

Prefrontal Cortex K_{Ca2} Channels Regulate $mGlu_5$ -Dependent Plasticity and Extinction of Alcohol-Seeking Behavior

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Identifying novel treatments that facilitate extinction learning could enhance cue-exposure therapy and reduce high relapse rates in alcoholics. Activation of $mGlu_5$ receptors in the infralimbic prefrontal cortex (IL-PFC) facilitates learning during extinction of cue-conditioned alcohol-seeking behavior. Small-conductance calcium-activated potassium (K_{Ca2}) channels have also been implicated in extinction learning of fear memories, and $mGlu_5$ receptor activation can reduce K_{Ca2} channel function. Using a combination of electrophysiological, pharmacological, and behavioral approaches, this study examined K_{Ca2} channels as a novel target to facilitate extinction of alcohol-seeking behavior in rats. This study also explored related neuronal and synaptic mechanisms within the IL-PFC that underlie $mGlu_5$ -dependent enhancement of extinction learning. Using whole-cell patch-clamp electrophysiology, activation of $mGlu_5$ in *ex vivo* slices significantly reduced K_{Ca2} channel currents in layer V IL-PFC pyramidal neurons, confirming functional downregulation of K_{Ca2} channel activity by $mGlu_5$ receptors. Additionally, positive modulation of K_{Ca2} channels prevented $mGlu_5$ receptor-dependent facilitation of long-term potentiation in the IL-PFC. Systemic and intra-IL-PFC treatment with apamin (K_{Ca2} channel allosteric inhibitor) significantly enhanced extinction of alcohol-seeking behavior across multiple extinction sessions, an effect that persisted for 3 weeks, but was not observed after apamin microinfusions into the prelimbic PFC. Positive modulation of IL-PFC K_{Ca2} channels significantly attenuated $mGlu_5$ -dependent facilitation of alcohol cue-conditioned extinction learning. These data suggest that $mGlu_5$ -dependent facilitation of extinction learning and synaptic plasticity in the IL-PFC involves functional inhibition of K_{Ca2} channels. Moreover, these findings demonstrate that K_{Ca2} channels are a novel target to facilitate long-lasting extinction of alcohol-seeking behavior.

Key words: addiction; alcohol; cues; extinction; infralimbic cortex; $mGlu_5$; SK channels

Significance Statement

Alcohol use disorder is a chronic relapsing disorder that is associated with compulsive alcohol-seeking behavior. One of the main causes of alcohol relapse is the craving caused by environmental cues that are associated with alcohol. These cues are formed by normal learning and memory principles, and the understanding of the brain mechanisms that help form these associations can lead to the development of drugs and/or behavior therapies that reduce the impact that these cues have on relapse in alcoholics.

Introduction

Relapse to alcohol is influenced by learning processes in which alcohol availability becomes associated with environmental cues. Exposure to these cues in alcoholics evokes maladaptive condi-

tioned responses that manifest as strong cravings and alcohol seeking observed in humans (Papachristou et al., 2012; Naqvi et al., 2015) and rodents (Bienkowski et al., 2000; Sinclair et al., 2012; Cannady et al., 2013). Cue-exposure therapy is a procedure proposed to reduce the effect of cues to elicit alcohol seeking through repeated noncontingent cue exposure. However, meta-analyses of cue-exposure therapy for alcohol-seeking revealed low efficacy (Childress et al., 1993; Conklin and Tiffany, 2002). Recent evidence suggests that cue-exposure therapy is enhanced when combined with acute pharmacotherapeutic treatment. Indeed, cognitive-enhancing agents facilitate inhibitory learning and reduce cue-induced craving and alcohol-seeking in humans (MacKillop et al., 2015) and rodents (Vengeliene et al., 2008; Nic Dhonnchadha and Kantak, 2011; Gass et al., 2014a). However, the cellular and molecular mechanisms that underlie extinction

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learning within the context of addiction have not been fully determined. Understanding of the neuronal substrates that regulate extinction/inhibitory learning is critical for the development of treatments that can enhance cue-exposure therapy and reduce relapse risk.

Small-conductance calcium-activated potassium (K_{Ca2}) channels are expressed throughout the brain (Stocker and Pedarzani, 2000) and have established roles in regulating neuronal intrinsic excitability, synaptic plasticity, and integration of dendritic signaling (Stocker et al., 1999; Blank et al., 2003; Cai et al., 2004; Villalobos et al., 2004; Fakler and Adelman, 2008; Adelman et al., 2012). Cortical and hippocampal K_{Ca2} channels also mediate learning and memory, as blocking or enhancing K_{Ca2} channel activity (via systemic or localized pharmacological manipulation) bidirectionally modulates performance in cognitive and associative learning tasks (Stackman et al., 2002; Deschaux and Bizot, 2005; Hammond et al., 2006; Brosh et al., 2007). Pharmacological inhibition of K_{Ca2} channels with apamin in the infralimbic prefrontal cortex (IL-PFC) facilitates extinction of cue-conditioned fear, whereas stimulating these channels with 1-ethyl-2-benzimidazolinone (1-EBIO) has the opposite effect (Criado-Marrero et al., 2014). This finding is intriguing given the significant overlap between the neurocircuitry involved in extinction of conditioned fear and cue-induced drug seeking (Peters et al., 2009; Gass and Chandler, 2013). Together, these findings suggest that IL-PFC K_{Ca2} channels may similarly regulate extinction of alcohol-seeking behavior.

Interestingly, there is a functional relationship between K_{Ca2} channels and the group I metabotropic glutamate receptor subtype 5 (mGlu₅). Activation of mGlu₅ enhances intrinsic neuronal excitability that appears to be attributable to reduced K_{Ca2} channel activity (Mannaioni et al., 2001; Sourdet et al., 2003). More recent evidence demonstrates that mGlu₅ and K_{Ca2} channels can coassemble to regulate neuronal activity (García-Negredo et al., 2014), and additional evidence suggests that the functional coupling may underlie neuroadaptations induced by fear extinction (Sepulveda-Orengo et al., 2013). mGlu₅ has been proposed as a promising molecular target for the facilitation of extinction learning based on preclinical evidence. Our previous findings have demonstrated that positive allosteric modulation of mGlu₅ in the IL-PFC by 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide (CDPPB) facilitates extinction of cue-conditioned alcohol seeking via potentiation of glutamatergic synaptic plasticity (Gass et al., 2014a). Similar findings have been observed on extinction of cocaine-seeking behavior after mGlu₅ activation (Gass and Olive, 2009; Cleva et al., 2011). Although there is an established role for mGlu₅ and K_{Ca2} channels to coregulate neuronal activity and fear extinction, no studies have examined how alcohol-seeking behavior may be regulated by this functional relationship.

Accordingly, we hypothesized that K_{Ca2} channel inhibition would facilitate extinction of cue-conditioned alcohol-seeking behavior. Given the relationship of K_{Ca2} channels and mGlu₅ receptors and their overlapping roles in mediating extinction behavior and synaptic plasticity within the IL-PFC, we hypothesized that mGlu₅-dependent facilitation of extinction learning is functionally regulated via control of K_{Ca2} channel activity. Electrophysiological procedures and behavioral pharmacology were used to examine the role of K_{Ca2} channels in modulating extinction of cue-conditioned alcohol-seeking behavior in rats. We also sought to determine whether IL-PFC K_{Ca2} channels could functionally regulate mGlu₅-mediated facilitation of synaptic plasticity and extinction learning.

Materials and Methods

Animals

All procedures were conducted in male Wistar rats weighing 250–275 g on arrival (Harlan). Rats were housed individually in standard polycarbonate cages where food and water were continuously available except during behavioral testing. Experiments were performed during the dark portion of a reverse 12 h light cycle (lights off at 9:00 A.M.). All procedures were approved by the Institutional Animal Care and Use Committee at the Medical University of South Carolina and were performed within the guidelines set forth by the National Research Council's *Guideline for the Care and Use of Mammals in Neuroscience and Behavioral Research* (2003).

Drugs

CDPPB was custom synthesized by Chemir Analytical Services, purified to >95% purity by liquid chromatography–mass spectrometry, and suspended in 10% (v/v) Tween 80 (Sigma-Aldrich). 3-((2-Methyl-4-thiazolyl)ethynyl)pyridine (MTEP) hydrochloride was purchased from Abcam and dissolved in sterile artificial CSF (ACSF) at a concentration of 5 μ g/ μ l. Apamin (Sigma-Aldrich) and (*RS*)-2-chloro-5-hydroxyphenylglycine sodium salt (CHPG; Tocris) were dissolved in either ACSF for electrophysiological recordings or 0.9% sterile saline for behavioral studies. 1-EBIO (Tocris) was dissolved in 0.1% DMSO.

Whole-cell patch-clamp recordings

Whole-cell patch-clamp recordings were recorded as described previously (Mulholland et al., 2011; Padula et al., 2015). Wistar rats (P60–P75) were deeply anesthetized with isoflurane gas and decapitated. Rat brains were rapidly removed and placed in an ice-cold dissection solution consisting of (in mM) 200 sucrose, 1.9 KCl, 1.2 NaH₂PO₄, 6.0 MgCl₂, 0.5 CaCl₂, 0.4 ascorbate, 10 glucose, and 25 NaHCO₃. Brains were then sectioned into coronal slices (200 μ m) containing the IL-PFC. Slices were transferred to an incubation chamber filled with ACSF solution containing (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1.3 MgCl₂, 2.0 CaCl₂, 0.4 ascorbate, 10 glucose, and 25 NaHCO₃ at 34°C for 30 min and then at room temperature for at least 30 min before recording at 25°C. The pH of all solutions listed above was adjusted to 7.4, and osmolality was measured to be ~300 mOsm. All external buffers were continuously aerated with 5% carbon dioxide/95% oxygen.

K_{Ca2} -mediated afterhyperpolarization currents (I_{AHP}) were recorded in layer V IL-PFC pyramidal neurons using borosilicate glass electrodes (2–3 M Ω) filled with an internal solution (pH 7.3 using KOH; osmolality, ~290 mOsm) containing (in mM) 140 K-methylsulfate, 8.0 NaCl, 1.0 MgCl₂, 0.05 EGTA, 10 HEPES, and 5 Mg₂ATP. To activate K_{Ca2} -mediated I_{AHP} , layer V IL-PFC pyramidal neurons were held at –70 mV in voltage-clamp mode, and 40 mV depolarizing voltage steps (400 ms duration) were applied with 2 s between steps. The protocol was repeated (three times) before and after a 10 min bath application of drugs. Peak amplitude (in picoamperes) of the K_{Ca2} -mediated I_{AHP} (up to 100 ms after each hyperpolarization) was measured, and each triplicate was averaged before and after drug treatment. Recordings were acquired with a Multiclamp 700B amplifier (Molecular Devices) and analyzed by computer using AxographX software (Axograph). Currents were filtered at 4 kHz and acquired at 10 kHz using an ITC-18 interface.

Examination of systemic mGlu₅ receptor activation on IL-PFC

I_{AHP} *ex vivo*

Naive Wistar rats were given subcutaneous injections of 30 mg/kg CDPPB or vehicle (10% Tween 80 v/v) 1 h before brain extraction for whole-cell patch-clamp recordings in the IL-PFC. I_{AHP} currents were evoked as described above.

Extracellular field potential recordings

For field potential recordings, acute coronal slices containing the IL-PFC were prepared exactly as previously described above (Padula et al., 2015), with the exception that slices were 350 μ m in thickness and ACSF contained a CaCl₂ concentration of 2.5 mM. ACSF-filled theta-glass stimulating pipettes were placed in layer II/III, and ACSF-filled recording pipettes (0.3–2 M Ω) were placed in layer V of the IL-PFC. Field EPSPs (fEPSPs) were recorded at 30°C. Input/output curves were generated to

determine the maximum stimulation intensity for each recording, and stimulation was adjusted to 50–60% maximum intensity for the duration of each recording. During the baseline period, slices were stimulated at a rate of 0.02 Hz. All drugs were applied for 10 min before high-frequency stimulation (HFS) consisting of five trains (50 Hz stimulation, 2 s duration) with a 30 s intertrain interval (Moussawi et al., 2009). After HFS, fEPSPs were evoked once per minute (0.02 Hz). Synaptic strength/potential was measured as the change in the initial onset of the fEPSP (slope) after HFS, relative to the normalized baseline slope, and expressed as a percentage of baseline. Long-term potentiation (LTP) recordings were amplified (gain of 10–50), filtered (0.2–1.2 kHz), and digitized at 10 kHz using an ITC-18 interface.

Self-administration apparatus

Alcohol self-administration, extinction, and reinstatement test sessions were conducted in Plexiglas chambers (32 cm wide × 25 cm deep × 11 cm high; model ENV-008, Med Associates) located in melamine sound-attenuating cubicles. Each cubicle was equipped with an exhaust fan to provide air circulation and mask external noise. Mounted on one wall of the self-administration chamber were two response levers that flanked a liquid receptacle connected to a single-speed syringe infusion pump with polyethylene tubing. Responses on one lever, designated the active lever, resulted in delivery of the liquid reinforcer (see below), whereas responses on the other (designated inactive) lever had no programmed consequences. Located above the active lever was a 2.5-cm-diameter stimulus light, which was illuminated for 1.5 s during each reinforcer delivery. Located atop the chambers was a house light to provide general illumination and a Sonalert speaker that emitted a tone (2900 Hz, ~65 dB) for 1.5 s during each reinforcer delivery. Chambers were interfaced to a PC that controlled experimental sessions and recorded data using commercially available software (MED-PC IV, MED Associates). Of the 42 rats trained to self-administer alcohol in an operant task and then subsequently exposed to extinction training, 1 was removed from the study before the commencement of extinction training because of failure to successfully acquire alcohol self-administration.

Self-administration training

The two-bottle choice (ethanol vs water) procedure was conducted to initiate drinking as described previously (Simms et al., 2008; McGuier et al., 2015). Rats were given 24 h intermittent access to alcohol (20%, v/v) on Monday, Wednesday, and Friday for 1 week. The day after the last two-bottle choice session, the rats were placed in operant chambers and trained to self-administer a 20% alcohol solution on an FR1 schedule of reinforcement during daily sessions (30 min) as described previously (Gass et al., 2014a,b). Active lever presses activated a syringe pump to deliver ~45 μ l of alcohol over a 1.5 s period. During reinforcer delivery, the stimulus light above the active lever was illuminated and the tone was presented. After each reinforcer delivery, a 4 s timeout period was initiated during which additional active lever presses were recorded but had no programmed consequences. After stable responding for 20% alcohol (~10–12 sessions) was reached, alcohol concentration was reduced to 10% to maximize the number of lever responses during subsequent operant sessions (12–16 sessions).

Extinction procedures

Extinction procedures and treatment with the mGlu₅-positive allosteric modulator CDPPB, the SK channel activator 1-EBIO, and/or the K_{Ca2} channel blocker apamin commenced after maintenance criteria for the alcohol solution was reached. Maintenance criteria included the following: less than 20% variation in the number of active lever presses across three consecutive sessions, a minimum of 30 reinforcers per session, and at least 12 sessions with 10% alcohol as the reinforcer. Extinction training was conducted in 30 min daily sessions in the presence of alcohol-associated cues (e.g., presentation of the light/tone stimulus), since such procedures produce drug-seeking behavior that is more resistant to extinction than that observed in the absence of drug-associated cues (Ranaldi and Roberts, 1996; Feltenstein and See, 2006). No alcohol was given during extinction sessions, and presses on the inactive lever during extinction were recorded but produced no programmed consequences.

For groups exposed to extinction training, rats were administered vehicle (saline or 10% Tween 80 v/v), apamin (0.2 mg/kg, i.p., in saline; 5 min pretreatment), or CDPPB (30 mg/kg, s.c., in 10% Tween 80 v/v; 20 min pretreatment) before each extinction training session according to their group assignment and returned to their home cages. The dose of apamin was chosen based on effectiveness in previously published behavioral studies in rats (Deschaux et al., 1997; Deschaux and Bizot, 2005). The dose of CDPPB was based on our previous studies that showed facilitation of extinction learning (Gass and Olive, 2009; Cleva et al., 2011). Rats were placed in the self-administration apparatus after treatment, and lever-pressing behavior was recorded. Extinction criteria were met when the number of active lever presses was <20% (for 2 consecutive days) of those observed on the average of the last 2 d of active alcohol self-administration.

Spontaneous recovery procedure

After each rat treated with systemic apamin (intraperitoneally) reached extinction criteria, it was returned to its home cage for 3 weeks before testing in a single spontaneous recovery session (30 min) using previously published procedures (Rodd-Henricks et al., 2002; Dhaher et al., 2010; Hauser et al., 2015). During the spontaneous recovery test, rats were placed in the operant chamber with levers extended, the house/well light illuminated, and the house fan started. All conditions remained the same as extinction sessions (i.e., active lever presses resulted in a 1.5 s presentation of the light–tone stimulus that was previously paired with alcohol delivery, but no alcohol was delivered). Active and inactive lever presses were recorded.

Locomotor activity procedure

A separate cohort of rats with a history of alcohol self-administration was treated with apamin to determine any effects on locomotor activity in an open field using previously described methods (McGuier et al., 2015). Rats were habituated to a 57 × 58 × 63 cm opaque acrylic box for 1 h per day for 2 consecutive days. On the third day, rats were treated with 0.9% sterile saline or 0.2 mg/kg apamin (intraperitoneally) 15 min before a 30 min locomotor session. Activity was digitally recorded with an overhead video camera under red light. Total movement was automatically scored using EthoVision XT software (Noldus Information Technology). Locomotor data were binned into 5 min periods.

Stereotaxic surgery and microinjection procedures

Rats were assigned to microinjection experiments to examine whether inhibition of K_{Ca2} channels within the IL-PFC ($n = 52$) or prelimbic prefrontal cortex (PrL-PFC; $n = 12$) would facilitate extinction of alcohol-seeking behavior. A separate group of rats ($n = 25$) was tested to determine whether administering the K_{Ca2} channel activator (1-EBIO) directly in the IL-PFC would prevent the facilitating effects of systemic CDPPB on the extinction of alcohol-seeking behavior.

Microinjection cannula implantation

Cannulae implantation and microinjections were done as described previously (Gass et al., 2014a). Rats were anesthetized with isoflurane vaporized in medical-grade breathing air at a flow rate of 0.4 L/min and placed in a stereotaxic instrument (Kopf Instruments). Bilateral microinjection guide cannulae (26 ga outer diameter, Plastics One) were aimed to terminate 1 mm dorsal to the IL-PFC. The stereotaxic coordinates used for the IL-PFC were (in millimeters from bregma and skull surface) anteroposterior +3.24, mediolateral \pm 0.6, and dorsoventral -3.8 , and coordinates for the PrL-PFC were anteroposterior +3.24, mediolateral \pm 0.6, and dorsoventral -2.2 (Paxinos and Watson, 2005). Microinjection cannulae were secured to the skull with stainless steel screws and dental cement. Removable obturators (33 ga outer diameter) were inserted in the full length of the guide cannulae to limit obstruction by tissue and contamination by external debris. The wound was treated with topical 2% xylocaine and 2% triple antibiotic ointments and sutured closed using 3-0 Vicryl sutures. After surgery, all rats were given carprofen (2.5 mg/kg, s.c., daily for 5 d) for postoperative pain management. After recovery from surgery, rats were trained to self-administer alcohol following our standard methods.

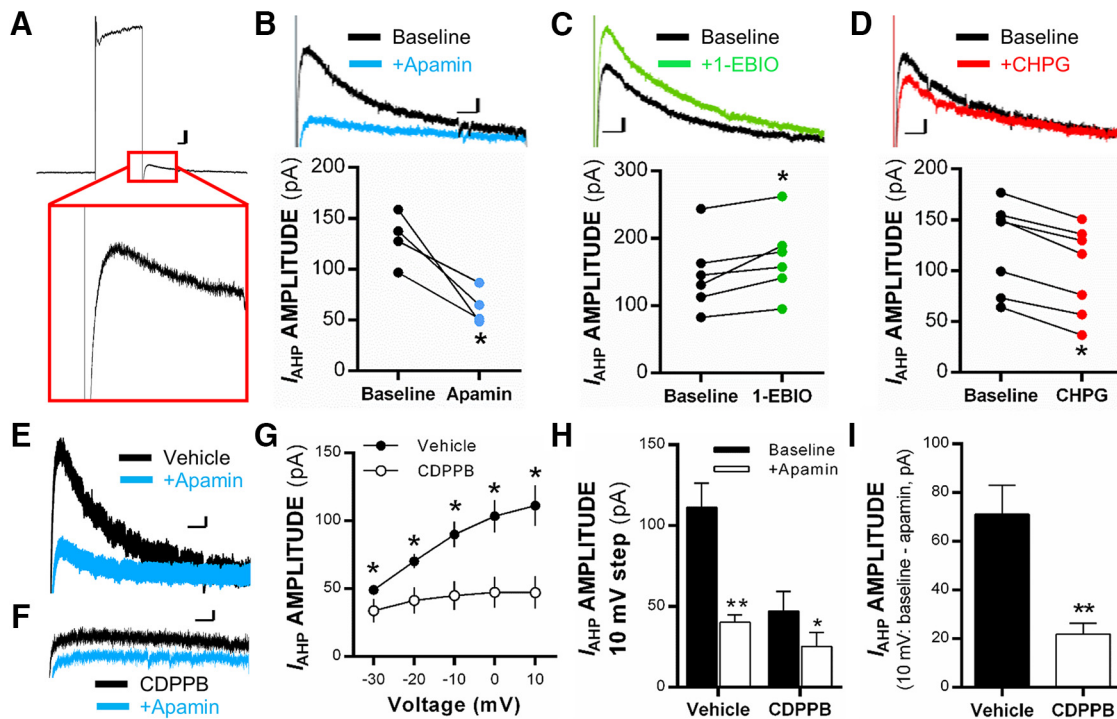


Figure 1. Localized and systemic mGlu₅ receptor activation reduces I_{AHP} in pyramidal neurons of the IL-PFC. **A**, Representative traces showing peak K_{Ca2} -mediated I_{AHP} (outward potassium current) after being evoked by 400 ms depolarizing voltage steps in voltage clamp. **B–D**, Individual triplicate mean values and representative traces for K_{Ca2} -mediated I_{AHP} after a voltage step from -70 to $+10$ mV in the presence of ACSF (baseline) or after bath application of 100 nM of apamin (**B**), 400 μ M of 1-EBIO (**C**), or 100 μ M CHPG (**D**; $n = 4–7$ neurons) in individual layer V pyramidal neurons of the IL-PFC. **E, F**, Representative traces of evoked I_{AHP} from rats treated systemically with vehicle (**E**) or CDPPB (**F**) before and after bath application of 100 nM apamin. **G**, Mean values of peak I_{AHP} current amplitude (in picoamperes) evoked in pyramidal neurons during the afterhyperpolarization phase of voltage steps after systemic treatment with 30 mg/kg CDPPB (subcutaneously) or vehicle ($n = 4–6$ neurons per treatment). **H**, Mean values of peak I_{AHP} current amplitude (in picoamperes) evoked at the 10 mV voltage step before and after 100 nM apamin bath application in neurons from systemic CDPPB- or vehicle-treated rats. **I**, Mean values for the apamin-sensitive portion of I_{AHP} amplitude (in picoamperes) at the 10 mV voltage step in neurons of rats treated with CDPPB or vehicle. Calibration: 20 pA, 100 ms. Values are expressed as group triplicate means \pm SEM. *Significance was determined at $p < 0.05$, paired t test or two-way ANOVA.

Microinjection procedure

Rats were assigned to treatment groups: intra-IL-PFC apamin [0, 0.1, 1, and 10 μ M; based on the study by Criado-Marrero et al. (2014)], intra-PrL-PFC apamin (0 and 10 μ M), or systemic vehicle (subcutaneously)/vehicle, CDPPB (30 mg/kg, s.c.)/vehicle, vehicle/1-EBIO (9.5 μ g/ μ l), and CDPPB (30 mg/kg, s.c.)/1-EBIO (9.5 μ g/ μ l); $n = 6–7$ rats per treatment group (EBIO dose based on Hopf et al., 2010). Importantly, CDPPB was not used for microinjection studies because of poor solubility. Thus, 1-EBIO was selected for localized microinjections to block systemic actions of CDPPB via positive modulation of IL-PFC K_{Ca2} channel activity. This dose of CDPPB (30 mg/kg) was chosen based on previous studies showing effective enhancement of extinction learning (Gass et al., 2014a) and lack of effects on locomotor behavior (Vales et al., 2010; Cleva et al., 2011; Kufahl et al., 2013; Besheer et al., 2014). Immediately after the microinjection, each rat received a systemic injection of CDPPB (or vehicle, subcutaneously). For apamin microinjection experiments, apamin was microinjected bilaterally 5 min before extinction sessions. Bilateral microinjections were performed 25 min before each systemic CDPPB/IL-PFC EBIO extinction session. Rats were lightly restrained, and obturators were removed. Sterile 33 ga microinjection needles (Plastics One) were connected via microbore tubing to two 10 μ l syringes (Hamilton). Syringes were mounted on a microinfusion pump (Harvard Apparatus) set to deliver fluids at a flow rate of 0.5 μ l/min. Microinjection needles were inserted bilaterally to a depth 1 mm beyond the ventral tip of the guide cannula. Drug solutions were infused in a volume of 0.5 μ l per side over a 1 min period. Microinjection needles were left in place for an additional 60 s period to allow drug diffusion. Next, injectors were removed and obturators were replaced.

Histological verification of microinjection sites

Verification of cannula placement was determined using previously published methods (Paxinos and Watson, 2005; Gass et al., 2014a). After

behavioral procedures, rats were anesthetized with isoflurane and killed by decapitation. Brains were then removed, immersed in 10% (v/v) formalin for at least 1 week at 4°C, and immersed in a 30% (w/v) sucrose solution for at least 72 h at 4°C, followed by immersion in 15% (w/v) sucrose for at least 72 h at 4°C. Brains were then cut into 40 μ m coronal sections on a cryostat (Leica CM1900, Leica Microsystems), mounted onto microscope slides, and stained with cresyl violet for histological verification of cannula placement under light microscopy.

Statistical analyses

Electrophysiology experiments. Averaged triplicate group mean peak I_{AHP} values (in picoamperes) elicited by depolarizing voltage steps during baseline and after bath application of drugs were analyzed by paired t tests or two-way ANOVAs where appropriate. LTP determined by percentage changes in normalized fEPSP slope after HFS was analyzed using a mixed model procedure (SAS) with treatment and time (20–30 and 50–60 min) as between- and within-subject factors, respectively.

Extinction learning experiments. For experiments involving analysis of extinction behavior, lever presses on the last 2 d of active self-administration of 10% alcohol (i.e., maintenance) were averaged and compared with each day of extinction training using a mixed two-way repeated-measures ANOVA with treatment (vehicle/apamin) as the between-subjects factor and experimental day (extinction session) as the within-subjects factor. An independent-samples t test or ANOVA was used to analyze the number of sessions required to reach extinction criteria.

Spontaneous recovery of alcohol-seeking behavior. For the analysis of spontaneous recovery, the number of lever presses recorded during alcohol self-administration, the last day of the extinction training, and during the spontaneous test were compared using a mixed two-way ANOVA with treatment (control/apamin) as the between-subjects factor and experimental phase (self-administration, extinction, spontaneous recovery)

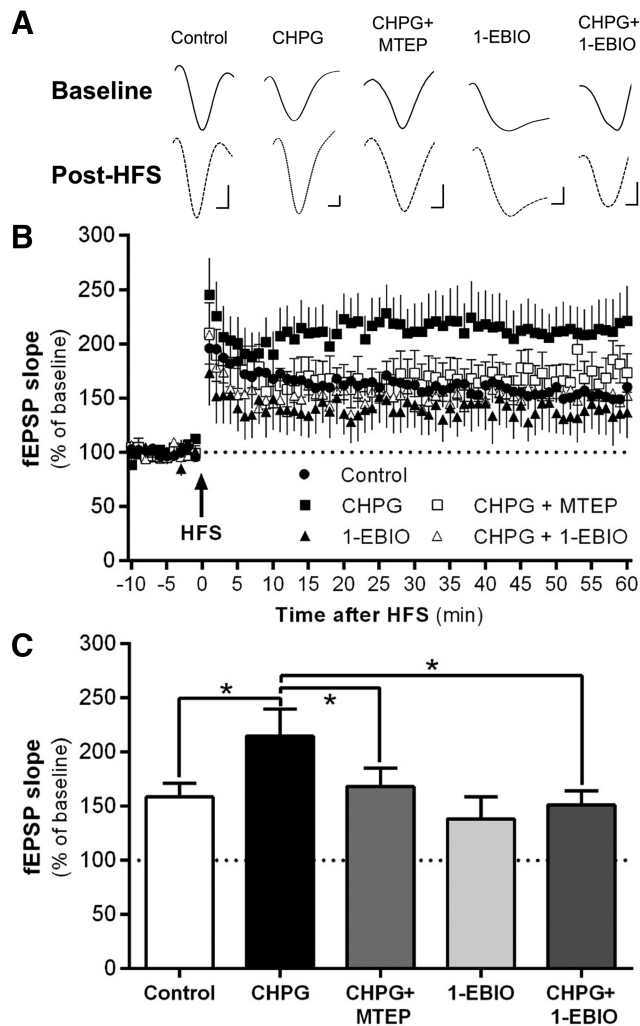


Figure 2. $mGlu_5$ -dependent enhancement of synaptic plasticity is regulated by K_{Ca2} channels within the IL-PFC. **A**, Representative traces of normalized fEPSPs after bath application of ACSF (control), 100 μM CHPG, 100 μM CHPG plus 10 μM MTEP, 400 μM 1-EBIO, or 1 mM CHPG plus 400 μM 1-EBIO. Slices were stimulated in layer II/III and recorded once per minute in layer V of the IL-PFC. Calibration: 100 μV , 1 ms. **B**, Mean normalized fEPSP slope values before bath application of ACSF, CHPG, CHPG plus MTEP, CHPG plus 1-EBIO, and 1-EBIO after 50 Hz HFS. **C**, Collapsed binned analysis of the post-HFS normalized fEPSP slope values at 20–30 and 50–60 min time intervals ($n = 10$ –12 slices per treatment group). Values are expressed as group means \pm SEM. *Significance was determined at $p < 0.05$, mixed model procedure.

ery) as the within-subjects factor. Data from this study were analyzed based on the stage of the experiment using SPSS software (version 22.0, SPSS) and Prism (version 6, GraphPad Software).

Results

Examination of functional coupling of IL-PFC K_{Ca2} channels and $mGlu_5$ receptors

Previous studies have shown that $mGlu_5$ receptor activation alters K_{Ca2} channel activity in brain regions such as the hippocampus and somatosensory cortex (Mannaioni et al., 2001; Sourdet et al., 2003). We further explored this functional coupling between K_{Ca2} channels and $mGlu_5$ receptors in the IL-PFC. In voltage clamp, 400 ms depolarizing voltage steps were applied to layer V IL-PFC pyramidal neurons to isolate K_{Ca2} channel-mediated I_{AHP} (Fig. 1A). As expected, bath application of 100 nM apamin (K_{Ca2} channel inhibitor) blocked the peak I_{AHP} in IL-PFC pyramidal neurons relative to baseline (paired $t_{(3)} = 4.24$, $p = 0.02$, $n = 4$ neurons/4 rats; Fig. 1B), whereas bath application of 400

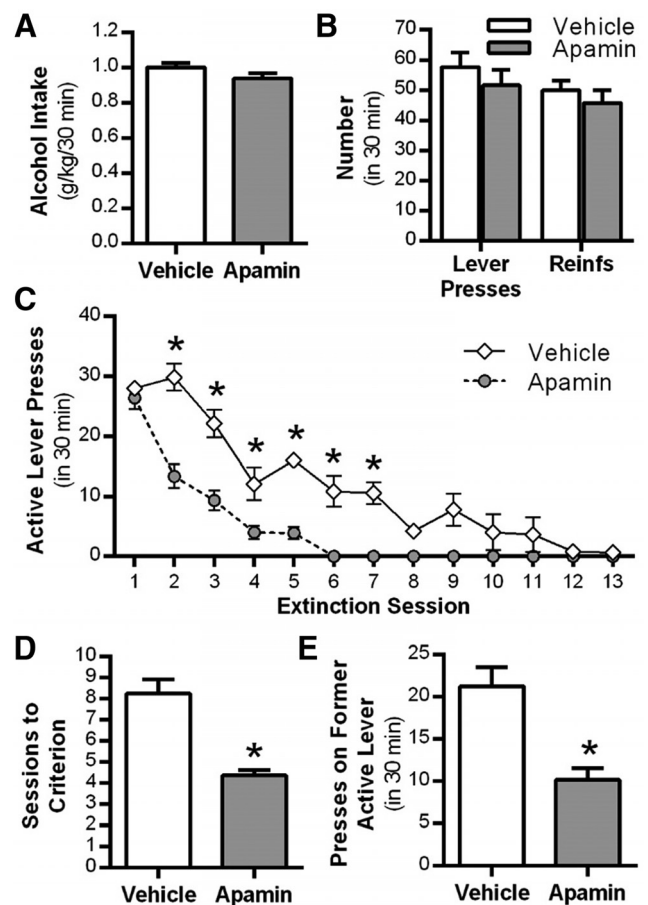


Figure 3. Systemic K_{Ca2} channel inhibition facilitates extinction of alcohol-seeking behavior. **A**, Alcohol consumption during the maintenance phase of self-administration procedure. **B**, Mean number of lever presses and reinforcers after a history of operant alcohol self-administration. **C**, Mean active lever presses during extinction learning sessions after daily treatment with vehicle or the K_{Ca2} channel inhibitor apamin. During extinction learning, lever pressing resulted in the presentation of alcohol cues, but no alcohol was delivered. **D**, Mean number of sessions to reach extinction criteria after vehicle or apamin treatment. **E**, Mean number of previously active lever presses after a single pavlovian spontaneous recovery test session conducted 3 weeks after extinction training and drug treatment ($n = 12$ vehicle group vs $n = 11$ apamin treatment group). Values are expressed as group means \pm SEM. *Significance was determined at $p < 0.05$, t test or two-way ANOVA.

μM 1-EBIO (K_{Ca2} channel-positive modulator) significantly increased I_{AHP} in IL-PFC pyramidal neurons relative to baseline (paired $t_{(5)} = 3.40$, $p < 0.02$; $n = 6$ neurons/4 rats; Fig. 1C). Bath application of the $mGlu_5$ receptor agonist CHPG significantly reduced I_{AHP} relative to baseline ($t_{(6)} = 10.1$, $p < 0.01$; $n = 7$ neurons/3 rats; Fig. 1D), demonstrating a functional reduction in K_{Ca2} channel activity via $mGlu_5$ activation in IL-PFC projection neurons.

To further examine the functional coupling of $mGlu_5$ receptors and K_{Ca2} channels, naive rats were given injections of vehicle or the $mGlu_5$ -positive modulator CDPBB (30 mg/kg, s.c.), and *ex vivo* slices containing the IL-PFC were prepared 60 min later for examination of I_{AHP} (Fig. 1E,F). Systemic activation of $mGlu_5$ with CDPBB resulted in a significant main effect of treatment ($F_{(1,40)} = 37.38$, $p < 0.001$). *Post hoc* analysis indicated that systemic CDPBB treatment ($n = 6$ neurons/3 rats) significantly reduced K_{Ca2} -mediated I_{AHP} amplitude at each voltage step relative to vehicle control cells ($n = 4$ neurons/3 rats, $p < 0.001$; Fig. 1G). An examination of K_{Ca2} -mediated I_{AHP} (at 10 mV) before and

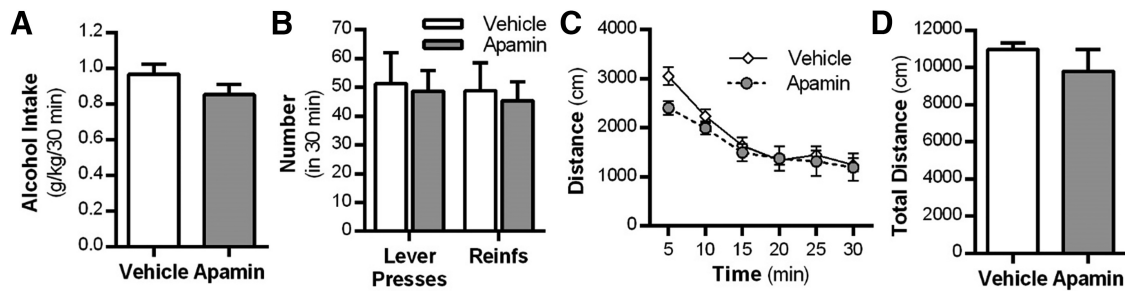


Figure 4. K_{Ca2} channel inhibition does not affect locomotor behavior in rats with a history of alcohol self-administration. **A**, Alcohol intake during voluntary alcohol self-administration. **B**, Mean number of lever presses and reinforcers (Reinfo.) after a history of operant alcohol self-administration. **C**, Cumulative distance traveled after treatment with vehicle or the K_{Ca2} channel inhibitor apamin in an open-field test of locomotor activity. **D**, Total distance traveled after vehicle or apamin treatment ($n = 5$ vehicle group vs $n = 6$ apamin treatment group). Values are expressed as group means \pm SEM.

after 100 nM apamin in IL-PFC pyramidal neurons from rats treated with systemic vehicle or CDPPB revealed a significant interaction of apamin \times CDPPB treatments ($F_{(1,8)} = 20.20$, $p = 0.002$). *Post hoc* analysis showed that neurons from rats treated with systemic CDPPB had significantly reduced I_{AHP} currents and displayed reduced sensitivity to bath application of apamin compared with neurons from vehicle-treated rats ($p = 0.006$; Fig. 1H). Additional analysis of I_{AHP} amplitude at the 10 mV step after systemic CDPPB (vs vehicle) demonstrated that the apamin-sensitive component of the I_{AHP} (subtracted from baseline) was reduced in pyramidal neurons from rats treated with CDPPB ($t_{(8)} = 4.49$, $p = 0.002$; Fig. 1I). These data confirm our *ex vivo* findings by showing that systemic activation of mGlu₅ receptors reduces K_{Ca2} channel activity within layer V IL-PFC pyramidal neurons.

Next, we determined whether K_{Ca2} channels functionally regulate mGlu₅-mediated synaptic plasticity of inputs onto IL-PFC neurons. Analysis revealed a main effect of treatment on a normalized fEPSP slope ($F_{(4,48)} = 3.84$, $p = 0.009$; Fig. 2B). *Post hoc* analysis revealed that treatment with CHPG significantly increased normalized fEPSP slope relative to control treatment ($p = 0.008$; Fig. 2C), an effect that was blocked by coapplication of the selective mGlu₅ antagonist MTEP ($p = 0.03$ relative to CHPG treatment; Fig. 2C). Interestingly, coapplication of the K_{Ca2} -positive modulator 1-EBIO prevented CHPG potentiation of LTP in IL-PFC slices ($p = 0.004$) (Fig. 2C). Importantly, the normalized fEPSP slope was not significantly reduced in slices treated with 1-EBIO alone ($p = 0.32$ vs controls; Fig. 2C), demonstrating that reversal of CHPG-induced enhancement of synaptic plasticity by combined treatment with 1-EBIO was not the result of an inability to induce LTP after positive modulation of K_{Ca2} channels.

Examination of K_{Ca2} channels in regulating extinction of alcohol-seeking behavior

Given the significant role of K_{Ca2} channels in modulating learning and memory (Stackman et al., 2002; Deschaux and Bizot, 2005; Hammond et al., 2006; Brosh et al., 2007) and regulation of cue-conditioned fear extinction (Criado-Marrero et al., 2014), we examined the effect of systemic administration of the K_{Ca2} channel inhibitor apamin on the extinction of alcohol-seeking behavior in rats. As shown in Figure 3A, no differences were observed in the amount of alcohol consumed ($t_{(10)} = 1.6$, $p = 0.13$). Also, there were no differences in the number of lever presses or reinforcements between groups during the final alcohol self-administration sessions before extinction ($F_{(1,42)} = 0.1$, $p = 0.835$, $n = 12$ control, $n = 11$ apamin; Fig. 3B). Apamin

administered before each daily extinction session resulted in a significant treatment \times session interaction ($F_{(12,252)} = 11.5$, $p = 0.001$, $n = 11$ –12 per group). *Post hoc* analyses revealed that rats treated with apamin had significantly fewer responses on the previously active lever compared with vehicle-treated rats during extinction sessions 2–7 ($*p < 0.05$; Fig. 3C). Rats treated with apamin also required significantly fewer extinction sessions to reach extinction criteria compared with vehicle-treated rats ($t_{(21)} = 5.1$, $p = 0.001$; Fig. 3D). To determine the persistence of the enhanced extinction learning produced by apamin, we assessed spontaneous recovery of alcohol-seeking behavior after 3 weeks in the home cage. Rats previously treated with apamin during extinction training showed a significant reduction in lever presses on the previously alcohol-reinforced lever ($t_{(21)} = 4.1$, $p = 0.001$; Fig. 3E) compared with vehicle-treated controls.

A potential concern with the apamin treatment was the possibility that deficits in motor behavior would be empirically perceived as an enhancement of extinction learning. Therefore, in a separate set of rats with a history of alcohol self-administration, we monitored locomotor activity in response to apamin (0.2 mg/kg, i.p.) administration. No differences were observed in alcohol intake ($t_{(10)} = 1.6$, $p = 0.13$; Fig. 4A) or the number of lever presses or number of reinforcements between groups ($F_{(1,42)} = 0.698$, $p = 0.044$; $n = 5$ control, $n = 6$ apamin; Fig. 4B) during the final alcohol self-administration sessions before locomotor testing. Apamin did not alter binned activity ($F_{(5,45)} = 1.698$, $p = 0.155$) or overall total distance traveled ($t_{(9)} = 0.88$, $p = 0.401$) in rats previously trained to self-administer alcohol (Fig. 4C,D).

Given that the IL-PFC is a critical site that regulates inhibitory control of behavioral responding (Peters et al., 2009; Gass and Chandler, 2013; Barker et al., 2014) and the current results showing that systemic K_{Ca2} inhibition facilitates extinction learning (Fig. 3), we investigated the effects of daily intra-IL-PFC K_{Ca2} apamin pretreatment on extinction of alcohol-seeking behavior. No significant differences were observed in the amount of alcohol consumed ($F_{(3,23)} = 0.6$, $p = 0.595$; Fig. 5A) or in the number of lever presses or reinforcements between groups during the final alcohol self-administration sessions before extinction ($F_{(3,40)} = 0.014$, $p = 0.998$; $n = 6$ per group; Fig. 5B). Intra-IL-PFC apamin (10 μ M) administered before each daily extinction session resulted in a significant treatment \times session interaction ($F_{(27,180)} = 2.6$, $p = 0.007$; Fig. 5C). *Post hoc* analyses revealed that rats given microinjections of 10 μ M apamin had significantly fewer responses on the previously active lever compared with vehicle-treated rats on multiple extinction sessions ($*p < 0.05$; Fig. 5C). Rats given microinjections of 10 μ M apamin also required significantly fewer extinction sessions to reach extinction criteria com-

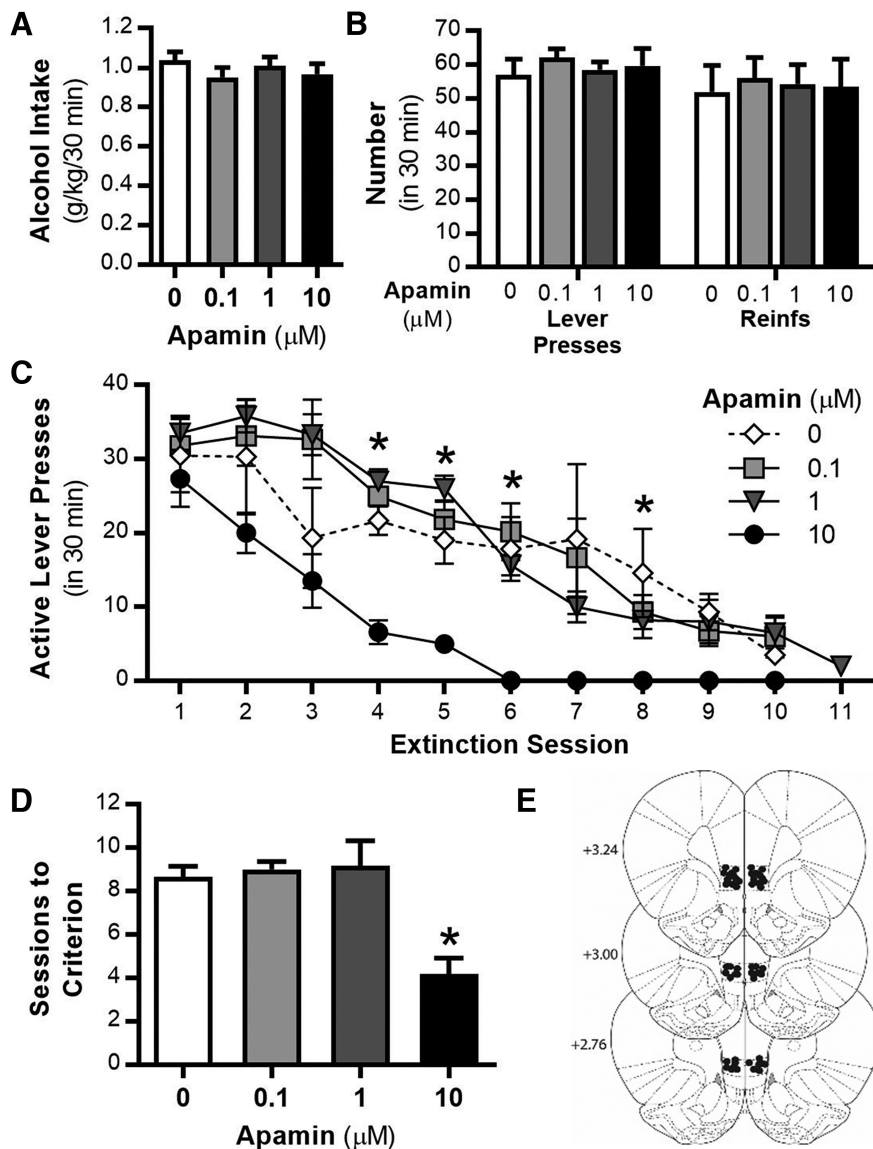


Figure 5. Inhibition of $K_{Ca}2$ channels in the IL-PFC facilitates extinction of alcohol-seeking behavior. **A**, Alcohol consumption during operant alcohol self-administration sessions. **B**, Mean number of lever presses and reinforcers (Reinf) after a history of operant alcohol self-administration. **C**, Mean active lever presses during extinction learning sessions after daily IL-PFC microinjections of the $K_{Ca}2$ channel inhibitor apamin (0, 0.1, 1, and 10 μ M). During extinction learning, lever pressing resulted in the presentation of alcohol cues, but no alcohol was delivered. **D**, Mean number of sessions to reach extinction criteria after vehicle or apamin microinjections. **E**, Diagram of coronal rat brain sections showing the location of microinjector tips (dark circles) in the IL-PFC. Illustrations were adapted from the atlas of Paxinos and Watson (2005). Numbers along the left side of each section represent the distance (in millimeters) of that section from bregma; $n = 6$ per treatment group. Values are expressed as group means \pm SEM. *Significance was determined at $p < 0.05$, t test or two-way ANOVA.

pared with vehicle-treated rats ($F_{(3,20)} = 32.9$, $p < 0.0001$; Fig. 5D). These data demonstrate that $K_{Ca}2$ channel inhibition within the IL-PFC is required for the facilitation of alcohol cue-associated extinction learning.

The PrL-PFC is a key region within the circuitry that processes and initiates cue-mediated reward seeking (Peters et al., 2008; Gourley and Taylor, 2016). Given the current findings demonstrating that IL-PFC $K_{Ca}2$ channel inhibition facilitates extinction learning (Fig. 5), we tested for region specificity by evaluating the effects of daily intra-PrL-PFC apamin pretreatment on extinction of alcohol-seeking behavior. No significant differences were observed in the amount of alcohol consumed ($t_{(10)} = 1.5$, $p = 0.171$; Fig. 6A) or the number of lever presses or

reinforcements between groups during the final alcohol self-administration sessions before extinction ($F_{(1,20)} = 0.05$, $p = .814$; $n = 6$ per group; Fig. 6B). Intra-PrL-PFC apamin (10 μ M) administered before each daily extinction session did not significantly alter lever responses on the previously active lever ($F_{(9,90)} = 0.3$, $p = 0.940$; Fig. 6C). Furthermore, there was no effect on sessions to reach extinction criterion ($t_{(10)} = 0.3$, $p = 0.734$; Fig. 6D). These data demonstrate that $K_{Ca}2$ channel inhibition within the IL-PFC, but not the PrL-PFC, is specifically required for the facilitation of alcohol cue-associated extinction learning.

$K_{Ca}2$ channel– $mGlu_5$ receptor interactions and extinction of alcohol-seeking behavior

Our current data indicate that $mGlu_5$ activation functionally reduces IL-PFC $K_{Ca}2$ channel activity (Fig. 1) and that this functional reduction in $K_{Ca}2$ activity is a requisite for $mGlu_5$ -dependent enhancement of LTP *ex vivo* (Fig. 2). Therefore, we examined the role of IL-PFC $K_{Ca}2$ channels in regulating $mGlu_5$ -dependent extinction of alcohol-seeking behavior by microinjecting 1-EBIO into the IL-PFC in rats treated with systemic CDPPB. No significant differences were observed in alcohol intake ($F_{(3,23)} = 1.2$, $p = 0.31$; Fig. 7A) or the number of lever presses and reinforcements between groups during the final alcohol self-administration sessions before extinction ($F_{(3,42)} = 0.04$, $p = 0.988$; Fig. 7B). An analysis of combined systemic and intra-IL-PFC drug treatment on active lever presses during extinction sessions resulted in a significant treatment \times session interaction ($F_{(27,171)} = 2.8$, $p = 0.001$; Fig. 7C). *Post hoc* analysis revealed that the systemic CDPPB treatment group responded significantly fewer times on the active lever (relative to vehicle/vehicle treatment) during extinction sessions 2, 3, 4, 6, and 7 ($*p < 0.05$), and this effect was blocked IL-PFC 1-EBIO microinfusions (Fig. 7C).

Moreover, systemic CDPPB/vehicle produced a significant decrease in the number of sessions to reach extinction criteria relative to control treatment ($F_{(3,19)} = 7.8$, $p = 0.001$; Fig. 7D), an effect reversed by $K_{Ca}2$ channel activation with intra-IL-PFC 1-EBIO. These data indicate that $K_{Ca}2$ channel inhibition in the IL-PFC is required for $mGlu_5$ -mediated facilitation of alcohol cue-associated extinction learning.

Discussion

The major findings from these studies demonstrate that both systemic and intra-IL-PFC inhibition of $K_{Ca}2$ channels facilitates extinction of cue-associated alcohol-seeking behavior and reveal a novel role for these channels in alcohol cue-related inhibitory

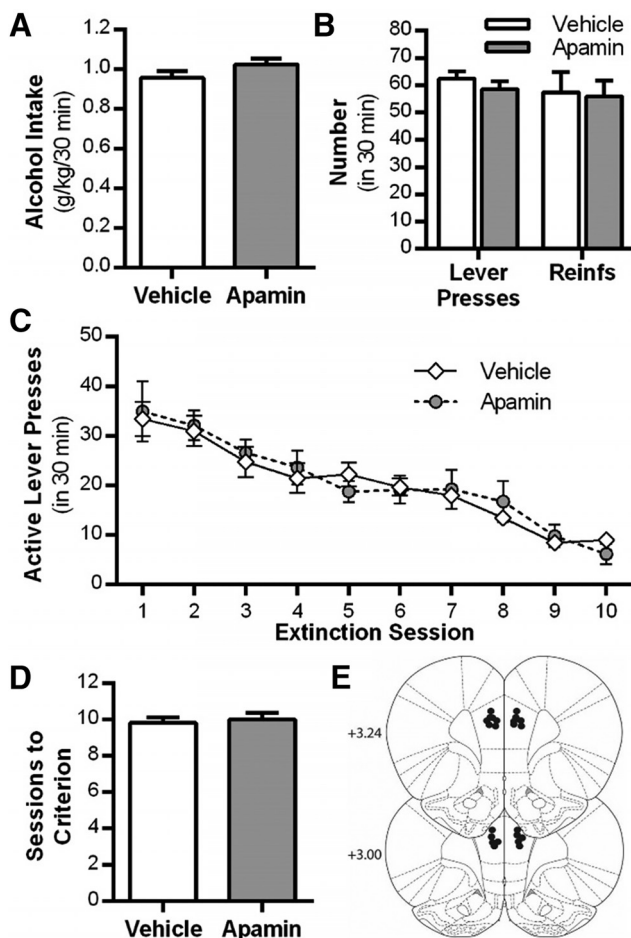


Figure 6. Inhibition of K_{Ca2} channels in the PrL-PFC does not alter extinction of alcohol-seeking behavior. *A*, Alcohol consumption during the maintenance phase of the self-administration procedure. *B*, Mean number of lever presses and reinforcers (Reinfo) after a history of operant alcohol self-administration. *C*, Mean active lever presses during extinction learning sessions after daily PrL-PFC microinjections of the K_{Ca2} channel inhibitor apamin (0 and 10 μ M). *D*, Mean number of sessions to reach extinction criteria after vehicle or apamin microinjections. *E*, Diagram of coronal rat brain sections showing the location of microinjector tips (dark circles) in the PrL-PFC. Illustrations were adapted from the atlas of Paxinos and Watson (2005). Numbers along the left side of each section represent the distance (in millimeters of that section from bregma; $n = 6$ per treatment group). Values are expressed as group means \pm SEM.

learning. K_{Ca2} inhibition in the PrL-PFC did not significantly alter extinction learning, revealing a subregion-specific role for IL-PFC K_{Ca2} channels to regulate extinction of alcohol seeking. We also demonstrate that K_{Ca2} inhibition is required for mGlu₅-dependent facilitation of extinction. Furthermore, results of the current study show that K_{Ca2} channels functionally couple with mGlu₅ receptors in the IL-PFC to regulate K_{Ca2} channel currents in layer V pyramidal neurons and to regulate mGlu₅-dependent enhancement of LTP. Together, these data provide strong pre-clinical evidence that facilitation of extinction of alcohol-seeking behavior involves functional inhibition of K_{Ca2} channels, through either direct or indirect activation of mGlu₅ receptors. In addition, these data implicate the IL-PFC as a critical site of action in K_{Ca2} -mediated facilitation of extinction learning.

K_{Ca2} channel inhibition and facilitated extinction learning

An intriguing finding in this study was the long-lasting (3 weeks) attenuation of alcohol seeking in rats previously treated with

systemic apamin during extinction training. This effect was not attributable to reductions in locomotor activity as apamin treatment did not significantly alter spontaneous locomotor behavior in the open field. New memory formation is thought to be the underlying mechanism that mediates extinction learning. During extinction training, newly formed memories compete with associations formed during previous long-term repeated pairings of alcohol, drugs, or fearful stimuli with environmental stimuli (Bouton, 2000; Rescorla, 2004; Hutton-Bedbrook and McNally, 2013). The current findings showing that systemic K_{Ca2} channel inhibition facilitated extinction learning are congruent with several studies demonstrating that K_{Ca2} channels play a critical role in the formation of new memories (Stackman et al., 2002; Deschaux and Bizot, 2005; Hammond et al., 2006; Brosh et al., 2007). We hypothesize that K_{Ca2} inhibition likely enhances the ability of the new memory to compete with older cue-associated memories that previously motivated alcohol-seeking behavior. The mechanism underlying K_{Ca2} channel-dependent enhancement of learning and memory is attributed, at least in part, to increased neuronal intrinsic excitability after reductions in K_{Ca2} channel function as evidenced by increased neuronal spiking after pharmacological block of K_{Ca2} channels (Bond et al., 2004; Criado-Marrero et al., 2014). Thus, these persistent effects of K_{Ca2} inhibition on alcohol seeking provide strong evidence that K_{Ca2} channels are an ideal target for pharmacological enhancement of inhibitory learning.

The results of the present study also show that reduced K_{Ca2} channel activity within the IL-PFC is selective in controlling extinction of alcohol-seeking behavior. Traditionally, the IL-PFC has been shown to play a more critical role in inhibiting, as opposed to the PrL-PFC, which initiates drug/reward-seeking behavior (Peters et al., 2008; Gass and Chandler, 2013; Barker et al., 2014) but see (Moorman and Aston-Jones, 2015). The current data provide the first preclinical evidence that inhibition of K_{Ca2} channels in the IL-PFC, but not the PrL-PFC, facilitates extinction of alcohol-seeking behavior. These data are also congruent with previous work showing facilitation of fear extinction after IL-PFC microinjection with apamin (Criado-Marrero et al., 2014). Whereas apamin was thought to be highly selective for K_{Ca2} channels, recent evidence has shown that apamin blocks $K_{V1.3}$ channels (Voos et al., 2017). It is possible that apamin enhances extinction learning by reducing $K_{V1.3}$ channel activity. This argument is weakened, however, because of our results demonstrating that 1-EBIO blocks CDPPB's effects on LTP and extinction learning. To our knowledge, 1-EBIO does not affect $K_{V1.3}$ channel activity, suggesting that the ability of apamin to facilitate extinction learning is mediated by inhibition of K_{Ca2} , but not $K_{V1.3}$, channel activity. Thus, reductions in K_{Ca2} channel activity in the IL-PFC may functionally regulate inhibitory learning processes in multiple behavioral and preclinical models of extinction therapy.

Behavioral and physiological implications of functional coupling between mGlu₅ receptors and K_{Ca2} channels

Given the established role of IL-PFC K_{Ca2} channels and mGlu₅ receptors in modulating extinction of cue-conditioned fear (Sepulveda-Orengo et al., 2013; Criado-Marrero et al., 2014), we examined IL-PFC K_{Ca2} channel regulation of mGlu₅-dependent extinction of cue-conditioned alcohol-seeking behavior. Site-specific activation of IL-PFC K_{Ca2} channels attenuated mGlu₅-dependent enhancement of extinction learning. These data also extend previous findings showing that mGlu₅ activation facilitates extinction learning (Gass et al., 2014a) by elucidating a req-

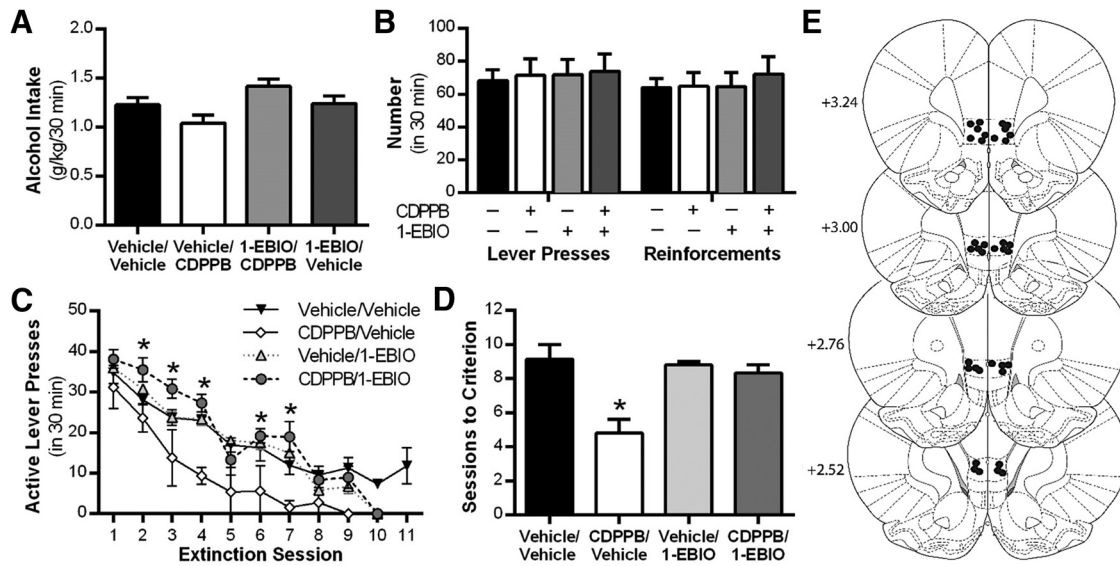


Figure 7. Activation of $K_{Ca}2$ channels in the IL-PFC blocks $mGlu_5$ receptor-mediated facilitation of alcohol-seeking behavior. **A**, Alcohol consumption during operant alcohol self-administration sessions. **B**, Mean number of lever presses and reinforcers after a history of operant alcohol self-administration. **C**, Mean number of active lever presses during extinction learning sessions after systemic treatment with vehicle or CDPPB ($mGlu_5$ -positive modulator) or in combination with local infusion of vehicle or 1-EBIO into the IL-PFC. **D**, Number of sessions to reach extinction criteria after systemic treatment with 1-EBIO or CDPPB combined with local infusion of vehicle or 1-EBIO into the IL-PFC. **E**, Diagram of coronal rat brain sections showing the location of microinjector tips (dark circles) in the IL-PFC. Illustrations were adapted from the atlas of Paxinos and Watson (2005). Values are expressed as group means \pm SEM ($n = 7$ per group). *Significance was determined at $p < 0.05$, one- or two-way ANOVA.

uisite for reduced activity of IL-PFC $K_{Ca}2$ channels to enhance extinction of alcohol-seeking behavior after positive modulation of $mGlu_5$ receptors. It is important to note that other forms of extinction learning, such as context-dependent extinction, are also regulated by $mGlu_5$ receptors. $mGlu_5$ receptor activity is critical for extinction of context-dependent fear memories (Riedel et al., 2000) and drug-seeking behavior (Gass and Olive, 2009; Chesworth et al., 2013; Kim et al., 2015). It is not fully clear, however, whether functional coupling of $mGlu_5$ receptors and $K_{Ca}2$ channels plays a specific role in context-dependent extinction learning, and future studies are needed to determine this relationship.

The aforementioned behavioral data are in parallel with the electrophysiological findings that demonstrate functional coupling of $mGlu_5$ receptors and $K_{Ca}2$ channels within the IL-PFC. Indeed, activation of $mGlu_5$ receptors by local bath application and systemic treatment reduced $K_{Ca}2$ -mediated I_{AHP} in deep-layer IL-PFC pyramidal neurons. This finding is consistent with previous work showing that group I activation reduces $K_{Ca}2$ channel-mediated currents (Fontanez-Nuin et al., 2011; García-Negredo et al., 2014; Tigaret et al., 2016). Although the mechanism is unknown, this effect may involve removal of $K_{Ca}2$ channels from the plasma membrane after cyclic AMP-dependent protein kinase A (PKA) phosphorylation of $K_{Ca}2$ channels (Ren et al., 2006; Lin et al., 2008). Indeed, several studies have shown that positive modulation of $mGlu_5$ receptors increases PKA activation (Lanté et al., 2006; Dell'anno et al., 2013). Other evidence demonstrates that G_q -mediated hydrolyzes of PIP₂ (phosphatidylinositol bisphosphate) regulates calcium sensitivity of $K_{Ca}2$ channels via uncoupling of calmodulin binding (Zhang et al., 2014) and suggests that $mGlu_5$ activation may be reducing $K_{Ca}2$ channel sensitivity to intracellular calcium levels. Thus, inhibitory learning may be enhanced by IL-PFC $K_{Ca}2$ channel endocytosis or reduced calcium sensitivity after $mGlu_5$ activation. Regardless of the intracellular signaling mechanism, these data support the hypothesis that the reduction in $K_{Ca}2$ channel-mediated I_{AHP} in the IL-PFC

by activation of $mGlu_5$ receptors is a key mechanism that enhances inhibitory learning.

Our previous work, as well as the work from other groups, has shown that facilitation of extinction learning has been associated with enhanced plasticity in the medial PFC (Herry and Garcia, 2002; Gass et al., 2014a; Zhong et al., 2015). In the present study, IL-PFC LTP was significantly and robustly enhanced after $mGlu_5$ activation. This effect of enhanced LTP is similar to what others have observed after $mGlu_5$ activation in the hippocampus (O'Leary and O'Connor, 1998; Ayala et al., 2009). Importantly, $mGlu_5$ -dependent facilitation of LTP was reversed by $K_{Ca}2$ channel activation, demonstrating that $K_{Ca}2$ channel inhibition is required for $mGlu_5$ -dependent enhancement of synaptic plasticity. A recent study demonstrated that activation of hippocampal $mGlu_1$, but not $mGlu_5$, enhances spike-timing-dependent synaptic plasticity via inhibition of $K_{Ca}2$ channels (Tigaret et al., 2016). Although these findings appear at odds with our results, they may be explained by different plasticity induction protocols and differing $mGlu_1$ expression within the PFC relative to $mGlu_5$ (Ferrer-Luna and Shigemoto, 2006). Another study reported that $K_{Ca}2$ overexpression inhibits LTP induction in the hippocampus (Hammond et al., 2006). However, the concentration of 1-EBIO used in the present study did not significantly inhibit induction of IL-PFC synaptic plasticity, suggesting that overexpression of $K_{Ca}2$ channels may result in much stronger regulation of synaptic plasticity compared with $K_{Ca}2$ expression in intact neurons. Together, the current findings indicate a critical role for $K_{Ca}2$ channels in modulating $mGlu_5$ -dependent synaptic plasticity in the IL-PFC.

In conclusion, the findings of the present study show that apamin treatment during extinction learning facilitated a persistent reduction in alcohol-seeking behaviors and provide strong evidence that $K_{Ca}2$ channels are a novel therapeutic target for the enhancement of cue-exposure treatment in alcoholics. Furthermore, the functional coupling between $K_{Ca}2$ channels and $mGlu_5$ receptors is a critical regulator of behavioral and synaptic plastic-

ity within an important extinction-related brain region, the IL-PFC. Finally, these data add to the growing body of literature demonstrating the beneficial role of cognitive enhancement in facilitating extinction cue-exposure therapy for the treatment of alcohol use disorder.

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