Published in final edited form as:

Addict Biol. 2012 September ; 17(5): 920–933. doi:10.1111/j.1369-1600.2012.00460.x.

Stress history increases alcohol intake in relapse: Relation to phosphodiesterase 10A

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Abstract

Stressful experiences in humans can result in elevated alcohol drinking, as exemplified in many individuals with post-traumatic stress disorder. However, how stress history, rather than acute stressors, influences alcohol intake remains uncertain. To model the protracted effects of past stress, male Wistar rats were subjected to light-cued footshock stress (Stress History) or light cues alone (Control) prior to their acquisition of alcohol self-administration (1-h sessions, fixed ratio 1–3, 100 μ l of 10% v/v alcohol as reinforcer). Stress history did not alter mean alcohol intake during acquisition of self-administration, but it increased preference for the alcohol-paired lever over the inactive lever. Following an extinction period, rats with a history of stress exposure and low baseline alcohol intake showed a 2-fold elevation in alcohol self-administration, as compared to low-drinking rats with no stress history. Similar effects were not seen in rats self-administering 0.1% sucrose. Analysis of mRNA levels of phosphodiesterase 10A (PDE10A), a dual-specificity cAMP and cGMP hydrolyzing enzyme, showed that stress history increased *Pde10a* mRNA levels in the basolateral amygdala and, in low drinking rats, the prelimbic prefrontal cortex (plPFC). *Pde10a* mRNA levels in the plPFC correlated directly with greater alcohol self-administration during the relapse-like phase, and greater BLA *Pde10a* mRNA levels correlated with increased ethanol preference after acquisition. The data demonstrate that stress history sensitizes otherwise low alcohol drinkers to consume more alcohol in a relapse-like situation, and identify stress-induced neuroadaptations in amygdala and prefrontal cortical *Pde10a* expression as changes that may drive heightened alcohol intake and preference in susceptible individuals.

Keywords

alcohol or ethanol; phosphodiesterase 10A; post-traumatic stress disorder; relapse; stress; trauma

Introduction

Alcohol abuse and dependence, health problems that affect ~10% of the population (Grant et al., 2004; Ross, 1995), show high comorbidity with anxiety disorders, including post-traumatic stress disorder (PTSD). One-quarter of all patients with substance abuse disorders also suffer from anxiety disorders (Driessen et al., 2008). Conversely, within the ~8% of the population suffering from PTSD (McCarthy and Petrakis, 2010), about half suffer from alcohol use disorders (Breslau et al., 2003; Perkonig et al., 2000). PTSD also is associated

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Authors' Contributions

MLL and EPZ conceptualized and designed the research; MLL performed the experiments; MLL and EPZ analyzed data and wrote the manuscript.

with increased risk of stress-induced alcohol relapse (Norman et al., 2007). However, few existing treatments address the comorbid PTSD-alcoholism diagnosis, resulting in poor treatment outcomes (Brown et al., 1995; Brown and Wolfe, 1994). Understanding the neuroadaptations responsible for past stress-induced alterations in alcohol intake is key for developing new therapies.

Many studies have examined the acute effects of stress on ongoing alcohol intake (Le and Shaham, 2002; Miczek et al., 2008; Pohorecky, 1981), but few have examined the distal effects of stress history on alcohol self-administration over a prolonged time course, which may be relevant to the comorbidity of PTSD with alcoholism. In existing rodent models, footshock stress has yielded inconsistent effects on alcohol intake, with both increases (Casey, 1960; Chester et al., 2008; Funk et al., 2004; Matthews et al., 2008; Mills et al., 1977; Opsahl and Hatton, 1972; Vengeliene et al., 2003; Volpicelli et al., 1990) and decreases (Brunell and Spear, 2005; Caplan and Puglisi, 1986) observed. Stress may have a greater impact on those with low initial preference for alcohol (Volpicelli et al., 1990). Whether alcohol access is provided in a “safe” context also may partly explain discrepant outcomes; alcohol intake was reduced in models involving continuous housing in the shock environment (Caplan and Puglisi, 1986; Casey, 1960; Opsahl and Hatton, 1972), unless shock delivery was signaled by a discrete cue (Cicero et al., 1968; Opsahl and Hatton, 1972). Conversely, alcohol intake increased once stress-experienced rats were returned to normal housing (Caplan and Puglisi, 1986; Vengeliene et al., 2003) or, in the stress-paired environment, beginning 1 week after the final footshock and lasting several weeks (Casey, 1960). The data suggest that a past history of stress may persistently impact alcohol self-administration.

Because patients with PTSD are more prone to relapse (Norman et al., 2007), the effects of stress history on alcohol-seeking or consumption following abstinence also are of special interest. Studies to date have examined the ability of stress or stress-conditioned stimuli to influence alcohol relapse behavior in their immediate aftermath. For example, footshock during alcohol deprivation blunted alcohol intake upon renewed access (Dayas et al., 2004). In contrast, footshock reinstated responding on a previously alcohol-paired lever in the immediate post-stress interval (Le et al., 1998), an effect augmented by cues of alcohol delivery (Liu and Weiss, 2002). Presentation of a footshock-conditioned stimulus also acutely precipitated alcohol-directed responding when presented with alcohol-paired cues in nondependent rats and, synergistically, in post-dependent rats (Liu and Weiss, 2003). These studies support the hypothesis that stress and stress-conditioned stimuli acutely influence alcohol relapse behavior. They do not, however, address the hypothesis, tested in the present study, that a past history of stress (i.e., as a pre-existing condition) has more distal effects that promote the acquisition of, maintenance of, or relapse to alcohol self-administration behavior.

Traumatic stress can induce lasting neuroadaptations, evidenced in its ability to modulate future alcohol-consummatory and seeking behavior in humans. The present study therefore tested the hypothesis that a past history of stress altered expression of phosphodiesterase 10A (PDE10A) in the amygdala and prefrontal cortex, brain regions that subserve stress responses and reward processing. PDE10A, a dual-specificity cAMP and cGMP hydrolyzing enzyme (Fujishige et al., 1999; Loughney et al., 1999; Soderling et al., 1999), was chosen as a candidate both because its activity directly regulates neuronal activity and because behavioral effects of PDA10A inhibition on conditioned avoidance (Grauer et al., 2009; Siuciak et al., 2006; Siuciak et al., 2008), prepulse inhibition (Grauer et al., 2009; Weber et al., 2009), social anxiety (Collimore et al., 2010; Feldman and Vengrober, 2011), and appetitive behavior (Piccart et al., 2011) appear therapeutically relevant to PTSD symptomatology. Based on pharmacological findings (Grauer et al., 2009; Siuciak et al.,

2006), PDE10A expression was hypothesized to be increased by stress history in the amygdala and prefrontal cortex of rats, with expression predicted to correlate positively with maintenance and/or relapse-like alcohol self-administration.

Materials and Methods

Subjects

Adult male Wistar rats (Charles River, Raleigh, NC; 200–225 g on arrival) were individually housed in a humidity- and temperature-controlled vivarium (12-h reversed light/dark cycle; dark at 8am or 10am) with food and water available *ad libitum*. Procedures conformed to the *National Institutes of Health Guide for the Care and Use of Research Animals* and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Behavioral Testing Chambers

Operant self-administration and footshock stress sessions were performed in 30.5 × 25 × 30.5 cm chambers (Coulbourn Instruments, Whitehall, PA) with front and rear walls constructed of Plexiglas and ceiling, modular side walls and rod floors of stainless steel. The right chamber wall contained 2 retractable levers (Med Associates, St. Albans, VT) located 2.1 cm above the floor and 2.1 cm from the nearest wall and an acrylic liquid reinforcer receptacle vertically aligned with and horizontally centered between the 2 levers. For footshock sessions, the reward receptacle was replaced with a standard wall panel. Custom software recorded lever presses and controlled reinforcer delivery. Reinforcers were delivered at a 0.1 ml/0.76 sec rate by Razel syringe pumps (St. Albans, VT). Behavioral chambers were enclosed in light- and sound-attenuating cubicles equipped with fans (Med Associates, St. Albans, VT).

Acquisition of operant self-administration behavior

Figure 1 shows the timeline of behavioral testing. Rats were initially provided 2-bottle choice access to reinforcers for 48 h in the home cage, with 1 bottle each of water and reinforce (10% alcohol [v/v] or 1% or 0.1% sucrose [w/v]). Rats then received 1-h, 2-bottle choice access, time-matched to future operant sessions, for 4 days. Sucrose rats were run in 2 cohorts, receiving 1% and 0.1% sucrose, respectively, during home cage access, due to the observation during cohort 1 that 0.1% sucrose yielded operant responding rates that better approximated those for 10% alcohol. Rats ($n=24$ /stress condition/reinforcer) then acquired operant self-administration behavior prior to stress exposure during a single 12-h session, in which active lever responses yielded delivery of 0.1 ml water while inactive lever responses generated no scheduled consequences. The training session spanned one dark cycle with chow available *ad libitum* and yielded rapid acquisition of operant self-administration behavior. These procedures allowed for analysis of stress effects on subsequent operant alcohol self-administration without the confounding influence of stress-induced neophobia or prior operant alcohol self-administration. Footshock sessions commenced 24 h after completion of the water training phase.

Footshock stress exposure

Rats received 30-min footshock sessions on 3 consecutive days. During each session, rats received 60 footshocks, delivered on a variable interval schedule with an average 30-s ITI (range 11–50 s). Shock delivery was discretely signaled via a 5-s house light cue (Cicero et al., 1968; Opsahl and Hatton, 1972) that was not presented during self-administration sessions and that co-terminated with the 1-s 0.4 mA footshocks. Because footshock and operant sessions were performed in similar chambers, efforts were made to differentiate the

contexts to model the human situation, in which alcohol is consumed apart from the trauma environment (see Supplemental Methods). Rats were assigned to Control (light cues alone) vs. Stress History (light cues co-terminating with footshocks) groups matched on 3 measures: (1) home cage intake of alcohol or sucrose during the final 1-h 2-bottle choice access period; (2) active lever presses during the 12-h water self-administration session; and (3) preference for the active lever during the water self-administration session, defined as (Active Lever Presses)/(Total Lever Presses).

Acquisition, maintenance, extinction, and relapse of self-administration

Rats acquired operant self-administration of alcohol (0.1 ml 10% alcohol [v/v] reinforcer, FR1 schedule) beginning 38–42 h after the final footshock session, with test sessions every 2–3 days. After 5 sessions at FR1 (post-stress day 14), the ratio requirement was increased to FR3, which was maintained for 8 sessions, generating a stable self-administration baseline. Lever pressing behavior was then extinguished over 15 sessions (post-stress days 33–55) during which responses at the active lever had no scheduled consequences. On post-stress Day 56, alcohol or sucrose access was renewed for one session under a progressive ratio schedule (1,1,2,2,3,3,4,4,6,6,8,8,10,10,12,12,14,14,17,17, etc.) (Walker and Koob, 2007) followed by reacquisition of self-administration under an FR3 schedule (“relapse”) for 9 sessions.

Sucrose self-administration was performed as for alcohol, but with 0.1% (w/v) sucrose used as the reinforcer, a concentration that maintains similar levels of responding as 10% (v/v). Because the appropriate matching concentration was uncertain at the outset of the study, cohort 1 acquired FR1 operant self-administration initially with a 1% (w/v) sucrose reinforcer that was reduced to 0.3% and then 0.1% (w/v) concentrations during subsequent FR1 test sessions. Both cohorts received 0.1% (w/v) reinforcers from the fourth FR1 session through the remainder of the study. To ensure that the exposure to elevated sucrose concentrations in early training phases was not a significant factor, cohort 2 received 0.1% (w/v) sucrose during all phases of the study. Performance of the two sucrose cohorts responding for 0.1% (w/v) sucrose did not differ from one another.

Reverse transcription and quantitative PCR

48 h after the final relapse self-administration session, rats were anesthetized with isoflurane and immediately euthanized by decapitation. Punches of the regions of interest were collected from 2mm slices on a chilled stage, RNA stabilized with RNAlater (Qiagen Inc., Valencia, CA), and stored at -80°C for subsequent processing. *Pde10a* expression levels were determined by quantitative polymerase chain reaction (qPCR) as described in Supplemental Methods, with *Cyclophilin A* (*Cyp*) as the internal standard.

Data Analysis

Statistical analyses were performed using Systat 12.0 (Chicago, IL). Behavioral data were analyzed using analyses of variance (ANOVA), with repeated measures where indicated, followed by *post hoc* comparisons using Tukey’s test.

Subdivision of rats according to the reinforcing efficacy of ethanol or sucrose prior to extinction training was performed via median split. Pre-extinction FR3 was selected as the baseline for subdivision, rather than home cage alcohol intake, for several reasons. First, within controls, there was little correlation of home cage alcohol intake to operant alcohol self-administration, contrary to common assumptions. For example, no significant correlation was observed of home cage alcohol intake during the final 1 h home cage access session to alcohol responding averaged across the 5 FR1 days in control rats ($R=0.26$, $p=0.24$, $n=23$) or to pre-extinction FR3 self-administration ($R=0.13$, $p=0.55$, $n=23$).

Conversely, a very strong correlation was observed in controls between pre-extinction FR3 and relapse self-administration ($R=0.89$, $p<0.001$, $n=23$). Together these factors indicated that home cage intake was a poor predictor of alcohol's reinforcing effects in an operant situation and of relapse self-administration performance. In addition, because home cage alcohol intake is conceptually distinct from the operant situation of the relapse phase, home cage intake does not sufficiently capture baseline differences in alcohol's reinforcing effects, and could thereby adversely impact conclusions regarding group differences in relapse-like alcohol self-administration. According to a 5-session average of pre-extinction FR3 intake, the medians fell at 0.5 g/kg for alcohol and 7 ml/kg for sucrose. Rats were thus classified as "Low Drinkers" or "High Drinkers" based on whether ethanol or sucrose reinforcers generated below- or above-median performance, respectively, in the operant self-administration context. To determine changes in alcohol or sucrose self-administration during relapse, data were analyzed by ANCOVA with mean FR3 intake across the last 5 pre-extinction sessions used as the covariate.

Gene expression data were normalized using *z*-scores and analyzed by ANCOVA with *Cyp* expression levels controlled as a covariate. Relationships between *Pde10a* and behavioral measures were assessed using partial correlation, controlling for *Cyp* levels. Alpha-levels for exploratory correlations were Bonferroni-corrected, as indicated, to account for multiple hypothesis tests. Detailed descriptions of statistical analyses can be found in Supplemental Methods.

Results

Acquisition of self-administration behavior

As desired, most rats acquired self-administration behavior within either the single 12-h water self-administration session (45/48) provided before stress exposure (Figure 1) or within the first 3 FR1 sessions (2/3 remaining rats). Acquisition was defined as over 100 active lever presses during the 12-h water session or 15 active lever presses during FR1 alcohol self-administration with better than chance preference for the active vs. inactive lever. The one rat that failed to acquire operant behavior (see Supplemental Methods) was eliminated from analyses. Rats were assigned to Stress History ($n = 24$) vs. Control ($n = 23$) conditions matched for 1-h home cage alcohol intake ($M \pm SEM$: Control, 0.57 ± 0.08 ; Stress History, 0.55 ± 0.08 g/kg, $p=0.92$) as well as active lever presses (329 ± 23 vs. 331 ± 18 , $p=0.94$) and active lever preference (0.82 ± 0.02 vs. 0.84 ± 0.02 , $p=0.49$) during the 12-h water self-administration session.

Stress history increases relapse-like alcohol self-administration in baseline Low Drinkers

Repeated measures ANOVA of weight-normalized alcohol intake across self-administration days (Table 1) yielded a main effect of the within-subjects factor Day ($F_{22,990}=23.73$, $p<0.001$), without effects of Stress History ($p=0.92$) or interaction between factors ($p=0.41$). Because baseline avidity for alcohol has been suggested to moderate the acute effects of stress on alcohol preference (Volpicelli et al., 1990), we sought to determine whether the effects of stress history on alcohol self-administration differed between baseline High vs. Low Drinkers. For this subgroup susceptibility analysis, rats were divided into baseline High ($M \pm SEM$ 0.76 ± 0.04 g/kg) vs. Low (0.25 ± 0.03 g/kg) Drinkers. Stress history selectively increased relapse-like alcohol lever responding (Figure 2A, Stress History $\times$ Day, $F_{37,814}=2.93$, $p<0.001$) and intake (Figure 2C, Stress History $\times$ Day, $F_{22,484}=2.24$, $p<0.005$) in Low Drinkers without altering the corresponding measures in High Drinkers (Figure 2B,D; Stress History $\times$ Day $F_{37,777}=0.16$, $p=0.69$ and $F_{22,462}=0.41$, $p=0.99$, respectively). Within Low Drinkers, *post hoc* tests showed significant increases in Stress History vs. Control rats for alcohol lever pressing during all "relapse" sessions (Figure 2A, p 's <0.05)

and intake during “relapse” sessions 2–6 (Figure 2C, $p<0.05$). As an index of change in alcohol intake from baseline to “relapse”, analysis of alcohol intake across the 9 “relapse” sessions with FR3 baseline as a covariate yielded a main effect of Stress History ($F_{1,44}=4.99$, $p<0.05$) as well as a Stress History $\times$ Baseline Drinking interaction ($F_{1,44}=4.99$, $p<0.05$). Subgroup analysis demonstrated a main effect of Stress History within baseline Low Drinkers (Figure 2E, $F_{1,21}=13.08$, $p<0.005$) but not High Drinkers (Figure 2F, $F_{1,19}=0.12$, $p=0.73$).

As expected, the within-subjects factor Day was a significant main effect for ethanol responses and intake in both Low Drinkers ($F_{37,814}=25.53$, $p<0.001$; $F_{22,484}=14.82$, $p<0.001$) and High Drinkers ($F_{37,777}=69.03$, $p<0.001$; $F_{22,462}=13.48$, $p<0.001$), reflecting the different ratio requirements of the various self-administration phases. Together, the results demonstrate that stress history selectively elevated relapse-like alcohol self-administration in those subjects that previously had exhibited a low level of responding for alcohol.

Effects of stress history on natural reinforcers: sucrose self-administration—

In order to determine the specificity of these findings for alcohol, rats were trained with 0.1% sucrose substituted as the reinforcer. As for alcohol, main effects of Day were observed due to changes in sucrose intake over the various training phases (Table 1; $F_{22,968}=39.40$, $p<0.001$). Unlike for alcohol, however, no Stress History effects or Stress History $\times$ Day interactions were seen (F s <0.50 , p 's >0.97). Importantly, no significant effects were observed during the “relapse” phase (F s <1.75 , p 's >0.19).

Moreover, as shown in Figure 3, subgroup analysis according to FR3 baseline sucrose self-administration did not show significant effects involving Stress History in either Low (Figure 3A,C) or High (Figure 3B,D) Drinkers (F s <0.62 , p 's >0.62). Similarly, Stress History effects were not observed for FR3 baseline-covaried sucrose intake during the “relapse” phase among Low (Figure 3E, $F_{1,21}=0.023$, $p=0.88$) or High Drinkers (Figure 3F, $F_{1,21}=2.24$, $p=0.15$), with no Stress History $\times$ Day interactions observed (F s <0.92 , p 's >0.51). As expected, significant effects of Day for active lever presses and weight-normalized intake were observed across the entirety of the study for both Low Drinkers (responses: $F_{37,777}=14.93$, $p<0.001$; intake: $F_{22,462}=27.03$, $p<0.001$) and High Drinkers (responses: $F_{37,777}=21.96$, $p<0.001$; intake: $F_{22,462}=29.66$, $p<0.001$), reflecting the different ratio requirements. The data indicate that the elevated relapse-like self-administration observed in Stress History Low Drinkers is specific for alcohol and does not generalize to sucrose, a natural reinforcer.

Stress history modulation of inactive lever responses—While the above results suggest a more pronounced effect of stress history in otherwise low drinkers, analysis of operant responding at the inactive lever showed a somewhat different pattern. Inactive lever responding was reduced in Stress History rats as compared to Controls, yielding main effects of Stress History ($F_{1,45}=7.81$, $p<0.01$) and Day ($F_{37,1665}=17.16$, $p<0.001$), as well as a significant interaction between the factors ($F_{37,1665}=1.61$, $p<0.05$). *Post hoc* testing demonstrated reduced inactive lever pressing among Stress History rats across all pre-extinction days.

Subgroup analysis showed a more pronounced reduction of inactive lever responding in Stress History High Drinkers (Figure 4A,B; main effect of Stress History, High Drinkers: $F_{1,19}=7.20$, $p<0.05$; Low Drinkers: $F_{1,21}=1.56$, $p=0.23$). When data were averaged by training phase (i.e. FR1, FR3, extinction, “relapse”), High Drinkers displayed significant Stress History-dependent reductions in inactive lever pressing, with main effects of Stress History ($F_{1,21}=6.62$, $p<0.05$) and Training Phase ($F_{3,63}=36.55$, $p<0.001$) without significant

interaction ($F_{3,63}=2.25$, $p=0.09$). *Post hoc* comparisons demonstrated that Stress History rats depressed the inactive lever significantly less during both FR1 and FR3 phases (FR1: $p<0.005$; FR3: $p<0.01$ vs. Control). These effects were specific for alcohol as a reinforcer, because rats self-administering sucrose, whether High or Low Drinkers, showed no significant Stress History effects for inactive lever responding (Figure 4C,D, $F_s<0.41$, $p's>0.53$ for Group effects, $F_s<1.61$, $p's>0.19$ for interaction terms). Thus, stress history reduced operant responses at the non-reinforced lever within the context of alcohol but not sucrose self-administration, an effect somewhat more pronounced in rats that self-administered higher quantities of alcohol prior to extinction training.

Pde10a expression in stress-responsive regions of the limbic system

Stress history facilitated relapse-like alcohol self-administration approximately 8 weeks post-stressor, suggesting the presence of long-term neuroadaptations. To investigate the relation of *Pde10a* to stress and alcohol self-administration history, brain punches were collected 48 h after the final relapse session, a time point corresponding to the expected onset of the next session. In the nucleus accumbens, a region highly enriched in PDE10A, no significant differences were observed between Control and Stress History rats (Figure S1A, $F_{1,40}=1.55$, $p=0.22$) or between High and Low Drinking subgroups (Figure S1B, $F_{1,40}=0.02$, $p=0.89$), nor was there any interaction between the factors (Figure S1C,D, $F_{1,40}=0.17$, $p=0.68$). Thus, the focus was shifted to other regions of the limbic system implicated in regulating stress- and reward-directed behaviors – namely, the amygdala and medial prefrontal cortex (mPFC).

Association of stress and alcohol drinking history with amygdala *Pde10a* expression—Three amygdala nuclei— basolateral (BLA), central (CeA) and medial (MeA) – were analyzed for alterations in *Pde10a* expression. In the BLA, higher *Pde10a* mRNA levels were present in Stress History rats as compared to Controls (Figure 5A), with a main effect of Stress History ($F_{1,39}=4.96$, $p<0.05$) but no significant effect of Baseline Drinking Level and no interaction between the factors ($F_s<0.66$, $p's>0.42$). Though the Stress History $\times$ Drinking Level interaction did not attain significance for BLA *Pde10a*, precluding a strong conclusion of differential effects, the main effect of Stress History was significant in High, but not Low, Drinkers if analyzed separately. The effect of Stress History on BLA *Pde10a* mRNA levels was regionally specific; no group differences were observed in the CeA or MeA (Figure 5B,C).

Correlational analyses further showed that higher BLA *Pde10a* levels were associated with greater baseline preference for the alcohol-paired lever in High Drinkers (Figure 5E, $r=0.48$, $p<0.05$), more so than Low Drinkers (Figure 5D, $r=0.31$, $p=0.15$). In contrast to the BLA, higher CeA *Pde10a* levels tended to correlate with greater baseline alcohol intake among Low Drinkers (Figure 5F, $r=0.58$, $p=0.063$) but not High Drinkers (Figure 5G, $r=0.33$, $p=1$). No significant correlations with behavior were found for MeA *Pde10a* expression levels. Thus, stress history increased BLA *Pde10a* expression, and amygdala *Pde10a* (CeA, BLA) expression correlated directly with baseline alcohol self-administration behaviors.

mPFC subregion-specific correlations of *Pde10a* with relapse-like alcohol intake—*Pde10a* levels were also measured in 3 mPFC subdivisions— prelimbic (plPFC), infralimbic (ilPFC), and anterior cingulate (ACC) cortices. In the plPFC, a significant interaction between Stress History and Drinking Level was observed (Table 2, $F_{1,35}=4.30$, $p<0.05$) in the absence of significant main effects of Stress History or Baseline Drinking ($F_s<0.18$, $p's>0.68$). Follow-up subgroup analyses showed that stress history tended to increase plPFC *Pde10a* expression in Low Drinkers ($F_{1,17}=3.53$, $p<0.08$), without reliably altering it in High Drinkers ($F_{1,13}=0.113$, $p=0.74$). Moreover, greater plPFC *Pde10a*

expression correlated with greater relapse-like alcohol intake in Low Drinkers (Figure 6A, $r=0.59$, $p<0.01$), the group that displayed Stress History-induced elevations in relapse-like alcohol self-administration. This correlation was not observed in High Drinkers (Figure 6B, $r=-0.03$, $p=0.90$).

Analysis of ilPFC *Pde10a* showed a significant effect of Baseline Drinking Level (Table 2, $F_{1,37}=4.40$, $p<0.05$), with no main effect of Stress History or Stress History×Drinking Level interaction (F 's <0.42 , p 's >0.52), reflecting higher ilPFC *Pde10a* expression in Low Drinkers than in High Drinkers. Accordingly, greater ilPFC *Pde10a* expression showed a trend toward greater relapse-like alcohol intake in High, but not Low, Drinkers (Figure 6C,D; Low: $r=0.13$, $p=0.57$; High: $r=0.58$, $p=0.08$).

No significant group effects or correlations with behavioral measures were found for ACC *Pde10a* (Table 2), including no correlation to relapse-like alcohol intake. Correlations not explicitly discussed were tested and found to be non-significant. Together the data indicate that stress history may increase plPFC *Pde10a* expression in Low Drinkers in direct relation to increased relapse-like alcohol self-administration. ilPFC *Pde10a*, in contrast, associated with greater relapse-like drinking in High Drinkers, but lower baseline self-administration.

Discussion

The present results show that a history of stress promotes greater alcohol self-administration in rats with an otherwise low motivation to work for alcohol reinforcement by producing lasting increases in the magnitude of relapse-like alcohol self-administration. From the onset of alcohol self-administration, stress history also reduced responding at the inactive lever, particularly in High Drinkers. The effects of stress history were not seen for a sucrose reinforcer, suggesting that past stress directed behavior towards alcohol reinforcement specifically. As predicted, stress history also led to increased *Pde10a* expression 12 weeks post-stress in the BLA, with a similar trend seen in the plPFC of Low Drinkers. In otherwise low-drinking rats, greater plPFC *Pde10a* mRNA levels correlated directly with greater relapse-like alcohol self-administration. In High Drinkers, greater BLA *Pde10a* mRNA correlated with increased preference ratios for alcohol. The behavioral results resonate with clinical reports that patients with PTSD are at increased risk for alcohol relapse (Norman et al., 2007) and the molecular findings, while correlational, suggest that neuroadaptation of BLA and plPFC *Pde10a* systems plays a role in the enduring behavioral consequences of past stress.

Role of baseline drinking levels in stress history effects on preference and relapse

Previous studies in rodents have yielded mixed results regarding the ability of stress to acutely modulate ongoing alcohol consumption. Here, we instead sought to determine whether a history of past stress, potentially relevant to the etiology of PTSD, influenced future patterns of alcohol self-administration. Under the current conditions, stress history did not change mean alcohol consumption across a subsequent 30-day period, including acquisition and initial maintenance of alcohol self-administration. However, greater focus on the alcohol-paired lever, as compared to the inactive lever, was observed even during the earliest phase of operant alcohol training in rats with a stress history. As stress group assignment was balanced for inactive lever responses during the 12-h operant water session, this elevated alcohol lever preference suggests that, particularly among rats self-administering high quantities of alcohol, stress history may focus behavior on the alcohol-paired lever, to the exclusion of non-reinforced lever pressing. An alternative interpretation is that stress history suppressed behavior, except that which resulted in ethanol delivery.

Furthermore, after extinction of the operant response, alcohol self-administration increased in previously low-drinking Stress History rats. This finding is of keen interest given the increased propensity for alcohol relapse among human PTSD patients (Norman et al., 2007). Whether stress history potentiated the reinforcing effects of alcohol in relation to the passage of time, abstinence, or the process of extinction remains to be determined. The specificity of the effects of stress history on baseline Low Drinkers conceivably could reflect a “ceiling” effect on self-administration of High Drinkers. Perhaps baseline High Drinkers were innately stressed, such that further exogenous stress had little incremental effect; indeed, multiple alcohol-preferring rat lines selectively bred from Wistar stock show high intrinsic anxiety (Ciccocioppo et al., 2006; Colombo et al., 1995; Hwang et al., 2004). Alternatively, as baseline drinking levels were divided based on FR3 self-administration, perhaps the motivation to self-administer alcohol was increased during relapse in baseline Low Drinkers while High Drinkers experienced high motivation both at baseline and during relapse. Whatever the basis, the effects of stress history are meaningful in that they may convert otherwise low drinkers to high drinkers, placing them at risk for transition towards alcoholism.

Role for PDE10A in post-stress alcohol drinking and behavioral sequelae

A possible mechanism for the dissimilar effects of stress history on Low vs. High Drinkers may be differential expression of *Pde10a* in the amygdala and mPFC. Stress history differentially increased *Pde10a* expression in Low vs. High Drinkers, with greater pLPFC *Pde10a* expression correlating with greater relapse-like alcohol intake, and greater BLA *Pde10a* expression correlating with increased alcohol preference ratios in High Drinking rats. PDE10A inhibits a variety of intracellular signaling cascades, resulting in reduced activity of neurons that express higher levels of the enzyme (Threlfell et al., 2009). Thus, the present results suggest that reduced pLPFC neuronal activity may promote increased alcohol intake during relapse. Consistent with this hypothesis, patients with post-traumatic stress disorder and detoxified alcoholics show decreased gray matter volume in a region of the PFC containing the pLPFC (Kroes et al., 2011; Rando et al., 2011), a structural difference that correlates with the latency and severity of alcohol relapse (Rando et al., 2011). Because the pLPFC sends predominantly excitatory projections to the BLA (Brinley-Reed et al., 1995), where stress history elevated *Pde10a*, decreased overall activity of the PFC-BLA circuit may promote increased alcohol self-administration.

The action of stress history to increase BLA and pLPFC *Pde10a* expression might be involved not only in post-stress alcohol relapse behavior, but also in other long-term behavioral consequences of past stress. For example, it is known that systemic inhibition or genetic deletion of PDE10A reduces conditioned avoidance responding (Grauer et al., 2009; Siuciak et al., 2006; Siuciak et al., 2008), which is heightened in patients with PTSD (Foa et al., 1995). PDE10A inhibitors also reduce deficits in prepulse inhibition (Grauer et al., 2009; Weber et al., 2009), a sign of deficient sensory gating that may contribute to “positive” symptoms of PTSD, including flashbacks and abnormal startle responses (Holstein et al., 2010). Systemic PDE10A inhibition or deletion increases social behaviors (Grauer et al., 2009; Sano et al., 2008), whereas traumatic stress is associated with social anxiety and withdrawal (Collimore et al., 2010; Feldman and Vengrober, 2011). Finally, PDE10A inhibition reduces appetitive responding for food (Piccart et al., 2011), whereas past trauma increases not only alcohol drinking, but also binge eating and abuse of other drugs (Harrington et al., 2010; Schiff et al., 2007). The effect of specifically inhibiting PDE10A activity within the BLA and pLPFC on post-stress behavioral endpoints warrants further study. The collective observations implicate this enzyme as an intriguing target for medications development to address comorbid alcohol use and stress-related disorders.

Conclusion

Patients suffering from comorbid PTSD and alcohol use disorders face significant obstacles to maintaining sobriety, as relapse risk is greatly elevated among alcoholics with comorbid PTSD. Here we show that a history of stress facilitated relapse-like alcohol self-administration, lending face validity to the current behavioral model for evaluating pharmacotherapies for comorbid stress and alcohol use disorders. Stress history also increased *Pde10a* expression in direct relation to increased relapse-like alcohol self-administration and alcohol preference in the mPFC and BLA, respectively. These data support further investigation of the therapeutic potential of drugs targeting PDE10A activity for alcohol abuse or other behavioral sequelae in patients with stress-related disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Stephanie Dela Cruz, Jeanette Helfers, David Howard, Sujin Park, John Sekab, Jaimie Shubin, and Elizabeth Wolf for technical assistance. Financial support was received from the Pearson Center for Alcoholism and Addiction Research and National Institutes of Health grants DK26741, from the National Institute of Diabetes and Digestive and Kidney Diseases, and AA06420 and AA018914, from the National Institute on Alcohol Abuse and Alcoholism.

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Figure 1.

Rapid acquisition model allows for stress exposure prior to operant alcohol training. Rats received 48-h home cage access to 10% EtOH (v/v) and water. The following day, rats received 1-h 2-bottle access time-matched to future operant schedule. 1-h 2-bottle sessions continued for the next 5 days, excluding the day on which rats were trained for active/inactive lever discrimination in a single 12-h session with 0.1 ml water delivered for each active lever press. Once all rats had completed the water training session, they received 3 days of 30-min cued footshock or cue only exposure. After a day of rest, operant alcohol training commenced for 5 sessions at FR1, 8 sessions at FR3, 15 sessions of extinction and 9 sessions of renewed alcohol access (relapse-like reacquisition training) at FR3.

Figure 2.

A history of stress increases relapse-like self-administration in previously Low Drinking rats. Rats were trained in 1-h operant self-administration sessions with 0.1 ml 10% alcohol (v/v) delivered after 1 (FR1) or 3 (FR3) depressions of the active lever, as indicated. Low (A, C, E) and High (B, D, F) Drinkers were subdivided by a median split of pre-extinction baseline (FR3) self-administration (0.5 g/kg intake). (A, B) Number of presses on the active lever and (C, D) body weight-normalized alcohol intake (g/kg) are shown across all sessions, spanning the acquisition (FR1 and FR3), extinction, and reacquisition (FR3 "RELAPSE") phases of operant training. (E, F) Alcohol intake during relapse-like sessions controlled for baseline FR3 alcohol self-administration via ANCOVA analysis. Data are

expressed as $M(A-D)$ or LSMeans (E-F, covarying for baseline self-administration) $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ vs. Control, $n=10-14$ per group.

Figure 3.

Stress history does not significantly alter sucrose self-administration, regardless of pre-extinction baseline. Rats were trained in 1-h operant self-administration sessions with 0.1 ml of 0.1 % sucrose (*w/v*) delivered after 1 (FR1) or 3 (FR3) depressions of the active lever, as indicated. Low (A, C, E) and High (B, D, F) Drinkers were subdivided by a median split of pre-extinction baseline (FR3) self-administration (7 ml/kg intake). (A, B) Number of presses on the active lever and (C, D) body weight-normalized alcohol intake (ml/kg) are shown across all sessions, spanning the acquisition (FR1 and FR3), extinction, and reacquisition (FR3 "RELAPSE") phases of operant training. (E, F) Sucrose intake during relapse-like sessions controlled for FR3 baseline self-administration via ANCOVA analysis. Data are

expressed as M (A–D) or LSMeans (E–F, covarying for baseline self-administration) $\pm$ SEM. $n=10$ –14 per group.

Figure 4.

A history of stress exposure reduces inactive lever pressing during the acquisition phase of operant alcohol self-administration. Average inactive lever presses by training phase for alcohol (A,B) and sucrose (C, D) self-administration, separated by low (A, C) and high (B, D) baseline levels of self-administration. Data are expressed as 5-day $M \pm SEM$. ** $p < 0.01$ vs. Control, $n=10-14$ per group.

Figure 5.

Differential modulation of *Pde10a* expression by stress and alcohol drinking history in the basolateral (BLA) and central (CeA) nuclei of the amygdala. At the conclusion of relapse-like alcohol self-administration, Stress History rats showed increased *Pde10a* in the BLA (A) but not CeA (B) or MeA (C). Drinking status was also related to differences in *Pde10a* expression, with significant direct correlations between BLA *Pde10a* and baseline alcohol lever preference in High Drinkers (E), but not Low Drinkers (D). CeA *Pde10a* showed a trend towards positive correlation with baseline FR3 alcohol intake in Low Drinkers (F) but not High Drinkers (G). Data are expressed as LSMeans $\pm$ SEM for *Pde10a*, controlled for *Cyp* covariate effects following z-score transformation of gene expression data (A–C) or as

partial regression plots of the normalized active lever preference (D, E) or operant alcohol intake (F, G) during the pre-extinction FR3 baseline period versus amygdala *Pde10a* expression, controlling for *Cyp* levels (D–G). $n = 23$ – 24 per group (A–C), or 20 – 24 (D–F). * $p < 0.05$, # $p < 0.07$ (vs. Control where applicable).

Figure 6.

Pde10a in the medial prefrontal cortex correlates with relapse-like alcohol intake. plPFC *Pde10a* expression shows a strong positive correlation with relapse-like alcohol intake in Low Drinkers (A) but not High Drinkers (B). Conversely, ilPFC *Pde10a* showed a significant positive relationship to relapse-like alcohol intake for High Drinkers (D) but not Low Drinkers (C). Data are presented as partial regression plots of the normalized 9-day mean relapse-like operant alcohol intake versus mPFC *Pde10a* expression, controlling for *Cyp* levels. $n = 18-23$. * $p < 0.05$, ** $p < 0.01$.

Table 1

Average reinforcer ($M \pm \text{SEM}$) intake by experimental phase of self-administration.

Group	FR1	FR3 Baseline	FR3 Relapse	Relapse Fold Change
<i>Alcohol(g/kg)</i>				
Control	0.86 $\pm$ 0.07	0.52 $\pm$ 0.07	0.49 $\pm$ 0.06	1.01 $\pm$ 0.18
Shock	0.78 $\pm$ 0.07	0.48 $\pm$ 0.06	0.56 $\pm$ 0.05	2.24 $\pm$ 0.85
<i>Sucrose (ml/kg)</i>				
Control	28.0 $\pm$ 3.3	13.4 $\pm$ 3.5	5.4 $\pm$ 1.0	0.60 $\pm$ 0.10
Shock	28.5 $\pm$ 2.8	12.8 $\pm$ 2.4	6.8 $\pm$ 1.2	0.66 $\pm$ 0.08

Table 2Standardized (z-score) prefrontal cortical *Pde10a* expression by Stress History and Drinking Group

Region	Low Drinkers		High Drinkers	
	Control	Stress History	Control	Stress History
plPFC	-0.22 ± 0.24	0.29 ± 0.21 *	0.13 ± 0.23	-0.33 ± 0.25
ilPFC	0.12 ± 0.30 [#]	0.42 ± 0.25 #	-0.36 ± 0.28	-0.29 ± 0.30
ACC	-0.01 ± 0.16	-0.04 ± 0.14	0.10 ± 0.15	-0.04 ± 0.16

Note: Values are expressed as least square means ± SEM, controlling for *Cyp* expression, wherein least square means were calculated from analysis of z-scored *Pde10a* data with *Cyp* as a covariate and Stress History and Drinking Group as factors.

* $p < 0.05$, Stress History × Drinking Group interaction;

[#] $p < 0.05$, main effect of Drinking Group.