking in rats. Eur J Neurosci 26:487–498.

864 Gobira PH, Aguiar DC, Moreira FA (2013) Effects of compounds that interfere with the
865 endocannabinoid system on behaviors predictive of anxiolytic and panicolytic activities in the
866 elevated T-maze. Pharmacol Biochem Behav 110:33–39.

867 Hayashi T, Huganir RL (2004) Tyrosine phosphorylation and regulation of the AMPA receptor by

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

868 Src family tyrosine kinases. J Neurosci 24:6152–6160.

869 He K, Song L, Cummings LW, Goldman J, Huganir RL, Lee H-K (2009) Stabilization of Ca2+-
870 permeable AMPA receptors at perisynaptic sites by GluR1-S845 phosphorylation. Proc Natl
871 Acad Sci 106:20033–20038.

872 Hearing MC, Schochet TL, See RE, McGinty JF (2010) Context-driven cocaine-seeking in
873 abstinent rats increases activity-regulated gene expression in the basolateral amygdala and
874 dorsal hippocampus differentially following short and long periods of abstinence.
875 Neuroscience 170:570–9.

876 Hodge J, Bow JP, Plyler KS, Vemuri VK, Wisniecki A, Salamone JD, Makriyannis A, McLaughlin
877 PJ (2008) The cannabinoid CB1 receptor inverse agonist AM 251 and antagonist AM 4113
878 produce similar effects on the behavioral satiety sequence in rats. Behav Brain Res 193:298–
879 305.

880 Hong I, Kim J, Kim J, Lee S, Ko H-G, Nader K, Kaang B-K, Tsien RW, Choi S (2013) AMPA
881 receptor exchange underlies transient memory destabilization on retrieval. Proc Natl Acad
882 Sci USA 110:8218–8223.

883 Kapur A, Zhao P, Sharir H, Bai Y, Caron MG, Barak LS, Abood ME (2009) Atypical
884 responsiveness of the orphan receptor GPR55 to cannabinoid ligands. J Biol Chem
885 284:29817–29827.

886 LeDoux J (2007) The amygdala. Curr Biol 17:868–874.

887 Lee JLC (2004) Independent cellular processes for hippocampal memory consolidation and
888 reconsolidation. Science 304:839–843.

889 Lee JLC, Di Ciano P, Thomas KL, Everitt BJ (2005) Disrupting reconsolidation of drug memories
890 reduces cocaine-seeking behavior. Neuron 47:795–801.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Lee JLC, Milton AL, Everitt BJ (2006) Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. J Neurosci 26:5881–5887.

Lewis DJ, Misanin JR, Miller RR (1968) Recovery of memory following amnesia. Nature 220:704–705.

Li L, Carter J, Gao X, Whitehead J, Tourtellotte WG (2005) The neuroplasticity-associated arc gene is a direct transcriptional target of early growth response (Egr) transcription factors. Mol Cell Biol 25:10286–10300.

Lopez J, Gamache K, Schneider R, Nader K (2015) Memory retrieval requires ongoing protein synthesis and NMDA receptor activity-mediated AMPA receptor trafficking. J Neurosci 35:2465–2475.

Makino H, Malinow R (2009) AMPA receptor incorporation into synapses during LTP: The role of lateral movement and exocytosis. Neuron 64(3):381–390.

McLaughlin PJ, Winston K, Swezey L, Wisniecki A, Aberman J, Tardif DJ, Betz AJ, Ishiwari K, Makriyannis A, Salamone JD (2003) The cannabinoid CB1 antagonists SR 141716A and AM 251 suppress food intake and food-reinforced behavior in a variety of tasks in rats. Behav Pharmacol 14:583–588.

McDonald AJ (1984) Neuronal organization of the lateral and basolateral amygdaloid nuclei in the rat. J Comp Neurol 222:589–606.

Miller CA, Marshall JF (2005) Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. Neuron 47:873-84.

Milton AL, Lee JLC, Butler VJ, Gardner R, Everitt BJ (2008) Intra-amygdala and systemic antagonism of NMDA receptors prevents the reconsolidation of drug-associated memory and impairs subsequently both novel and previously acquired drug-seeking behaviors. J

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

914 Neurosci 28:8230–8237.

915 Milton AL, Merlo E, Ratano P, Gregory BL, Dumbreck JK, Everitt BJ (2013) Double dissociation
916 of the requirement for GluN2B- and GluN2A-containing NMDA receptors in the
917 destabilization and restabilization of a reconsolidating memory. J Neurosci 33:1109–1115.

918 Moreira FA, Crippa JAS (2009) The psychiatric side-effects of rimonabant. Braz J Psychiatry
919 31:145–153.

920 Nadel L, Moscovitch M (1997) Memory consolidation, retrograde amnesia and the hippocampal
921 complex. Curr Opin Neurobiol 7:217–227.

922 Nader K, Schafe GE, Le Doux JE (2000) Fear memories require protein synthesis in the amygdala
923 for reconsolidation after retrieval. Nature 406:722–726.

924 Nakazawa T, Komai S, Watabe AM, Kiyama Y, Fukaya M, Arima-Yoshida F, Horai R, Sudo K,
925 Ebine K, Delawary M, Goto J, Umemori H, Tezuka T, Iwakura Y, Watanabe M, Yamamoto
926 T, Manabe T (2006) NR2B tyrosine phosphorylation modulates fear learning as well as
927 amygdaloid synaptic plasticity. EMBO J 25:2867–2877.

928 National Research Council. 2011. Guide for the Care and Use of Laboratory Animals: Eighth
929 Edition. Washington, DC: The National Academies Press.

930 Ortinski PI, Vassoler FM, Carlson GC, Pierce RC (2012) Temporally dependent changes in
931 cocaine-induced synaptic plasticity in the nucleus accumbens shell are reversed by D1-like
932 dopamine receptor stimulation. Neuropsychopharmacology 37:1671–1682.

933 Paoletti P, Bellone C, Zhou Q (2013) NMDA receptor subunit diversity: Impact on receptor
934 properties, synaptic plasticity and disease. Nat Rev Neurosci 14:383–400.

935 Ramirez DR, Bell GH, Lasseter HC, Xie X, Traina SA, Fuchs RA (2009) Dorsal hippocampal
936 regulation of memory reconsolidation processes that facilitate drug context-induced cocaine-

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

seeking behavior in rats. Eur J Neurosci 30:901–912.

Ramirez S, Liu X, Lin PA, Suh J, Pignatelli M, Redondo RL, Ryan TJ, Tonegawa S (2013) Creating a false memory in the hippocampus. Science 341:387–391.

Rao-Ruiz P, Rotaru DC, van der Loo RJ, Mansvelder HD, Stiedl O, Smit AB, Spijker S (2011) Retrieval-specific endocytosis of GluA2-AMPARs underlies adaptive reconsolidation of contextual fear. Nat Neurosci 14:1302–1308.

Rao-Ruiz P, Schmitz LJMM, Smit AB, Spijker S (2015) AMPA receptor plasticity in retrieval, reconsolidation and post-retrieval extinction of memories. In: Novel Mechanisms of Memory, pp 21–41. Cham: Springer International Publishing.

Rescorla RA (2004) Spontaneous recovery. Learn Mem 11:501–9.

Rich MT, Torregrossa MM (2018) Molecular and synaptic mechanisms regulating drug-associated memories: Towards a bidirectional treatment strategy. Brain Res Bull 141:58–71.

Rich MT, Huang YH, Torregrossa MM (2019) Plasticity at thalamo-amygdala synapses regulates cocaine-cue memory formation and extinction. Cell Rep. 26(4):1010–1020.

Richards BA, Frankland PW (2017) The persistence and transience of memory. Neuron 94:1071–1084.

Richardson BD, Rossi DJ (2017) Recreational concentrations of alcohol enhance synaptic inhibition of cerebellar unipolar brush cells via pre- and postsynaptic mechanisms. J Neurophysiol 118:267–279.

Roy DS, Arons A, Mitchell TI, Pignatelli M, Ryan TJ, Tonegawa S (2016) Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease. Nature 531:508–512.

Sah P, Faber ESL, Lopez De Armentia M, Power J (2003) The amygdaloid complex: anatomy

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

959 and physiology. Physiol Rev 83:803–834.

960 Saladin ME, Gray KM, McRae-Clark AL, Larowe SD, Yeatts SD, Baker NL, Hartwell KJ, Brady
961 KT (2013) A double blind, placebo-controlled study of the effects of post-retrieval propranolol
962 on reconsolidation of memory for craving and cue reactivity in cocaine dependent humans.
963 Psychopharmacology (Berl) 226:721–737.

964 Sanderson JL, Gorski JA, Dell'Acqua ML (2016) NMDA receptor-dependent LTD requires
965 transient synaptic incorporation of Ca2+-permeable AMPARs mediated by AKAP150-
966 Aachored PKA and calcineurin. Neuron 89(5):1000–15.

967 Schutz-Geschwender A, Zhang Y, Holt T, Mcdermitt D, Olive DM, Biosciences L (2004)
968 Quantitative, two-color western blot detection with infrared fluorescence. LI-COR Biosci
969 800:1–8.

970 Seely KA, Brents LK, Franks LN, Rajasekaran M, Zimmerman SM, Fantegrossi WE, Prather PL
971 (2012) AM-251 and rimonabant act as direct antagonists at mu-opioid receptors: Implications
972 for opioid/cannabinoid interaction studies. Neuropharmacology 63:905–15.

973 Stern CAJ, de Carvalho CR, Bertoglio LJ, Takahashi RN (2018) Effects of cannabinoid drugs on
974 aversive or rewarding drug-associated memory extinction and reconsolidation.
975 Neuroscience 370:62–80.

976 Stringfield SJ, Higginbotham JA, Wang R, Berger AL, McLaughlin RJ, Fuchs RA (2017) Role of
977 glucocorticoid receptor-mediated mechanisms in cocaine memory reconsolidation.
978 Neuropharmacology 123:349–358.

979 Theberge FR, Milton AL, Belin D, Lee JL, Everitt BJ (2010) The basolateral amygdala and nucleus
980 accumbens core mediate dissociable aspects of drug memory reconsolidation. Learn Mem
981 17:444–453.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Tronson NC, Taylor JR (2007) Molecular mechanisms of memory reconsolidation. Nat Rev Neurosci 8:262–275.

Turrigiano G, Long-term NR, Lüscher C, Malenka RC, Harris KM, Weinberg RJ, Smart TG, Paoletti P, Südhof C (2014) Homeostatic synaptic plasticity: Local and global mechanisms for stabilizing. Cell 135:422–435.

Turrigiano GG (2008) The self-tuning neuron: Synaptic scaling of excitatory synapses. Cell 135:422–435.

Wang H, Treadway T, Covey DP, Cheer JF, Lupica CR (2015) Cocaine-induced endocannabinoid mobilization in the ventral tegmental area. Cell Rep 12:1997–2008.

Wells AM, Arguello AA, Xie X, Blanton MA, Lasseter HC, Reittinger AM, Fuchs RA (2013) 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 38:753–762.

Wells AM, Lasseter HC, Xie X, Cowhey KE, Reittinger AM, Fuchs R a. (2011) Interaction between the basolateral amygdala and dorsal hippocampus is critical for cocaine memory reconsolidation and subsequent drug context-induced cocaine-seeking behavior in rats. Learn Mem 18:693–702.

Wells AM, Xie X, Higginbotham JA, Arguello AA, Healey KL, Blanton M, Fuchs RA (2016) Contribution of an SFK-mediated signaling pathway in the dorsal hippocampus to cocaine-memory reconsolidation in Rats. Neuropsychopharmacology 41:675–685.

Yu LL, Wang XXY, Zhao M, Liu Y, Li YQ, Li FQ, Wang XXY, Xue YX, Lu L (2009) Effects of cannabinoid CB1 receptor antagonist rimonabant in consolidation and reconsolidation of methamphetamine reward memory in mice. Psychopharmacology (Berl) 204:203–211.

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.130419; this version posted June 3, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

1005 Yu Y-J, Huang C-H, Chang C-H, Gean P-W (2016) Involvement of protein phosphatases in the
1006 destabilization of methamphetamine-associated contextual memory. Learn Mem 23:486–
1007 493.

1008