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Riluzole Impairs Cocaine Reinstatement and Restores Adaptations in Intrinsic Excitability and GLT-1 Expression

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Adaptations in glutamate signaling within the brain's reward circuitry are observed following withdrawal from several abused drugs, including cocaine. These include changes in intrinsic cellular excitability, glutamate release, and glutamate uptake. Pharmacological or optogenetic reversal of these adaptations have been shown to reduce measures of cocaine craving and seeking, raising the hypothesis that regulation of glutamatergic signaling represents a viable target for the treatment of substance use disorders. Here, we tested the hypothesis that administration of the compound riluzole, which regulates glutamate dynamics in several ways, would reduce cocaine seeking in the rat self-administration and reinstatement model of addiction. Riluzole dose-dependently inhibited cue- and cocaine-primed reinstatement to cocaine, but did not affect locomotor activity or reinstatement to sucrose seeking. Moreover, riluzole reversed bidirectional cocaine-induced adaptations in intrinsic excitability of prelimbic (PL) and infralimbic (IL) pyramidal neurons; a cocaine-induced increase in PL excitability was decreased by riluzole, and a cocaine-induced decrease in IL excitability was increased to normal levels. Riluzole also reversed the cocaine-induced suppression of the high-affinity glutamate transporter 1 (EAAT2/GLT-1) in the nucleus accumbens (NAc). GLT-1 is responsible for the majority of glutamate uptake in the brain, and has been previously reported to be downregulated by cocaine. These results demonstrate that riluzole impairs cocaine reinstatement while rectifying several cellular adaptations in glutamatergic signaling within the brain's reward circuitry, and support the hypothesis that regulators of glutamate homeostasis represent viable candidates for pharmacotherapeutic treatment of psychostimulant relapse.
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INTRODUCTION

Identification and reversal of cocaine-induced cellular adaptations that drive drug seeking represent a strategic approach toward development of pharmacotherapeutic treatments for cocaine relapse. Considerable evidence indicates that withdrawal from chronic cocaine leads to numerous adaptations in signaling at glutamatergic excitatory projections onto medium spiny neurons (MSNs) in the nucleus accumbens (NAc), including changes in intrinsic excitability, synaptic strength and plasticity, and glutamate uptake (Kalivas and Volkow, 2011; Kourrich *et al*, 2015; Uys and Reissner, 2011). Reversal of these adaptations has revealed therapeutic potential for inhibition of cocaine relapse at glutamatergic synapses, particularly those originating from the prefrontal cortex (Javitt *et al*, 2011; McCullumsmith and Sanacora, 2015; Roberts-Wolfe and Kalivas, 2015).

Riluzole (Rilutek, Sanofi-Aventis) is an inhibitor of voltage-gated Na+ channels, and is FDA approved for treatment of amyotrophic lateral sclerosis (Meininger *et al*, 2000; Traynor *et al*, 2006). The multiple cellular targets of riluzole render it an intriguing candidate for inhibition of relapse to cocaine. Firstly, riluzole has been ascribed neuroprotective and anticonvulsive properties because of reduced glutamate release subsequent to Na+ channel inhibition (for review see Machado-Vieira *et al*, 2009; Pittenger *et al*, 2008). Riluzole can also influence intrinsic excitability through modulation of potassium channels (Ahn *et al*, 2006; Duprat *et al*, 2000; Pittenger *et al*, 2008; Xu *et al*, 2001). Of relevance, an increase in cellular excitability in the dorsomedial prefrontal cortex (dmPFC) has previously been reported following withdrawal from noncontingent cocaine administration (Dong *et al*, 2005; Hearing *et al*, 2013; Nasif *et al*, 2005a, b, but see also Otis and Mueller, 2017).

Secondly, in addition to these effects on cellular excitability, numerous studies have reported that riluzole administration also leads to upregulation of expression and activity of glutamate transporters, in particular GLT-1, both *in vitro* and *in vivo* (Banasr *et al*, 2010; Brothers *et al*, 2013; Carbone *et al*, 2012; Frizzo *et al*, 2004; Fumagalli *et al*, 2008; Gourley *et al*, 2012; Liu *et al*, 2011). Cocaine withdrawal-dependent decreases in expression and activity of glutamate transporter GLT-1 in the NAc have been described (Fischer *et al*, 2013; Knackstedt *et al*, 2010), and pharmacological restoration of

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GLT-1 leads to decreased cue-primed as well as cocaine-primed reinstatement in the rat self-administration model of cocaine addiction (Knackstedt *et al*, 2010; Reissner *et al*, 2014, 2015; Sari *et al*, 2009). GLT-1 is responsible for ~90% of glutamate uptake in the brain, and is important for protection against excitotoxicity as well as for shaping glutamatergic transmission (Danbolt, 2001; Murphy-Royal *et al*, 2017; Robinson and Jackson, 2016; Tzingounis and Wadiche, 2007). Given the important role for GLT-1 in glutamatergic signaling, and the suppressive effect of cocaine on GLT-1 expression and activity, current theory posits that modulation of NAc GLT-1 function can influence glutamatergic drive in the NAc that serves as a limbic motor integrator within the brain's reward circuitry (Roberts-Wolfe and Kalivas, 2015; Spencer and Kalivas, 2017). Accordingly, normalization of function would normalize transmission and the drive toward drug seeking as behavioral output.

Thirdly, riluzole has also been reported to inhibit glutamate release from presynaptic neurons because of Ca^{2+} channel inhibition (Cheah *et al*, 2010; Lamanauskas and Nistri, 2008; Siniscalchi *et al*, 1997); cocaine-primed reinstatement leads to potentiated release of glutamate within the NAc (McFarland *et al*, 2003). Thus, riluzole is pharmacologically well positioned to reverse several of the cocaine-induced changes in glutamatergic signaling that are postulated to influence drug seeking, including intrinsic excitability, glutamate release, and glutamate uptake. In order to more fully explore this potential, we sought to investigate the effects of riluzole on cue- and cocaine-primed reinstatement in the rat self-administration model of cocaine abuse. In addition, because of the fundamental role for corticostriatal glutamatergic transmission in cocaine seeking, we subsequently assessed the effects of riluzole on cocaine-induced adaptations in intrinsic excitability in the mPFC and on GLT-1 levels in the NAc.

MATERIALS AND METHODS

Animal Care and Surgical Procedures

Male Sprague-Dawley rats (Harlan, Boston, MA; ~ 280 g at the time of surgery) were kept on a 12 h reverse light cycle. Before surgery, rats were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine (7 mg/kg) together with ketorolac analgesic (0.28–0.32 mg/kg). Chronic indwelling catheters were implanted into the right jugular vein for the administration of intravenous (i.v.) cocaine, as described previously (Scofield *et al*, 2016). Rats were given 5–7 days of recovery before behavioral training. Catheters were flushed daily with a prophylactic antibiotic (timentin 10.0 mg/0.1 ml, i.v., GlaxoSmithKline, Research Triangle Park, NC or gentamicin 0.5 mg/0.1 ml, i.v., Hospira, Lake Forest, IL) followed by heparinized saline (100 mg/ml, 0.1 ml i.v., Fresenius Kabi USA). Catheter testing for patency was performed periodically with propofol (1 mg/0.1 ml, i.v.). All experiments were approved by the institutional animal care and use committee of University of North Carolina at Chapel Hill.

Behavioral Training

All operant training was performed in a standard rat operant chamber (Med Associates, St Albans, VT). Before self-administration training, rats received a food training session (at least 6 h) in which an active lever press resulted in the administration of a single food pellet (45 mg/pellet, BioServ) until a criterion of 100 pellets received was met. Rats subsequently received 6 food pellets of standard rat chow per day (Envigo Teklad laboratory animal diet). Self-administration of cocaine (or saline) was performed in 2 h sessions on a fixed-ratio 1 (FR1) schedule of reinforcement. Each reinforced active lever press resulted in a drug infusion (5 mg/ml cocaine hydrochloride; 0.2 mg per infusion, i.v.; NIDA), a 5 s compound stimulus (cue light and tone, 70 dB, 2.5 kHz), and a 20 s time-out period. Criteria for self-administration were 10 days of at least 10 infusions per session. Rats were divided into treatment groups based on active lever presses during the last 3 days of self-administration. After self-administration of cocaine (or saline), rats entered the extinction phase (16 days: 2 h/day in the same operant boxes as self-administration) where an active lever no longer elicited the tone, cue light, and the infusion of cocaine. Following extinction, rats received a 2 h cue-primed (light and tone) reinstatement test, followed by 3 days of extinction and a 2 h cocaine-primed reinstatement test (10 mg/kg, i.p.). Throughout extinction training and reinstatement testing, rats received either vehicle or riluzole (1 or 4 mg/kg, i.p.; Toronto Research Chemicals) 30 min before each session. Riluzole was similarly given 30 min before all analyses (locomotor testing, sucrose reinstatement, electrophysiology, tissue preparation). Riluzole was prepared daily, in saline containing 2% Tween-80 (Sigma item P6474) and mixed at room temperature for at least 1 h before use. Saline containing 2% Tween-80 used to prepare riluzole was used as vehicle. In a separate set of experiments, rats were trained to self-administer sucrose (FR5; 45 mg/pellet, Test-Diet, Richmond, IL) in the same operant chambers used for cocaine studies. Sucrose-administering rats were trained in these operant chambers for the same number of days of self-administration and extinction as cocaine-administering rats. Cue-primed sucrose reinstatement (2 h) was performed under an FR1 schedule for presentation of cues. Rats did not receive surgery but were administered riluzole (4 mg/kg) or vehicle in an identical manner as for cocaine experiments.

Locomotor Activity

Rats were trained in self-administration and extinction as described, receiving riluzole (4 mg/kg) or vehicle 30 min before each extinction session and before locomotor testing. No reinstatement test was performed on rats used for locomotor testing. On test day rats were placed in open field chamber within a sound-attenuating chamber (Med Associates). Distance traveled (cm) was measured using activity motoring software (Med Associates) over a 2 h period.

Whole-Cell Patch-Clamp Electrophysiology

Measurements of neuronal excitability were taken 24 h after the last day of extinction. At 30 min after injection of riluzole (4 mg/kg) or vehicle, rats were deeply anesthetized with pentobarbital (65 mg/kg) and perfused transcardially with oxygenated cold modified artificial cerebral spinal fluid (aCSF)–NMDG-HEPES recovery solution and decapitated. Coronal slices (235 μm) of the medial prefrontal cortex

(mPFC) were taken using a vibratome (Leica VT1200S) in ice-cold cutting solution (in mM): 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1 NaH_2PO_4, 25 glucose, 6 $MgCl_2$, 1.5 $CaCl_2$, and 1 kynurenic acid. Slices were incubated at 32 °C for at least 10 min in oxygenated NMDG-HEPES recovery solution and then 1 h in oxygenated HEPES holding aCSF solution at room temperature (Ting *et al*, 2014). Slices were transferred to the recording chamber equipped with an upright microscope with differential interference contrast (DIC) water immersion optics and perfused at 2–3 ml/min with 32 °C aCSF. Whole-cell current-clamp recordings were performed in pyramidal neurons from layer V located in the prelimbic cortex (PL) and infralimbic cortex (IL) at a holding potential of − 70 mV. Action potentials were evoked by injecting 800 ms depolarizing current pulses with the range of 20–320 pA at 10 pA increments with an intertrial interval of 5 s, as described previously (Santini *et al*, 2008; Sepulveda-Orengo *et al*, 2013). Recordings were filtered at 4 kHz (MultiClamp700B; Molecular Devices), digitized at 10 kHz, and saved to a computer using pClamp10 (Molecular Devices). Membrane potentials (V_m) were not corrected for the junction potential. The input resistance (R_{in}) was measured from a 5 mV, 50 ms depolarizing pulse in voltage-clamp mode at a holding potential of − 60 mV. The fast afterhyperpolarizing potential (fAHP) was obtained from traces showing at least three spikes. The fAHP was measured between the second and third evoked spikes by subtracting the voltage at the peak of the fAHP from the threshold potential for spike initiation (Figures 2f and 3e, insert). Cells with less than three spikes were not used for the fAHP calculation. The medium afterhyperpolarizing potentials (mAHPs) and slow afterhyperpolarizing potentials

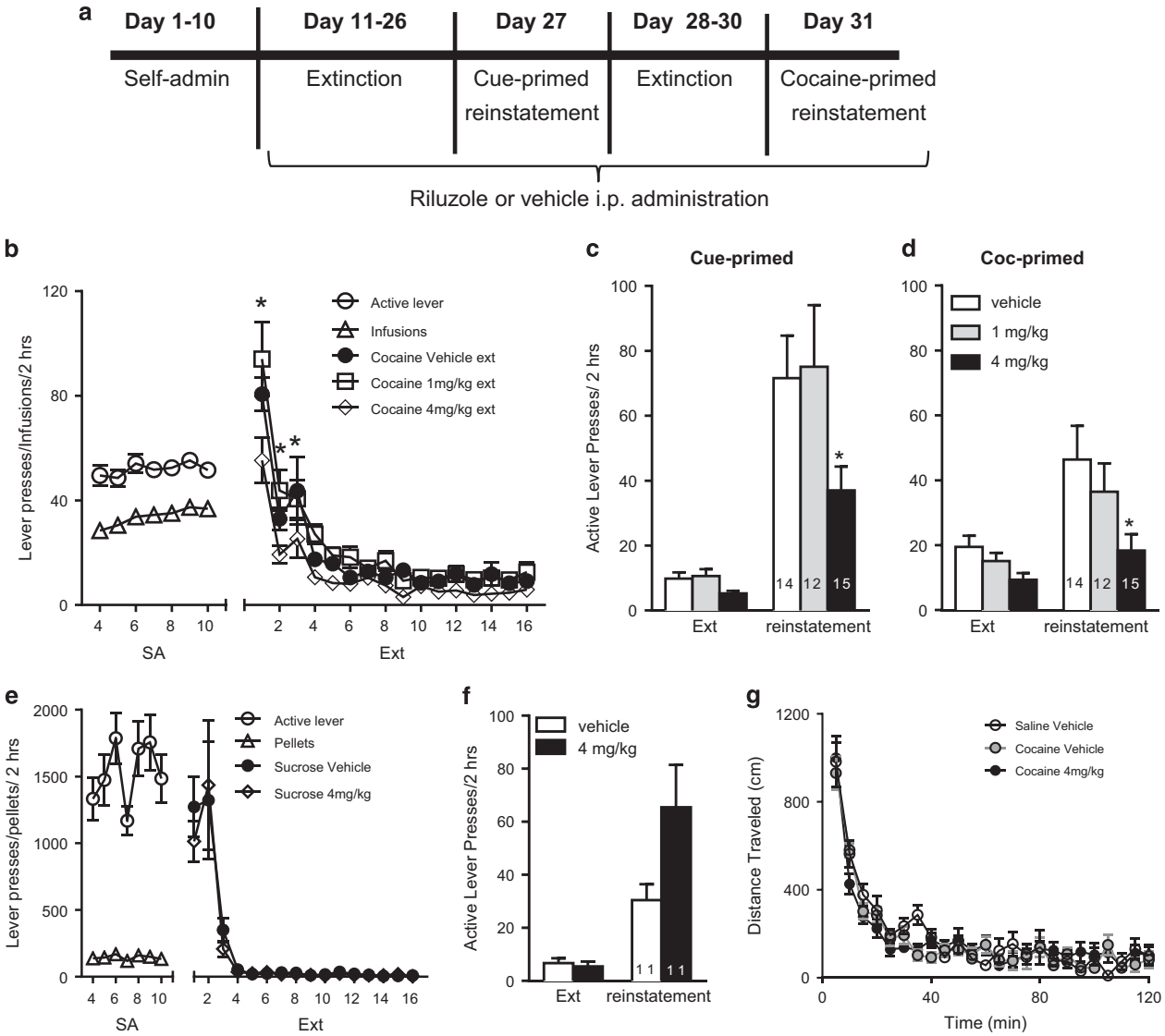

Figure 1 Riluzole inhibits cocaine reinstatement without impairment on locomotor activity or sucrose reinstatement. (a) Schematic of self-administration (SA), extinction (Ext), and reinstatement testing. At 30 min before each extinction or reinstatement session, rats were injected intraperitoneally (i.p.) with 4 mg/kg riluzole, 1 mg/kg riluzole, or vehicle. (b) Active lever presses and infusions for all rats across groups during the last 7 days of cocaine SA and throughout extinction training. Extinction pressing is shown separately for respective treatment groups. *Indicates 4 mg/kg riluzole-treated significantly different from vehicle-treated. (c, d) Active lever presses for each group during the last day of extinction, and during cue- and cocaine-primed reinstatement tests. (e) Active lever pressing and number of sucrose pellets during sucrose SA and Ext. (f) Active lever presses during the last day of Ext and during the cue-primed sucrose reinstatement test. (g) Open field locomotor activity of Saline–Vehicle, Cocaine–Vehicle, and Cocaine–4 mg/kg rats.

(sAHPs) were measured after the end of the 800 ms pulse. The mAHP was measured as the peak of the AHP, and the sAHP was measured as the average potential during a 50 ms period beginning 280 ms after the end of the 800 ms depolarizing pulse (Faber and Sah, 2002; Santini *et al*, 2008; Sepulveda-Orengo *et al*, 2013). For these experiments, the internal solution contained the following (in mM): 140 K-Glu, 10 HEPES, 12 KCl, 0.2 EGTA, 10 HEPES, 5 biocytin, 0.3 GTP, and 4 ATP. pH was adjusted to 7.4 with KOH (300 mOsm). For morphological identification and localization of IL and PL neurons, 5 mM biocytin was included in the recording solution and slices were fixed overnight at 4 °C in 4% paraformaldehyde. Standard avidin-biotin peroxidase (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) procedure was performed to visualize the neurons with bright-field microscopy as described previously (Porter *et al*, 2001). Neurons that did not have pyramidal shape were excluded from the analysis.

Western Blot

At 24 h after the last extinction session, rats were treated with a final administration of vehicle or riluzole (4 mg/kg) and rapidly decapitated 30 min later for preparation of nucleus accumbens tissue. Tissue predominantly containing core, but probably also some shell, was homogenized by hand, and a P2 crude membrane preparation was prepared as previously described (Knackstedt *et al*, 2010; Reissner *et al*, 2014). A subset of rats used for western analysis was subjected to locomotor testing immediately before rapid decapitation (Figure 1g). P2 membrane pellets were stored at − 80 °C until use. Pellets were suspended in 35 µl of 1× RIPA buffer supplemented with 1.0% SDS and 1 : 100 Halt protease inhibitors containing EDTA (Thermo Scientific). Samples were centrifuged for 10 min at 14 000 *g* and supernatant was taken for protein determination by the BCA method (Thermo Scientific). Samples were heated at 50 °C for 30 min, and equal µg of protein was separated per lane on 10% Criterion Tris-HCl gels (Bio-Rad) and transferred 1.5 h at 150 mA onto PVDF membranes. Membranes were blocked for 1 h at room temperature in Licor Odyssey Tris blocking solution and incubated with primary antibodies overnight at 4 °C (Millipore AB1783, 1 : 2000; Enzo ADI-SPA-860-D, 1 : 4000). Secondary antibody incubation was performed for 1.5 h at room temperature (800CW anti-guinea pig and 680RP anti-rabbit, Licor, 1 : 15 000 each). Westerns were imaged and quantified on a Licor Odyssey Fc imager. GLT-1/calnexin ratio was measured and normalized to samples from saline-administering rats.

Statistical Analysis

Behavioral and electrophysiology data were analyzed using a two-way repeated measures analysis of variance (ANOVA). Western blots for GLT-1 were analyzed with a two-way ANOVA. A three-factor, mixed model, repeated measures ANOVA was used for analysis of action potentials elicited across increasing current intensities in pyramidal neurons from saline- and cocaine-extinguished rats treated with vehicle or riluzole. Significant main effects were followed by Fisher's (PLSD) *post hoc* tests. The electrophysiological data such as interspike interval (ISI), number of spikes evoked, and AHPs were analyzed using Clampfit 10 (Axon Instruments, Union City, CA). Two-way, repeated measures ANOVA was used for planned comparisons between specific properties of PL and IL neurons (Table 1). Values are reported as mean ± SEM, and significance was considered as $p \leqslant 0.05$ ($^*p < 0.05$).

RESULTS

Riluzole Administration Impairs Cocaine Reinstatement

The timeline for behavioral training and riluzole or vehicle administration is shown in Figure 1a. No differences were observed between treatment groups in active lever presses or infusions during self-administration ($F_{(2, 38)} = 0.7125$, $p > 0.05$; $F_{(2, 38)} = 0.1213$, $p > 0.05$, respectively); Figure 1b

Table 1 Electrophysiological Properties of PL and IL Neurons

Groups	V_m(mV)	R_{in}(MΩ)	Rheobase (pA)	Threshold (mV)	sAHP (mV)	mAHP (mV)
PL						
Saline–Vehicle	− 66.8 ± 1.6	106.0 ± 13.3	117.7 ± 12.3*	31.2 ± 1.4	− 0.5 ± 0.1	− 0.8 ± 0.2
Saline–4 mg/kg	− 71.8 ± 1.3	90.1 ± 6.7	90.1 ± 7.8	30.9 ± 1.2	− 0.8 ± 0.3	− 1.6 ± 0.5
Cocaine–Vehicle	− 69.9 ± 1.3	87.2 ± 7.7	101.8 ± 10.1	29.8 ± 1.1	− 0.6 ± 0.2	− 1.1 ± 0.2
Cocaine–4 mg	− 65.7 ± 1.3	81.8 ± 5.0	110.8 ± 10.0	31.5 ± 1.3	− 0.8 ± 0.2	− 1.1 ± 0.2
IL						
Saline–Vehicle	− 65.8 ± 1.3	93.9 ± 5.6	81.9 ± 5.2*	31.9 ± 0.8	− 1.8 ± 0.3	− 2.2 ± 0.3
Saline–4 mg/kg	− 68.1 ± 1.8	115.0 ± 10.0	72.0 ± 9.5	30.4 ± 1.3	− 1.0 ± 0.4	− 1.6 ± 0.4
Cocaine–Vehicle	− 66.6 ± 1.0	108.9 ± 13.3	94.8 ± 9.8	30.7 ± 0.9	− 1.46 ± 0.3	− 2.2 ± 0.4
Cocaine–4 mg	− 68.5 ± 1.5	90.8 ± 6.8	78.0 ± 6.7*	28.5 ± 1.0	− 1.4 ± 0.2	− 2.3 ± 0.3

Two-way ANOVA showed no difference between the groups for any measure per brain subregion ($p > 0.05$). Two-way ANOVA showed a treatment main effect in the amount of depolarizing current necessary to evoke an action potential (rheobase) between subregions ($F_{(3, 190)} = 5.128$, $p < 0.05$). Tukey's *post hoc* multiple comparisons test showed that PL–Saline–Vehicle neuron rheobase is significantly different compared with IL neuron rheobase from Saline–Vehicle and Cocaine–4 mg/kg groups ($^*p < 0.05$).

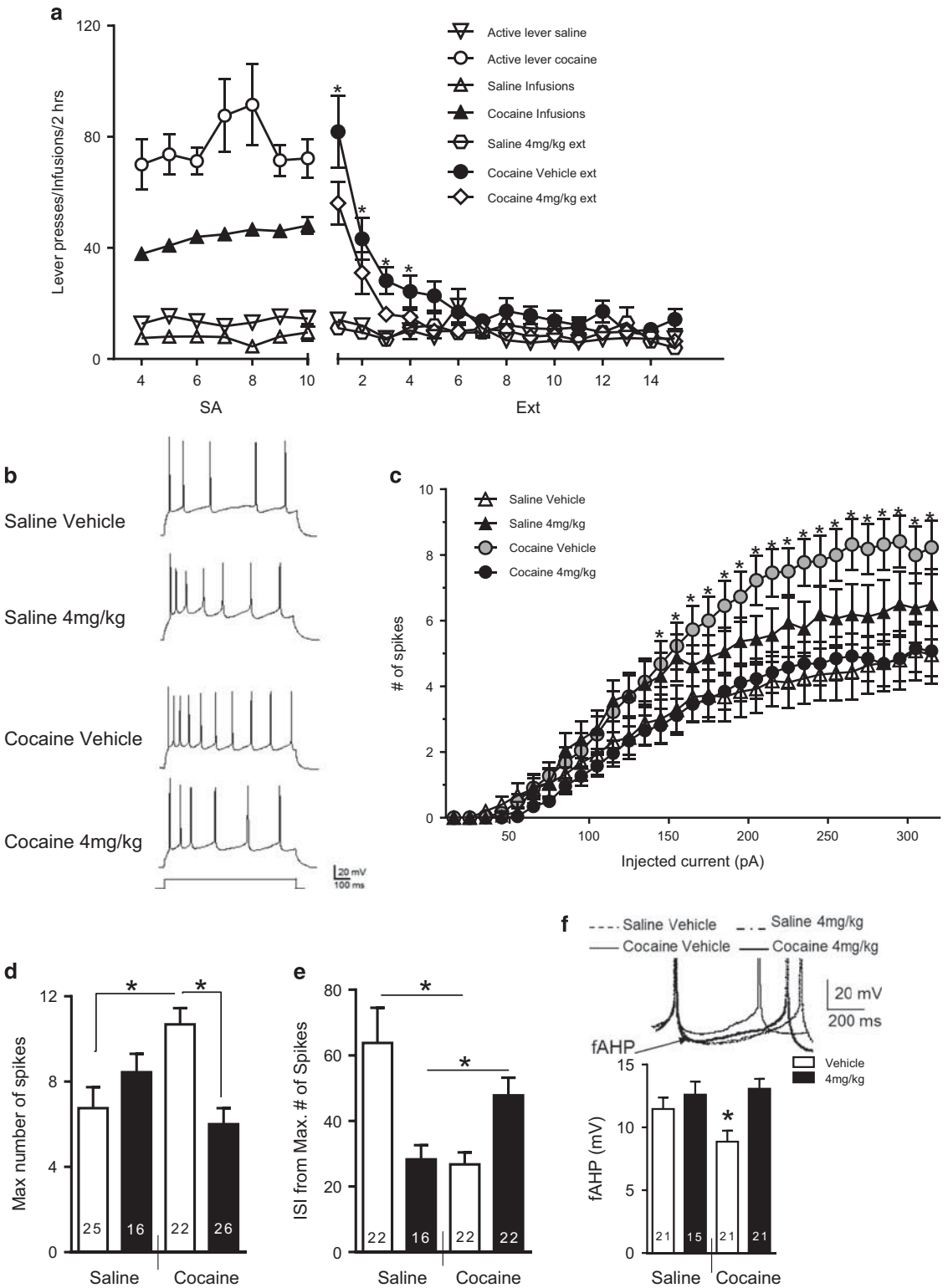

Figure 2 Riluzole prevents cocaine-induced intrinsic changes in PL pyramidal neuron. (a) Active lever presses and infusions during cocaine or saline SA for all rats from which neurons were recorded in PL and IL. Before each extinction (Ext) session, rats were injected i.p. with 4 mg/kg riluzole (Cocaine–4 mg/kg, $N=14$; Saline–4 mg/kg, $N=8$) or vehicle (Cocaine–Vehicle, $N=12$; Saline–Vehicle, $N=13$). *Indicates significant difference between Cocaine–Vehicle and Cocaine–4 mg/kg groups. (b) Example traces from each group. (c) Number of spikes evoked by depolarizing steps of increasing current in the four groups (Cocaine–4 mg/kg, $N=14$, $n=26$; Saline–4 mg/kg, $N=8$, $n=16$; Cocaine–Vehicle, $N=12$, $n=22$; Saline–Vehicle, $N=13$, $n=25$). (d) Bar graph shows the maximum number of evoked spikes at any current step. (e) Duration of the first ISI from maximum number of spikes. (f) Group data of the fAHP measured after the second evoked spike. For (d–f), *x* axis indicates the self-administration group, and legend indicates treatment with riluzole or vehicle. In (f), $*p<0.05$ as compared with all other groups in (f). N = number of rats; n = number of cells.

shows the combined self-administration behavior from rats in the three groups. Main effects of both time and treatment were observed across extinction (treatment: $F_{(2, 38)} = 7.508$, $p < 0.05$; time: $F_{(14, 532)} = 64.20$, $p < 0.05$), with significantly fewer active lever presses observed in 4 mg/kg riluzole-treated rats, as compared with vehicle-treated rats, on extinction days 1 through 3 ($p < 0.05$). Rats treated with 4 mg/kg but not 1 mg/kg riluzole during extinction demonstrated a reduction of both cue- and cocaine-primed reinstatement (Figure 1c and d). Two-way, repeated measures ANOVA indicated all rats reinstated cocaine seeking compared with extinction responding and that there was an effect of treatment (Cue: $F_{(1, 38)} = 50.28$, $p < 0.001$; $F_{(2, 38)} = 3.125$, $p = 0.05$, Coc: $F_{(1, 38)} = 20.51$, $p < 0.0001$; $F_{(2, 38)} = 4.125$, $p < 0.05$ respectively). The *post hoc* comparisons revealed a significant attenuation of active lever presses in the 4 mg/kg riluzole group compared with vehicle in cue- as well as cocaine-primed reinstatement ($p < 0.05$ and $p < 0.005$). In addition, 4 mg/kg riluzole group had significantly reduced cocaine seeking compared with the 1 mg/kg riluzole in cocaine-primed reinstatement ($p < 0.05$). No differences were observed between treatment groups in active lever presses on the last day of extinction training before cue- or cocaine-primed reinstatement ($p > 0.05$ and $p > 0.05$). Thus, riluzole dose-dependently impairs cocaine reinstatement. Henceforth, the effective dose (4 mg/kg) of riluzole was used in the following experiments.

Riluzole Administration Does Not Attenuate Sucrose Reinstatement or Locomotor Activity

In order to investigate the effects of riluzole on nondrug reward, a separate cohort of rats was used to assess the effects of riluzole on reinstatement to sucrose seeking (Figure 1e and f). In contrast to cocaine extinction, no differences were observed between Sucrose–Vehicle and Sucrose–4 mg/kg riluzole in active lever presses across days of extinction ($F_{(1, 224)} = 0.2138$, $p > 0.05$). In addition, in contrast to cocaine, 4 mg/kg riluzole did not suppress cue-primed reinstatement to sucrose. In fact, a nonsignificant trend toward an increase in reinstatement was observed following administration of riluzole. Two-way, repeated measures ANOVA indicated a significant main effect of reinstatement ($F_{(1, 20)} = 23.88$, $p < 0.0001$; Figure 1f), but no effect of riluzole ($F_{(1, 20)} = 3.771$, $p = 0.0664$.

Because sedative effects of riluzole can be observed at high doses (Lourenco Da Silva *et al*, 2003; Sugiyama *et al*, 2012), and to further ensure that effects on cocaine reinstatement were not because of a sedative effect, locomotor testing was subsequently performed in an additional cohort of rats. This was particularly important, given the effect of riluzole on cocaine extinction pressing. Rats were trained on self-administration and extinction to cocaine, and were treated with vehicle or 4 mg/kg riluzole across extinction in a manner identical to those tested for reinstatement. No differences in locomotor activity or distance traveled over 2 h in an open field were observed ($F_{(2, 26)} = 0.4277$, $p > 0.05$; Figure 1g). Accordingly, the effective dose of riluzole against cocaine reinstatement had no effect on cue-primed sucrose reinstatement or locomotor activity.

Riluzole Administration Prevents Cocaine-Induced Hyperexcitability in PL Neurons

The PL region of the mPFC is necessary for cocaine-reinforced responses (Gipson *et al*, 2013; McFarland and Kalivas, 2001), and increased PL excitability has been previously reported following noncontingently administered cocaine (Dong *et al*, 2005; Hearing *et al*, 2013; Nasif *et al*, 2005a, b). Because riluzole is known to block action potential firing, we hypothesized that riluzole would rectify cocaine-induced hyperexcitability. Rats were trained in saline or cocaine self-administration, and treated with either vehicle or 4 mg/kg riluzole, respectively, as for previous experiments. Whole-cell slice electrophysiology recordings were performed 24 h after the last extinction section. Self-administration and extinction behavior for rats used in electrophysiology studies is shown in Figure 2a. Similarly, as observed in behavior experiments, there was no difference between rats during cocaine self-administration ($F_{(1, 120)} = 0.2677$, $p > 0.05$, comparing cocaine-administering groups only), but an effect of treatment was observed across extinction ($F_{(3, 43)} = 9.11$, $p < 0.05$). Significantly fewer active lever presses were observed in Cocaine–4 mg/kg riluzole-treated rats compared with vehicle-treated cocaine-administering rats on days 1–3 and day 5 of extinction.

In agreement with previous studies, we observed a cocaine-dependent increase in intrinsic excitability of PL pyramidal neurons that was reversed by administration of riluzole (Figure 2b and c). A three-way mixed model ANOVA with current as the repeated measures factor and group (cocaine *vs* saline self-administering rats) and treatment (vehicle *vs* 4 mg/kg riluzole) as between-subjects factors was used to examine differences in the number of spikes in the PL (Figure 2c). There was a main effect of current ($F_{(32, 2720)} = 132.97$, $p < 0.001$), and the current × group interaction ($F_{(32, 2720)} = 1.92$, $p < 0.05$), current × group × treatment interaction ($F_{(32, 2720)} = 20.7$, $p < 0.001$), and group × treatment interactions were significant ($F_{(1, 85)} = 8.91$, $p < 0.05$). The *post hoc* comparisons showed that within saline self-administering rats, there was no significant difference between vehicle and 4 mg/kg riluzole-treated rats ($p = 0.17$). Within cocaine self-administering rats, there was a significant difference between treatment groups ($p < 0.05$). Cocaine self-administering rats treated with vehicle showed a significantly greater number of spikes than riluzole-treated rats. When comparing number of spikes from vehicle-treated rats, there was a significant difference between saline and cocaine self-administering rats ($p < 0.05$), with cocaine self-administering rats showing significantly more spikes than saline self-administering rats. However, within riluzole-treated rats, there was no significant difference in spikes between saline and cocaine self-administering rats ($p = 0.15$).

In addition to the number of evoked spikes, we examined neuronal excitability parameters such as the ISI and AHPs. The AHP is divided in three types, sAHP, mAHP, and fAHP, that contribute differentially to neuronal excitability. The fAHP occurs within the first 10 ms following an action potential, and contributes to the repolarization of the neuron. The sAHP and mAHP are more prolonged AHPs, lasting several hundred ms to several seconds, and mediate firing

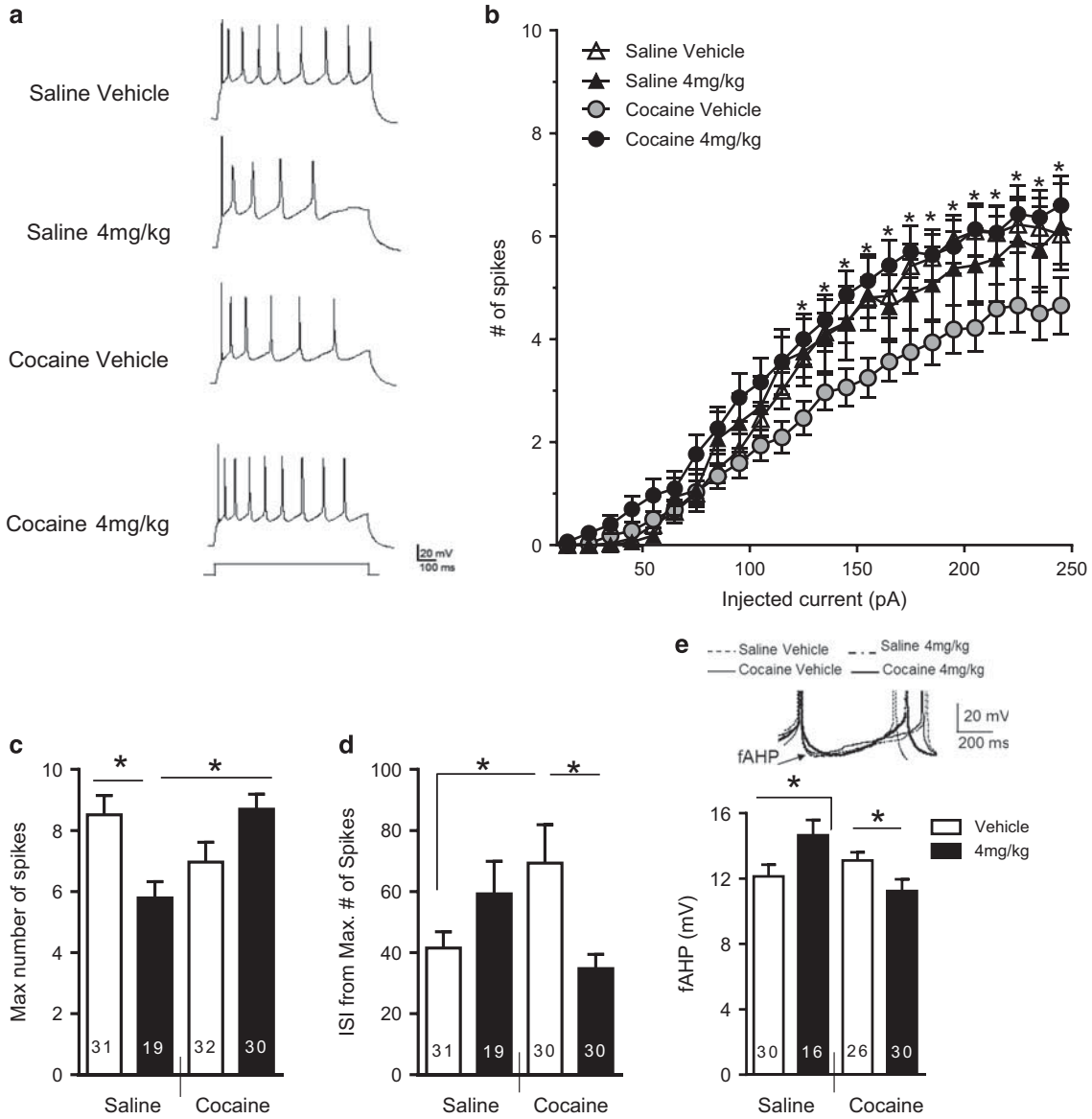

Figure 3 Riluzole reverses cocaine-induced decrease in intrinsic excitability in IL pyramidal neurons. (a) Example traces from each group. (b) Number of spikes evoked by depolarizing steps of increasing current (Cocaine–4 mg/kg, $N=14$, $n=30$; Saline–4 mg/kg, $N=8$, $n=19$; Cocaine–Vehicle, $N=12$, $n=32$; Saline–Vehicle, $N=13$, $n=31$). (c) Maximum number of evoked spikes at any current step. (d) Group of data showing the duration of the first ISI from two spike traces. (e) Group data of the fAHP measured after the second evoked spike. *$p < 0.05$ *post hoc* comparisons as indicated by the bar.

frequency and spike-frequency adaptations (Bean, 2007; Faber and Sah, 2003).

No differences were observed in sAHP, mAHP, input resistance (R_{in}), threshold at action potential, resting membrane potential (V_m), or the amount of depolarizing current necessary to evoke an action potential (rheobase) among the groups (Table 1). As shown in Figure 2e, neurons from the Cocaine–Vehicle group exhibited a significantly smaller ISI from maximum fired spikes compared with the Saline–Vehicle group that was reverse by riluzole. Two-way ANOVA indicated an interaction ($F_{(1, 79)}=16.41$, $p<0.001$), and *post hoc* comparisons showed that the ISI number from maximum evoked spikes in the Cocaine–Vehicle group was significantly smaller than in the Cocaine–4 mg/kg and Saline–Vehicle ($p<0.05$), but not than Saline–4 mg/kg ($p=0.8821$). In addition, *post hoc* comparisons showed that the ISI number from maximum evoked spikes in the Saline–4 mg/kg was significantly smaller than in the Saline–Vehicle ($p<0.05$), indicating an effect of riluzole on this excitability measure in saline-experienced rats. In addition, we measured the fAHPs evoked by the second spike (Figure 2f) as previously described (Duvarci and Pare, 2007). Neurons from the Cocaine–Vehicle group had smaller fAHPs than neurons from either the Cocaine–4 mg/kg, Saline–Vehicle, or Saline–4 mg/kg groups (main effect of group ($F_{(1, 80)}=4.14$, $p<0.05$), *post hoc* comparisons between: Cocaine–Vehicle and Cocaine–4 mg/kg ($p<0.005$), Cocaine–Vehicle and Saline–Vehicle ($p<0.05$), Cocaine–Vehicle and Saline–4 mg/kg ($p<0.05$). Collectively, these results indicate that riluzole prevents the cocaine-induced increase in PL pyramidal neuron excitability that correlates with the normalization of maximum number of spikes, ISI, and fAHP.

Riluzole Administration Prevents Cocaine-Induced Hypoexcitability in IL Neurons

Whereas the PL is generally associated with goal-directed behavior, the IL subregion of the mPFC is known to be important in cocaine extinction learning (LaLumiere *et al*, 2010; Moorman *et al*, 2014). Therefore, we also tested the effects of cocaine history and riluzole administration on neuronal excitability in the IL. Interestingly, and opposed to results obtained from the PL, excitability in the IL neurons following extinction from cocaine self-administration was significantly reduced, an effect that was reversed by systemic riluzole administration (Figures 3a–e). A three-way mixed ANOVA with current as the repeated measures factor and group (cocaine *vs* saline self-administering rats) and treatment (vehicle *vs* 4 mg/kg riluzole) as between-subjects factors was used to examine differences in the number of spikes in the IL. There was a main effect of current ($F_{(25, 2700)} = 228.43$, $p < 0.001$), and the current × treatment interaction ($F_{(25, 2700)} = 1.70$, $p < 0.05$), the current × group × treatment interaction ($F_{(25, 2700)} = 8.51$, $p < 0.001$), and group × treatment interaction were significant ($F_{(1, 108)} = 8.32$, $p < 0.05$). The *post hoc* comparisons showed that within saline self-administering rats, there was no significant difference in the number of spikes in the IL between vehicle and 4 mg/kg riluzole-treated rats ($p = 0.25$). However, within cocaine self-administering rats, there was a significant difference between treatment groups ($p < 0.05$). Cocaine self-administering rats treated with riluzole showed a significantly greater number of spikes than cocaine self-administering rats treated with vehicle. When comparing within treatment groups, *post hoc* comparisons show that in vehicle-treated rats, there was a significant difference between saline and cocaine self-administering rats ($p < 0.05$). Cocaine self-administering rats treated with vehicle showed significantly fewer spikes than saline self-administering rats treated with vehicle. Within riluzole-treated rats, there was also a significant difference in spikes between saline and cocaine self-administering rats ($p = 0.05$). Cocaine self-administering rats treated with riluzole showed significantly more spikes than saline self-administering rats treated with riluzole.

The maximum number of evoked spikes in IL neurons in the Cocaine–Vehicle group was lower than Cocaine–4 mg/kg group but not than Saline–Vehicle or Saline–4 mg/kg (Figure 3c). Two-way ANOVA indicated an interaction between self-administration and treatment (($F_{(1, 108)} = 13.15$, $p < 0.001$), *post hoc* comparisons showed Cocaine–Vehicle lower than Cocaine–4 mg/kg ($p < 0.05$) but not lower than Saline–4 mg/kg ($p = 0.22$) or Saline–Vehicle ($p = 0.056$). The maximum number of spikes was significantly reduced in the Saline–4 mg/kg group, as compared with the Saline–Vehicle group (Figure 3c). In addition, we measured the ISI from maximum number of evoked spikes and fAHPs (Figure 3d and e). Two-way ANOVA showed a significant interaction for both measurements ($F_{(1, 107)} = 8.35$, $p < 0.01$, $F_{(1, 99)} = 10.38$, $p < 0.01$). The *post hoc* comparisons indicated that the Cocaine–Vehicle group had a higher ISI than the Cocaine–4 mg/kg ($p < 0.05$) and Saline–Vehicle groups ($p < 0.05$) but not than Saline–4 mg/kg ($p > 0.05$), and that neurons from the Cocaine–4 mg/kg group had smaller fAHPs than neurons from Cocaine–Vehicle ($p < 0.05$) and Saline–4 mg/kg groups ($p < 0.05$), but not than Saline–Vehicle ($p > 0.05$). There were no differences in sAHP, mAHP, input resistance, resting membrane potential, threshold of action potential, or the amount of depolarizing current necessary to evoke an action potential (rheobase) among the groups (Table 1). These results show that riluzole prevents cocaine-induced decreased excitability in IL and normalizes maximum number of spikes, ISI, and fAHP.

Because different effects were observed in neuronal excitability from cocaine-extinguished rats in the PL and IL, we sought to determine whether preexisting differences in membrane properties might exist between neurons in these regions. No differences were observed in resting membrane potential between the PL and IL among treatment groups (Table 1); however, the amount of depolarizing current necessary to evoke an action potential (rheobase) among the groups was significantly different between PL and IL neurons from saline extinguished rats (Table 1), indicating subregion differences in neuronal properties.

Riluzole Restores the Cocaine-Induced Decrease in NAc GLT-1 Expression

We next examined the effect of riluzole on GLT-1 expression in the NAc from rats following self-administration and extinction (Figure 4). A two-way ANOVA showed significant main effects of cocaine and treatment ($F_{(1, 54)} = 5.92$, $p < 0.05$, $F_{(1, 54)} = 10.74$, $p < 0.01$, respectively). The *post hoc* comparisons revealed that the Cocaine–Vehicle group had lower NAc GLT-1 expression as compared with the Saline–Vehicle ($p < 0.01$), Saline–4 mg/kg ($p < 0.01$), and Cocaine–4 mg/kg groups ($p < 0.05$). This result replicates previous results that indicate a cocaine-induced decrease in GLT-1 expression in the NAc, and show that this can be normalized by riluzole.

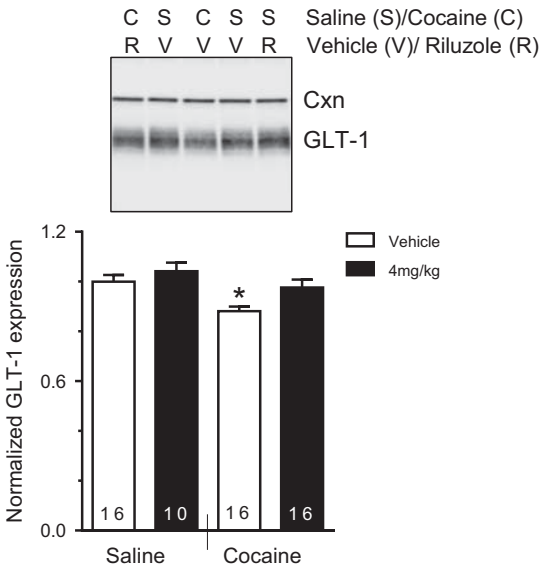

Figure 4 Riluzole blocks cocaine-induced decrease in GLT-1. Normalized GLT-1/Cxn expression. Expression levels are normalized to Saline–Vehicle. Representative western blots showed bands corresponding to GLT-1 and calnexin (Cxn, control loading protein) expression from Saline–Vehicle, Saline–4 mg/kg, Cocaine–Vehicle, and Cocaine–4 mg/kg groups. *$P < 0.05$ compared with all other groups. The *x* axis indicates self-administration group, and legend indicates treatment group.

DISCUSSION

Riluzole Administration Reduces Cocaine Reinstatement, But Not Sucrose Reinstatement

Results presented herein indicate that riluzole impairs cocaine reinstatement, normalizes cellular excitability within the mPFC, and restores the cocaine-induced decrease in GLT-1 in the nucleus accumbens. Behaviorally, our results indicate that administration of riluzole dose-dependently impairs cocaine reinstatement, but does not affect sucrose reinstatement. In support of the behavioral effect of riluzole on cocaine seeking, it has recently been shown that microinjection of riluzole directly into the laterodorsal tegmental nucleus (LDT) blocked expression of cocaine conditioned place preference (CPP) (Kamii *et al*, 2015). These findings collectively suggest that an FDA-approved medication carries potential as a substance abuse therapeutic agent, and also shed light on the effects of cocaine self-administration on cellular excitability.

Riluzole Restores Cocaine-Induced Adaptations in Intrinsic Excitability in the mPFC

Although a substantial body of literature exists on the synaptic consequences of drug self-administration (Bowers *et al*, 2010; Korpi *et al*, 2015; Luscher and Malenka, 2011; Wolf, 2016), less is known about how drugs of abuse affect intrinsic excitability (Kourrich *et al*, 2015), in particular in the PFC. We show that pyramidal neuron excitability is increased in the PL of cocaine-extinguished rats, but decreased in the IL. Increased PL excitability following noncontingent cocaine injections and withdrawal has previously been reported, dependent on a reduction in voltage-gated potassium channels and an increase in voltage-sensitive Ca2+ channels (Dong *et al*, 2005; Hearing *et al*, 2013; Nasif *et al*, 2005a, b; Nasif *et al*, 2005b, but see also Otis and Mueller, 2017). Moreover, an increase in PL neuron excitability in cocaine-extinguished rats has been reported in abstract form (Buchta and Riegel, 2015). Our results similarly show that cocaine self-administration and extinction leads to increased intrinsic excitability in PL neurons by a reduction of the fAHP. The fAHP is predominantly (although not exclusively) governed by the conductance of voltage-gated and Ca^{2+}-dependent potassium channels (Oh *et al*, 2010). Interestingly, it has been shown that decreased PL excitability accompanies resistance to punishment induced by cocaine self-administration (Chen *et al*, 2013). In this case, deficits in PL neuronal activity were observed after prolonged exposure to cocaine (>8 weeks of extended access), in contrast to the more moderate exposure paradigms utilized here and elsewhere (Dong *et al*, 2005; Nasif *et al*, 2005b). Thus, it may well be that more 'recreational' exposure to cocaine results in differential effects on PL excitability than is observed in paradigms associated with more compulsive behaviors.

This is, to our knowledge, the first report that cocaine exposure leads to a decrease in IL intrinsic excitability. Moreover, the divergent effects of cocaine self-administration and extinction on PL and IL neuron excitability underscores proposed functional differences in these regions with regard to reward seeking or inhibition of responses (Gourley and Taylor, 2016; Moorman *et al*, 2014). Comparisons of properties of PL and IL neurons under various treatment conditions indicated no baseline differences in resting membrane potential, but a significant difference in rheobase between PL and IL neurons in Saline–Vehicle and Cocaine–4 mg/kg groups was observed, suggesting an intrinsic difference in excitability between these populations that is independent of resting potential (Table 1). Given that neurons from Saline–Vehicle and Cocaine–Vehicle groups did not differ in fAHP, this suggests that cocaine may decrease neuronal excitability by reducing Na+ channel currents in IL neurons. Furthermore, riluzole restored the bidirectional changes in intrinsic excitability induced by cocaine experience. Interestingly, although riluzole reversed the cocaine-induced increase and decrease in excitability in the PL and IL, respectively, it had marginal effects on measures of excitability in saline-experienced rats. For example, there was no significant effect of riluzole on response to increasing current stimulation in either PL or IL neurons (Figures 2c and 3b). However, we did observe a significant decrease in ISI in the PL (Figure 2e) and in the max number of spikes recorded in neurons from saline-experienced rats in the IL (Figure 3c). These inconsistent effects in control rats may reflect the fact that riluzole was administered systemically 30 min before preparation of tissue for recording, and is a reversible inhibitor of Na+ channels *in vitro* (Cao *et al*, 2002; Siniscalchi *et al*, 1997). These observations underscore the likelihood that the effect of riluzole on neuronal excitability in cocaine-extinguished rats is a reflection of amelioration of drug-induced adaptations.

Although we do not know the precise mechanism(s) responsible for the effects of riluzole on reinstatement, it is notable that in addition to acting as a Na+ channel inhibitor, riluzole also modulates activation and inactivation of potassium channels, inhibits activation of voltage-gated Ca^{2+} channels and persistent sodium channels, increases AMPA receptor surface expression (GluA1 and GluA2) and AMPA-induced depolarization, increases BDNF, activates GABA receptor, and increases glutamate uptake by increasing GLT-1 transporter function and expression (Du *et al*, 2007; Pittenger *et al*, 2008). We believe that the pharmacological effects of riluzole in the PL and IL may rely on differentially weighted mechanisms of action. One mechanism by which riluzole may renormalize PL hyperexcitability is by modulating persistent sodium channels and Ca^{2+}-dependent potassium channels. One mechanism by which riluzole may renormalize cocaine-induced IL hypoexcitability is by increasing the expression of BDNF and AMPA receptor subunits. Future electrophysiology experiments designed to assess pre- and postsynaptic components of synaptic transmission and plasticity, including paired-pulse ratio and AMPA/NMDA ratio, will be used to elucidate the precise way in which riluzole affects reinstatement behavior.

Furthermore, evidence indicates that BDNF can enhance intrinsic excitability (Guo *et al*, 2017), and in fact infusion of BDNF into the ventral hippocampus selectively increased the firing rate of IL, but not PL neurons, in fear conditioned rats (Rosas-Vidal *et al*, 2014). Interestingly, riluzole decreased the fAHP, suggesting that inhibition of K+ channels contribute to normalized intrinsic excitability of IL neurons. Future studies will be important to assess how both fundamental cellular properties and their responsiveness to

learning and to reward contribute to the complex functional outputs from these regions.

Riluzole Restores NAc GLT-1 Expression in Cocaine-Extinguished Rats

Numerous studies have demonstrated that riluzole not only modulates Na+ and K+ channels that are important in the neuronal excitability, but also increases glutamate uptake via GLT-1 expression and activity (Banasr *et al*, 2010; Brothers *et al*, 2013; Carbone *et al*, 2012; Frizzo *et al*, 2004; Fumagalli *et al*, 2008; Gourley *et al*, 2012; Liu *et al*, 2011). Furthermore, it has been thoroughly demonstrated that cocaine leads to decreased GLT-1 expression and activity in the NAc (Fischer *et al*, 2013; Fischer-Smith *et al*, 2012; Knackstedt *et al*, 2010; Reissner *et al*, 2014). The present study shows that the cocaine-induced reduction in NAc GLT-1 is reversed by riluzole, but that NAc GLT-1 expression is unaffected by riluzole treatment in saline-administering rats. Interestingly, it has also previously been shown that ceftriaxone, which increases NAc GLT-1 expression in cocaine-extinguished rats, does not affect GLT-1 expression in cocaine-naive rats (Knackstedt *et al*, 2010). Importantly, riluzole can also increase glutamate uptake by enhancing GLT-1 function without a change in expression (Carbone *et al*, 2012; Fumagalli *et al*, 2008).

In summary, riluzole can impair cocaine reinstatement and reverse several cocaine-induced adaptations within the reward circuitry, indicating promise for pharmacotherapeutic intervention for cocaine relapse. A single, small-scale human trial showed that riluzole lacked effect on cocaine use when taken during cocaine dependence (Ciraulo *et al*, 2005). Importantly, this was a small study in which medication compliance was not verified. Accordingly, given findings from preclinical literature, we propose that use of riluzole as pharmacological intervention, and particularly targeting multiple components of glutamatergic signaling, carries potential for intervention against cocaine relapse.

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The authors declare no conflict of interest.

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