

# Chronic alcohol produces neuroadaptations to prime dorsal striatal learning

Lauren DePoy<sup>a</sup>, Rachel Daut<sup>a</sup>, Jonathan L. Brigman<sup>a</sup>, Kathryn MacPherson<sup>a</sup>, Nicole Crowley<sup>b</sup>, Ozge Gunduz-Cinar<sup>a</sup>, Charles L. Pickens<sup>a</sup>, Resat Cinar<sup>c</sup>, Lisa M. Saksida<sup>d</sup>, George Kunos<sup>c</sup>, David M. Lovinger<sup>b</sup>, Timothy J. Bussey<sup>d</sup>, Marguerite C. Camp<sup>a</sup>, and Andrew Holmes<sup>a,1</sup>

<sup>a</sup>Laboratory of Behavioral and Genomic Neuroscience, National Institute on Alcoholism and Alcohol Abuse, National Institutes of Health, Bethesda, MD 20814; <sup>b</sup>Department of Psychology and Medical Research Council and Wellcome Trust Behavioural and Clinical Neuroscience Centre, University of Cambridge, Cambridge CB2 3EB, United Kingdom; <sup>c</sup>Laboratory of Physiological Studies, National Institute on Alcoholism and Alcohol Abuse, National Institutes of Health, Rockville, MD 20852; and <sup>d</sup>Laboratory for Integrative Neuroscience, National Institute on Alcoholism and Alcohol Abuse, National Institutes of Health, Rockville, MD 20852

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**Drug addictions including alcoholism are characterized by degradation of executive control over behavior and increased compulsive drug seeking. These profound behavioral changes are hypothesized to involve a shift in the regulation of behavior from prefrontal cortex to dorsal striatum (DLS). Studies in rodents have shown that ethanol disrupts cognitive processes mediated by the prefrontal cortex, but the potential effects of chronic ethanol on DLS-mediated cognition and learning are much less well understood. Here, we first examined the effects of chronic EtOH on DLS neuronal morphology, synaptic plasticity, and endocannabinoid-CB1R signaling. We next tested for ethanol-induced changes in striatal-related learning and DLS in vivo single-unit activity during learning. Mice exposed to chronic intermittent ethanol (CIE) vapor exhibited expansion of dendritic material in DLS neurons. Following CIE, DLS endocannabinoid CB1 receptor signaling was down-regulated, and CB1 receptor-dependent long-term depression at DLS synapses was absent. CIE mice showed facilitation of DLS-dependent pairwise visual discrimination and reversal learning, relative to air-exposed controls. CIE mice were also quicker to extinguish a stimulus–reward instrumental response and faster to reduce Pavlovian approach behavior under an omission schedule. In vivo single-unit recording during learning revealed that CIE mice had augmented DLS neuronal activity during correct responses. Collectively, these findings support a model in which chronic ethanol causes neuroadaptations in the DLS that prime for greater DLS control over learning. The shift to striatal dominance over behavior may be a critical step in the progression of alcoholism.**

cannabinoid | abuse | touchscreen | PFC | dependence

**A**lcoholism is a highly prevalent disorder with a massive and growing impact on public health (1). The course of alcoholism and other chronic drug addictions has been conceptualized as involving the progressive deterioration of executive control of behavior and the corresponding emergence of compulsive drug seeking (2, 3). At the neural systems level, this shift is associated with a “devolution” away from prefrontal cortical (PFC) regulation of behavior in favor of greater control by subcortical regions including the dorsal striatum (DS) (2, 4).

Consistent with this scheme, alcohol-dependent individuals show impairments on PFC-mediated cognitive measures, such as impulse control and reversal learning (5, 6), but exaggerated neural responses in the DS when, for example, presented with alcohol-associated cues (7–9). Along similar lines, rodents chronically exposed to ethanol (EtOH) exhibit deficits in PFC- and hippocampal-mediated tasks, including spatial and reversal learning, set-shifting, and fear extinction, that in some cases are linked to cortical cell death (10–17).

These observations are consistent with an EtOH-induced impairment in various PFC-related cognitive behaviors but do not address the possibility of concomitant changes in processes

mediated by the DS. In this context, recent studies in rats have found that chronic EtOH alters synaptic plasticity in the DS and causes functional alterations in key plasticity mechanisms, including cannabinoid-CB1 and NMDA-GluN2B receptors (18–21). In addition, responding for EtOH develops habit-like properties after prolonged operant training in a manner reversed by selective lesions of the lateral part of the DS (DLS) (22–24). These findings suggest that, following a long history of EtOH-seeking, behavior falls under the control of the DLS. However, it is still not fully clear whether chronic exposure to EtOH is itself sufficient to bias DLS-mediated control of behavior, how such priming of behavior is driven by alterations in the structure and plasticity of DLS, and which mechanisms might subservise these effects.

Here, using a mouse model, we demonstrate that chronic EtOH exposure produces profound neuroadaptations in DLS, including neuronal dendritic expansion, down-regulation of endocannabinoid-CB1R signaling, and abnormal CB1R-dependent synaptic plasticity (long-term depression). In tandem, we find that chronic EtOH exposure leads to a facilitation of multiple forms of operant-based learning that are indicative of an up-regulation of DLS function and that were coupled to enhanced learning-related in vivo single-unit activity. Taken together, our findings support a shift to dorsal striatal processing and striatal control of behavior as a result of chronic exposure to EtOH.

## Significance

**Alcoholism is characterized by a progressive degradation of executive control and an increase in compulsive alcohol seeking that is hypothesized to involve a shift from prefrontal cortex to dorsal striatal (DLS) control over behavior. Here, we show that mice exposed to chronic intermittent alcohol exhibited expansion of dendritic material in DLS neurons, coupled with loss of endocannabinoid CB1 receptor signaling and CB1-mediated long-term depression in the DLS. Behaviorally, chronic alcohol exposure facilitated various forms of DLS-dependent learning and augmented in vivo DLS neuronal activity as correct learned choices were made. These findings support a model in which chronic ethanol causes DLS neuroadaptations that prime for greater striatal control over behavior, potentially contributing to the progression of alcoholism.**

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<sup>1</sup>To whom correspondence should be addressed. E-mail: Andrew.Holmes@mail.nih.gov.

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## Results and Discussion

**Chronic Intermittent EtOH Exposure.** Male C57BL/6J mice were exposed to a chronic intermittent ethanol (CIE) procedure designed to simulate the repeated episodes of heavy drinking and withdrawal that characterize alcohol abuse (25, 26). CIE involved 16-h continuous EtOH exposure followed by an 8-h withdrawal. A cycle of CIE consisted of four exposures (Monday–Friday), followed by a longer 80-h withdrawal (Saturday–Sunday). There were two cycles in total. Representative blood EtOH concentrations (BECs) from sentinel mice ( $n = 11$ ) were in the range of  $175 \pm 25$  mg/dL and did not decrease (i.e., exhibit metabolic tolerance) from the first to the second cycle (Fig. 1A).

To confirm that this CIE procedure increased EtOH consumption, as reported in prior studies (8), we tested mice for EtOH drinking in a two-bottle choice test over 12 d, beginning 3 d after CIE (i.e., postacute withdrawal). CIE mice exhibited significantly increased EtOH drinking relative to baseline levels recorded over 2 wk before exposure, and compared with air-exposed controls (ANOVA group  $\times$  time interaction:  $F_{2,36} = 6.19$ ,  $P < 0.01$ , followed by Fisher's least significant differences (LSD) post hoc tests,  $n = 10$ ). Increased drinking in CIE mice was evident during the first 1–6 d of post-CIE measurement (see Fig. 1B and Fig. S1 for detailed timeline). Air controls also showed an increase in drinking relative to preexposure baseline ( $t$  test:  $t(9) = 4.83$ ,  $P < 0.01$ ), likely due to an EtOH deprivation effect. By days 7–12 postexposure, consumption had returned to baseline in the air group but remained significantly elevated in CIE mice ( $t$  test:  $t(9) = 2.72$ ,  $P < 0.05$ ) (Fig. 1B). These data show that CIE produced lasting increases in EtOH drinking.

**CIE Causes DLS Dendritic Hypertrophy.** Chronic psychostimulant exposure increases dendritic arborization in the PFC and ventral striatum (27). We recently found that four cycles of CIE produced dendritic hypertrophy in mouse PFC neurons (10), but it is not known whether neurons in the DLS are also affected. We therefore exposed mice to two cycles of CIE and collected brains 3 d later for impregnation with a Golgi–Cox stain to visualize the dendritic arbor of DLS neurons, as previously described (10, 28).

Sholl analysis revealed CIE-induced increases in dendritic length (ANOVA group  $\times$  distance-from-soma interaction:  $F_{11,143} = 3.41$ ,  $P < 0.01$ , group effect:  $F_{1,13} = 5.60$ ,  $P < 0.05$ , distance-from-soma effect:  $F_{11,143} = 286.88$ ,  $P < 0.01$ ,  $n = 7$ –8 mice per group,  $n = 8$  neurons per mouse) (Fig. 2A) and number of dendritic branches (ANOVA group  $\times$  distance-from-soma interaction:  $F_{11,143} = 1.95$ ,  $P < 0.05$ ) (Fig. S2). There was a significant increase in the overall amount of dendritic material in DLS neurons after CIE, relative to air exposure. Moreover, CIE produced a significant increase in the length ( $t$  test:  $t(13) = 6.60$ ,  $P < 0.05$ ) and number ( $t$  test:  $t(13) = 5.91$ ,  $P < 0.05$ ) of terminal dendritic branches (Fig. 2B–D).

These data show that DLS neurons undergo significant and selective dendritic remodeling following chronic exposure to

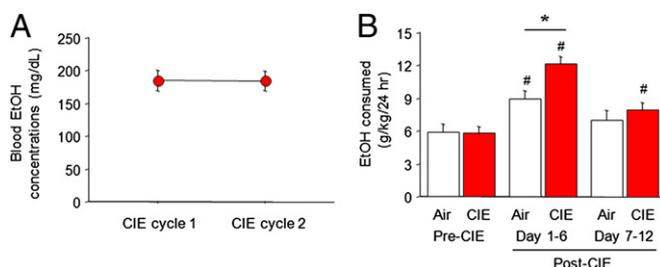
EtOH. In addition, the CIE-induced dendritic hypertrophy was restricted to DLS neurons and did not extend to cortical or limbic regions. The dendritic arbors of pyramidal neurons in the orbitofrontal cortex and the basolateral amygdala (Fig. S3) or (as previously shown in ref. 10) the infralimbic and prelimbic subregions of medial PFC all showed no differences between mice exposed to two cycles of CIE and air controls.

### Altered DLS Synaptic Plasticity and CB1R Down-Regulation After CIE.

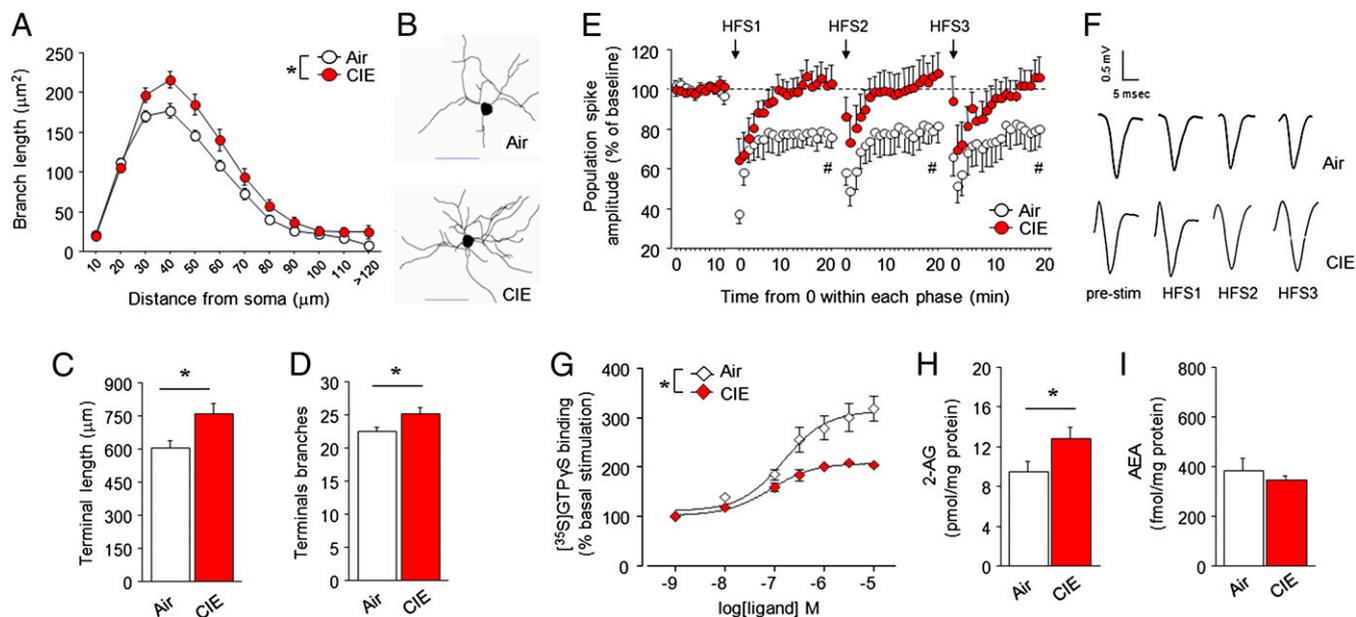
To test whether the CIE-induced structural abnormalities in DLS neurons were accompanied by functional changes, we examined long-term depression (LTD) at cortical glutamatergic inputs to DLS synapses, a key form of synaptic plasticity in the DLS (29). Three days after CIE, mice were assessed for LTD via local afferent high-frequency stimulation (HFS) of cortical inputs to DLS, comprising  $2 \times 1$ -s 100-pulse trains applied three times, once every 20 min. There was a borderline significant effect of CIE on LTD of evoked field potentials (ANOVA effect of group:  $F_{1,18} = 4.39$ ,  $P = 0.05$ , followed by Fisher's LSD post hoc tests,  $n = 10$ ). In air controls, HFS significantly reduced population spike amplitude after each of the three sets of trains whereas HFS completely failed to induce LTD in CIE mice (Fig. 2E and F). There was also partial attenuation of the efficacy (population spike magnitude) of synaptically induced activation in DLS following CIE (ANOVA group  $\times$  stimulation amplitude interaction:  $F_{14,182} = 1.70$ ,  $P = 0.058$ ) (Fig. S4). These data extend prior reports of loss of corticostriatal LTD in rats measured 1 d after chronic EtOH exposure (30), by showing that this effect persists into the postacute-withdrawal period.

Current findings are reminiscent of the loss of (glutamate-dependent) LTD seen following chronic exposure to psychostimulant drugs of abuse that occurs in the DS as well as the nucleus accumbens (31–34). By limiting synaptic rescaling, drug-induced loss of LTD has been posited to impair the responsiveness of accumbal circuits to new (e.g., cortically derived) environmental information, and thereby contribute to behavioral inflexibility in addiction (33). In light of the current findings in the DS after chronic alcohol, LTD loss may be a mechanism that is common to multiple drugs of abuse and would appear to be pervasive across striatal regions. At least in the case of chronic alcohol and the DS, deficient LTD may also be coupled with changes in other forms of striatal plasticity, including *N*-methyl-D-aspartate (NMDAR)-mediated LTP (35). Previous studies have found that chronic EtOH up-regulates NMDAR function and plasticity in the DS (albeit medially), in a manner that underlies dependence drinking (20, 21, 36). Moreover, various forms of learning, including some of the tasks used in the current study, are critically dependent upon DS NMDARs (37, 38). Thus, chronic alcohol may disrupt LTD-mediated plasticity in response to cortical input while at the same time unmasking NMDAR-dependent LTP and strengthening the associability of a subset of DS synapses.

The potential effect of CIE on NMDAR-mediated LTP in the DS remains to be tested. The mechanisms underlying the absence of LTD after CIE are also unclear but could reflect either a basal enhancement of LTD that occluded induction by HFS or an impairment of mechanisms subserving this form of plasticity in DLS. Endocannabinoid (ECB) signaling through CB1 receptors (CB1R) (19, 29) is a critical, obligatory mechanism for LTD in DLS, and blockade of CB1R in the DLS impairs certain forms of learning mediated by this region (39). CB1R signaling is also linked to our finding of DLS dysmorphology by prior work showing that loss of CB1R causes dendritic hypertrophy (40). We therefore asked whether CIE-induced alterations in plasticity were associated with changes in ECB signaling by performing CB1R agonist (R-WIN55230-2)-stimulated [ $^{35}$ S] GTP $\gamma$ S binding in DLS membranes (41) 3 d after CIE. There was a significant dose-dependent reduction in agonist-stimulated binding in DLS samples from CIE mice, relative to air controls (ANOVA group  $\times$  dose interaction:  $F_{6,111} = 4.00$ ,  $P < 0.01$ ,  $n = 9$ ) (Fig. 2G). The maximum effect of the agonist ( $E_{max}$ ) (air =  $314 \pm 14$ ,



**Fig. 1.** Chronic intermittent EtOH exposure. (A) Representative blood EtOH concentrations obtained in sentinel mice on the first to the second CIE cycle. (B) CIE increased EtOH drinking in a two-bottle choice test. Air controls showed a transient increase in drinking (EtOH deprivation effect). Data are means  $\pm$  SEM, \* $P < 0.05$  CIE vs. air, # $P < 0.05$  vs. pre-CIE/same group.



**Fig. 2.** CIE causes DLS dendritic hypertrophy and altered DLS synaptic plasticity. (A) CIE increased dendritic length. (B) DLS neuron reconstructions in air and CIE mice. (Scale bar: 50  $\mu\text{m}$ .) (C and D) CIE increased terminal dendritic length and number. (E) CIE prevented induction of LTD induced by local afferent high-frequency stimulation (HFS, denoted by arrows) of cortical inputs to DLS. (F) Traces from E. (G) CIE decreased CB1R agonist-stimulated functional binding in DLS. (H and I) CIE increased levels of 2-AG, not AEA, in DLS. Data are means  $\pm$  SEM, \* $P < 0.05$  CIE vs. air, # $P < 0.05$  vs. pre-HFS1 baseline.

CIE =  $208 \pm 4$ ,  $t$  test:  $t(16) = 3.79$ ,  $P < 0.01$ ), but not the negative logarithm of the half-maximal effective concentration ( $\text{pEC}_{50}$ ) (air =  $6.8 \pm 0.2$ , CIE =  $7.1 \pm 0.1$ ), values were significantly decreased after CIE. These data are in line with the decreased CB1R binding and signaling previously reported in the DLS of rats chronically exposed to EtOH (42–44), and in the DS of alcohol-dependent patients (45, 46).

Decreases in CB1R functional binding after chronic EtOH exposure are associated with elevated levels of the ECB 2-arachidonoylglycerol (2-AG) and, in some studies, anandamide (AEA) (18, 19). To test whether similar changes were present after CIE, we quantified DLS levels of 2-AG and AEA (3 d after CIE) via liquid chromatography-tandem mass spectrometry. We detected significantly higher levels of 2-AG ( $t$  test:  $t(17) = 5.12$ ,  $P < 0.05$ ,  $n = 9$ –10) (Fig. 2H), not AEA ( $t$  test:  $P > 0.05$ ) (Fig. 2I), in the DLS of CIE-exposed mice, relative to air controls. There were no comparable changes in ECBs in other brain regions (medial PFC, dorsal hippocampus), indicating changes specific to DLS (Fig. S5). Taken together, these data suggest that CIE increases 2-AG levels in the DLS, leading to a compensatory down-regulation of CB1R signaling and a resultant loss of CB1R-mediated plasticity (LTD). CB1R down-regulation may also account for CIE-induced DLS dendritic dysmorphology, given prior studies have demonstrated that CB1R gene deletion is sufficient to cause similar dendritic hypertrophy in other brain regions (e.g., amygdala) (40).

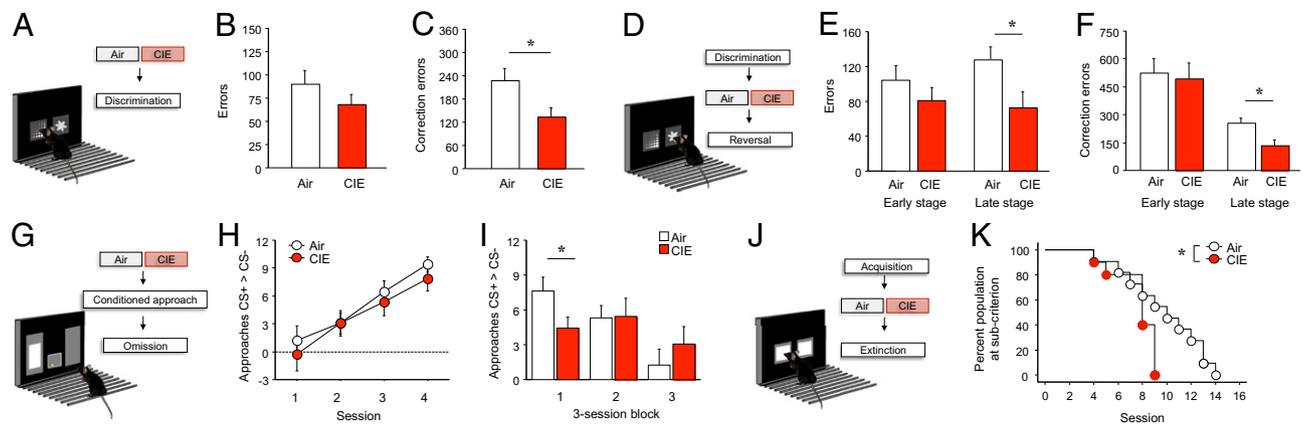
**CIE Facilitates DLS-Dependent Learning.** We next examined whether the structural and functional effects of CIE in the DLS affected learning processes previously shown to be mediated by this brain region (37, 47). We first assessed the effects of CIE on pairwise visual discrimination learning (Fig. 3A). This task requires mice to make a discriminative response to stimuli on a touch-sensitive computer screen to obtain food reward and is severely impaired by discrete excitotoxic lesions of the DLS (47). To reach a criterion of 85% correct responding, CIE mice made significantly fewer repetitive (“correction”) errors ( $t$  test:  $t(22) = 2.38$ ,  $P < 0.05$ ,  $n = 12$ ) and slightly fewer errors overall ( $t$  test:  $t(22) = 1.16$ ,  $P > 0.05$ ), compared with air controls (Fig. 3B and C). This improvement in discrimination learning in CIE mice was

independent of changes in reaction times (air =  $4.17 \pm 0.45$ , CIE =  $4.00 \pm 0.29$  s) or reward retrieval latencies (air =  $2.20 \pm 0.20$ , CIE =  $1.84 \pm 0.10$  s), discounting gross alterations in motivation or locomotor activity.

Another cohort was tested on a reversal learning variant of the task, whereby mice had to reduce responding to the previously rewarded stimulus and learn to respond to the second, newly rewarded stimulus (Fig. 3D). Performance on this task is also highly sensitive to lesions of the DLS (47). CIE mice showed improved learning, relative to air controls, making significantly fewer errors ( $t$  test:  $t(22) = 2.42$ ,  $P < 0.05$ ,  $n = 11$ –13) and correction errors ( $t$  test:  $t(22) = 2.83$ ,  $P < 0.01$ ) (Fig. 3E and F). Notably, the CIE-induced improvements emerged during the late phase of reversal when performance was already above chance levels and new learning predominates over perseveration (48, 49). This effect on late reversal coincides with the phase of reversal learning when the DLS is most strongly activated, as evidenced by high immediate-early gene activity (37).

These data are consistent with a CIE-induced facilitation of learning processes mediated by the DLS. The precise nature of the learning processes that are altered remains to be determined. One possibility is that CIE promotes DLS-mediated forms of stimulus–response (S–R) learning, such as habit formation (23, 50). Prior work has shown that rats given prolonged access to EtOH develop habit-like imperviousness to drinking taste-adulterated EtOH (51). Moreover, instrumental responding for EtOH becomes insensitive to reinforcer devaluation following extended behavioral training (23, 52). We previously found that EtOH-naïve mice that well-trained on the reversal task show habit-like insensitivity to reinforcer devaluation (47). When probed for reinforcer devaluation when performing at chance levels, neither CIE mice nor air controls reduced performance or errors rates following malaise-induced devaluation, suggesting that both groups may have already developed habit-like behavior (Fig. S6). Although these data cannot address whether CIE enhanced habit-like behavior in this task, they do suggest that CIE did not facilitate reversal by promoting action–outcome learning, which would be predicted to increase sensitivity to devaluation.

To further elucidate the behavioral effects of CIE, we tested another cohort for Pavlovian conditioned approach (53) (Fig.



**Fig. 3.** CIE facilitates DLS-dependent learning. (A–C) CIE before pairwise visual discrimination training decreased the number of errors and correction errors to criterion performance. (D–F) CIE before reversal training decreased the number of errors and correction errors to criterion performance, specifically during the late testing stage when percent correct performance was greater than chance. (G–I) CIE before Pavlovian conditioned approach testing produced a more rapid decrease in approaches during omission training. (J and K) CIE before stimulus–reward extinction training produced faster extinction. Data are means  $\pm$  SEM, \* $P < 0.05$  CIE vs. air.

3G). Following repeated presentation of a touchscreen stimulus that noncontingently signaled reward, CIE mice and air controls similarly increased their approaches to the stimulus over a second stimulus never associated with reward (ANOVA session effect:  $F_{3,69} = 23.31$ ,  $P < 0.01$ ,  $n = 12–13$ ) (Fig. 3H). After attaining the conditioned approach response, we tested mice under an omission schedule in which stimulus approaches had to be withheld to obtain a reward. Although both groups decreased approaches during omission training, CIE mice were significantly quicker to do so than air controls (ANOVA group  $\times$  session interaction:  $F_{2,46} = 4.35$ ,  $P < 0.05$ ) (Fig. 3I). This finding demonstrates that CIE improved the ability not just to associate and respond to a stimulus to obtain reward (as in discrimination and reversal learning), but also to learn to inhibit responses to stimuli no longer associated with reward.

We further tested learned inhibition of responding by measuring the extinction of the response to a visual stimulus that mice had previously (pre-CIE) learned to respond to, to obtain reward (Fig. 3J). CIE mice took significantly fewer sessions than air-exposed controls to reduce responding to a  $\geq 77\%$  extinction criterion (survival analysis:  $\chi^2 = 3.94$ ,  $df = 1$ ,  $P < 0.05$ ,  $n = 10–11$ ) (Fig. 3K). Thus, CIE improved learned inhibition under extinction, suggesting that CIE might have enabled better predictions about the absence of reward. More generally, these behavioral results taken together demonstrate that the striatal neuroadaptations resulting from CIE are associated with improvements in learning across a range of diverse behavioral settings.

**CIE Augments Learning-Related DS Single-Unit Activity.** We next sought to more directly connect the behavioral effects of CIE to changes in the function of neurons in the DS. To this end, we conducted in vivo single-unit neuronal recordings as mice performed the reversal learning task. After being trained to discrimination criterion, mice had 16-channel multielectrode arrays implanted into the DS, with one row of 8 channels targeting the most lateral part of DS and another row targeting the more medial area (Fig. 4A), and were then exposed to CIE. As no differences were observed between learning-related activity recorded from the two rows, data were collapsed for analysis.

Mice began reversal training 3 d after CIE. To assess how single-unit activity changes as reversal learning progressed, recordings were made on three separate sessions, corresponding to the start (“early”), midpoint (“mid”), and end (“late”) stages of training, when percent correct performance was poor, at chance or at criterion, respectively. In this way, CIE-exposed mice and air controls were matched for learning performance at each recording stage (Fig. 4B). Yoking the groups in this manner allowed for comparison of single-unit activity, while avoiding confounding

effects of group differences in performance or elapsed time from CIE exposure (time since CIE: early training: air =  $4 \pm 1$  d, CIE =  $3 \pm 1$ , midtraining: air =  $12 \pm 1$ , CIE =  $11 \pm 1$ , late-training: air =  $26 \pm 3$ , CIE =  $23 \pm 2$ ).

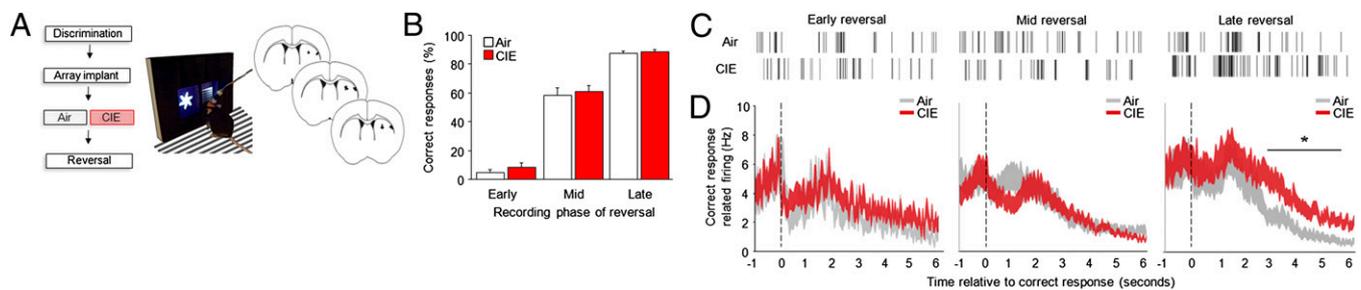
At each training stage, recordings were made from seven CIE mice and six air controls (79–108 neurons per group). Baseline firing rate did not differ between the CIE (CIE =  $4.4 \pm 0.3$  Hz) and air (air =  $4.3 \pm 0.3$  Hz) groups. To measure single-unit firing associated with learning, activity was aligned to touchscreen responses (1 s preresponse/6 s postresponse) and then segregated according to whether the response was correct or incorrect. Individual cells were categorized as event-related if there was a significant ( $t$  test) change in firing after a response was made, relative to preresponse baseline. The firing patterns in these units were broken out into the three learning stages and presented in perievent histograms (Fig. 4C).

Inspection of the histograms for all three stages revealed an increase in single-unit activity immediately before a correct response being made, followed by a rapid decrease and a second peak in activity  $\sim 2$  s postresponse. There was subsequently a steady decrease to below preresponse levels over the next 4 s. As learning progressed across stages, this decrease became more marked and the variability in activity lessened, suggesting greater coherence in activity within the network. The magnitude and dynamic profile of activity was essentially the same in CIE mice and air controls during the early stage (ANOVA effect of time:  $F_{139,12093} = 8.64$ ,  $P < 0.01$ ). On the midstage, there was a slight right-shifted delay in the postresponse peak in the CIE mice (ANOVA effect of time:  $F_{139,14317} = 26.16$ ,  $P < 0.01$ ). However, significant differences emerged on the late stage, with the CIE units showing sustained firing across the latter part of the epoch, relative to air controls, after mice made a correct response (ANOVA time  $\times$  CIE interaction:  $F_{139,11537} = 1.26$ ,  $P < 0.05$ ). Post hoc analysis of the data in 1-s blocks revealed significantly higher firing in CIE than air controls during the final 3 s of the postresponse epoch (Fig. 4D). This increased firing in CIE mice during late reversal did not reflect a general hyperexcitability of recorded units because analysis of unit activity after incorrect responses was not different between groups during the late (or early or mid) stage (Fig. S7).

These data provide an in vivo correlate of the improved late-stage reversal learning after CIE and support the notion that CIE facilitates this and other forms of learning by strengthening DLS encoding of behavior.

## Conclusions

The current findings show that chronic intermittent alcohol exposure causes a down-regulation of CB1R signaling and plasticity



**Fig. 4.** CIE augments learning-related DS single-unit activity. (A) Multielectrode arrays were implanted in DLS and a medial/DLS intermediate zone before CIE and reversal. (B) Recordings were made on early, mid, and late stages of reversal, matching CIE and air groups for percent correct performance. (C) Example raster plots during each state of reversal. (D) CIE produced a sustained increase in unit firing during late reversal. Data are means  $\pm$  SEM, \* $P < 0.05$  CIE vs. air.

(LTD) that may underpin DLS neuronal dendritic hypertrophy and contribute to the alcohol-induced facilitation and enhanced neuronal encoding of DLS-mediated behaviors. These data provide insight into how chronic alcohol can profoundly reshape processes in the DLS to drive changes in learning and the regulation of rewarded behavior.

In addition to an up-regulation of DS function, current models posit loss of PFC control over behavior in addiction (2, 4). The current study does not address parallel changes in PFC with CIE, and it remains possible that, rather than causing the degradation in cortical contribution to behavior, chronic ethanol strengthens functional cortical input to the DS. This account cannot be definitely excluded but seems a less likely scheme for various reasons. Previous studies have shown that CIE impairs PFC-dependent behavior (e.g., Pavlovian fear extinction) (10) and that lesioning the PFC facilitates DS-mediated learning in a manner very similar to that currently observed following CIE (47). In addition, the fact that synaptically induced activation of DS neurons after cortical stimulation was not increased, but rather partially decreased, argues against increased cortical drive with CIE. Further studies on the cortical changes caused by CIE will be valuable to help clarify these issues and determine the relative roles of PFC and DS in the behavioral changes produced by chronic alcohol.

There is growing evidence that drugs of abuse produce long-lasting changes in the function and plasticity of corticostriatal circuits (33, 54). In line with this view, the behavioral and neural alterations produced by CIE were apparent postacute withdrawal and in some cases were manifest for many weeks after CIE. As such, these data demonstrate how a history of chronic alcohol exposure causes persistent alterations that may maintain long-lasting shifts in the neural control over behavior. Similar changes could potentially contribute to the development of alcohol dependence in humans. Chronic psychostimulant exposure produces changes similar to those we observed after CIE, including the promotion of habitual, corticostriatal dendritic dysmorphology (27, 55) and, in the case of human abusers, striatal enlargement (56). Thus, neuroadaptations in the DS may reflect a general feature of chronic drug abuse and a key mechanism by which drugs catalyze the progression from abuse to addiction.

## Materials and Methods

For a description, see *SI Materials and Methods*.

**Subjects.** Subjects were male C57BL/6J mice (at least 8 wk of age at the beginning of experimentation) obtained from The Jackson Laboratory.

**CIE Exposure.** Chronic alcohol exposure was via a vapor inhalation procedure previously described for C57BL/6J mice (57, 58). Briefly, exposure lasted 16 h per day (in at 1700 hours, 2 h before start of the 12-h circadian dark phase, out at 0900 hours), followed by 8-h withdrawal. There were four consecutive days of intermittent exposure (Monday–Friday) and then a longer, 80-h

withdrawal (Friday–Monday). This complete weekly cycle was repeated two times. Air controls received an injection of 71.6 mg of pyrazole, to control for this treatment, before placement in dedicated air chambers (located adjacent to the EtOH chambers), which received vaporized air at the same exchange rate as the EtOH chambers.

**EtOH Drinking.** To confirm that our CIE procedure increased drinking (57, 59), we measured EtOH consumption using a 2-bottle choice paradigm (60).

**Neuronal Dendritic Morphology.** The dendritic morphology and spine density of neurons in DLS, basolateral amygdala (BLA), and orbitofrontal cortex using Glaser and Van der Loos' modified Golgi stain (61).

**Brain Slice Electrophysiology.** Extracellular field recordings were performed as previously described (38).

**CB1R Agonist-Stimulated [<sup>35</sup>S]GTP $\gamma$ S Binding.** The binding assay was performed based on previously described procedures (62).

**Endocannabinoid Levels.** Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) levels were quantified by liquid chromatography/tandem mass spectrometry, using multiple-reactions monitoring, as previously described (63), from tissue punches from DLS, BLA, PFC, and dorsal hippocampus.

**Discrimination and Reversal Learning.** Mice were trained to discriminate between 2 novel stimuli presented in a spatially pseudorandomized manner over 30-trial sessions, with the correct vs incorrect designation switched for reversal training (64, 65).

**Reversal Learning.** Three days after CIE, mice were trained on a reversal task in which the designation of correct and incorrect stimulus acquired on discrimination was reversed.

**Pavlovian Conditioned Approach and Omission Training.** Disappearance of a rectangle in one window coincided with delivery of a food reward in the magazine [conditioned stimulus (CS)+], whereas disappearance of a rectangle in the other window had no programmed consequences (CS−). Mice were tested for approaches to the CS+ (53). Omission training was identical to approach training, except that food was not delivered.

**Stimulus–Reward Extinction.** Mice first learned to make 30 correct responses to a white rectangle on the touchscreen within 12 min, 30 s. Extinction of the stimulus–response behavior was tested (66, 67).

**In Vivo Single-Unit Recordings.** Fixed 8  $\times$  2 arrays of microelectrodes (35  $\mu$ m diameter tungsten, 150  $\mu$ m electrode spacing within a row, 1,000  $\mu$ m spacing between the two rows) were implanted (lengthwise anteroposterior) targeting DS. Recordings were made during early (R1), mid (R2) and late reversal (R3).

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