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Permalink

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Journal

Translational Psychiatry, 15(1)

ISSN

2158-3188

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Publication Date

2025-10-01

DOI

10.1038/s41398-025-03650-7

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ARTICLE OPEN



Traumatic brain injury exacerbates alcohol consumption and neuroinflammation with decline in cognition and cholinergic activity

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Traumatic brain injury (TBI) is a global health challenge that substantially contributes to disability and is responsible for 30% of injury-related deaths. Annually, over 50 million TBIs occur worldwide, with many adult TBI patients in emergency departments presenting with alcohol in their system. TBI is also a known risk factor for alcohol abuse, yet its interaction with alcohol consumption remains poorly understood. In this study, we demonstrate that the fluid percussion injury (FPI) model of TBI in male C57BL/6 mice significantly increases alcohol consumption and impairs cognitive function. FPI markedly reduced the number and activity of striatal cholinergic interneurons (CINs) while increasing striatal microglial cells. Notably, depleting microglial cells by systemic PLX 5622 administration enhanced cholinergic activity, as measured by electrophysiology and acetylcholine biosensing. These findings suggest that TBI promotes alcohol consumption and impairs cognitive abilities through microglia activation and reduced cholinergic function. This research provides critical insight into the mechanisms linking TBI with increased alcohol use and cognitive deficits, potentially guiding future therapeutic strategies.

Translational Psychiatry (2025)15:403; <https://doi.org/10.1038/s41398-025-03650-7>

INTRODUCTION

Traumatic brain injury (TBI), a major cause of death and disability worldwide, impacts over 50 million people annually and accounts for approximately 30% of injury-related deaths [1, 2]. TBI often results in cognitive impairment, depression, and an increased risk of developing neuropsychiatric diseases, including Parkinson's and Alzheimer's diseases, and substance abuse disorders. Notably, alcohol consumption significantly raises TBI risk, with many patients intoxicated during injury [1–7]. This interplay between alcohol and TBI complicates recovery, as alcohol use can impede neurological healing, and TBI may increase alcohol and substance use [6, 8–13].

The fluid percussion injury (FPI) model of TBI increases alcohol consumption in rats, specifically in males [14–16]. FPI also induces neuroinflammation in the brain, including the striatum, potentially disrupting striatal circuits [17–22]. Concurrent alcohol exposure further exacerbates this pro-inflammatory state [23]. Microglia play a crucial role in regulating alcohol consumption, as PLX 5622-mediated microglial depletion has been shown to prevent increased alcohol intake [24]. Despite the profound impact of TBI on cognitive and addictive behaviors, the mechanisms remain poorly understood.

Medium spiny neurons (MSNs) in the dorsomedial striatum (DMS) and their cortical inputs play a key role in regulating alcohol-seeking behaviors and cognitive flexibility [25–27]. DMS cholinergic interneurons (CINs) make up less than 5% of the striatal neurons,

but they are crucial for cognition and substance use disorders [25, 28–34]. CINs modulate striatal activity and microglia function [35], the latter of which contributes to the neuroinflammatory response after TBI. Microglia activation and chronic neuroinflammation have also been linked to cholinergic dysfunction [36–39].

Thus, this study tested the hypothesis that FPI in mice increases alcohol intake, impairs striatal CIN function, and causes cognitive impairment. Additionally, we investigated whether microglial ablation could improve striatal cholinergic function. The results show that FPI increased alcohol preference and induced a hypo-cholinergic state in the striatum. Crucially, microglial depletion using PLX 5622 enhanced cholinergic activity, indicating that microglia could contribute to striatal cholinergic dysfunction following FPI. These results highlight the role of microglial mechanisms in FPI-induced striatal dysfunction and increased alcohol consumption. Furthermore, this study identifies the striatal cholinergic system as a potential therapeutic target to mitigate the neurological effects of TBI, including increased alcohol abuse and cognitive deficits.

RESULTS

FPI increases voluntary alcohol intake and preference

To investigate the effect of TBI on alcohol consumption, mice trained to consume alcohol using the intermittent-access two-

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Received: 1 March 2025 Revised: 7 August 2025 Accepted: 11 September 2025

Published online: 16 October 2025

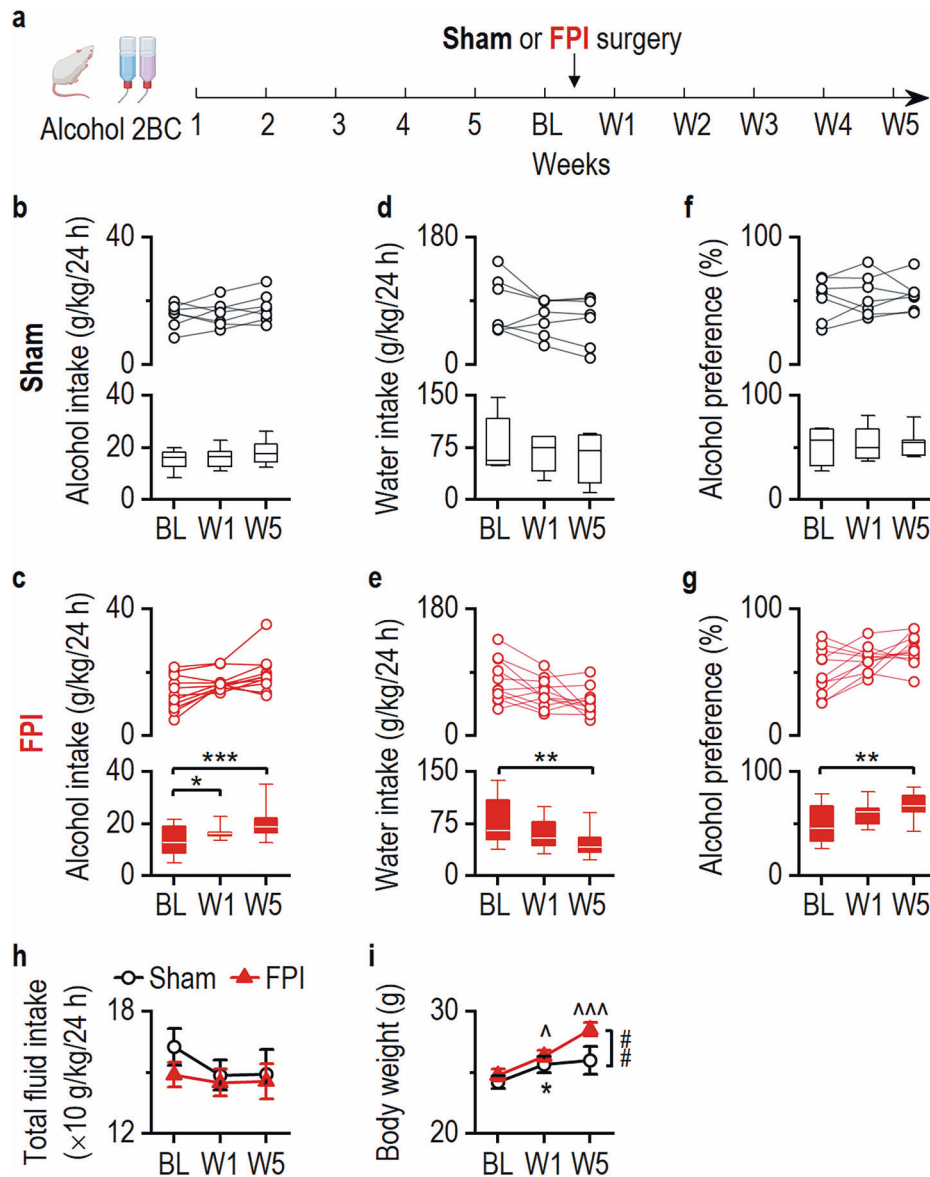


Fig. 1 FPI increases alcohol preference in male mice. **a** Wild-type male C57/BL6 mice were trained to consume 20% alcohol using the intermittent-access two-bottle choice drinking protocol for six weeks, followed by either FPI (red) or sham (black) surgery. **b** Alcohol intake did not change in the sham group during weeks 1 (W1) and 5 (W5) post-surgery. **c** FPI significantly heightened alcohol intake at W1 and W5 post-surgery. **d** Water intake remained stable post-surgery in the sham group. **e** A significant decline in water intake was observed at W1 and W5 post-surgery in the FPI group. **f** Alcohol preference remained unchanged in the sham group post-surgery. **g** FPI led to a marked increase in alcohol preference at W1 and W5 post-treatment. **h** Total fluid intake was consistent for both groups before and after their respective surgeries. **i** FPI, but not sham surgery, led to an increase in body weight at W5 post-surgery. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $\wedge p < 0.05$, $\wedge\wedge\wedge p < 0.001$ versus baseline (BL) in the same group; $\#\# p < 0.01$ versus sham. One-way RM ANOVA followed by Tukey post-hoc test (b–g), Two-way RM ANOVA followed by Tukey post-hoc test (h, i).

bottle choice drinking procedure received either sham or FPI. After surgery, mice resumed alcohol consumption (Fig. 1a). The sham group exhibited no significant change in alcohol intake one week (W1) or five weeks (W5) after the procedure (Fig. 1b; $F_{(2,12)} = 1.51$; $q = 0.58$, $p > 0.05$ (W1); $q = 2.35$, $p > 0.05$ (W5)). However, the FPI group increased their alcohol consumption both at W1 and W5 post-FPI (Fig. 1c; $F_{(2,20)} = 11.25$; $q = 3.72$, $p < 0.05$ (W1); $q = 6.69$, $p < 0.001$ (W5)), indicating that FPI significantly enhanced voluntary alcohol intake.

Water intake did not change in the sham group during W1 and W5 post-surgery (Fig. 1d; $F_{(2,12)} = 2.53$, $p > 0.05$). However, it was reduced in the FPI group at W5 post-FPI compared to baseline (Fig. 1e; $F_{(2,20)} = 6.75$, $p < 0.01$ (W5)), suggesting a shift in fluid preference towards alcohol. Indeed, while alcohol preference did

not change for the sham group post-surgery (Fig. 1f; $F_{(2,12)} = 0.19$, $p > 0.05$), the FPI group exhibited an increase in alcohol preference at W5 post-FPI relative to baseline (Fig. 1g; $F_{(2,20)} = 6.37$, $p < 0.01$ (W5)). Total fluid intake (alcohol and water combined) remained comparable between groups (Fig. 1h; $F_{(1,16)} = 0.51$, $p > 0.05$) and throughout the study period (Fig. 1h; $F_{(2,32)} = 1.48$, $p > 0.05$).

The sham group exhibited a modest increase in body weight at W1 post-surgery (Fig. 1i; $q = 3.59$, $p < 0.05$). However, the increase in body weight was more robust in the FPI group both at W1 and W5 post-injury (Fig. 1i; $q = 3.99$, $p < 0.05$ (W1 versus BL); $q = 9.51$, $p < 0.001$ (W5 versus BL); $q = 5.52$, $p < 0.01$ (W5 versus W1)), with higher body weights observed in the FPI group at W5 as compared to the sham group (Fig. 1i; $q = 3.91$, $p < 0.01$ (W5)). This weight gain in the FPI group, concomitant with increased alcohol preference, aligns with

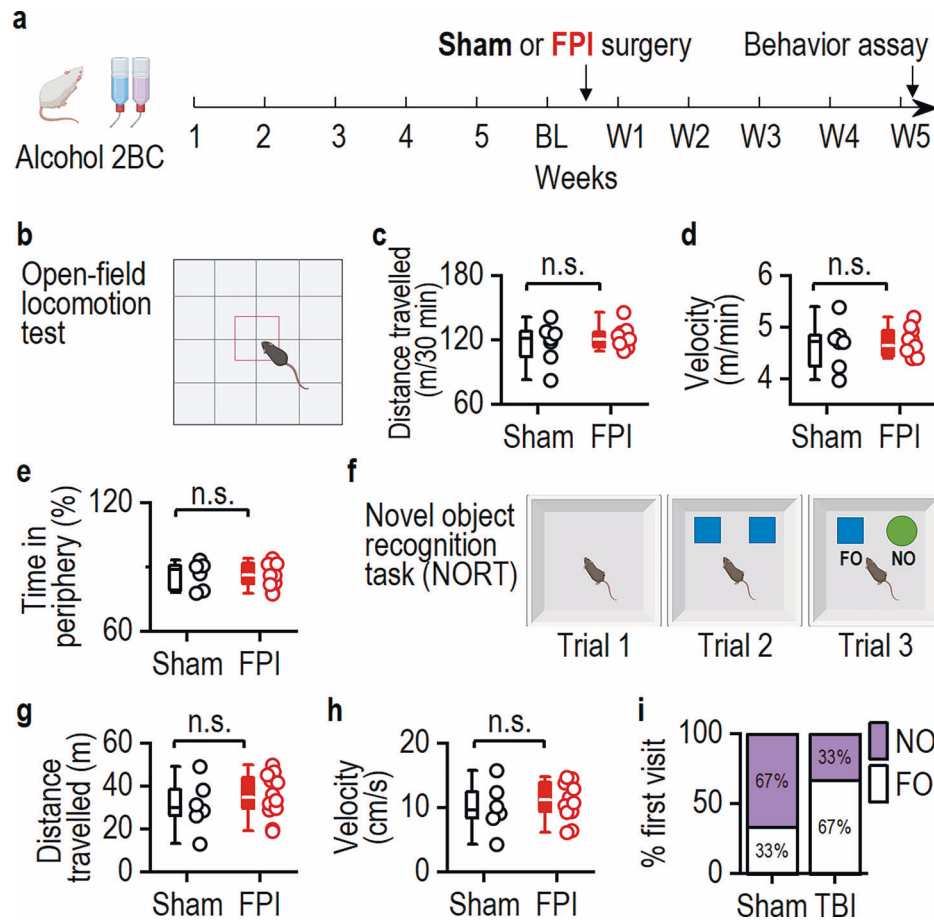


Fig. 2 FPI causes cognitive but not motor deficits in mice with an alcohol-drinking history. **a** Experimental timeline. **b–e** No significant changes were observed in total distance traveled (**c**), movement velocity (**d**), or time spent in the periphery of the open field chamber (**e**) after FPI. **f–h** In the NORT, FPI did not affect total distance traveled (**g**) or velocity of movement (**h**). **i** The percent of FPI-treated mice that visit the novel object first was less than that of sham controls, indicating a cognitive deficit. n.s. (not significant; **c, d, e, g, h**). * $p < 0.05$ (**i**). unpaired t-test (**c, d, e, g, h, i**).

studies showing weight gain following TBI [40–43]. The increase in body weight could be attributed to (a) higher ethanol calorific value; (b) increase in food consumption; (c) reduced home cage physical activity; (d) increased fat storage via changes in hormonal regulation, metabolic pathways, or gut microbiota.

FPI causes cognitive but not motor impairment

Given that TBI and chronic alcohol intake both affect cognitive function [25, 44], the combined effect of FPI and alcohol intake on locomotion, exploration, and cognitive function was assessed using an open-field and novel object recognition task (NORT) at five weeks post-surgery (Fig. 2a). The open-field test revealed no differences in total distance traveled (Fig. 2b, c; $t_{(16)} = -0.60$; $p > 0.05$), locomotion velocity (Fig. 2d; $t_{(16)} = -0.18$; $p > 0.05$), or time spent in the periphery of the arena (Fig. 2e; $t_{(16)} = 0.13$; $p > 0.05$).

In the NORT (Fig. 2f), which measures reference memory, no differences in total distance traveled (Fig. 2g; $t_{(16)} = 0.8103$; $p > 0.05$) or velocity (Fig. 2h; $t_{(16)} = 0.7093$; $p > 0.05$) were observed between groups. Sham mice, but not the FPI mice, preferentially visited the novel object first, indicating an impairment in the FPI mice (Fig. 2i; $p < 0.05$). Therefore, FPI and alcohol exposure did not affect motor performance at this time point but caused cognitive impairment in the NORT.

FPI reduces cholinergic activity in the DMS

Striatal CINs release acetylcholine, which mediates cognitive flexibility, and attention [28, 29]. Importantly, striatal acetylcholine

also regulates addictive behaviors, including alcohol consumption [25, 29–32]. Given the association between cognition and CIN activity, we investigated the effect of FPI on CINs using three groups of ChAT-eGFP mice: naïve, FPI, and a group with both alcohol consumption and FPI treatment. Five weeks post-FPI, total striatal CINs were counted. The FPI-only and the alcohol+FPI groups exhibited fewer striatal CINs per slice compared to the naïve controls (Fig. 3a, b; $t_{(1, 44)} = -2.98$, $p < 0.01$ (FPI); $t_{(1, 48)} = -2.21$, $p < 0.05$ (alcohol+FPI); Supplementary Fig. 1a, b). There was no difference in CIN counts between the FPI-only and the alcohol+FPI groups (Fig. 3b; $t_{(1, 42)} = -1.09$, $p > 0.05$), suggesting that FPI leads to a reduction in total striatal CINs, which is not significantly exacerbated by concurrent alcohol exposure.

The activity of DMS CINs was assessed using ChAT-eGFP mice that received either FPI or sham surgery. One week post-surgery, cell-attached recordings showed that the firing frequency of DMS CINs was reduced in the FPI group compared to sham controls (Fig. 3c; $t_{(28)} = 2.44$, $p < 0.05$). Additionally, current-clamp recordings revealed a trend towards fewer evoked action potentials in CINs in the FPI group (Fig. 3d; $F_{(1, 43)} = 3.43$, $p = 0.07$). No difference in resting membrane potential was observed between groups (Fig. 3e; $t_{(43)} = -0.59$, $p > 0.05$). Notably, FPI led to an increase in the characteristic sag response in DMS CINs, indicative of changes in the hyperpolarization-induced cation current, I_h (Fig. 3f; $t_{(36)} = -4.18$, $p < 0.001$). These results suggest that FPI reduces cholinergic activity in the DMS, impacting CIN function.

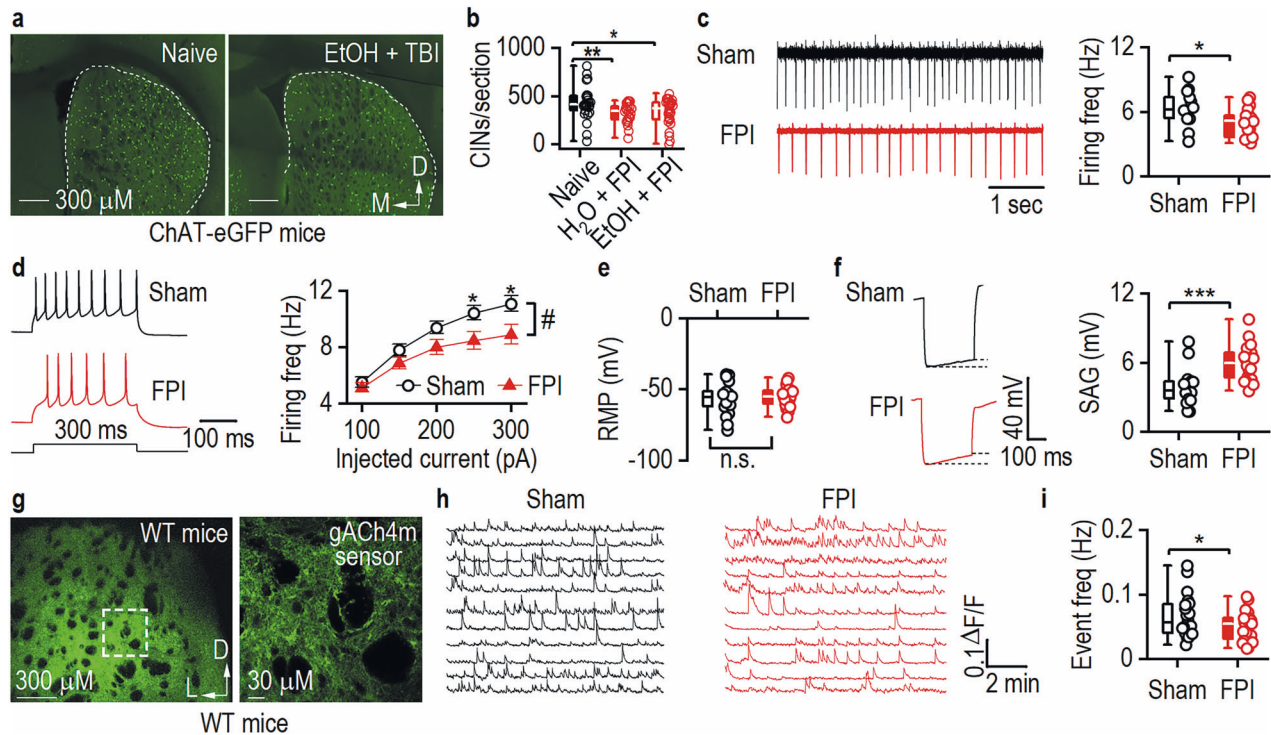


Fig. 3 FPI suppresses DMS cholinergic activity. **a** Confocal micrographs showing striatal sections from the EtOH+FPI and naive groups, with CINs in green. Bilateral striatal sections were prepared 5 weeks post-FPI. D, dorsal; M, medial. **b** FPI significantly decreased the number of CINs per slice in both water and alcohol-exposed groups compared to naive controls. **c** FPI reduced the spontaneous firing rates of DMS CINs. Bilateral DMS slices were prepared 1-week post-FPI. **d** FPI reduced the number of evoked action potentials in DMS CINs. **e** FPI did not significantly alter the resting membrane potential of DMS CINs. **f** FPI significantly increased the SAG in DMS CINs. **g** Confocal micrographs show acetylcholine sensor expression in the striatum of wild-type mice. D, dorsal; L, lateral. **h–i** FPI reduced the frequency of acetylcholine events in the dorsomedial striatum. n.s. (not significant; e), $^{\#}p = 0.07$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Two-way RM ANOVA followed by Tukey post-hoc test (**d**), unpaired t-test (**b**, **c**, **e**, **f**, **i**).

Given that FPI reduces CIN activity, which is a key driver of striatal acetylcholine release, we next examined whether FPI could also affect DMS acetylcholine release. An adeno-associated virus (AAV) encoding a green acetylcholine sensor (gACh4m) was infused in the DMS of WT mice (Fig. 3g). Two weeks after infusion, mice underwent either FPI or sham surgeries. After one week, acetylcholine release in the DMS was assessed using live tissue confocal recordings (Fig. 3h). A reduction in the frequency of spontaneous ACh events in the FPI group was observed compared to sham controls (Fig. 3i; $t_{(45)} = 2.099$, $p < 0.05$). These findings suggest that FPI reduces DMS cholinergic activity, which may contribute to the cognitive deficits following FPI.

No between-group differences were observed in AMPA-induced currents in CINs (Supplementary Fig. 1c; $t_{(16)} = -0.73$, $p > 0.05$). Similarly, FPI did not affect the frequency (Supplementary Fig. 1d; $t_{(26)} = 1.37$, $p > 0.05$) or amplitude (Supplementary Fig. 1e; $t_{(26)} = -0.29$, $p > 0.05$) of spontaneous inhibitory postsynaptic currents (sIPSCs) in CINs. Additionally, there were no differences in the frequency (Supplementary Fig. 1f; $t_{(21)} = -0.42$, $p > 0.05$) or amplitude (Supplementary Fig. 1g; $t_{(21)} = -0.62$, $p > 0.05$) of spontaneous excitatory postsynaptic currents (sEPSCs). The lack of changes in sEPSCs or sIPSCs suggests FPI may not affect glutamatergic or GABAergic transmission in the CINs.

FPI increases chronic microglial activation, but not astrocyte activation in the ipsilateral and contralateral striatum

Increased microglial activation in the ipsilateral cortex has been previously observed following FPI [17]. This initial microglial response is subsequently followed by broader bilateral microglial activation, including in the striatum [17, 19, 45–47]. Given the

critical role of microglia in neuroinflammation following TBI or alcohol exposure [48], we investigated how TBI influences microglial activity in the striatum of alcohol-drinking mice. Iba-1-labeled microglial cells in the striatum were analyzed at W5 following either sham or FPI (Fig. 4a, b).

Quantitative microglial analysis in the dorsal striatum of the ipsilateral hemisphere revealed an increase in microglial optical density in FPI mice as compared to sham controls (Fig. 4c; $F_{(1,66)} = 23.70$, $p < 0.001$). Post-hoc Tukey test identified elevations in optical density in all medial subregions of the ipsilateral striatum in the FPI mice (Fig. 4c; dorsal medial, $q = 3.58$, $p < 0.05$; central medial, $q = 3.08$, $p < 0.05$; ventral medial, $q = 5.18$, $p < 0.001$). These elevations were not observed in the lateral subregions of the ipsilateral striatum (Fig. 4c; dorsal lateral, $q = 1.76$, $p = 0.22$; central lateral, $q = 1.55$, $p = 0.28$; ventral lateral, $q = 1.72$, $p = 0.23$), suggesting more prominent changes in the medial compared to the lateral subregions. Microglial optical density in the entire ipsilateral DMS was elevated in the FPI-treated group, as compared to sham controls (Fig. 4d; $t_{(11)} = -2.38$, $p < 0.05$). Notably, more ipsilateral microglial cells were observed in the FPI mice (Fig. 4e; $t_{(11)} = -2.22$, $p < 0.05$).

In the contralateral hemisphere, microglial optical density was elevated after FPI in all striatal subregions analyzed (Fig. 4f; $F_{(1,66)} = 37.40$, $p < 0.001$; dorsal medial, $q = 3.29$, $p < 0.05$; dorsal lateral, $q = 3.35$, $p < 0.05$; central medial, $q = 3.13$, $p < 0.05$; central lateral, $q = 4.34$, $p < 0.01$; ventral medial, $q = 4.16$, $p < 0.01$; ventral lateral, $q = 2.92$, $p < 0.05$). Overall, in the total contralateral DMS, microglial optical intensity was higher in the FPI group, as compared to controls (Fig. 4g; $t_{(11)} = -2.89$, $p < 0.05$). A trend towards an increase in the number of microglial cells in the contralateral striatum after FPI was also noted (Fig. 4h;

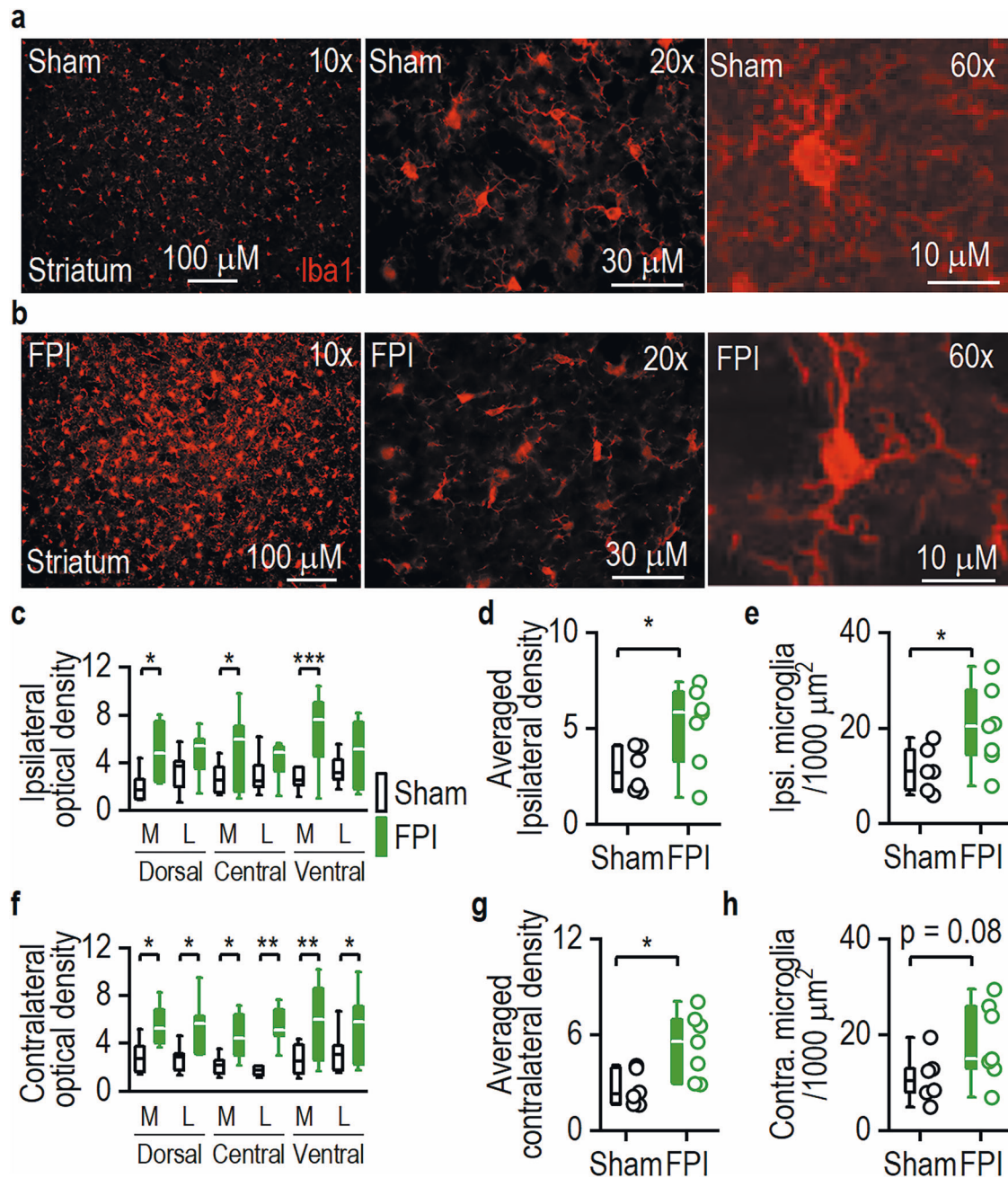


Fig. 4 FPI increases microglial levels in the striatum. **a, b** Confocal micrographs display Iba-1-stained coronal striatum sections from sham plus alcohol (top, **a**) and FPI plus alcohol (bottom, **b**) groups. **c–e** In the ipsilateral striatum, FPI significantly increased the microglial optical density in the medial regions (**c**) and across the entire ipsilateral DMS (**d**). Additionally, there was an increase in the total number of microglial cells on the ipsilateral side (**e**). **f–h** In the contralateral striatum, FPI significantly enhanced the optical density across all subregions (**f**) and in the entirety of the contralateral DMS (**g**). There was a trend towards an increase in the number of microglial cells in the contralateral striatum (**h**). Iba-1, Ionized calcium-binding adaptor molecule 1; M, Medial; L, Lateral. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Two-way RM ANOVA followed by Tukey post-hoc test (**c, f**) unpaired t-test (**d, e, g, h**).

$t_{(11)} = -1.91$, $p = 0.08$). Collectively, these results are consistent with a previous study that showed increased bilateral microglial activation at 30 days post-FPI [17].

Astrocytes in the basolateral amygdala have been shown to regulate alcohol consumption after FPI [15, 49]. To assess whether FPI affects striatal astrocytes in alcohol-drinking mice, GFAP-labeled astrocytes were analyzed (Fig. 5a, d). No differences in astrocyte optical density were observed in the subregions of the ipsilateral striatum between the FPI and sham groups (Fig. 5b;

$F_{(1,66)} = 1.52$, $p > 0.05$) and within the overall ipsilateral DMS (Fig. 5c; $t_{(11)} = -0.72$, $p > 0.05$). Similarly, no differences in astrocyte optical density between the FPI and sham groups were observed in the subregions of the contralateral striatum (Fig. 5e; $F_{(1,66)} = 0.87$, $p > 0.05$), or in the overall contralateral DMS (Fig. 5f; $t_{(11)} = 0.01$, $p > 0.05$). Although quantitative analysis of the number of astrocytes was not performed, these findings suggest that FPI does not affect chronic astrocyte activation in the striatum of alcohol-drinking mice.

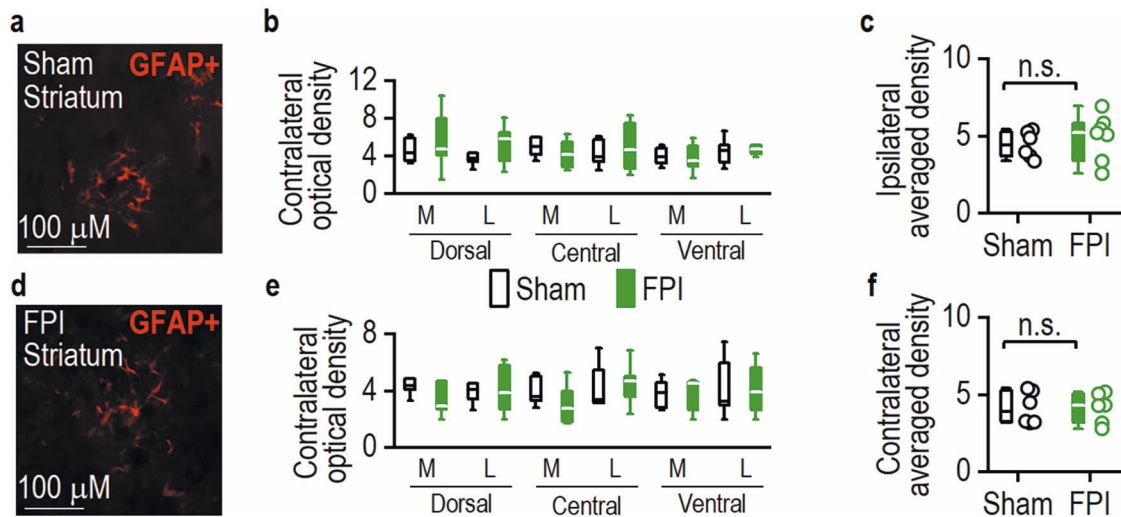


Fig. 5 FPI does not alter astrocytes in the striatum. **a** Confocal micrographs show GFAP-stained coronal striatum sections from the sham plus alcohol group. **b–c** In the ipsilateral striatum, FPI did not significantly alter astrocyte optical densities in striatal subregions (**b**) or the average density of astrocytes in the entire ipsilateral DMS (**c**). **d** Confocal micrographs of GFAP-stained coronal striatum sections from the FPI plus alcohol group. **e–f** In the contralateral striatum, FPI did not significantly alter astrocyte optical densities in striatal subregions (**e**) or the average density of astrocytes in the entire contralateral DMS (**f**). n.s. (not significant; **c, f**). Two-way RM ANOVA followed by Tukey post-hoc test (**b, e**), unpaired t-test (**c, f**).

PLX 5622-induced microglia depletion increases striatal acetylcholine

Microglial depletion has been shown to mitigate brain damage following TBI by reducing neuronal loss and reversing TBI-related inflammation [20, 50]. Additionally, microglial cells are in close proximity to cholinergic projection neurons in the basal forebrain [51], suggesting a potential modulatory role on cholinergic function. We investigated whether microglial depletion influences cholinergic function in the striatum. We observed microglial cells in close proximity to CINs in the DMS (Supplementary Fig. 2a). Given that FPI combined with alcohol consumption increased striatal microglia and decreased acetylcholine activity, we hypothesized that microglial activation modulates CIN activity. To test this, PLX 5622 was administered twice daily for 1 week in naïve C57BL/6 mice, whereas controls received saline (Fig. 6a). Iba-1 staining confirmed a reduction in microglia in the brain (Fig. 6b), with a 60% decrease in striatal microglia observed after PLX treatment (Fig. 6c; $t_{(22)} = 6.506$, $p < 0.001$).

Subsequently, striatal CIN activity was assessed in ChAT-eGFP mice treated with either PLX 5622 or saline. Slice recordings from DMS CINs, conducted a day after the final PLX 5622 treatment, revealed that the spontaneous firing frequency was higher in the PLX group than in saline controls (Fig. 6d; $t_{(28)} = 4.184$, $p < 0.001$). Similarly, current injection evoked more action potentials in striatal CINs of the PLX group than those from the saline controls (Fig. 6e; $F_{(1,20)} = 25.36$, $p < 0.001$). Measurement of baseline striatal ACh activity using an ACh sensor one day following the last PLX 5622 treatment revealed a significantly higher frequency of spontaneous ACh events in the PLX-treated mice as compared to saline controls (Fig. 6f–h; $t_{(187)} = -2.521$, $p < 0.05$). However, no differences were observed in amplitude or half-width of these events (Amplitude: Supplementary Fig. 2b, $t_{(16)} = -1.413$, $p > 0.05$; Half-width: Supplementary Fig. 2c, $t_{(16)} = 0.0597$, $p > 0.05$).

Since basal forebrain cholinergic projection neurons (CPNs) also regulate cognition, we recorded their activity after PLX-induced microglia depletion in ChAT-eGFP mice. Systemic PLX 5622 administration led to reduced microglia density in the basal forebrain (Supplementary Fig. 3a, $t_{(16)} = 4.089$, $p < 0.001$). However, no differences in intrinsic excitability of CPNs were observed between PLX-administered mice and saline controls (Supplementary Fig. 3b, $F_{(1,29)} = 0.8879$, $p = 0.3538$). Additionally, no

differences in electrically evoked IPSCs and paired-pulse ratio of eIPSCs were observed between groups (eIPSCs: Supplementary Fig. 3c, $F_{(1,19)} = 0.0005$, $p = 0.9821$; PPR: Supplementary Fig. 3d, $F_{(1,29)} = 0.5439$, $p = 0.4667$).

These results suggest that microglia depletion enhances CIN activity and acetylcholine release in the striatum, supporting the hypothesis that microglial activation after FPI contributes to the injury-induced deficits in CIN activity.

DISCUSSION

In this study, we found that FPI increased alcohol preference in mice and reduced cognitive function. FPI reduced spontaneous and evoked striatal CIN activity and ACh release. FPI also resulted in chronic microglia but not astrocytic elevation in the striatum of alcohol-drinking mice. Interestingly, microglia depletion enhanced CIN activity. These results align with preclinical and clinical studies that report increased alcohol consumption following TBI [52–55]. Given the association between TBI and heightened risk of alcohol use disorder, these findings in the striatum post-FPI are highly significant.

Neuroinflammation, specifically the activity of microglial cells, plays a crucial role in increasing alcohol consumption [54]. Supporting this notion, minocycline administration, which targets microglial activation, prevented the rise in alcohol consumption induced by a mild closed-head FPI in juvenile mice [54]. Additionally, direct alcohol injection into the ventral striatum triggers minocycline-dependent microglial activation [54]. In rat models of lateral FPI combined with post-injury alcohol consumption, neuroinflammation was observed in the peri-injury cortex at 2–3 weeks post-FPI, correlating with increased alcohol intake and cognitive deficits [8, 15].

A closed-head TBI model demonstrated increased astrogliosis as early as 72 h post-injury, persisting for at least 7 days, including in the nucleus accumbens. This was linked to reduced alcohol consumption immediately after injury [16]. Conversely, a series of studies have demonstrated a role for astrocyte activation in the basolateral amygdala (BLA) of rats following FPI. The BLA regulates reward-seeking behavior, including drug and alcohol seeking behaviors [56–59]. The lack of striatal astrocyte activation in our study may be due to examining astrocytes at a more chronic post-

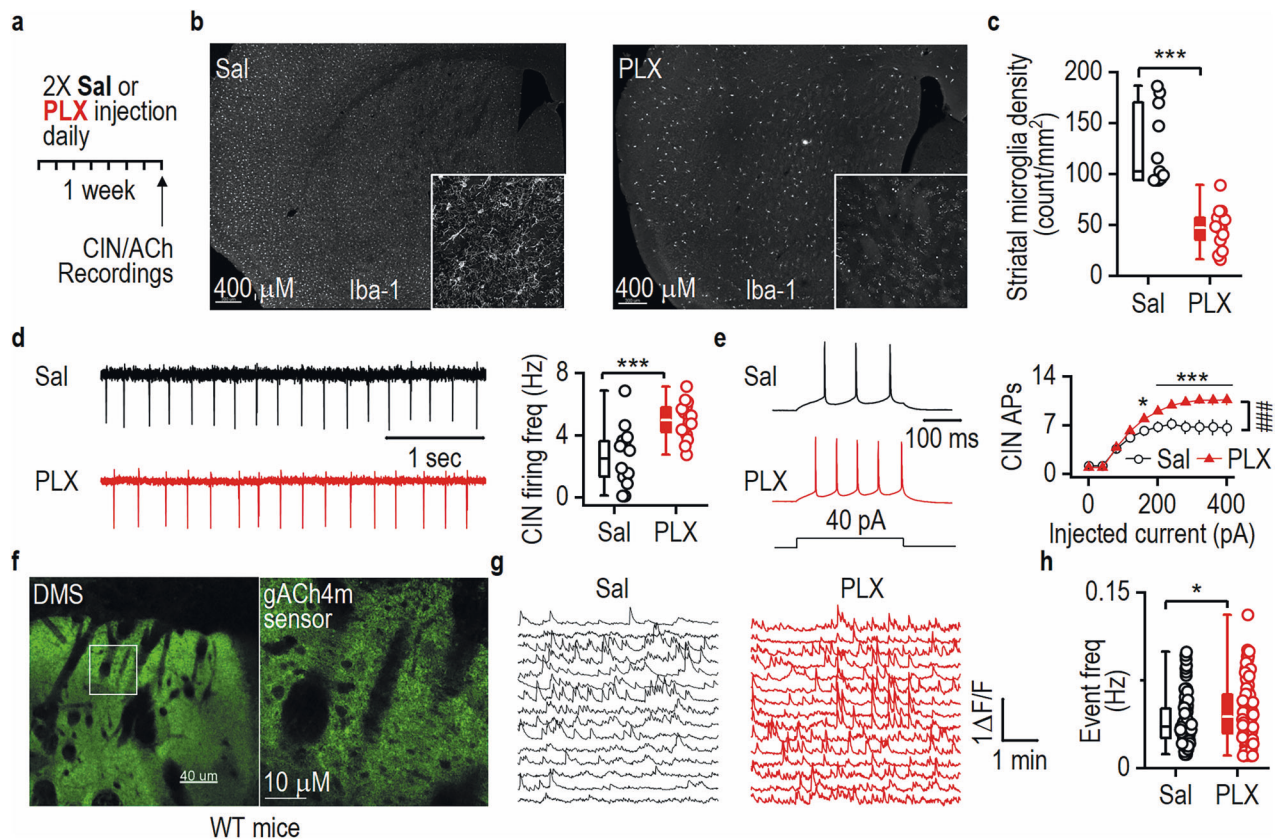


Fig. 6 Microglia depletion increases striatal acetylcholine activity. **a** Mice received injections of either PLX 5622 (50 mg/kg) or saline twice daily for one week. Electrophysiology and confocal recordings were performed one day after the last PLX injection. **b** Confocal micrographs illustrate the PLX-induced reduction in microglial cells; the right bottom box shows a magnified image from the striatum. **c** Summarized data showing microglia depletion after PLX 5622 administration. **d** PLX administration led to an increase in the spontaneous firing frequency of DMS CINs. **e** PLX treatment increased the number of current-evoked action potentials in DMS CINs. **f** An acetylcholine sensor (gACh4m) was expressed in the striatum of wild-type mice. **g–h** Live-tissue confocal recordings of striatal acetylcholine (**g**) revealed increased baseline acetylcholine events in PLX-treated mice compared to saline controls (**h**). * $p < 0.05$, *** $p < 0.001$, *** $p < 0.001$. Two-way RM ANOVA followed by Tukey post-hoc test (**e**), unpaired t-test (**c**, **d**, **h**, **j**, **k**).

TBI time point than in the studies mentioned above. While the possibility cannot be ruled out that earlier astrocytic changes may have contributed to some of the observed findings in the current study, the fact that PLX-induced microglial depletion improved CIN function supports a role for microglial cells in DMS dysfunction.

Most of the cortical and subcortical glia return to their resting appearance by 1 month after the injury [17]. However, alcohol seems to have exacerbated the microglial activation in the striatum, suggesting it may contribute to striatal dysfunction after TBI, possibly influencing the increased alcohol consumption in clinical populations [60]. PLX-induced microglial depletion in naïve mice is not specific to the striatum. However, it enhanced striatal CIN activity. The selective effect on striatal but not BF CPNs likely relates to the proximity of the striatum to the cortex, and the relatively fewer cortical inputs to BF CPNs. Retrograde rabies tracing studies support this interpretation, showing that BF CPNs receive fewer cortical inputs [61–63], whereas striatal CINs receive significant cortical inputs [64]. These differences in cortical connectivity may explain the region-specific impact of FPI on cholinergic function. Future studies should selectively deplete microglia following FPI, alcohol, and following FPI and alcohol, to fully elucidate the singular and collective influence on the role of microglial cells and striatal CINs.

The deficits noted in CINs post-FPI may underpin the cognitive impairments observed in the NORT. NORT depends on the functionality of multiple brain regions, including the

hippocampus, perirhinal cortex, and the mPFC, and is sensitive to disruptions in object recognition linked to the striatum [65–71]. In schizophrenia rodent models, VGLUT1, expressed across the medial prefrontal cortex (mPFC), striatum, and hippocampus, mediates object recognition deficits [72]. Moreover, altered corticostriatal communication underlies recognition deficits, characterized by reduced activity in the nucleus accumbens (NAc) and its desynchronization with the mPFC [73]. It is possible that FPI's effect on other brain regions could account for the behavioral outcomes observed in the current study, including those in the hippocampus, neocortex, and amygdala [8, 14, 15, 74]. Future studies should consider examining the changes in regional connectivity resulting from alcohol consumption and TBI.

NORT could also be linked to disrupted cholinergic signaling, which supports object recognition [75]. Striatal CINs are crucial for various cognitive processes [75–77] and particularly important for the acquisition of object recognition memory [78]. Thus, the FPI-induced changes in CINs likely underpin the cognitive impairments we identified. This possibility is supported by the fact that the PLX treatment improved cholinergic signaling in the striatum.

This investigation highlights connections between TBI, alcohol consumption, striatal microglia, and CIN activity. Chronic alcohol exposure selectively enhances DMS glutamatergic inputs, facilitating addictive behaviors [26, 79–81]. Elevated striatal microglia and reduced CIN activity underscore how brain inflammation might contribute to enhanced alcohol preference and cognitive

dysfunction following FPI. However, our study does not exclude the potential involvement of other contributing cellular mechanisms. For example, nigrostriatal plasticity, also observed after striatal stroke, has been implicated in elevated alcohol intake [82]. Future studies will investigate microglia-specific mechanisms in the striatum that are altered after FPI to identify molecular signatures of neuroinflammation linked to increased alcohol consumption and cognitive dysfunction. Rather than depleting microglia pharmacologically, we will use region-specific and pathway-targeted approaches to modulate microglial activity and assess their role in the TBI-induced increase in alcohol consumption.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice were group-housed at the Texas A&M University Health Science Center animal facility, maintained in a temperature-controlled (72 degrees Fahrenheit) and humidity-controlled (54 percent) environment. Post sham/FPI treatment, mice were single-housed. Male mice were used because TBI incidence in young adults is prominent in males, and previous studies do not report increased alcohol consumption after FPI in female rats [14]. Separate mouse cohorts were used to quantify CINs and microglia. Microglia depletion was performed in naïve mice. Sample size: Fig. 1: $n = 7$ (Sham), 11 (FPI); Fig. 2: $n = 6$ (Sham), 12 (FPI); Fig. 3: $n = 26$ (slices) and 3 (mice) (26/3, b, Naïve), 24/4 (b, H₂O + FPI), 24/3 (b, EtOH + FPI), $n = 13$ (neurons) and 3 (mice) (13/3, c, sham), 17/4 (c, FPI), 17/3 (d, e, f, sham), 28/4 (d, e, FPI), 21/3 (f, FPI), $n = 22$ (slices) and 4 mice (22/4, i, sham), 25/5 (i, FPI); Fig. 4: $n = 6$ (Sham), 7 (FPI); Fig. 5: $n = 6$ (Sham), 7 (FPI); Fig. 6: $n = 9$ slices from 3 mice (9/3, c, PLX, Sal), $n = 14$ (neurons) and 4 (mice) (14/4, d, e, Sal), 16/4 (d, e, PLX), $n = 91$ (ROIs) from 4 (mice) (91/4, h, Sal), 98/4 (h, PLX), $n = 4$ mice (j, k; Sal, PLX).

Intermittent-access to 20% alcohol 2-bottle-choice drinking procedure

Mice were trained to consume 20% alcohol for six weeks, following a method previously described [25, 26, 29, 83–87]. Alcohol consumption was monitored before mice were separated into FPI and sham groups, ensuring equal distribution of drinking tendency between groups. After sham or FPI induction, alcohol access continued for an additional five weeks. Behavioral testing was performed over the following week. Alcohol preference: “EtOH solution” consumed (gm) × 100 / (“EtOH solution” consumed (gm) + Water consumed (gm)). Alcohol preference was computed on alternate days and then averaged for a week. Alcohol intake (in g/kg/24 h) is calculated from 20% of the EtOH solution consumed (since we used a diluted 20% EtOH solution), whereas the water intake value (in g/kg/24 h) is calculated from 100% of the water consumed.

Fluid percussion injury

Mice were subjected to lateral FPI as previously described [17, 88, 89]. Briefly, mice underwent a sterile craniotomy over the left parietal cortex. The injury involved a 12–16 ms pressure pulse delivered at ~1.5–1.8 atm from the FPI apparatus (Custom Design & Fabrication, Richmond, VA). Following injury, the craniotomy is allowed to heal openly, and the incision sites were closed with sterile sutures. Sham mice were connected to the FPI apparatus without receiving an injury.

Novel object recognition test (NORT)

NORT assesses memory and exploratory behavior in rodents [66]. The test included three successive trials with a 1-h inter-trial interval (ITI). Each trial was conducted in a white Plexiglas open-field box (60 × 45 cm). First trial: Mice were allowed to explore the empty box for 1 h. Second trial: Two similar objects were placed in the open field box. Third trial: one object from the training trial was replaced with a novel object, while the other object (familiar object) remained. All trials were videotaped for automatic and manual scoring. Locomotor activity in the first trial was assessed using a Kinder Scientific SmartFrame Open Field System. Object exploration was scored based on whether the mouse's nose contacted an object.

Tissue processing and histology

Mice were euthanized via transcardial perfusion as previously described [29, 74, 89–91]. Following perfusion and a 24-h post-fixation period *in situ*, brains were extracted, post-fixed for an additional 24–48 h in 4% paraformaldehyde (PFA), and subsequently transferred to phosphate-buffered saline (PBS). Brains were sectioned in the coronal plane using a Vibratome (LEICA VT 1200) at 50-μm intervals. Serial sections were collected sequentially in 12-well plates and stored at 4 °C in a 0.01% PBS solution. Astrocytes were stained using an anti-GFAP-Cy3 conjugated antibody (1:500; Sigma #C9205), and microglia were labeled with primary anti-Iba-1 (1:400; WAKO labs #019-19741) and secondary goat anti-rabbit IgG (1:200; Invitrogen #A21428). After staining, sections were mounted onto glass slides, covered with VECTASHIELD (Vector Labs, Burlingame, CA #H140010), and sealed with nail polish to secure the coverslips. Striatal slices were imaged using confocal microscopy.

Images focusing on the DMS (bregma 1.42 to −0.10 mm) [92] were analyzed using a stereology-based probe for quantitative analysis. Within the boxes randomly selected for counting, cells were counted if more than 60% of their perikaryon was in the plane of focus. The study included a total of 7 FPI and 6 sham animals, with quantifications coming from 3–4 slices per animal containing the DMS (note: only 1 animal had 2 slices), and the results were averaged for each animal. All analyses were performed with the rater blinded to the condition of the mice to minimize bias. In addition to quantitative stereological analysis, semi-quantitative densitometry analysis of GFAP and Iba-1+ cells was performed using Image J software (ImageJ).

For quantifying striatal CINs, striatal sections from ChAT-eGFP mice (naïve, H₂O + FPI, EtOH + FPI) were imaged using a confocal laser-scanning microscope (Fluoroview-1200, Olympus). Image processing was performed using Imaris 8.3.1 (Bitplane, Zurich, Switzerland). All GFP+ cells in the striatum from bregma 2 to −2.25 mm were manually counted for all groups using Imaris 8.3.1. The counts were then compiled based on the three groups and statistically analyzed.

Electrophysiology

Electrophysiological recordings were conducted as previously described [25, 27, 29, 85, 87, 93–96]. Specifically, DMS coronal sections (thickness: 250 μm) were prepared at a speed of 0.14 mm/s in an ice-cold cutting solution containing (in mM) 40 NaCl, 148.5 sucrose, 4.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 0.5 CaCl₂, 7 MgSO₄, 10 dextrose, 1 sodium ascorbate, 3 myo inositol, and 3 sodium pyruvate, continuously oxygenated with 95% O₂ and 5% CO₂. After cutting, slices were incubated for 45 min in a 1:1 mixture of cutting and external solution containing (in mM) 125 NaCl, 4.5 KCl, 2.5 CaCl₂, 1.3 MgSO₄, 1.25 NaH₂PO₄, 25 NaHCO₃, 15 sucrose and 15 glucose, and saturated with 95% O₂ and 5% CO₂.

The potassium-based intracellular solution was used for all cell-attached and current-clamp recordings in Figs. 3c–f and 6d–e; this contained (in mM) 123 potassium gluconate, 10 HEPES, 0.2 EGTA, 8 NaCl, 2 MgATP and 0.3 NaGTP, with the pH adjusted to 7.3 using KOH. Cesium intracellular solution was used for all other whole-cell recordings; this contained (in mM) 119 CsMeSO₄, 8 TEA.Cl, 15 HEPES, 0.6 ethylene glycol tetraacetic acid (EGTA), 0.3 Na₃GTP, 4 MgATP, 5 QX-314 and 7 phosphocreatine, with the pH adjusted to 7.3 using CsOH. The recording bath temperature was maintained at 32 °C, with a perfusion speed set to 2–3 mL/min.

To inhibit glutamatergic transmission for sIPSC recordings, NBQX (10 μM) and AP5 (50 μM) were used. sEPSC recordings were conducted by blocking all GABAergic transmission using picrotoxin (100 μM). CINs in slices were identified based on color and were held at −60 mV. Spontaneous cell-attached CIN firing was recorded for 5 min to calculate the average frequency. sIPSCs and sEPSCs were recorded for 3 min to determine average frequency and amplitude. Sag was calculated at a current injection of −400 pA. To measure AMPA-induced current, AMPA (5 μM) was applied via bath perfusion for 1 min, with holding currents recorded at a frequency of 0.2 Hz [26, 95, 97].

Live tissue confocal

Acute brain slices were imaged in a custom-made chamber using an Olympus Fluoview FV3000 confocal microscope, with flowing aCSF and saturated with 95% O₂ and 5% CO₂ as previously described [30, 96]. The microscope was equipped with a 10× NA 0.3 and a 40× NA 0.8 water immersion objective, and 488 and 561 nm lasers. The imaging sample rate was 2–3 frames per second. Imaging parameters, including laser intensity, HV, gain, offset, and aperture diameter, were consistently maintained across sessions.

PLX 5622 preparation

To prepare the 5 mg/mL (12.65 mM) solution of PLX 5622 (CHEMGOOD, C-1521), it was initially dissolved in dimethyl sulfoxide (DMSO, Sigma, D8418) to create a DMSO-PLX 5622 stock solution. The solvent for the DMSO-PLX5622 was 20% ethoxylated hydrogenated castor oil (MedChemExpress, HY-126403) in saline. The final solution involved diluting the DMSO-PLX5622 stocking solution in the 20% ethoxylated hydrogenated castor oil in saline solvent, resulting in a mixture of 5% DMSO-PLX5622 and 95 20% ethoxylated hydrogenated castor oil in saline. The mixture was ultrasonicated until transparent. 0.1 ml of PLX 5622 was injected per 10 g bodyweight of mice per injection.

Statistics

Data were analyzed using Student's t-test, one-way analysis of variance (ANOVA), or two-way ANOVA with repeated measures (two-way RM ANOVA), followed by the Tukey post-hoc test. Significance was determined at $p < 0.05$ with a 95% confidence interval. Data is plotted as box and whisker plots, representing the 25th and 75th percentiles and the minimum and maximum values, respectively. The horizontal line on the box plot represents the median. Analysis was conducted using SigmaPlot 12.5 software.

DATA AVAILABILITY

Data will be available from the corresponding author upon reasonable request.

REFERENCES

- Taylor CA, Bell JM, Breiding MJ, Xu L. Traumatic brain injury-related emergency department visits, hospitalizations, and deaths - United States, 2007 and 2013. *MMWR Surveill Summ*. 2017;66:1–16.
- Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung YC, Punchak M, et al. Estimating the global incidence of traumatic brain injury. *J Neurosurg*. 2018;130:1080–97.
- Clausen T, Martinez P, Towers A, Greenfield T, Kowal P. Alcohol consumption at any level increases risk of injury caused by others: data from the Study on Global AGEing and Adult Health. *Subst Abus*. 2015;9(Suppl 2):125–32.
- Cuthbert JP, Harrison-Felix C, Corrigan JD, Kreider S, Bell JM, Coronado VG, et al. Epidemiology of adults receiving acute inpatient rehabilitation for a primary diagnosis of traumatic brain injury in the United States. *J Head Trauma Rehabil*. 2015;30:122–35.
- Taylor LA, Kreutzer JS, Demm SR, Meade MA. Traumatic brain injury and substance abuse: a review and analysis of the literature. *Neuropsychol Rehabil*. 2003;13:165–88.
- Lan CW, Fiellin DA, Barry DT, Bryant KJ, Gordon AJ, Edelman EJ, et al. The epidemiology of substance use disorders in US Veterans: a systematic review and analysis of assessment methods. *Am J Addict*. 2016;25:7–24.
- Dikmen SS, Machamer JE, Donovan DM, Winn HR, Temkin NR. Alcohol use before and after traumatic head injury. *Ann Emerg Med*. 1995;26:167–76.
- Teng SX, Katz PS, Maxi JK, Mayeux JP, Gilpin NW, Molina PE. Alcohol exposure after mild focal traumatic brain injury impairs neurological recovery and exacerbates localized neuroinflammation. *Brain Behav Immun*. 2015;45:145–56.
- Prasad RM, Doubinskaia I, Singh DK, Campbell G, Mace D, Fletcher A, et al. Effects of binge ethanol administration on the behavioral outcome of rats after lateral fluid percussion brain injury. *J Neurotrauma*. 2001;18:1019–29.
- Ponsford J, Whelan-Goodinson R, Bahar-Fuchs A. Alcohol and drug use following traumatic brain injury: a Prospective study. *Brain Inj*. 2007;21:1385–92.
- Kreutzer JS, Witol AD, Marwitz JH. Alcohol and drug use among young persons with traumatic brain injury. *J Learn Disabil*. 1996;29:643–51.
- Hibbard MR, Uysal S, Kepler K, Bogdany J, Silver J. Axis I psychopathology in individuals with traumatic brain injury. *J Head Trauma Rehabil*. 1998;13:24–39.
- Koponen S, Taiminen T, Hiekkänen H, Tenovu O. Axis I and II psychiatric disorders in patients with traumatic brain injury: a 12-month follow-up study. *Brain Inj*. 2011;25:1029–34.
- Stieler ZF, Fucich EA, Middleton JW, Hillard CJ, Edwards S, Molina PE, et al. Traumatic brain injury and alcohol drinking alter basolateral amygdala endocannabinoids in female rats. *J Neurotrauma*. 2021;38:422–34.
- Mayeux JP, Teng SX, Katz PS, Gilpin NW, Molina PE. Traumatic brain injury induces neuroinflammation and neuronal degeneration that is associated with escalated alcohol self-administration in rats. *Behav Brain Res*. 2015;279:22–30.
- Lowling JL, Susick LL, Caruso JP, Provenzano AM, Raghupathi R, Conti AC. Experimental traumatic brain injury alters ethanol consumption and sensitivity. *J Neurotrauma*. 2014;31:1700–10.
- Mukherjee S, Zeitouni S, Cavarsan CF, Shapiro LA. Increased seizure susceptibility in mice 30 days after fluid percussion injury. *Front Neurol*. 2013;4:28.
- Mukherjee S, Katki K, Arisi GM, Foresti ML, Shapiro LA. Early TBI-induced cytokine alterations are similarly detected by two distinct methods of multiplex assay. *Front Mol Neurosci*. 2011;4:21.
- Witcher KG, Dziabis JE, Bray CE, Gordillo AJ, Kumar JE, Eiferman DS, et al. Comparison between midline and lateral fluid percussion injury in mice reveals prolonged but divergent cortical neuroinflammation. *Brain Res*. 2020;1746:146987.
- Witcher KG, Bray CE, Chunchai T, Zhao F, O'Neil SM, Gordillo AJ, et al. Traumatic brain injury causes chronic cortical inflammation and neuronal dysfunction mediated by microglia. *J Neurosci*. 2021;41:1597–616.
- Liu M, Bachstetter AD, Cass WA, Lifshitz J, Bing G. Pioglitazone attenuates neuroinflammation and promotes dopaminergic neuronal survival in the nigrostriatal system of rats after diffuse brain injury. *J Neurotrauma*. 2017;34:414–22.
- van Bregt DR, Thomas TC, Hinzman JM, Cao T, Liu M, Bing G, et al. Substantia nigra vulnerability after a single moderate diffuse brain injury in the rat. *Exp Neurol*. 2012;234:8–19.
- Garcia-Baños A, Alegre-Zurano L, Cantacors L, Martín-Sánchez A, Valverde O. Role of cannabinoids in alcohol-induced neuroinflammation. *Prog Neuropsychopharmacol Biol Psychiatry*. 2021;104:110054.
- Warden AS, Wolfe SA, Khom S, Varodayan FP, Patel RR, Steinman MQ, et al. Microglia control escalation of drinking in alcohol-dependent mice: genomic and synaptic drivers. *Biol Psychiatry*. 2020;88:910–21.
- Ma T, Huang Z, Xie X, Cheng Y, Zhuang X, Childs MJ, et al. Chronic alcohol drinking persistently suppresses thalamostriatal excitation of cholinergic neurons to impair cognitive flexibility. *J Clin Invest*. 2022;132:e154969.
- Cheng Y, Huang CCY, Ma T, Wei X, Wang X, Lu J, et al. Distinct synaptic strengthening of the striatal direct and indirect pathways drives alcohol consumption. *Biol Psychiatry*. 2017;81:918–29.
- Ma T, Cheng Y, Roltsch Hellard E, Wang X, Lu J, Gao X, et al. Bidirectional and long-lasting control of alcohol-seeking behavior by corticostriatal LTP and LTD. *Nat Neurosci*. 2018;21:373–83.
- Chantranupong L, Beron CC, Zimmer JA, Wen MJ, Wang W, Sabatini BL. Dopamine and glutamate regulate striatal acetylcholine in decision-making. *Nature*. 2023;621:577–85.
- Gangal H, Xie X, Huang Z, Cheng Y, Wang X, Lu J, et al. Drug reinforcement impairs cognitive flexibility by inhibiting striatal cholinergic neurons. *Nat Commun*. 2023;14:3886.
- Huang Z, Chen R, Ho M, Xie X, Gangal H, Wang X, et al. Dynamic responses of striatal cholinergic interneurons control behavioral flexibility. *Sci Adv*. 2024;10:eadn2446.
- Williams MJ, Adinoff B. The role of acetylcholine in cocaine addiction. *Neuropsychopharmacology*. 2008;33:1779–97.
- Hikida T, Kitabatake Y, Pastan I, Nakanishi S. Acetylcholine enhancement in the nucleus accumbens prevents addictive behaviors of cocaine and morphine. *Proc Natl Acad Sci USA*. 2003;100:6169–73.
- Lee JH, Ribeiro EA, Kim J, Ko B, Kronman H, Jeong YH, et al. Dopaminergic regulation of nucleus accumbens cholinergic interneurons demarcates susceptibility to cocaine addiction. *Biol Psychiatry*. 2020;88:746–57.
- Essoh A, Gangal H, Huang Z, Chen R, Xie X, Wang X, et al. Alcohol attenuates CRF-induced excitatory effects from the extended amygdala to dorsostriatal cholinergic interneurons. *eLife*. 2025;14:RP107145.
- Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature*. 2003;421:384–8.
- Gamage R, Rossetti I, Niedermayer G, Münch G, Buskila Y, Gyengesi E. Chronic neuroinflammation during aging leads to cholinergic neurodegeneration in the mouse medial septum. *J Neuroinflammation*. 2023;20:235.
- Kékesi O, Liang H, Münch G, Morley JW, Gyengesi E, Buskila Y. The differential impact of acute microglia activation on the excitability of cholinergic neurons in the mouse medial septum. *Brain Struct Funct*. 2019;224:2297–309.
- Ni H, Liao Y, Zhang Y, Lu H, Huang Z, Huang F, et al. Levistilide A ameliorates neuroinflammation via inhibiting JAK2/STAT3 signaling for neuroprotection and cognitive improvement in scopolamine-induced Alzheimer's disease mouse model. *Int Immunopharmacol*. 2023;124(Pt A):110783.
- Giovannini MG, Scali C, Prosperi C, Bellucci A, Vannucchi MG, Rosi S, et al. Beta-amyloid-induced inflammation and cholinergic hypofunction in the rat brain in vivo: involvement of the p38MAPK pathway. *Neurobiol Dis*. 2002;11:257–74.
- Driver S, Juengst S, McShan EE, Bennett M, Bell K, Dubiel R. A randomized controlled trial protocol for people with traumatic brain injury enrolled in a healthy lifestyle program (GLB-TBI). *Contemp Clin Trials Commun*. 2019;14:100328.
- Dreer LE, Ketchum JM, Novack TA, Bogner J, Felix ER, Corrigan JD, et al. Obesity and overweight problems among individuals 1 to 25 years following acute

- rehabilitation for traumatic brain injury: a NIDILRR Traumatic Brain Injury Model Systems Study. *J Head Trauma Rehabil.* 2018;33:246–56.
42. Brown RM, Tang X, Dreer LE, Driver S, Pugh MJ, Martin AM, et al. Change in body mass index within the first-year post-injury: a VA Traumatic Brain Injury (TBI) model systems study. *Brain Inj.* 2018;32:986–93.
 43. Driver S, Reynolds M, Douglas M, Bennett M. Describing weight loss attempts and physical activity among individuals with TBI prior to participation in a weight-loss program. *J Head Trauma Rehabil.* 2018;33:E36–e43.
 44. Brunner M, Hemsley B, Togher L, Palmer S. Technology and its role in rehabilitation for people with cognitive-communication disability following a traumatic brain injury (TBI). *Brain Inj.* 2017;31:1028–43.
 45. Donat CK, Scott G, Gentleman SM, Sastre M. Microglial activation in traumatic brain injury. *Front Aging Neurosci.* 2017;9:208.
 46. Petersen A, Soderstrom M, Saha B, Sharma P. Animal models of traumatic brain injury: a review of pathophysiology to biomarkers and treatments. *Exp Brain Res.* 2021;239:2939–50.
 47. Gottlieb A, Toledano-Furman N, Prabhakara KS, Kumar A, Caplan HW, Bedi S, et al. Time dependent analysis of rat microglial surface markers in traumatic brain injury reveals dynamics of distinct cell subpopulations. *Sci Rep.* 2022;12:6289.
 48. Loane DJ, Kumar A. Microglia in the TBI brain: the good, the bad, and the dysregulated. *Exp Neurol.* 2016;275(Pt 3):316–27.
 49. Nwachukwu KN, Evans WA, Sides TR, Trevisani CP, Davis A, Marshall SA. Chemogenetic manipulation of astrocytic signaling in the basolateral amygdala reduces binge-like alcohol consumption in male mice. *J Neurosci Res.* 2021;99:1957–72.
 50. Henry RJ, Ritzel RM, Barrett JP, Doran SJ, Jiao Y, Leach JB, et al. Microglial depletion with CSF1R inhibitor during chronic phase of experimental traumatic brain injury reduces neurodegeneration and neurological deficits. *J Neurosci.* 2020;40:2960–74.
 51. Stratoulis V, Ruiz R, Kanatani S, Osman AM, Keane L, Armengol JA, et al. ARG1-expressing microglia show a distinct molecular signature and modulate postnatal development and function of the mouse brain. *Nat Neurosci.* 2023;26:1008–20.
 52. Jacotte-Simancas A, Molina PE, Gilpin NW. Repeated mild traumatic brain injury and JZL184 produce sex-specific increases in anxiety-like behavior and alcohol consumption in wistar rats. *J Neurotrauma.* 2023;40:2427–41.
 53. Fucich EA, Mayeux JP, McGinn MA, Gilpin NW, Edwards S, Molina PE. A novel role for the endocannabinoid system in ameliorating motivation for alcohol drinking and negative behavioral affect after traumatic brain injury in rats. *J Neurotrauma.* 2019;36:1847–55.
 54. Karelina K, Nicholson S, Weil ZM. Minocycline blocks traumatic brain injury-induced alcohol consumption and nucleus accumbens inflammation in adolescent male mice. *Brain Behav Immun.* 2018;69:532–9.
 55. Weil ZM, Karelina K, Gaier KR, Corrigan TE, Corrigan JD. Juvenile traumatic brain injury increases alcohol consumption and reward in female mice. *J Neurotrauma.* 2016;33:895–903.
 56. Ambroggi F, Ishikawa A, Fields HL, Nicola SM. Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. *Neuron.* 2008;59:648–61.
 57. Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, et al. Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature.* 2011;475:377–80.
 58. Balleine BW, Delgado MR, Hikosaka O. The role of the dorsal striatum in reward and decision-making. *J Neurosci.* 2007;27:8161–5.
 59. Nimitvilai-Roberts S, Lopez MF, Woodward JJ. Effects of chronic ethanol exposure on dorsal medial striatal neurons receiving convergent inputs from the orbitofrontal cortex and basolateral amygdala. *Neuropharmacology.* 2025;267:110303.
 60. Adams RS, Larson MJ, Corrigan JD, Horgan CM, Williams TV. Frequent binge drinking after combat-acquired traumatic brain injury among active duty military personnel with a past year combat deployment. *J Head Trauma Rehabil.* 2012;27:349–60.
 61. Do JP, Xu M, Lee SH, Chang WC, Zhang S, Chung S, et al. Cell type-specific long-range connections of basal forebrain circuit. *Elife.* 2016;5:e13214.
 62. Hu R, Jin S, He X, Xu F, Hu J. Whole-brain monosynaptic afferent inputs to basal forebrain cholinergic system. *Front Neuroanat.* 2016;10:98.
 63. Klug JR, Engelhardt MD, Cadman CN, Li H, Smith JB, Ayala S, et al. Differential inputs to striatal cholinergic and parvalbumin interneurons imply functional distinctions. *Elife.* 2018;7:e35657.
 64. Guo Q, Wang D, He X, Feng Q, Lin R, Xu F, et al. Whole-brain mapping of inputs to projection neurons and cholinergic interneurons in the dorsal striatum. *PLoS One.* 2015;10:e0123381.
 65. Denninger JK, Smith BM, Kirby ED. Novel object recognition and object location behavioral testing in mice on a budget. *J Vis Exp.* 2018;141:e58593.
 66. Lueptow LM. Novel object recognition test for the investigation of learning and memory in mice. *J Vis Exp.* 2017;126:e55718.
 67. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process.* 2012;13:93–110.
 68. Leger M, Quiedeville A, Bouet V, Haelewyn B, Boulouard M, Schumann-Bard P, et al. Object recognition test in mice. *Nat Protoc.* 2013;8:2531–7.
 69. Warburton EC, Brown MW. Findings from animals concerning when interactions between perirhinal cortex, hippocampus and medial prefrontal cortex are necessary for recognition memory. *Neuropsychologia.* 2010;48:2262–72.
 70. Balderas I, Rodriguez-Ortiz CJ, Bermudez-Rattoni F. Retrieval and reconsolidation of object recognition memory are independent processes in the perirhinal cortex. *Neuroscience.* 2013;253:398–405.
 71. Dere E, Huston JP, De Souza Silva MA. The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neurosci Biobehav Rev.* 2007;31:673–704.
 72. Piyabhan P, Wetchateng T. Cognitive enhancement effects of Bacopa monnieri (Brahmi) on novel object recognition and VGLUT1 density in the prefrontal cortex, striatum, and hippocampus of sub-chronic phencyclidine rat model of schizophrenia. *J Med Assoc Thai.* 2013;96:625–32.
 73. Asif-Malik A, Dautan D, Young AMJ, Gerdjikov TV. Altered cortico-striatal crosstalk underlies object recognition memory deficits in the sub-chronic phencyclidine model of schizophrenia. *Brain Struct Funct.* 2017;222:3179–90.
 74. Arisi GM, Foresti ML, Katki K, Shapiro LA. Increased CCL2, CCL3, CCL5, and IL-1 β cytokine concentration in piriform cortex, hippocampus, and neocortex after pilocarpine-induced seizures. *J Neuroinflammation.* 2015;12:129.
 75. Okada K, Hashimoto K, Kobayashi K. Cholinergic regulation of object recognition memory. *Front Behav Neurosci.* 2022;16:996089.
 76. Kitabatake Y, Hikida T, Watanabe D, Pastan I, Nakanishi S. Impairment of reward-related learning by cholinergic cell ablation in the striatum. *Proc Natl Acad Sci USA.* 2003;100:7965–70.
 77. Amaya KA, Smith KS. Spatially restricted inhibition of cholinergic interneurons in the dorsolateral striatum encourages behavioral exploration. *Eur J Neurosci.* 2021;53:2567–79.
 78. Palmer D, Creighton S, Prado VF, Prado MAM, Choleris E, Winters BD. Mice deficient for striatal Vesicular Acetylcholine Transporter (VACHT) display impaired short-term but normal long-term object recognition memory. *Behav Brain Res.* 2016;311:267–78.
 79. Wang J, Lanfranco MF, Gibb SL, Yowell QV, Carnicella S, Ron D. Long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum play a crucial role in alcohol consumption and relapse. *J Neurosci.* 2010;30:10187–98.
 80. Wang J, Lanfranco MF, Gibb SL, Ron D. Ethanol-mediated long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum. *Channels (Austin).* 2011;5:205–9.
 81. Vierkant V, Xie X, Huang Z, He L, Bancroft E, Wang X, et al. Optogenetic inhibition of light-captured alcohol-taking striatal engrams facilitates extinction and suppresses reinstatement. *Alcohol Clin Exp Res (Hoboken).* 2024;48:1728–39.
 82. Huang CCY, Ma T, Roltsch Hellard EA, Wang X, Selvamani A, Lu J, et al. Stroke triggers nigrostriatal plasticity and increases alcohol consumption in rats. *Sci Rep.* 2017;7:2501.
 83. Wang J, Cheng Y, Wang X, Roltsch Hellard E, Ma T, Gil H, et al. Alcohol elicits functional and structural plasticity selectively in dopamine D1 receptor-expressing neurons of the dorsomedial striatum. *J Neurosci.* 2015;35:11634–43.
 84. Bito-Onon JJ, Simms JA, Chatterjee S, Holgate J, Bartlett SE. Varenicline, a partial agonist at neuronal nicotinic acetylcholine receptors, reduces nicotine-induced increases in 20% ethanol operant self-administration in Sprague-Dawley rats. *Addict Biol.* 2011;16:440–9.
 85. Xie X, Lu J, Ma T, Cheng Y, Woodson K, Bonifacio J, et al. Linking input- and cell-type-specific synaptic plasticity to the reinforcement of alcohol-seeking behavior. *Neuropharmacology.* 2023;237:109619.
 86. Vierkant V, Xie X, Wang X, Wang J. Experimental models of alcohol use disorder and their application for pathophysiological investigations. *Curr Protoc.* 2023;3:e831.
 87. Lu J, Cheng Y, Wang X, Woodson K, Kemper C, Disney E, et al. Alcohol intake enhances glutamatergic transmission from D2 receptor-expressing afferents onto D1 receptor-expressing medium spiny neurons in the dorsomedial striatum. *Neuropsychopharmacology.* 2019;44:1123–31.
 88. Tobin RP, Mukherjee S, Kain JM, Rogers SK, Henderson SK, Motal HL, et al. Traumatic brain injury causes selective, CD74-dependent peripheral lymphocyte activation that exacerbates neurodegeneration. *Acta Neuropathol Commun.* 2014;2:143.
 89. Newell-Rogers MK, Rogers SK, Tobin RP, Mukherjee S, Shapiro LA. Antagonism of macrophage Migration Inhibitory Factor (MIF) after traumatic brain injury ameliorates astrogliosis and peripheral lymphocyte activation and expansion. *Int J Mol Sci.* 2020;21:7448.

90. Venkatasamy L, Nizamutdinov D, Jenkins J, Shapiro LA. Vagus nerve stimulation ameliorates cognitive impairment and increased hippocampal astrocytes in a mouse model of gulf war illness. *Neurosci Insights*. 2021;16:26331055211018456.
91. Iannucci J, Nizamutdinov D, Shapiro LA. Neurogenesis and chronic neurobehavioral outcomes are partially improved by vagus nerve stimulation in a mouse model of Gulf War illness. *Neurotoxicology*. 2022;90:205–15.
92. Franklin KBJ, Paxinos G. *The mouse brain in stereotaxic coordinates*. 3 edn. San Diego: Academic Press; 2007.
93. Wang W, Xie X, Zhuang X, Huang Y, Tan T, Gangal H, et al. Striatal mu-opioid receptor activation triggers direct-pathway GABAergic plasticity and induces negative affect. *Cell Rep*. 2023;42:112089.
94. Cheng Y, Xie X, Lu J, Gangal H, Wang W, Melo S, et al. Optogenetic induction of orbitostriatal long-term potentiation in the dorsomedial striatum elicits a persistent reduction of alcohol-seeking behavior in rats. *Neuropharmacology*. 2021;191:108560.
95. Cheng Y, Wang X, Wei X, Xie X, Melo S, Miranda RC, et al. Prenatal exposure to alcohol induces functional and structural plasticity in dopamine D1 receptor-expressing neurons of the dorsomedial striatum. *Alcohol Clin Exp Res*. 2018;42:1493–502.
96. Purvines W, Gangal H, Xie X, Ramos J, Wang X, Miranda R, et al. Perinatal and prenatal alcohol exposure impairs striatal cholinergic function and cognitive flexibility in adult offspring. *Neuropharmacology*. 2025;279:110627.
97. Ungless MA, Whistler JL, Malenka RC, Bonci A. Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature*. 2001;411:583–7.

ACKNOWLEDGEMENTS

This work was supported by NIAAA R01AA027768 (JW), U01AA025932 (JW), R01AA030293 (JW), and R01NS104282 (LAS).

AUTHOR CONTRIBUTIONS

HG designed experiments, performed histology, behavioral, electrophysiological experiments, analyzed data, and wrote the manuscript; JI performed FPI surgeries, histology, behavioral experiments, analyzed data and revised the manuscript; YH and WD performed behavioral experiments; WD wrote the early draft of the manuscript; SM performed FPI surgeries; RC and WP performed confocal imaging; XW performed animal breeding; XX, AR and GJ performed data analysis; KM and KO assisted with behavior and histology; VV revised the manuscript; LS and JW designed experiments, supervised the project, and wrote the manuscript. We also thank Gabriella Hollingsworth for assistance with behavior and histology.

COMPETING INTERESTS

The authors have declared that no conflict of interest exists.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All animal care and experimental procedures were approved by the Texas A&M University Institutional Animal Care and Use Committee and were conducted in agreement with the National Research Council Guide for the Care and Use of Laboratory Animals (AUP# #2020-0140 and AUP# 2022-0198). Informed consent was obtained from all participants.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41398-025-03650-7>.

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