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Inhibitors of cellular stress overcome acute effects of ethanol on hippocampal plasticity and learning

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A B S T R A C T

Ethanol intoxication can produce marked changes in cognitive function including states in which the ability to learn and remember new information is completely disrupted. These defects likely reflect changes in the synaptic plasticity thought to underlie memory formation. We have studied mechanisms contributing to the adverse effects of ethanol on hippocampal long-term potentiation (LTP) and provided evidence that ethanol-mediated LTP inhibition involves a form of metaplasticity resulting from local metabolism of ethanol to acetaldehyde and untimely activation of *N*-methyl-D-aspartate receptors (NMDARs), both of which are neuronal stressors. In the present studies, we sought to understand the role of cellular stress in LTP defects, and demonstrate that ethanol's effects on LTP in the CA1 hippocampal region are overcome by agents that inhibit cellular stress responses, including ISRIB, a specific inhibitor of integrated stress responses, and GW3965, an agonist that acts at liver X receptors (LXRs) and dampens cellular stress. The agents that alter LTP inhibition also prevent the adverse effects of acute ethanol on one trial inhibitory avoidance learning. Unexpectedly, we found that the LXR agonist but not ISRIB overcomes effects of ethanol on synaptic responses mediated by *N*-methyl-D-aspartate receptors (NMDARs). These results have implications for understanding the adverse effects of ethanol and possibly for identifying novel paths to treatments that can prevent or overcome ethanol-induced cognitive dysfunction.

1. Introduction

Alcohol intoxication results in adverse effects on cognition. During milder intoxication (blood alcohol levels (BAL) of 5–20 mM) individuals exhibit motor incoordination and diminished reaction times. Greater intoxication (BAL ~ 20–40 mM) results in slowed thinking and altered learning; at BAL above 40 mM some individuals develop acute memory "blackouts" (White, 2003; Abrahao et al., 2017). The latter are periods in which individuals are awake but have no recollection of their actions. A blackout involves an acute defect in memory processing and can have devastating consequences. Mechanisms contributing to alcohol-induced memory blackouts are likely complex but thought to involve defects in the synaptic plasticity underlying learning and memory, particularly defects in long-term potentiation (LTP) (Abrahao et al., 2017; Chandler et al., 1998; Zorumski et al., 2014). In addition to acute effects, chronic alcohol use can result in persistent impairments in cognition (Oslin and Cary, 2003; Parada et al., 2011). Taken together the short and longer-term adverse effects of ethanol prompt efforts to define mechanisms involved in memory defects and to identify ways to prevent or overcome memory dysfunction.

To probe mechanisms by which ethanol impairs memory, we have examined effects on learning-related synaptic plasticity in the CA1 region of the hippocampus, and found that ethanol impairs long-term potentiation (LTP) and homosynaptic long-term depression (LTD) by distinct mechanisms (Izumi et al., 2005b, 2007; Tokuda et al., 2011). Effects on LTD involve partial antagonism of *N*-methyl-D-aspartate receptors (NMDARs) expressing GluN2B subunits (Izumi et al., 2005b). These effects reverse readily following ethanol washout. In contrast, effects on LTP are more complex and persistent, requiring high acute concentrations (~60 mM) to inhibit LTP completely. LTP block involves local metabolism of ethanol to acetaldehyde and synthesis of allopregnanolone (alloP), a GABA-enhancing 5α-reduced neurosteroid (Izumi et al., 2007; Tokuda et al., 2011). This LTP inhibition is overcome by agents that inhibit aldehyde dehydrogenase or neurosteroid synthesis (Tokuda et al., 2011, 2013a, 2013b). High ethanol's effects on LTP also involve untimely NMDAR activation resulting in a form of metaplasticity (Zorumski and Izumi, 2012; Zorumski et al., 2014). LTP block by high ethanol is prevented by co-administration of a full NMDAR antagonist (2-amino-5-phosphonovalerate, APV) during ethanol exposure (Tokuda et al., 2011).

Prior studies have found that several neuronal stressors including low glucose, ammonia and brief hypoxia inhibit LTP via untimely activation of NMDARs (Zorumski and Izumi, 2012). Prior work has also shown that ethanol-induced liver injury involves production of acetaldehyde and activation of endoplasmic reticulum (ER) stress responses (Magne et al., 2011). Given that we have previously observed a role for

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untimely NMDAR activation and acetaldehyde in ethanol's block of LTP (Zorumski et al., 2014), we sought to examine the possibility that acute ethanol-mediated LTP inhibition also involves neuronal ER stress responses (Gyamfi and Wan, 2010; Magne et al., 2011). In the present studies, we used novel pharmacological agents to manipulate effects of ethanol on NMDARs, and provide support for the hypothesis that cellular stress responses are key contributors to ethanol's acute effects on synaptic function and learning.

2. Materials and methods

2.1. Hippocampal slice preparation

Animal use followed NIH guidelines and was approved by the Washington University Institutional Animal Care and Use Committee. We prepared hippocampal slices from postnatal day (P) 28–32 Harlan Sprague-Dawley male albino rats (Indianapolis IN) (Tokuda et al., 2010, 2011). Dissected hippocampi were pinned at their ventral pole on a 3.3% agar base in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 5 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 22 $NaHCO_3$, 10 glucose, bubbled with 95% O_2–5% CO_2 at 4-6 °C. The dorsal two-thirds of the hippocampus were cut into 500 μm slices using a rotary tissue slicer. Acutely prepared slices were placed in an incubation chamber containing gassed ACSF for at least 1 h at 30 °C before further study.

2.2. Hippocampal slice physiology

At the time of study, slices were transferred to a submersion-recording chamber at 30 °C and perfused with ACSF at 2 ml/min. Extracellular recordings were obtained from the apical dendritic layer (*stratum radiatum*) of the CA1 region for monitoring excitatory postsynaptic potentials (EPSPs) with electrodes filled with 2 M NaCl (5–10 MΩ resistance).

EPSPs were evoked with 0.1 ms constant current pulses through a bipolar stimulating electrode in the Schaffer collateral (SC) pathway. Responses were monitored by applying single stimuli to the SC pathway every 60 s at half maximal intensity. After obtaining a control input-output curve and stable baseline recordings for at least 10 min, LTP was induced by a single 100 Hz × 1 s high frequency stimulation (HFS) at the same intensity stimulus. LTD was induced using repeated low frequency stimulation (LFS) consisting of 900 single pulses at 1 Hz. Input-output curves were repeated 60 min following HFS or LFS.

Isolated EPSPs mediated by NMDARs were recorded at low frequency (1/min) in ACSF containing 0.1 mM Mg^{2+} and 2.5 mM Ca^{2+} to promote NMDAR activation by Schaffer collateral stimulation, and 30 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to eliminate the contribution of AMPARs (Izumi et al., 2005b). NMDAR-mediated EPSPs isolated in this manner are completely inhibited by the NMDAR-antagonist, APV (Izumi et al., 2005b, 2006).

2.3. Behavioral studies

Rats were tested in a one-trial inhibitory avoidance learning task (Whitlock et al., 2006; Tokuda et al., 2010). The testing apparatus has two chambers, one of which is lit and the other is kept dark; both compartments have a floor of stainless steel rods (4 mm diameter, spaced 10 mm apart) through which an electrical shock could be delivered in the dark chamber (12 × 20 × 16 cm). The lit compartment (30 × 20 × 16 cm) was illuminated with four 13 W lights. Light intensity in the lit chamber was 1000 lx while that in the dark chamber was < 10 lx. On the first day of testing, rats were placed in the lit chamber and allowed to habituate to the apparatus by freely moving between chambers for 10 min. On the second day, rats were administered ISRIB (2.5 mg/kg ip), GW3965 (20 mg/kg ip), or vehicle (DMSO) 2 h prior to being administered ethanol or saline. Fifteen minutes prior to being placed in the apparatus, rats were treated with either saline or ethanol (2 g/kg ip). Again, animals were initially placed in the lit compartment and allowed to explore the apparatus freely for up to 300 s (5 min). When rats entered the dark chamber they were given a foot shock. Upon returning to the lit (safe) chamber, animals were removed from the apparatus and returned to their home cages. On the next day of testing, rats were placed in the lit chamber without any drug treatment and the latency to enter the dark compartment was recorded over a 300 s trial.

2.4. In vivo ethanol levels

Blood and CSF were obtained from animals15 min after ethanol administration. After deproteination of plasma with trichloroacetic acid, ethanol levels were determined as increased absorbance at 340 nm from consumption of NAD in the presence of alcohol dehydrogenase using Alcohol Reagent Set (Cat #A7504–150 from Pointe Scientific, Carton MI) (Izumi et al., 2005a).

2.5. Chemicals

For in vivo experiments, 5 mg ISRIB or 10 mg GW3965 was dissolved in 1 ml DMSO and 1 ml poly-ethylene glycol (PEG, Sigma Cat# 202398) and intraperitoneally injected with saline. The final concentration of solvents for the in vivo studies was 10% DMSO and 10% PEG. For ex vivo slice experiments, both agents were dissolved in DMSO and used at a final DMSO concentration of 0.1%. At this concentration DMSO alone has no effect on LTP (EPSPs: 151.4 ± 16.1% of baseline 60 min following HFS, $N = 5$) or LTD (44.6 ± 8.4% of baseline 60 min following LFS, N = 5). ISRIB was purchased from Tocris Bioscience (Bristol, UK). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO). For ex vivo studies, drugs were diluted to final concentrations in ACSF and administered by bath perfusion. Concentrations selected for study and the durations of drug administration were based on prior studies (DiPrisco et al., 2014; Sandoval-Hernandez et al., 2015; Sidrauski et al., 2013) and were selected for study in synaptic plasticity experiments based on having no significant effects on baseline transmission in naïve slices.

2.6. Experimental design & statistical analysis

Data were collected and analyzed using PClamp software (Axon Instruments, Union City CA; RRID:SCR_011323). Data are expressed as mean ± SEM 60 min following HFS or LFS, and are normalized to initial baseline recordings (taken as 100%). At 60 min following the induction stimulus, LTP and LTD are stably induced avoiding confounds with shorter term forms of plasticity. A two-tailed Student's *t*-test was used for comparisons between groups. In cases of non-normally distributed data, the non-parametric Wilcoxon Rank Sum Test was used. Statistical comparisons were based on data from input-output (I/O) curves at baseline and sixty minutes following HFS or LFS to determine the degree of change in EPSP slope at the 50% maximal point with $p < .05$ considered significant; an example of IO analysis is shown in Fig. 1B. Statistics were performed using commercial software (SigmaStat, Systat Software, Inc., Richmond City, CA; RRID:SCR_010285). Data in figures for ex vivo physiological studies are derived from continuous monitoring of synaptic responses at low frequency during the course of experiments and thus may differ from results described in the text, which are based on analyses of input-output curves. Because synaptic responses were monitored infrequently, short-term forms of plasticity following HFS are not observed in all figures.

3. Results

Because we previously found that the effects of ethanol on LTP are overcome by agents that inhibit local metabolism of ethanol to

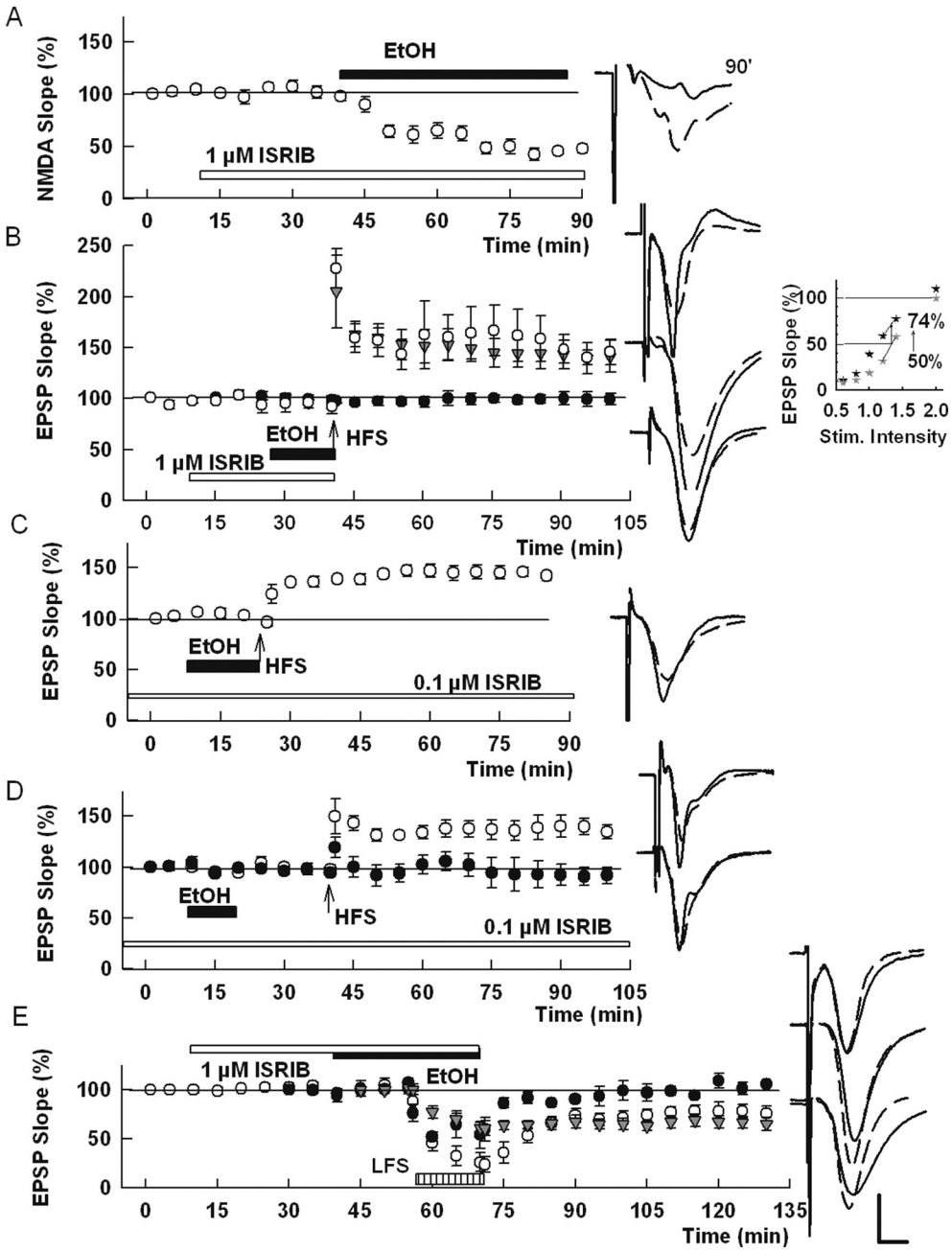

Fig. 1. An inhibitor of integrated stress responses does not alter NMDA EPSPs but overcomes effects of ethanol on CA1 synaptic plasticity. A. At 1 μM, ISRIB had no effect on NMDA EPSPs and did not alter the block of synaptic NMDARs by 60 mM ethanol. Traces to the right of the graph show representative NMDA EPSPs at the time denoted with baseline response shown with dashed line. B. 1 μM ISRIB overcame the acute effects of ethanol on LTP. Gray triangles show control LTP in naïve slices; black circles show effects of ethanol and white circles depict ethanol plus ISRIB. The graph to the right of the representative EPSP shows IO curves at baseline and 60 min following HFS to depict methods used for analyzing LTP and LTD experiments. In the example shown the stimulus that produced a half-maximal response at baseline gave a 74% maximal response 1 h after HFS resulting in a degree of LTP that was 148% of baseline. C. Longer administration of 0.1 μM ISRIB also overcame the acute effects of ethanol on LTP. D. When administered at 0.1 μM for a longer period, ISRIB also overcame the prolonged effects of ethanol on LTP. E. At 1 μM, ISRIB blocked the effects of 60 mM ethanol on LTD. Gray triangles show LTD in control slices; black circles show effects of 60 mM ethanol (administered during black bar); white circles show effects of ISRIB (administered during white bar). The hashed bar depicts administration of 1 Hz LFS. At 1 μM, ISRIB overcame the acute effects of ethanol on LTD. Traces in B-E show representative EPSPs taken 60 min following the conditioning stimulation (solid lines) with dashed lines showing baseline responses. In B, the middle traces depict LTP in a control slice and the lowest traces depict LTP inhibition by ethanol alone. In D, the lower traces depict LTP inhibition by ethanol. In E, the upper trace depicts LTD inhibition by ethanol alone and the middle traces show LTD in a control slice. The uppermost traces in B and the lowest traces in E depict LTP and LTD in control slices. Calibration 1 mV, 5 ms.

acetaldehyde (Tokuda et al., 2013a, 2013b) and acetaldehyde contributes to toxic effects of ethanol in liver via activation of endoplasmic reticulum (ER) stress responses (Magne et al., 2011), we explored whether agents that modulate cellular stress alter ethanol's effects on CA1 plasticity. In these experiments, we focused on ISRIB, an inhibitor of ER stress that enhances hippocampal plasticity in experimental models (DiPrisco et al., 2014; Sekine et al., 2015). At 1 μM, ISRIB had no effect on isolated NMDAR-mediated EPSPs (94.2 ± 6.3% of control after 30 min exposure, $N = 5$; Fig. 1A) or on the ability of 60 mM ethanol to inhibit NMDAR responses (50.7 ± 5.7% of baseline, $N = 5$ vs. 59.3 ± 4.9% in the presence of ethanol alone; $p > .05$; Fig. 1A). However, 1 μM ISRIB completely overcame the acute effects of 60 mM ethanol on LTP when administered 15 min prior to and during ethanol (EPSP slope: 144.6 ± 7.6% of baseline 60 min following HFS, $N = 5$; $p < .001$ vs. ethanol alone: 93.6 ± 2.4% of baseline EPSPs, N = 5, Fig. 1B). In control slices, HFS readily induced LTP and the degree of LTP in the presence of ISRIB plus ethanol was similar to this control LTP (136.6 ± 6.8% of baseline 60 min following HFS, $N = 6$; $p > .05$ vs. ethanol plus ISRIB; Fig. 1B).

A lower concentration of ISRIB (0.1 μM) also overcame the acute effects of ethanol on LTP when slices were pre-incubated with ISRIB for an hour or more prior to experiments and ethanol exposure (ESPS slope: 147.8 ± 4.7% of baseline 60 min following HFS, N = 5, p < .001 vs. ethanol alone; Fig. 1C). We previously showed that ethanol's block of LTP can persist for an hour or more following ethanol washout (Tokuda et al., 2011; Zorumski et al., 2014); this persisting effect of ethanol on LTP is also eliminated by the lower concentration of ISRIB (EPSP slopes: 95.5 ± 7.3% of baseline 60 min following HFS after treatment with 60 mM ethanol alone vs. 148.2 ± 15.7% in slices treated with ISRIB, $p < .05$ vs. ethanol, $N = 5$; Fig. 1D).

At 1 μM, we also found that ISRIB acutely overcame the ability of ethanol to inhibit homosynaptic LTD induced by 1 Hz LFS (Ethanol: 104.9 ± 4.9% of baseline, $N = 5$ vs. ISRIB: 71.2 ± 7.1%, N = 5; $p < .01$; Fig. 1D). In naïve slices, LFS induced robust LTD and this

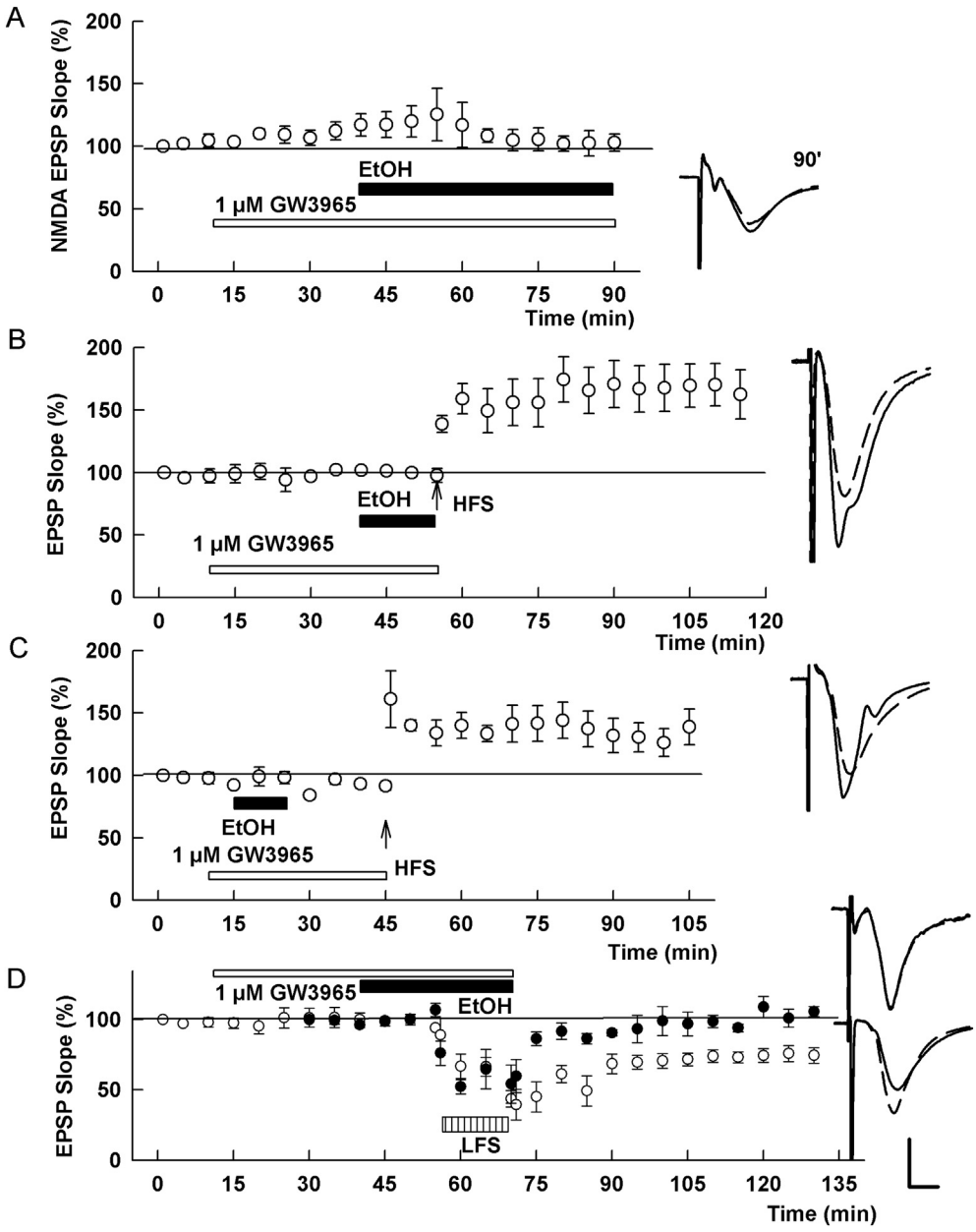

Fig. 2. An LXR agonist does not alter NMDA EPSPs alone but overcomes effects of ethanol on NMDAR-mediated synaptic responses and hippocampal plasticity. A. At 1 μM, GW3965 had no effect on isolated NMDAR-mediated EPSPs but blocked the ability of 60 mM ethanol to inhibit NMDAR EPSPs. Traces to the right the graph show representative EPSP at the times denoted with baseline response shown as dashed line. B,C. This same concentration of GW3965 overcame both the acute (B) and persistent (C) effects of ethanol on LTP. D. The LXR agonist also overcame the acute effects of 60 mM ethanol on LTD. Traces to the right of the graphs of B-D show representative EPSPs taken 60 min after the conditioning stimulation with baseline traces shown as dashed lines. The lower set of traces in E is with GW3965. Calibration: 1 mV, 5 ms.

degree of LTD did not differ significantly from ethanol plus ISRIB (EPSP slope: 53.3 ± 5.3% 60 min following LFS; $N = 6$, $p > .05$ vs. ethanol plus ISRIB; Fig. 1E).

The ability of ISRIB to overcome effects of ethanol on plasticity raises questions about whether other agents that modulate cellular stress can alter the adverse effects of ethanol on synaptic plasticity. Prior studies have found that agonists at liver X receptors (LXRs), a subtype of nuclear receptors, can dampen ER stress via effects on membrane phospholipid composition (Sun et al., 2016; Rong et al., 2013; Janowski et al., 1999). Our interest in the role of LXRs was also prompted by prior studies showing a role for 5α-reduced neurosteroids in the effects of ethanol on LTP (Izumi et al., 2007; Tokuda et al., 2011) and the ability of these steroids to modulate LXRs (Brinton, 2013; Mendel and MacLusky, 2018). To test whether activation of LXRs alters the effects of ethanol, we used GW3965, an agonist at both α and β LXR isoforms (Fessler, 2018). GW3965 alone had no effect on isolated NMDAR EPSPs at 1 μM (96.9 ± 5.7% of control after 30 min exposure, $N = 5$; Fig. 2A), but unlike ISRIB, prevented the effects of ethanol on NMDAR EPSPs (106.6 ± 9.7% of baseline, $N = 5$; $p < .001$ vs. ethanol alone; Fig. 2A)., GW3965 also overcame both the acute (EPSP slope: 158.7 ± 10.9%, N = 5; p < .001 vs. ethanol alone; Fig. 2B) and prolonged effects of ethanol on LTP (EPSP slope: 147.8 ± 8.7%, $N = 6$, $p < .01$ vs. ethanol; Fig. 2C). GW3965 also blocked the ability of acute ethanol to inhibit LTD (74.2 ± 3.2%, N = 5, p < .001 vs. ethanol; Fig. 2D).

Because our prior studies indicated that the effects of high ethanol on LTP likely involve local hippocampal production of acetaldehyde (Tokuda et al., 2013a, 2013b), we also examined whether cellular stress inhibitors could overcome the effects of a lower concentration of ethanol in combination with exogenous acetaldehyde. For these studies, we used concentrations of ethanol (20 mM) and acetaldehyde (60 μM) that inhibit LTP when applied together, but not individually (Tokuda et al., 2013b). In control slices, the combination of acetaldehyde and ethanol completely blocked LTP induction (97.6 ± 3.7% of baseline, $N = 5$, p < .01 vs. control LTP; Fig. 3A). As with high ethanol alone, we found that ISRIB overcame the effects of ethanol plus acetaldehyde (126.8 ± 3.3%, N = 5; p < .001 vs. ethanol + acetaldehyde; Fig. 3A). Similarly, the LXR agonist, GW3965, overcame the persistent

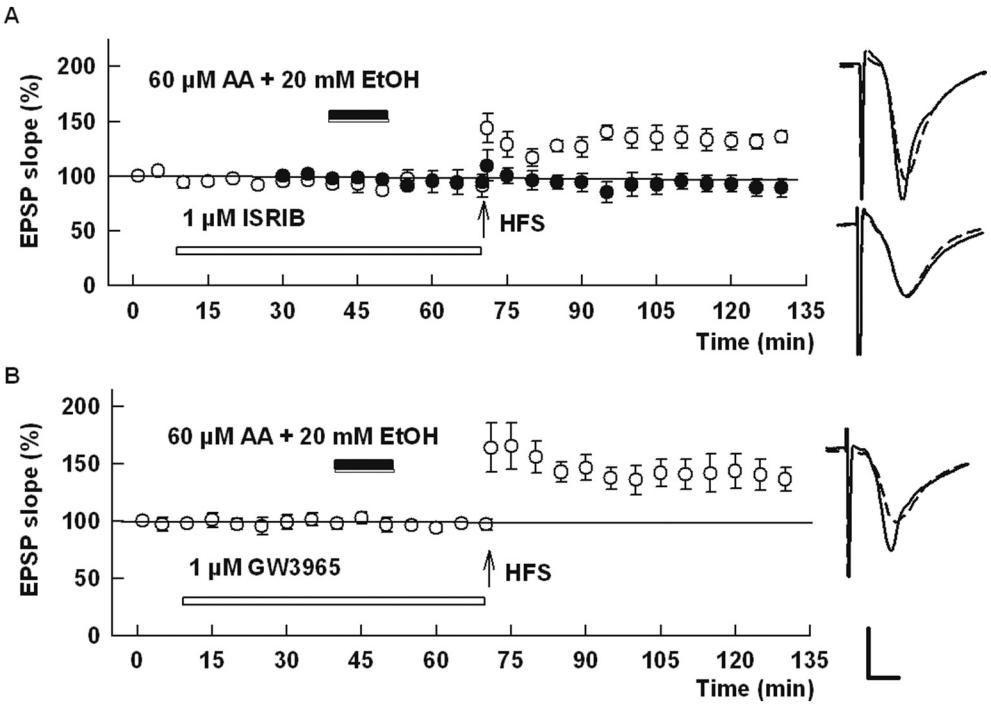

Fig. 3. ISRIB and GW3965 overcome the effects of acetaldehyde plus ethanol on LTP. A. When 60 μM acetaldehyde plus 20 mM ethanol (black bar) was administered for 15 min and then washed out 30 min prior to HFS, LTP was completely inhibited (black circles). In slices pretreated with ISRIB (white bar) before the ethanol plus acetaldehyde and maintained until HFS, LTP was readily induced (white circles). B. GW3965 (white bar) also overcame the prolonged effects of ethanol plus acetaldehyde on LTP. Traces to the right depict EPSPs at baseline (dashed lines) and 60 min following HFS (solid lines). In A, the lower traces depict LTP inhibition by ethanol and acetaldehyde. Calibration 1 mV, 5 ms.

effects of the drug combination on LTP (134.4 ± 13.5%, N = 5; $p < .05$ vs. ethanol + acetaldehyde, Fig. 3B).

Because ethanol-mediated LTP inhibition involves a form of NMDAR-dependent metaplasticity (Zorumski and Izumi, 2012; Zorumski et al., 2014), we also examined whether the effects of ISRIB and GW3965 extend to other forms of metaplastic LTP inhibition, focusing on LTP inhibition mediated by a low concentration of NMDA (Izumi et al., 1992a, 1992b). At 1 μM, NMDA completely blocked LTP when administered for 15 min prior to HFS (103.0 ± 5.5% 60 min following SC HFS, $N = 6$; Fig. 4A). This LTP inhibition was prevented by administration of either ISRIB (148.0 ± 17.0% change after HFS, $N = 5$, $p < .05$ vs. NMDA alone; Fig. 4B) or GW3965 (175.4 ± 11.6%, $N = 5$, $p < .001$ vs. NMDA alone, Fig. 4C).

To determine whether the ability of the stress inhibitors to overcome effects of ethanol on hippocampal plasticity translate into changes in behavior, we examined whether ISRIB and GW3965 alter acute amnesic effects of ethanol. For these studies, we used a one trial inhibitory avoidance learning task, a form of learning dependent upon hippocampal plasticity (Whitlock et al., 2006; Tokuda et al., 2010). At a dose of 2 g/kg ip that produces blood and CSF alcohol levels of 81.1 ± 15.3 mg/dl (17.6 ± 3.3 mM) and 89.6 ± 18.6 mg/dl (19.4 ± 4.0 mM, N = 6 each) 15 min following ethanol administration, ethanol markedly impaired learning, and animals much more readily entered the dark chamber the day following a foot shock in the dark chamber.

Saline-treated controls learned the task well and did not leave the lit chamber after receiving a shock in the dark chamber 24 h prior to testing (N = 5; not shown). Similarly pretreatment with solvents used for ISRIB and GW3965 (10% DMSO +10% PEG) had no effect on the ability of rats to learn the task (N = 5; Fig. 5). Rats treated with vehicle followed by ethanol were sedated (hypoactive) immediately after ethanol administration, but did not exhibit loss of righting reflexes (LORR). Hypoactivity persisted for at least one hour after ethanol exposure. At the time of testing, rats were awake with mild incoordination (staggering on moving in the test apparatus). In contrast to saline or vehicle-treated animals, rats that received ethanol 15 min before being shocked readily entered the dark chamber 24 h later (latency to enter dark chamber: 37.8 ± 12.5 s, $N = 8$, $p < .001$ vs. saline controls; 22.4 ± 14.8 s, N = 5, $p < .001$ vs. vehicle-treated controls, Fig. 5). Pretreatment of rats with 2.5 mg/kg ip ISRIB 2 h prior to ethanol completely prevented the adverse effects of ethanol on learning, and all ISRIB-treated animals remained in the lit compartment for the full 300 s test period ($p < .001$ vs. vehicle + ethanol, N = 5, Fig. 5). Pretreatment with GW3965 (20 mg/kg ip) also overcame the effects of ethanol on inhibitory avoidance learning (latency to enter the dark chamber: 242.0 ± 27.8 s, N = 6, $p < .001$ vs. vehicle + ethanol; Fig. 5). Neither ISRIB nor GW3965 had a clear effect on the hypoactivity or mild incoordination observed following ethanol, and, by themselves, did not alter learning in the one-trial task (ISRIB latency time: 291.4 ± 8.6 s, N = 5; GW3965 latency time: 274 ± 25.4 s, N = 5, Fig. 5).

4. Discussion

Ethanol inhibits NMDARs (Izumi et al., 2005b; Zorumski et al., 2014; Abrahao et al., 2017) and inhibits NMDAR-dependent synaptic plasticity in hippocampal slices (Zorumski et al., 2014). Thus, it has been thought that effects on NMDARs are critical for effects on LTP and LTD. However, prior studies found that ethanol is only a partial inhibitor of NMDARs at concentrations that inhibit synaptic plasticity (Izumi et al., 2005b), and effects of ethanol on plasticity involve GABA-A receptors (Izumi et al., 2005b; Zorumski et al., 2014). Additionally, effects of ethanol on CA1 LTP involve a form of metaplasticity in which untimely NMDAR activation dampens LTP induction (Zorumski and Izumi, 2012). This LTP inhibition is overcome paradoxically by complete NMDAR antagonism with APV administered during ethanol exposure (Izumi et al., 1992b; Tokuda et al., 2011). Effects on LTP require high concentrations of ethanol (60 mM) and are prevented by agents that inhibit the metabolism of ethanol to acetaldehyde (Tokuda et al., 2013a, 2013b). Because acetaldehyde has adverse effects on cellular function including activation of ER stress responses (Magne et al., 2011; Gyamfi and Wan, 2010), these observations raise the possibility that cellular stress responses could be important contributors to changes in hippocampal plasticity.

Here we provide evidence that GW3965, an agonist at both α and β LXRs, did not alter baseline NMDAR responses, but prevented the effects of ethanol on NMDA EPSPs. In contrast, the integrated stress response (ISR) inhibitor, ISRIB, had no effect on either baseline NMDAR

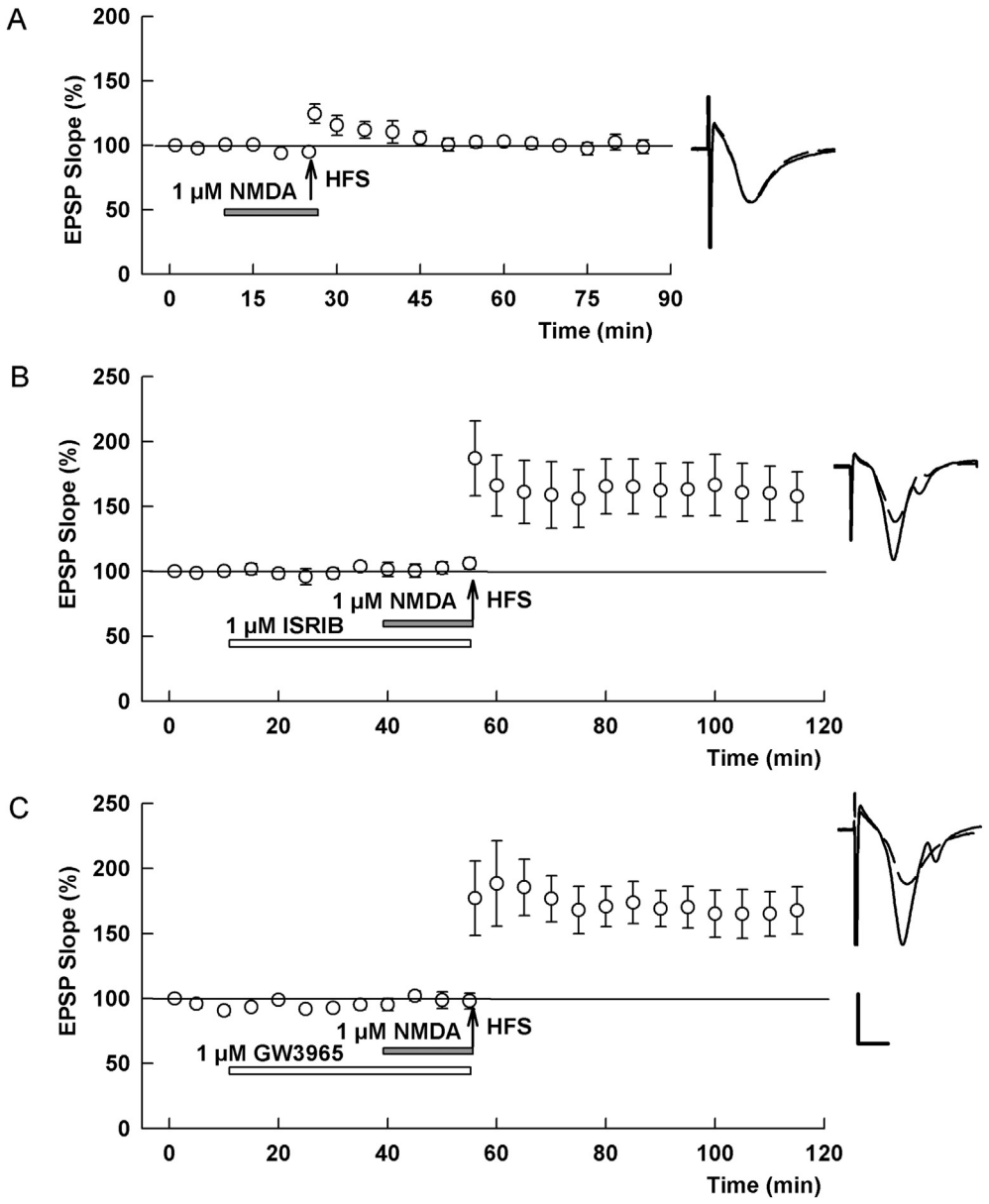

Fig. 4. ISRIB and GW3965 overcome the acute effects of NMDA on CA1 LTP. A. Consistent with what we have observed previously (24,25), 1 μM NMDA (administered for 15 min in this case to be consistent with acute administrations of ethanol) had no effect on baseline EPSPs but completely inhibited the ability of a single 100 Hz HFS (arrow) to induce LTP in the SC pathway. B, C. At a concentration of 1 μM, ISRIB (B) and GW3965 (C) overcame the block of LTP by NMDA. Traces show representative EPSPs taken 60 min after HFS with dashed lines depicting controls. Calibration bar: 1 mV, 5 ms.

EPSPs or on the block of these responses by ethanol. The mechanisms underlying the effects of the LXR agonist on ethanol-mediated block of NMDARs are uncertain, but functional interactions between NMDARs and LXR have been observed in retina, where activation of LXR is protective against NMDA-mediated toxicity via p38 MAP kinase, an enzyme that also contributes to NMDAR-mediated metaplasticity (Zheng et al., 2015; Izumi et al., 2008). Additionally, GW3965 and LXR activation can prevent adverse synaptic effects of beta amyloid in cultured hippocampal neurons (Baez-Becerra et al., 2018) and beta amyloid has been shown to activate ER stress responses in neurons (Barbero-Camps et al., 2014). There are two forms of LXR expressed in cells, α and β, with the β-isoform being predominant in neurons and contributing to synaptic development (Cai et al., 2018) and neurogenesis (Peng et al., 2018) in the hippocampus. Additionally, LXRs can modulate cellular function through both genomic and non-genomic mechanisms (Fessler, 2018). Based on our results, we propose that LXR activation has two actions that overcome the acute effects of ethanol on hippocampal plasticity – effects on ethanol-mediated NMDAR block and effects on ER stress; ISRIB shares only the effects on ER stress. Our observations further suggest that ER stress as well as NMDAR inhibition can contribute to effects of ethanol on LTD.

The ability of inhibitors of cellular stress responses to overcome effects of ethanol on hippocampal synaptic plasticity is also consistent with prior observations that acute ethanol results in local NMDAR-mediated production of GABA-enhancing 5α-reduced neurosteroids and reversal of effects on LTP by agents that inhibit either the synthesis or function of these neurosteroids (Izumi et al., 2007; Tokuda et al., 2011). The role of endogenous neurosteroids in dampening neuronal responses to stress, including neuroprotective effects (Ishikawa et al., 2014) suggests that altered synaptic plasticity may be a compensatory mechanism that helps to protect neurons from adverse effects of acute stress. In this case, as has been observed in other systems (Ishikawa et al., 2014, 2020), alloP serves as a neuroprotective mechanism that dampens neuronal excitability but results acutely in altered synaptic

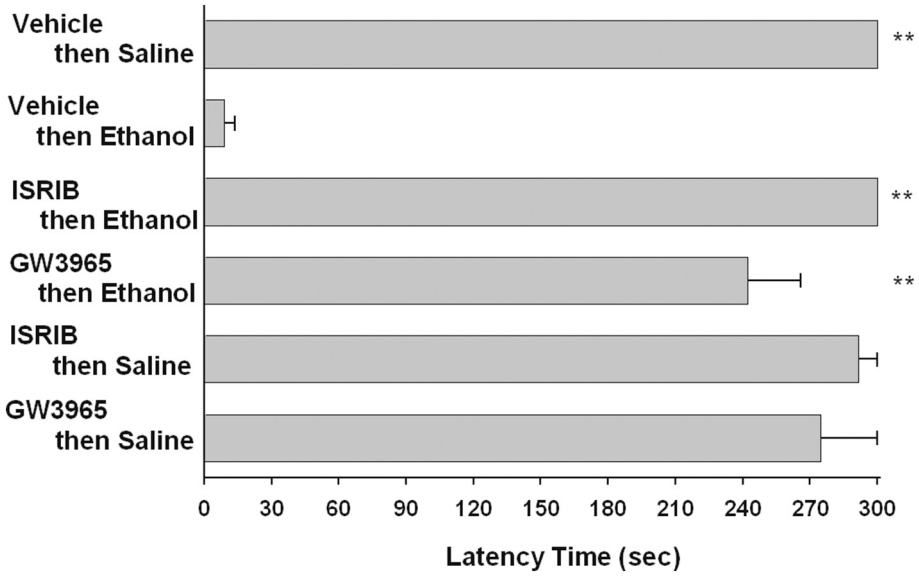

Fig. 5. ISRIB and GW3965 overcome the effects of ethanol on one trial inhibitory avoidance learning. A. The top bar depicts the latency for vehicle-treated rats (10% DMSO +10% PEG) to enter the dark chamber twenty four hours after receiving a shock upon entry into the dark compartment. These animals learned the task and failed to leave the lit chamber during the 300 s test. Rats treated with vehicle plus 2 g/kg ethanol 30 min prior to the conditioning trial showed a marked decrement in learning and readily entered the dark chamber. In contrast animals pretreated with ISRIB or GW3965 2 h prior to ethanol during the conditioning trial performed like untreated controls and readily learned the task. ISRIB and GW3965 alone had no effect on learning. ** $p < .001$ vs. vehicle + ethanol.

plasticity and learning (Izumi et al., 2005; Tokuda et al., 2011). We also note that ER stress can promote synthesis of pregnenolone from cholesterol, a first step in synthesis of alloP (Barbero-Camps, 2014). Our present and prior results also indicate that this scheme, particularly involvement of the ISR, is relevant to the direct adverse effects of NMDA on LTP (Fig. 4; Zorumski and Izumi, 2012).

The ability of ISRIB to overcome acute effects of ethanol on LTD was unexpected. In prior studies, ethanol block of LTD, unlike LTP inhibition, tracked closely with the timing of ethanol exposure and reversed rapidly following ethanol washout (Izumi et al., 2005b). Ethanol preferentially inhibits a subtype of ifenprodil-sensitive (GluN2B-expressing) synaptic NMDARs in the CA1 region of P30 rat hippocampus, and ifenprodil mimics the effects of ethanol on LTD (Izumi et al., 2005b, 2006). ISRIB had no effect on isolated NMDAR responses or on the ability of ethanol to inhibit NMDA EPSPs, but reversed effects of ethanol on LTD. In contrast, GW3965 overcame the effects of ethanol on NMDARs and prevented the block of LTD, consistent with a role of NMDAR block in the effects of ethanol on this form of plasticity.

Our present studies were stimulated by prior findings that acute block of LTP by ethanol involves local metabolism to acetaldehyde (Boyd et al., 2008; Tokuda et al., 2013a, 2013b), and the role of acetaldehyde in certain effects of ethanol including adverse effects on cellular function and survival (Quertemont et al., 2005; Boyd et al., 2008; Magne et al., 2011). In liver, ethanol activates both ER stress responses and nuclear receptors in the pathogenesis of liver injury (Magne et al., 2011; Gyamfli and Wan, 2010; Yang et al., 2014), and activation of ER stress responses are mediated by acetaldehyde. These actions are likely related in that LXR can dampen ER stress via changes in membrane phospholipids (Rong et al., 2013) and also possibly via more rapid non-genomic mechanisms (Fessler, 2018). ER stress can have adverse effects on synaptic function and learning in rodents both in vitro and in vivo (Sidrauski et al., 2013; Chou et al., 2017) and contributes to cognitive dysfunction in models of neuropsychiatric disorders (Zhu et al., 2019). ISRIB, an ISR inhibitor that acts by mechanisms distinct from LXR by disrupting a key step in ISR activation involving interactions between phosphorylated eukaryotic initiation factor 2α (eIF2α) and eIF2B (Sekine et al., 2015' Sidrauski et al., 2013), can promote synaptic plasticity and learning, and reverse synaptic dysfunction in neurocognitive disorders (DiPrisco et al., 2014; Zhu et al., 2019). Our results indicate that ISRIB and GW3965, likely acting via distinct (effects on NMDARs) but overlapping mechanisms (ER stress), overcome the adverse effects of acute high ethanol on hippocampal plasticity and one-trial learning. While the contributions of LTP and LTD to different forms of learning are uncertain, the form of one-trial learning we studied has been linked previously to changes in hippocampal LTP (Whitlock et al., 2006; Tokuda et al., 2010) suggesting that the behavioral effects observed here may be most relevant to ethanol-induced changes in LTP.

The present results provide strong support for the idea that acute adverse effects of ethanol on hippocampal function involve activation of cellular stress responses, and point the way to potential therapeutic approaches to treat and possibly prevent acute alcohol-induced memory impairment (memory "blackouts"). These amnestic episodes are associated with severe intoxication and/or intoxication in the context of use of other depressant drugs (opiates, benzodiazepines, anticholinergics). Memory blackouts can have devastating personal and societal effects, particularly among adolescents and young adults, and may predict further cognitive difficulties with ongoing alcohol use (White, 2003; Parada et al., 2011). There is also evidence that adverse brain effects of acute binge alcohol consumption (Crews et al., 2004), including defects in short-term memory, can persist in the alcohol hangover period when BAC levels have dropped to zero (Contreras et al., 2019; Gunn et al., 2018; Silvestre de Ferron et al., 2015; West et al., 2018). This latter state may be akin to the persisting adverse effects of ethanol on LTP that we observe after ethanol washout. This hypothesis would suggest that the persisting effects of ethanol on LTP are most critical for persisting effects on learning in the hangover period because changes in NMDAR-mediated synaptic responses and LTD appear to reverse once ethanol is eliminated (Izumi et al., 2005).

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