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A hippocampo-cortical pathway detects changes in the validity of an action as a predictor of reward

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Summary

Much research has been dedicated to understanding the psychological and neural bases of goal-directed action. Yet, the relationship between context and goal-directed action is not well understood. Here, we used excitotoxic lesions, chemogenetics, and circuit-specific manipulations to demonstrate a role for ventral hippocampus (vHPC) in the contextual learning that supports sensitivity to action-outcome contingencies, a hallmark of goal-directed action. We found that chemogenetic inhibition of ventral, but not dorsal, hippocampus attenuated sensitivity to instrumental contingency degradation. We then tested the hypothesis that this deficit was due to an inability to discern the relative validity of the action compared with the context as a predictor of reward. Using latent inhibition and Pavlovian context conditioning, we confirm that degradation of action-outcome contingencies relies on intact context-outcome learning and show that this learning is dependent on vHPC. Finally, we show that chemogenetic inhibition of vHPC terminals in the medial prefrontal cortex also impairs both instrumental contingency degradation and context-outcome learning. These results implicate a hippocampo-cortical pathway in adapting to changes in instrumental contingencies and indicate that the psychological basis of this deficit is an inability to learn the predictive value of the context. Our findings contribute to a broader understanding of the neural bases of goal-directed action and its contextual regulation.

Keywords: goal-directed behaviour, context, choice, rodent, DREADD, hippocampus, operant

INTRODUCTION

Every day we make decisions based on internal goals and on the expectation that a given action will lead to goal achievement. Such 'goal-directed' behaviour is critical for adapting to our environment and is defined as an intention to act that is driven by knowledge of both the causal relation between an action and its associated outcome, and the value or current desirability of that outcome^{1,2}. The involvement of the hippocampus in this behaviour is debated. Despite early demonstrations that dorsal hippocampus lesions rendered rats insensitive to changes in the causal consequences of their actions³, it was later shown that these deficits were caused by damage to fibres of passage rather than to the hippocampus itself⁴ and further studies confirmed that goal-directed behaviour was intact in rats with perturbed hippocampal activity⁵⁻⁷. Yet, there is increasing evidence that this behaviour is, in fact, impaired following hippocampal inhibition⁸⁻¹⁴.

A question then arises regarding the origin of the hippocampal contribution to goal-directed behaviour. When impairments are observed, they typically manifest as an insensitivity to the causal relationship between action and outcome rather than to outcome value. Rats with hippocampal damage or inhibition are able to select actions based on the current value of the associated outcome, as assessed via satiety-induced outcome devaluation^{3,5-8}, yet they can appear insensitive to degradation of the action→outcome (A→O) contingency through presentation of unsignalled outcomes, particularly when ventral hippocampus (vHPC) is manipulated^{8,9}. One interpretation of this dissociation is that sensitivity to degradation of the A→O contingency relies on the formation of context-outcome associations but selective satiation does not⁷.

Contingency degradation involves 'free' delivery of outcomes that are not earned by performing an action¹⁵ and is believed to generate context-outcome associations that compete with the A→O association^{3,16–18}, leading the action's causal relationship with the outcome to degrade. The context, understood as the environmental cues that surround a learning experience, is therefore capable of serving as a cue that enters into associative competition with the action^{19,20}. An inability to detect the current A→O contingency might then be attributed to a failure to encode the relative validity of the action compared with the context as a predictor of reward^{3,16–18}.

Here, we sought to determine the psychological mechanism that is supported by the hippocampus to produce goal-directed behaviour. We showed that dysfunction of ventral, but not dorsal, hippocampus attenuates sensitivity to degradation of the A→O relationship. We then demonstrate that adapting to a change in the A→O contingency requires intact context-outcome learning, and this learning is indeed vHPC-dependent. Chemogenetic silencing of vHPC terminals in the medial prefrontal cortex (mPFC) impaired both instrumental contingency degradation and context-outcome learning, indicating that hippocampal input to mPFC is required to learn the predictive value of the context and, thus, to successfully adapt to changes in action causality. Our findings demonstrate a fundamental role of context in disambiguating action causality and reveal vHPC as a vital player in the contextual regulation of goal-directed behaviour.

RESULTS

Inactivation of ventral, but not dorsal, hippocampus attenuates sensitivity to degradation of instrumental contingencies

We first investigated if vHPC is required for goal-directed behaviour by testing the impact of excitotoxic vHPC lesions on instrumental outcome-specific devaluation (Figure 1) and instrumental contingency degradation (Figure 2).

Rats with sham or vHPC lesions (Figure 1A, S1A) were trained to perform two actions for two distinct food outcomes while mildly food restricted. One of the outcomes was then devalued via sensory-specific satiety and rats were subsequently given an unrewarded choice test with the two actions (Figure 1B). Typically, rats will use outcome value to guide their choice and will perform the action associated with the valued (non-sated) outcome more than the action associated with the devalued outcome. Instrumental acquisition did not differ between Sham and Lesion groups (Figure 1C; session effect only: $F_{1,18} = 300.31$, $p < 0.01$) and, at test (Figure 1D), devaluation was intact for both groups (devaluation effect only: $F_{1,18} = 82.76$, $p < 0.01$). The rate of extinction on the valued lever across testing (Figure S2A) also did not differ between groups, indicating that extinction learning was unaffected²¹. vHPC lesions did not, therefore, affect the rats' ability to select the action associated with the more valuable outcome.

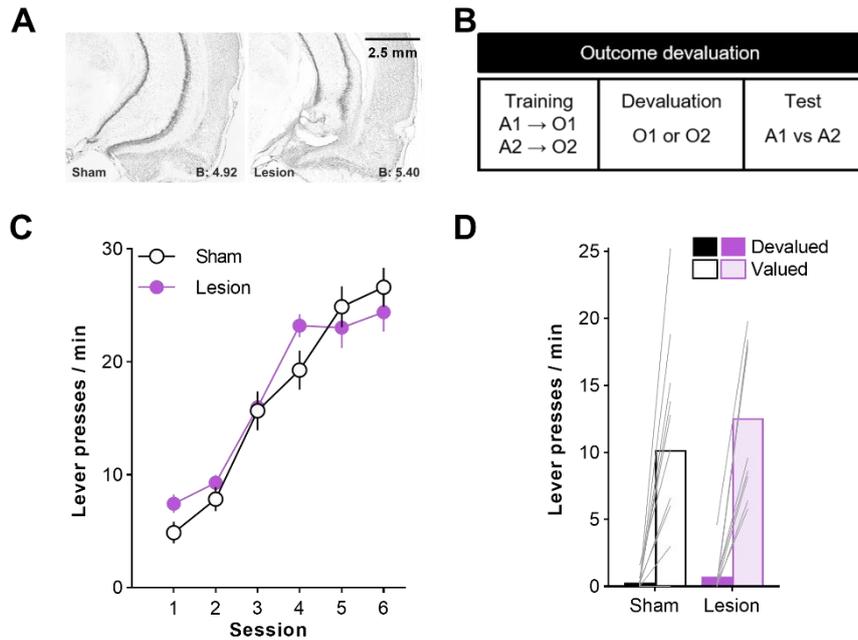


Figure 1. vHPC lesions leave instrumental outcome devaluation intact.

A. Representative image of sham and ventral hippocampus (vHPC) lesions (see also [Figure S1A](#)). Scale: 2.5 mm. Three rats were excluded from group Lesion due to unilateral lesions: Sham ($n = 11$) and Lesion ($n = 9$).

B. Rats were trained on two action→outcome associations (A1→O1; A2→O2). One of the outcomes was devalued via specific satiety (O1 or O2) and rats were then given an unrewarded choice test with the two actions (A1 and A2).

C. Lever pressing rate (\pm SEM) across instrumental training, averaged across the two levers.

D. Lever pressing rate during the outcome devaluation test ([see also Figure S2A](#)).

Having confirmed that rats with vHPC lesions can (1) learn A→O associations and (2) use outcome value to guide choice, we next assessed the rats' ability to adapt to a change in the causal relationship between an action and its outcome using instrumental contingency degradation (Figure 2A). Rats were re-trained on the same A→O associations (largest $F_{1,18} = 1.79$, $p=0.20$; data not shown). Then, one A→O association was degraded such that delivery of that outcome occurred independently of lever pressing (Degraded). The contingency was maintained for the other A→O association (Non-degraded). Across degradation (Figure 2B), rats with vHPC lesions

were less sensitive to the change in A→O contingency (group x lever interaction: $F_{1,18} = 8.78$, $p=0.01$) but both groups performed the degraded action less than the non-degraded action (simple effect for sham: $F_{1,18} = 58.00$, $p<0.01$, and lesion: $F_{1,18} = 8.37$, $p=0.01$). Post-hoc two-way ANOVAs revealed a significant difference on the degraded ($F_{1,18} = 6.93$, $p=0.02$) but not the non-degraded lever ($F_{1,18} = 0.47$, $p=0.50$), such that lesion rats pressed more on the degraded lever than shams.

During the choice retrieval test (Figure 2C, S2B), we again observed that degradation was attenuated for group Lesion (degradation effect: $F_{1,18} = 47.68$, $p<0.01$; group x lever interaction: $F_{1,18} = 4.96$, $p=0.04$; simple effect for Sham: $F_{1,18} = 46.32$, $p<0.01$ and Lesion: $F_{1,18} = 9.95$, $p=0.01$). This was also evident during a subsequent reacquisition test during which rats were re-trained on the two actions (Figure 2D). Shams pressed less on the previously degraded action relative to the previously non-degraded action but lesion rats did not (group x lever interaction: $F_{1,18} = 5.10$, $p=0.04$; simple effect for Sham but not Lesion: $F_{1,18} = 15.1$, $p<0.01$ and $F_{1,18} = 0.22$, $p=0.64$, respectively).

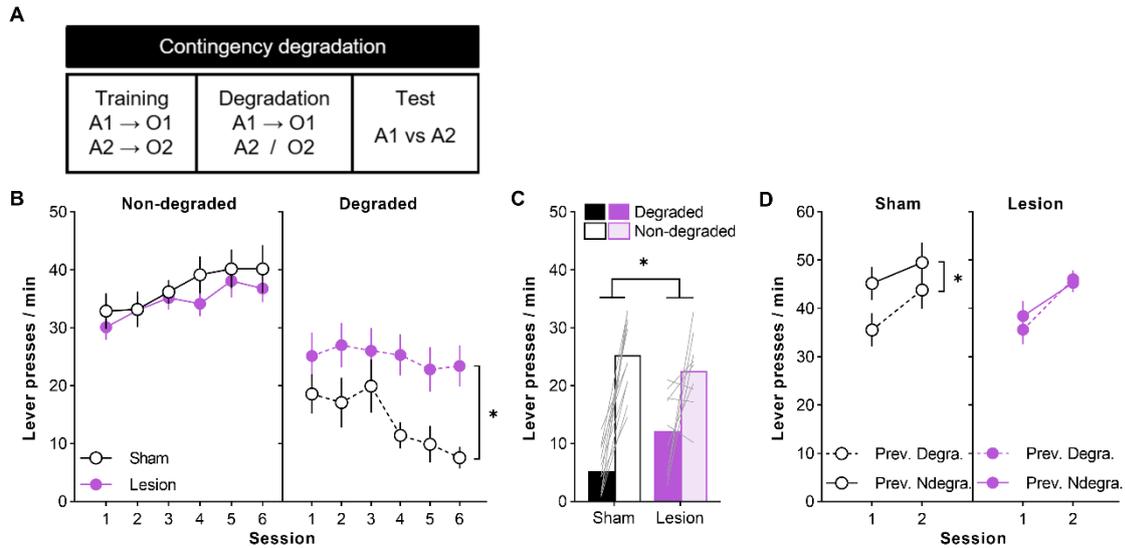


Figure 2. vHPC lesions impair sensitivity to instrumental contingency degradation.

- A.** Rats were re-trained on the action→outcome associations (A1→O1; A2→O2) and then one A→O contingency was degraded (e.g., A2 / O2) while the other was maintained (A1→O1). Rats were then given a choice test with the two actions.
- B.** Lever pressing rate (\pm SEM) across degradation training on the Non-degraded and Degraded levers. Food port entries are shown in Figure S3A. *significant group effect in post-hoc two-way ANOVA.
- C.** Lever pressing rate during the test (see also Figure S2B). *group x lever interaction.
- D.** Lever pressing rate (\pm SEM) during reacquisition on the previously degraded (Prev. Degra.) and previously non-degraded (Prev. Ndegra.) levers. *simple effect of lever.

We next examined the temporal specificity of the deficit in contingency degradation by selectively inhibiting vHPC during degradation training or during the retrieval test using an inhibitory hM4Di DREADD (designer receptor exclusively activated by a designer drug^{22,23}). Half of the rats were injected in vHPC with an adeno-associated virus carrying the hM4Di receptor (vHPC-hM4Di; Figure 3A), and the other half were injected with a GFP virus (vHPC-GFP). The DREADD ligand, CNO, or vehicle was injected before degradation training or test.

Instrumental training proceeded normally (Figure 3B; session effect only: $F_{1,52} = 336.84$, $p < 0.01$). During degradation (Figure 3C), there was an effect of degradation ($F_{1,52} = 157.46$, $p < 0.01$) and a three-way interaction that approached

significance ($F_{1,52} = 3.67$, $p=0.06$). When performance was expressed as a percentage of responding during the final instrumental training day, we observed that the vHPC-hM4Di group injected with CNO pressed more on the degraded lever than the vHPC-GFP group injected with CNO (Figure S4A). Food port entries also differed between groups (Figure S3B).

An analogous pattern was observed during the test (Figure 3D, S4B). Here, the vHPC-hM4Di group that received CNO during training failed to show a preference for the non-degraded lever (effect of degradation and virus: smallest $F_{1,52} = 12.15$, $p<0.01$; overall virus x degradation interaction: $F_{1,52} = 5.7$, $p=0.02$; post-hoc virus x degradation interaction for rats receiving CNO during degradation: $F_{1,52} = 5.37$, $p=0.02$, but not during test: $F_{1,52} = 1.07$, $p=0.31$; simple effect of degradation for all groups: $F_{1,52}$ values > 13.03 , p values < 0.01 , except the vHPC-hM4Di group that received CNO during degradation training: $F_{1,52} = 3.26$, $p=0.08$). Thus, impaired contingency degradation was observed when vHPC was inhibited during degradation training but not when it was inhibited during test, which indicates a specific role for vHPC during updating of the A→O contingency. **Extinction of responding on the non-degraded lever for groups receiving CNO at test was unaffected (Figure S2C).**

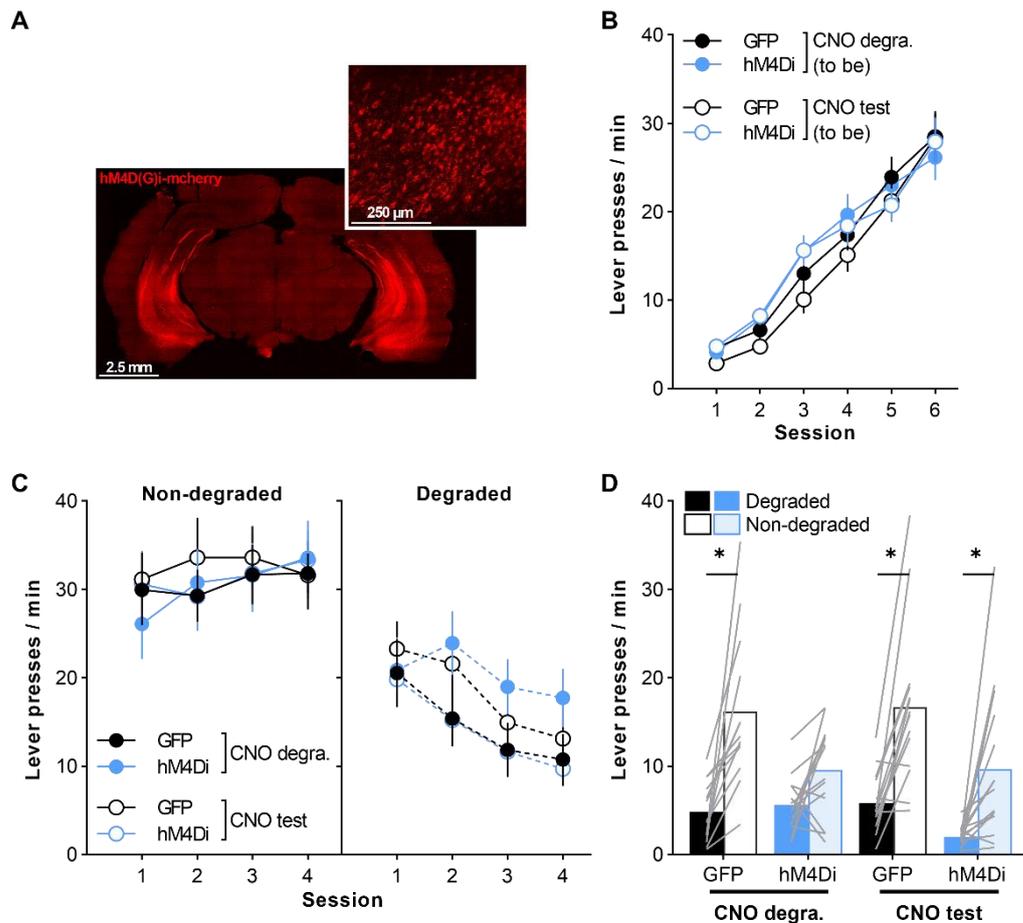


Figure 3. Silencing vHPC during degradation training diminishes sensitivity to instrumental contingency degradation.

A. Representative image of viral expression (AAV8-CamKII-hM4Di-mCherry) in ventral hippocampus (vHPC) (see also [Figure S1B](#)). Scale: 2.5 mm. Inset: magnification of the area of interest, transfected neurons appear in red (scale: 250 μ m). Two GFP and three hM4Di rats were excluded due to misplaced injections. Two GFP rats were also removed due to an experimenter mistake during degradation training, yielding these between-subject group sizes: CNO during degradation training (CNO degra.): GFP ($n = 13$), hM4Di ($n = 14$), and CNO during test (CNO test): GFP ($n = 14$), hM4Di ($n = 15$).

B. Lever pressing rate (\pm SEM) across instrumental training, averaged across the two levers.

C. Lever pressing rate (\pm SEM) across degradation training on the Non-degraded and Degraded levers (see also [Figure S4A](#)). Food port entries are shown in [Figure S3B](#).

D. Lever pressing rate during the test (see also [Figure S2C and S4B](#)). *simple effect of lever.

We then established the regional specificity of our results using the same chemogenetic approach to inhibit dorsal hippocampus (dHPC) during degradation training or test (Figure 4A). Instrumental training progressed smoothly (Figure 4B; session effect only: $F_{1,32} = 208.12$, $p < 0.01$). During degradation (Figure 4C), all groups performed the degraded action less than the non-degraded action (degradation effect only: $F_{1,32} = 108.44$, $p < 0.01$) and showed intact sensitivity to degradation during the test (Figure 4D; degradation effect only: $F_{1,32} = 35.02$, $p < 0.01$). Therefore, unlike vHPC inhibition, inhibition of dHPC did not impair sensitivity to contingency degradation, indicating a specific role for vHPC in detecting and encoding changes in action causality.

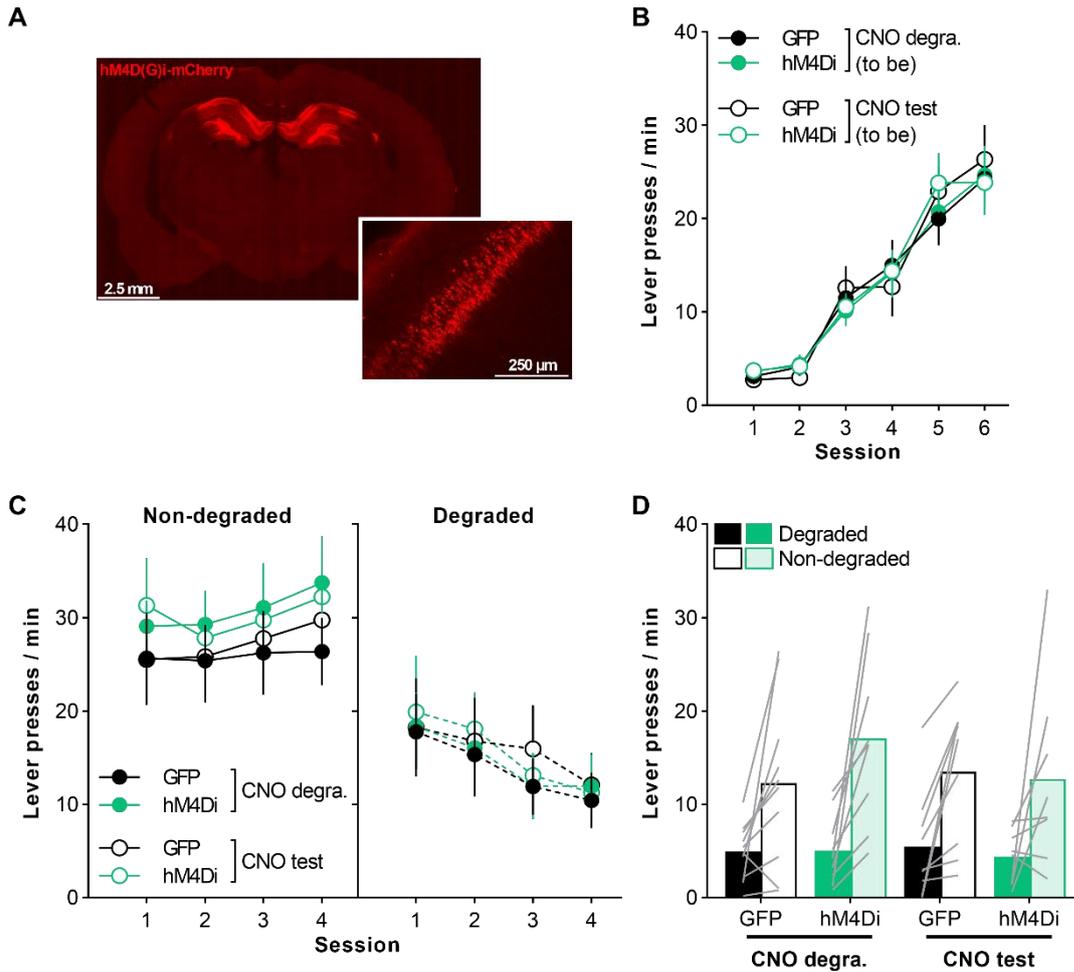


Figure 4. Silencing dHPC leaves sensitivity to instrumental contingency degradation intact.

A. Representative image of viral expression (AAV8-CamKII-hM4Di-mCherry) in the dorsal hippocampus (dHPC) (see also [Figure S1C](#)). Scale: 2.5 mm. Inset: magnification of the area of interest, transfected neurons appear in red (scale: 250 μ m). Two hM4Di rats were excluded due to misplaced injections: CNO at degradation training (CNO degra.): GFP ($n = 10$), hM4Di ($n = 9$), and CNO at test (CNO test): GFP ($n = 9$), hM4Di ($n = 8$).

B. Lever pressing rate (\pm SEM) across instrumental training, averaged across the two levers.

C. Lever pressing rate (\pm SEM) across degradation training on the Non-degraded and Degraded levers. Food port entries are shown in [Figure S3C](#).

D. Lever pressing rate during the test.

Sensitivity to contingency degradation relies on intact context-outcome learning

It has been proposed that reduced performance on a degraded action is the result of a decrease in the effectiveness of that action compared with the context as a predictor of reward^{3,4,7,16–18,24}. That is, to update the A→O causal contingency, one must learn that it is the context, and not the instrumental action, that now signals reward delivery. We reasoned that the reduced sensitivity to contingency degradation that we observed with vHPC inhibition may result from failing to learn that the context predicts reward (i.e., impaired context-outcome learning)^{3,4}. To test this, we used a new cohort of rats to see if reducing the predictive validity of the context via extensive context pre-exposure (latent inhibition of the context^{20,25,26}) would, like vHPC inhibition, impair instrumental contingency degradation.

Instrumental training proceeded without incident (Figure 5A; session effect only: $F_{1,13} = 266.11$, $p < 0.01$). Then, half of the rats were pre-exposed to the training context (group Exposed) whereas the remaining half (group Non-exposed) were not. For group Exposed, food port entries decreased across context pre-exposure (data not shown; $F_{1,6} = 13.33$, $p = 0.01$).

We then assessed the impact of this exposure on contingency degradation (Figure 5B). Context exposure did not affect degradation training itself (lever effect only: $F_{1,13} = 81.74$, $p < 0.01$). However, during the test (Figure 5C), we observed that context exposure had attenuated, but not abolished, sensitivity to contingency degradation (effect of lever: $F_{1,13} = 38.92$, $p < 0.01$; group x lever interaction: $F_{1,13} = 5.92$, $p = 0.03$; simple effect of degradation for group Exposed: $F_{1,13} = 6.79$, $p = 0.02$, and Non-exposed: $F_{1,13} = 40.28$, $p < 0.01$). These results indicate that context-outcome learning supports the updating of the A→O contingency and that pre-exposure to the context can, like vHPC inhibition, reduce sensitivity to contingency degradation.

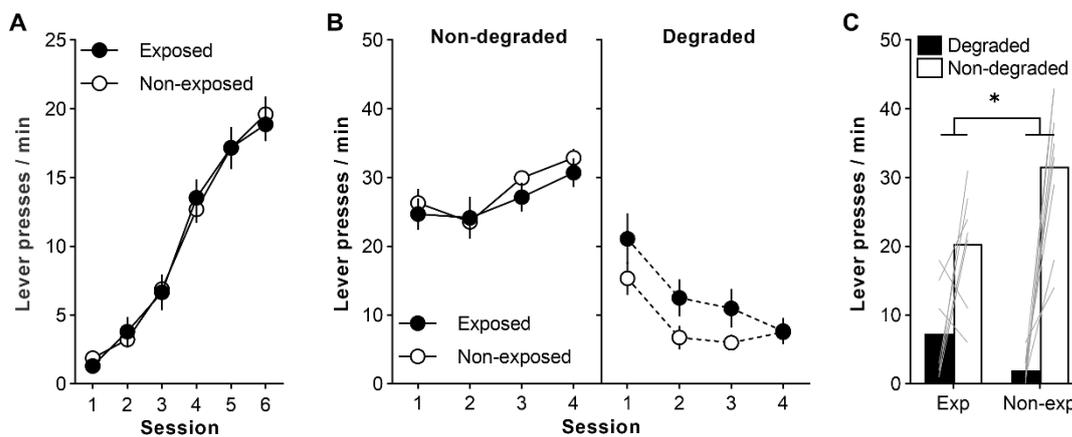


Figure 5. Context pre-exposure attenuates sensitivity to instrumental contingency degradation.

A. Lever pressing rate (\pm SEM) across instrumental training, averaged across the two levers.

B. Lever pressing rate (\pm SEM) across degradation training.

C. Lever pressing rate during the test for the Exposed (Exp) and Non-exposed (Non-exp) groups. One rat was excluded from group Exposed for performing > 2 standard deviations from the mean during the test: Exposed ($n = 7$); Non-exposed ($n = 8$).

*significant group \times lever interaction.

Context-outcome learning is vHPC-dependent

We next tested if vHPC is indeed required to encode context-outcome (C-O) associations. Rats with sham or vHPC excitotoxic lesions were trained in a Pavlovian context outcome devaluation task⁷ during which they learned to associate one context (e.g., context A) with one outcome (e.g., grain pellet) and another context (B) with a different outcome (e.g., sugar pellet). One of these outcomes was then devalued via sensory-specific satiety and rats were finally tested in each context (Figure 6A). The dependent variable was the number of food port entries. If rats had successfully learned the C-O associations, they should enter the food port less in the context associated with the devalued outcome relative to the context associated with the still valued outcome.

Food port entries across C-O training sessions did not differ between Sham and Lesion groups (Figure 6B; largest $F_{1,19} = 3.01$, $p=0.10$) however, during the unrewarded tests, it appeared that sham rats were sensitive to devaluation (entries in context paired with devalued outcome < entries in context paired with valued outcome) but vHPC lesion rats were not (Figure 6C), although unfortunately the interaction did not reach statistical significance (group x devaluation interaction: $F_{1,19} = 3.2$, $p=0.09$). Satiety-induced devaluation was effective as both groups consumed less of the devalued outcome compared to the valued outcome during a choice consumption test (data not shown; effect of outcome only: $F_{1,19} = 79.36$, $p<0.01$). Lesions of vHPC also had no impact on general exploration in an open field (Figure S5A).

We next attempted to replicate these results using our chemogenetic approach to inhibit vHPC during C-O training or during the retrieval test. Inhibition of vHPC had no observable effect across C-O training (Figure 6D; session effect only: $F_{1,54} = 26.17$, $p<0.01$). However, at test (Figure 6E), vHPC-GFP rats were sensitive to devaluation (entries in context paired with devalued outcome < entries in context paired with valued outcome), but sensitivity was abolished in vHPC-hM4Di rats that received CNO during training or during the retrieval test (effect of devaluation and virus: smallest $F_{1,54} = 4.41$, $p=0.04$; overall virus x devaluation interaction: $F_{1,54} = 10.22$, $p<0.01$; post-hoc virus x devaluation interaction for rats receiving CNO during training: $F_{1,54} = 5.77$, $p=0.02$; and during test: $F_{1,54} = 4.49$, $p=0.04$). Indeed, a simple effect of devaluation was detected for both vHPC-GFP groups (smallest $F_{1,54} = 10.24$, $p<0.01$) but not for vHPC-hM4Di groups (largest $F_{1,54} = 0.95$, $p=0.34$). Again, both groups consumed more of the valued outcome during the choice consumption test

(data not shown; outcome effect only: $F_{1,54} = 132.67$, $p < 0.01$). Thus, vHPC inhibition impaired the encoding and retrieval of context-outcome associations.

Next, we inhibited dHPC during C-O training or during test. We found no effect of dHPC inhibition on training (Figure 6F; session effect only: $F_{1,32} = 15.62$, $p < 0.01$). At test (Figure 6G), all groups showed intact devaluation except the dHPC-hM4Di group that received CNO at test (effect of devaluation: $F_{1,32} = 35.58$, $p < 0.01$; overall treatment x devaluation interaction: $F_{1,32} = 7.61$, $p = 0.01$; virus x devaluation interaction that approached significance: $F_{1,32} = 3.77$, $p = 0.06$; significant post-hoc treatment x devaluation interaction for hM4Di rats: $F_{1,32} = 5.53$, $p = 0.03$, but not for GFP rats: $F_{1,32} = 2.34$, $p = 0.14$; simple effect of devaluation for all groups: smallest $F_{1,32} = 8.48$, $p = 0.01$, except the dHPC-hM4Di group receiving CNO at test: $F_{1,32} = 0.08$, $p = 0.78$). Once again, the consumption test results indicated that satiety-induced devaluation was intact for all groups (data not shown; outcome effect only: $F_{1,32} = 121.50$, $p < 0.01$). Thus, dHPC inhibition impairs the retrieval, but not encoding, of C-O associations.

Overall, these results show that vHPC, but not dHPC, is required to learn C-O associations, which is a prerequisite for successful adaptation to contingency degradation. Both vHPC and dHPC were required for the retrieval of C-O associations. Neither vHPC or dHPC inhibition affected general exploration in an open field (Figure S5B-C).

A

Context outcome devaluation		
Training	Devaluation	Test
Context 1 → O1	O1 or O2	Context 1 vs
Context 2 → O2		Context 2

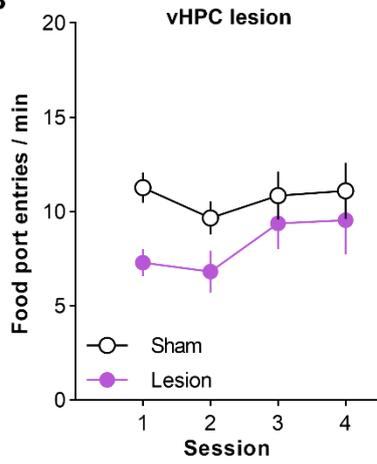
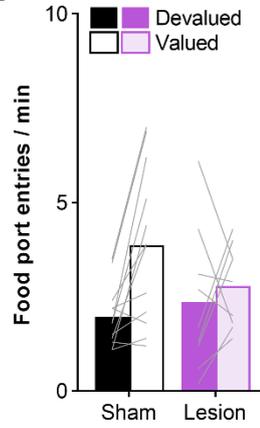
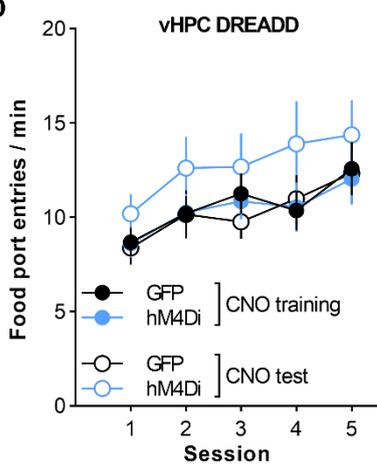
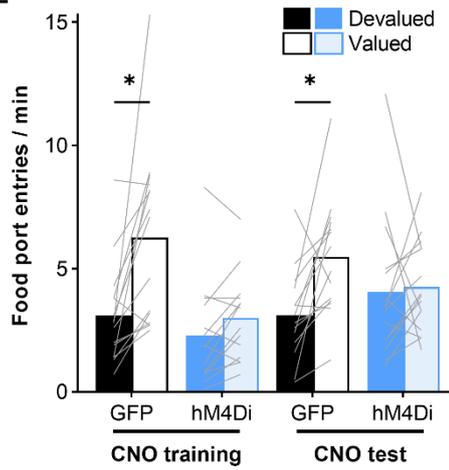
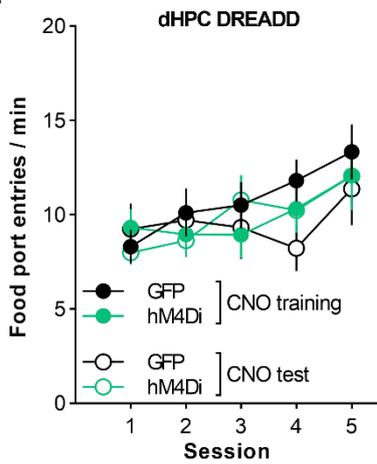
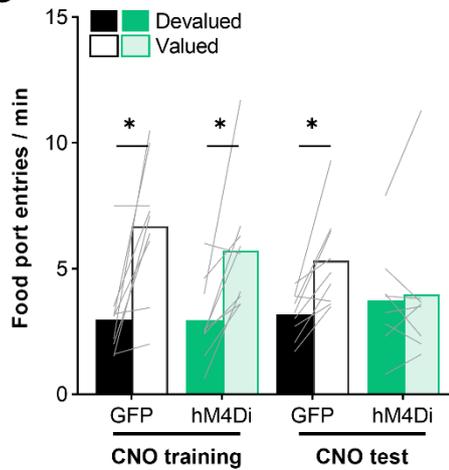
B**C****D****E****F****G**

Figure 6. vHPC, but not dHPC, encodes context-outcome associations.

A. Rats were trained to associate one context (e.g., context A) with one outcome (e.g., O1) and another context (B) with another outcome (O2). One outcome was then devalued via satiety and rats were tested in each context. The dependent variable was the number of food port entries.

B. Food port entries (\pm SEM) across context-outcome training, averaged across the two contexts. A new cohort of rats was used in this experiment (see Table S1). One rat was excluded from group Lesion due to a unilateral lesion: Sham ($n = 12$); Lesion ($n = 9$).

C. Food port entries during test, averaged across the two contexts.

D. Food port entries (\pm SEM) across context-outcome training, averaged across the two contexts for vHPC-GFP and vHPC-hM4Di rats (see Table S1): CNO at degradation training (CNO degrad.): GFP ($n = 15$), hM4Di ($n = 14$), and CNO at test (CNO test): GFP ($n = 14$), hM4Di ($n = 15$).

E. Food port entries during test averaged across the two contexts for vHPC-GFP and vHPC-hM4Di rats. *simple effect of devaluation.

F. Food port entries (\pm SEM) across context-outcome training, averaged across the two contexts for dHPC-GFP and dHPC-hM4Di rats (see Table S1): CNO at training (CNO degrad.): GFP ($n = 10$), hM4Di ($n = 9$), and CNO at test (CNO test): GFP ($n = 9$), hM4Di ($n = 8$).

G. Rate of food port entries during test, averaged across the two contexts, for dHPC-GFP and dHPC-hM4Di rats. *simple effect of devaluation

Inhibition of vHPC terminals in mPFC impairs sensitivity to A→O contingency degradation and context-outcome learning.

Finally, we asked how information encoded in vHPC makes contact with the broader neural circuit to support sensitivity to the causal relation between action and outcome. The medial prefrontal cortex (mPFC), particularly its dorsal subregion, is required to adapt to changes in A→O contingencies²⁷⁻²⁹ and receives direct projections from vHPC³⁰⁻³⁵. We thus hypothesized that input from vHPC to mPFC may be critical to update A→O contingencies. We used DREADD-mediated inhibition of vHPC terminals in mPFC by combining our viral injections in vHPC with intracerebral infusions of CNO or artificial cerebrospinal fluid (aCSF) in the mPFC (Figure 7A-C).

Rats were first trained in contingency degradation. CNO was microinjected across degradation training and aCSF was microinjected at test. This allowed us to

inhibit vHPC terminals in mPFC specifically during degradation training as we identified this as the critical period for vHPC involvement (see Figure 3). Performance across instrumental training did not differ between groups (Figure 7D; session effect only: $F_{1,13} = 41.57$, $p < 0.01$) and, across degradation training, both groups decreased their responding on the degraded lever relative to the non-degraded lever (Figure 7E; lever effect only: $F_{1,13} = 22.99$, $p < 0.01$). Importantly, during the choice test (Figure 7F), we observed that inhibition of vHPC terminals in mPFC during degradation training rendered rats unable to subsequently bias their choice towards the non-degraded action (effect of lever: $F_{1,13} = 5.13$, $p = 0.04$; group x lever interaction: $F_{1,13} = 4.95$, $p = 0.04$; simple effect for group GFP: $F_{1,13} = 10.80$, $p < 0.01$, but not group hM4Di: $F_{1,13} < 0.01$, $p = 0.98$). Activation of the vHPC projection to mPFC is therefore required to detect a change in the A→O contingency.

We then trained the same rats in context outcome devaluation. We microinjected CNO during training and aCSF at test to inhibit vHPC terminals in mPFC only during C-O learning. Inhibition of these terminals decreased food port entries across C-O training (Figure 7G; effect of group: $F_{1,14} = 4.81$, $p = 0.045$; and session: $F_{1,14} = 16.3$, $p < 0.01$; no interaction: $F_{1,14} = 0.11$, $p = 0.75$) however, both groups consumed all the pellets and, therefore, experienced the same amount of C-O pairings. Moreover, the latency to first food port entry across sessions did not differ between groups suggesting that both groups learned the predictive value of the contexts (data not shown; session effect only: $F_{1,14} = 5.81$, $p = 0.03$).

At test (Figure 7H), GFP rats showed selective devaluation and entered the food port more in the context associated with the valued outcome relative to the context associated with the devalued outcome, but this was not the case for group hM4Di (effect of group, devaluation, and group x devaluation interaction: smallest

$F_{1,14} = 4.85$, $p=0.045$; simple effect for group GFP: $F_{1,14} = 13.95$, $p<0.02$, but not for group hM4Di: $F_{1,14} = 0.13$, $p=0.73$). Given the difference between groups during C-O training, we also expressed the test data as a percentage of food port entries in each context on the final day of training. Again, group hM4Di failed to show selective outcome devaluation (Figure 7I; effect of devaluation: $F_{1,14} = 17.25$, $p=0.01$; group x devaluation interaction: $F_{1,14} = 6.04$, $p=0.03$; simple effect for group GFP: $F_{1,14} = 24.97$, $p<0.01$, but not for group hM4Di: $F_{1,14} = 1.28$, $p=0.28$). Sensitivity to selective satiety was intact for both groups during the consumption test (data not shown; effect of outcome only: $F_{1,14} = 38.08$, $p<0.01$). Finally, inhibition of vHPC terminals in mPFC did not impact general exploration in an open field (Figure S5D). These data show that CNO-hM4Di inactivation of vHPC terminals in mPFC impaired context-outcome learning.

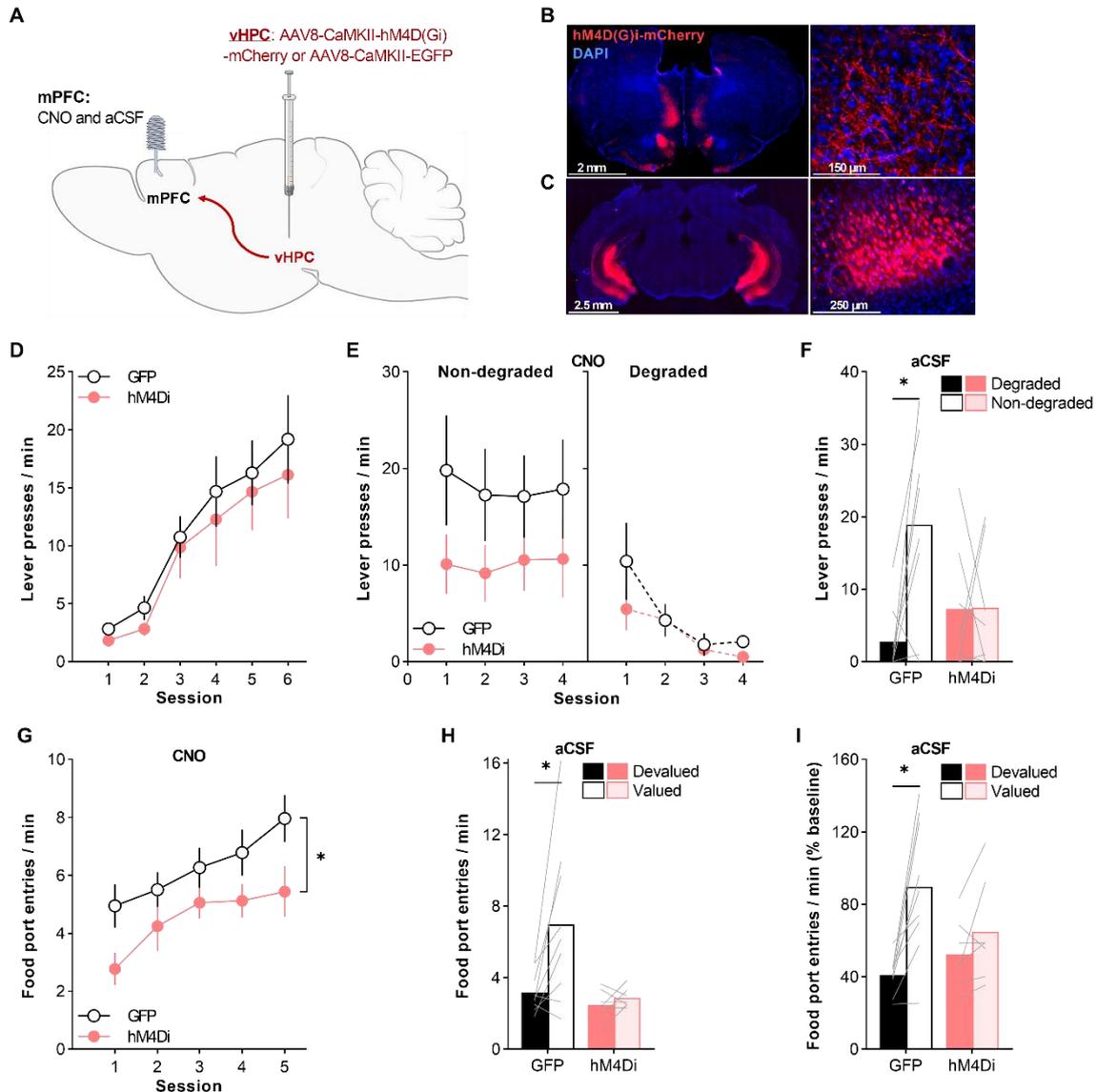


Figure 7. Inhibition of the vHPC terminals in mPFC impairs sensitivity to instrumental contingency degradation and context-outcome learning.

A. Strategy used to inhibit ventral hippocampus (vHPC) input to medial prefrontal cortex (mPFC). Rats were injected with the GFP or hM4Di virus in vHPC and a bilateral cannula was implanted above mPFC. CNO or artificial cerebrospinal fluid (aCSF) was microinjected in mPFC. Rats were trained in contingency degradation (GFP: $n = 8$; hM4Di: $n = 7$) followed by context-outcome devaluation (GFP: $n = 9$; hM4Di: $n = 7$). One GFP rat was excluded from contingency degradation for performing > 2 standard deviations from the mean.

B. Representative image of vHPC terminals in the mPFC (scale: 2 mm). Inset: magnification of vHPC efferent fibres in mPFC (scale: 150 μ m). The infusion cannula projected 1.5 mm from the tip of the guide (see Figure S1D).

C. Representative image of viral expression (AAV8-CaMKII-hM4Di-mCherry) in vHPC (see Figure S1E). Scale: 2.5 mm. Inset: magnification of the area of interest, transfected neurons appear in red (scale: 250 μ m).

D. Lever pressing rate (\pm SEM) across instrumental training, averaged across the two levers.

- E.** Lever pressing rate (\pm SEM) across degradation training, presented as performance on the Non-degraded and Degraded levers. Food port entries are shown in **Figure S3D**.
- F.** Lever pressing rate during the test. *simple effect of degradation.
- G.** Rate of food port entries (\pm SEM) across context-outcome training, averaged across the two contexts. *main effect of group
- H.** Rate of food port entries during test, averaged across the two contexts. *simple effect of devaluation.
- I.** Rate of food port entries during test, expressed as % baseline of responding on final training day, averaged across the two contexts. *simple effect of devaluation.

DISCUSSION

Our results demonstrate that perturbation of ventral hippocampus (vHPC) function impairs the monitoring of action→outcome (A→O) contingencies. This impairment is temporally and anatomically specific and results from a failure to calculate background rates of reinforcement based on the formation of context-outcome associations.

Contingency knowledge permits the distinction between dependent and adventitious relationships in the environment^{36–38} and allows us to assess the extent to which performance of an action is predictive of a given outcome. When the predictive status of an action is degraded, the functional validity of that action in relation to other available cues must be ascertained^{39–41}. The context may therefore contribute to goal-directed behaviour by serving as a predictive cue that enters into associative competition with the instrumental action^{16,24,42–44}. Here, we provide evidence for the relationship between contextual learning and the detection of action causality. Latent inhibition of contextual learning attenuated sensitivity to degradation, which provided the psychological basis for the deficits we observed following perturbation of vHPC activity, **at least in male rats**. Given its well-established role in contextual learning^{7,45–49,49,50}, it was indeed hypothesized that hippocampus may contribute to goal-directed behaviour by forming context-outcome

associations and thus allowing animals to differentiate between actions that are causal and those that are not^{3,4,7,36–38}.

Our results confirm that rats with vHPC lesions remain sensitive to outcome value^{4,8} but perturbation of vHPC rendered rats less sensitive to the causal consequences of their actions^{4,8,9}. Importantly, this deficit emerged when vHPC was inhibited during degradation training and not during test, indicating that vHPC is required only during the updating of the A→O contingency. Inactivation of vHPC had no effect on the rate of extinction during test suggesting that the deficit we observed in contingency degradation was not simply a failure of response inhibition (Figure S2). The deficit was also anatomically specific as inhibition of dorsal hippocampus (dHPC) did not affect degradation.

We then showed a specific role for vHPC in encoding context-outcome (C-O) associations^{45,51–53}. A dissociation was observed such that vHPC inhibition impaired the encoding *and* retrieval of C-O associations but dHPC inhibition affected only the retrieval. We recently obtained a similar pattern of results when context acts as a modulator (or occasion setter) of instrumental performance, rather than as a predictive cue⁵⁴. While this suggests that it is learning about the context that is affected and not simply cue-outcome learning, it remains to be confirmed if the functional dichotomy between dHPC and vHPC applies more generally to the encoding and retrieval of cue-outcome associations that are not necessarily contextual^{55,56}. Attempts to characterize the difference between dHPC and vHPC in contextual representations suggest that dHPC represents a precise spatial environment and vHPC is required to link this environmental context with relevant behavioral events. This appears consistent with the current results showing a role for vHPC, but not dHPC, in encoding context-outcome associations^{57,58,67}.

Interestingly, we show that neither dHPC or vHPC is required to retrieve the A→O association. This supports our conclusion that the formation of C-O associations is critical for the online updating of A→O contingencies but, once updated, the C-O association (and, thus, hippocampal activity) is no longer needed. It also suggests that the C-O association is not asserting an inhibitory effect over the intact A→O association. If this were the case, we would expect impaired degradation when vHPC was inhibited during the instrumental test; vHPC inhibition would impair retrieval of the dominant C-O association, which would 'reinstate' the degraded A→O contingency. Instead, it appears that the strengthening of the C-O association across degradation training results in a revaluation or weakening of the A→O association itself⁵⁹.

Other approaches might constitute a more direct test of our interpretations of these results. For example, inactivation of vHPC during context pre-exposure in our latent inhibition experiment may abolish the deficit in degradation. Alternatively, it has been proposed that pairing a discrete cue with outcome delivery during degradation should render this cue a more effective predictor of the outcome compared to either the action or the context³. As hippocampus is not required for cue-reward learning^{6,61}, rats with vHPC inhibition should not show a deficit in contingency degradation. Such experiments would provide additional support for the current findings that the hippocampus is involved in representing the content of contextual, but not instrumental, conditioning episodes⁷.

Bradfield and colleagues¹³ also showed that A→O learning was impaired following inhibition of dHPC. However, this impairment was only observed during initial A→O training when learning is thought to be context-dependent^{13,63,65,66}. With

additional training, it was indeed found that A→O learning was no longer dependent on dHPC activation nor was there a decrease in responding when rats were tested outside the training context. This appears consistent with findings that dHPC cells quickly represent distinct location and conjunctive representations of events in a new environment, while vHPC cell ensembles are shaped by learning to later distinguish between behaviourally meaningful contexts^{57,58,67}. Together, our results suggest that dorsal and ventral hippocampus both contribute to the contextual control of goal-directed behaviour but the psychological nature of their contribution is distinct.

vHPC shares anatomical connections with the broader neurocircuitry known to modulate goal-directed behaviour, including the basolateral amygdala⁶⁸, orbitofrontal cortex^{30,69}, and medial prefrontal cortex (mPFC)^{30–35}. Lesions of basolateral amygdala impair contingency degradation^{70,71} but these deficits likely reflect an inability to relate the sensory features of the outcome to their motivational value rather than a deficit in contingency learning⁷¹. It has also been proposed that vHPC input to medial orbitofrontal cortex (mOFC) supports goal-directed behaviour however, if true, it is again likely that this pathway relays information regarding the motivational properties of a rewarding outcome¹⁰. In addition, mOFC itself is not necessary to adapt to degradation of the A→O contingency⁷². Lateral OFC is also not required to learn A→O associations^{23,73,74} and studies examining the lateral OFC in instrumental contingency degradation have produced mixed results^{75–77}. Nevertheless, it has been reported that inactivation of vHPC neurons projecting to lateral OFC impairs degradation⁹, although the existence of collateral projecting vHPC neurons^{78–80} makes it unclear whether these deficits are specific to inhibition of the vHPC→lateral OFC pathway.

We found that information encoded in vHPC regarding the predictive value of the context is transmitted to mPFC to produce goal-directed behaviour. Dorsal mPFC has a well-established role in encoding the contingent A→O relationship^{27–29,81} and the vHPC→mPFC pathway is implicated in the contextual modulation of other behaviours, including fear^{34,82–85}. Here, hM4Di-mediated inhibition of vHPC terminals in mPFC impaired both instrumental contingency degradation and context-outcome learning, which suggests that the contextual representations encoded in vHPC are interlaced with initial A→O learning in dorsal mPFC. The hippocampus has been proposed as a comparator of expected versus actual outcomes and, in the event of a discrepancy between what is expected and what is obtained, it sends an error signal to mPFC to prevent the execution of the next action⁸⁶. Consistent with this suggestion, inhibition of vHPC input to mPFC prevented the updating of the A→O association, leading animals to persist on a futile behaviour.

The past years have seen a renewed interest in the relationship between context and goal-directed behaviour^{13,87–89}. We provide psychological and neurobiological evidence that vHPC contributes **to the contextual learning that may support goal-directed action in male rats**. We show that vHPC encodes the background rate of reward and transmits this information to mPFC, which allows the animal to detect the relative validity of the action compared with the context as a signal of reward. The origin of the hippocampal contribution to goal-directed action is thus inextricably linked with its role in the contextual regulation of acquired behaviour.

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AUTHOR CONTRIBUTIONS

Conceptualization, Formal analysis, Writing – original draft and review & editing: R.P. and S.L.P. *Investigation and Methodology:* R.P., A.F., and S.L.P. *Project administration, Resources, and Supervision:* S.L.P.

DECLARATION OF INTERESTS

The authors declare no competing interests.

MAIN-TEXT FIGURE LEGENDS

Figure 1. vHPC lesions leave instrumental outcome devaluation intact.

- A.** Representative image sham and ventral hippocampus (vHPC) lesions (see also [Figure S1A](#)). Scale: 2.5 mm. Three rats were excluded from group Lesion due to unilateral lesions: Sham ($n = 11$) and Lesion ($n = 9$).
- B.** Rats were trained on two action→outcome associations (A1→O1; A2→O2). One of the outcomes was devalued via specific satiety (O1 or O2) and rats were then given an unrewarded choice test with the two actions (A1 and A2).
- C.** Lever pressing rate (\pm SEM) across instrumental training, averaged across the two levers.
- D.** Lever pressing rate during the outcome devaluation test ([see also Figure S2A](#)).

Figure 2. vHPC lesions impair sensitivity to instrumental contingency degradation.

- A.** Rats were re-trained on the action→outcome associations (A1→O1; A2→O2) and then one A→O contingency was degraded (e.g., A2 / O2) while the other was maintained (A1→O1). Rats were then given a choice test with the two actions.
- B.** Lever pressing rate (\pm SEM) across degradation training on the Non-degraded and Degraded levers. Food port entries are shown in [Figure S3A](#). *significant group effect in post-hoc two-way ANOVA.
- C.** Lever pressing rate during the test ([see also Figure S2B](#)). *group x lever interaction.
- D.** Lever pressing rate (\pm SEM) during reacquisition on the previously degraded (Prev. Degra.) and previously non-degraded (Prev. Ndegra.) levers. *simple effect of lever.

Figure 3. Silencing vHPC during degradation training diminishes sensitivity to instrumental contingency degradation.

- A.** Representative image of viral expression (AAV8-CamKII-hM4Di-mCherry) in ventral hippocampus (vHPC) (see also [Figure S1B](#)). Scale: 2.5 mm. Inset: magnification of the area of interest, transfected neurons appear in red (scale: 250 μ m). Two GFP and three hM4Di rats were excluded due to misplaced injections. Two GFP rats were also removed due to an experimenter mistake during degradation training, yielding these between-subject group sizes: CNO during degradation training (CNO degrad.): GFP ($n = 13$), hM4Di ($n = 14$), and CNO during test (CNO test): GFP ($n = 14$), hM4Di ($n = 15$).
- B.** Lever pressing rate (\pm SEM) across instrumental training, averaged across the two levers.
- C.** Lever pressing rate (\pm SEM) across degradation training on the Non-degraded and Degraded levers ([see also Figure S4A](#)). Food port entries are shown in [Figure S3B](#).
- D.** Lever pressing rate during the test ([see also Figure S2C and S4B](#)). *simple effect of lever.

Figure 4. Silencing dHPC leaves sensitivity to instrumental contingency degradation intact.

- A.** Representative image of viral expression (AAV8-CamKII-hM4Di-mCherry) in the dorsal hippocampus (dHPC) (see also [Figure S1C](#)). Scale: 2.5 mm. Inset: magnification of the area of interest, transfected neurons appear in red (scale: 250 μ m). Two hM4Di rats were excluded due to misplaced injections: CNO at degradation

training (CNO degrad.): GFP ($n = 10$), hM4Di ($n = 9$), and CNO at test (CNO test): GFP ($n = 9$), hM4Di ($n = 8$).

B. Lever pressing rate (\pm SEM) across instrumental training, averaged across the two levers.

C. Lever pressing rate (\pm SEM) across degradation training on the Non-degraded and Degraded levers. Food port entries are shown in [Figure S3C](#).

D. Lever pressing rate during the test.

Figure 5. Context pre-exposure attenuates sensitivity to instrumental contingency degradation.

A. Lever pressing rate (\pm SEM) across instrumental training, averaged across the two levers.

B. Lever pressing rate (\pm SEM) across degradation training.

C. Lever pressing rate during the test for the Exposed (Exp) and Non-exposed (Non-exp) groups. One rat was excluded from group Exposed for performing > 2 standard deviations from the mean during the test: Exposed ($n = 7$); Non-exposed ($n = 8$).

*significant group x lever interaction.

Figure 6. vHPC, but not dHPC, encodes context-outcome associations.

A. Rats were trained to associate one context (e.g., context A) with one outcome (e.g., O1) and another context (B) with another outcome (O2). One outcome was then devalued via satiety and rats were tested in each context. The dependent variable was the number of food port entries.

B. Food port entries (\pm SEM) across context-outcome training, averaged across the two contexts. A new cohort of rats was used in this experiment ([see Table S1](#)). One rat was excluded from group Lesion due to a unilateral lesion: Sham ($n = 12$); Lesion ($n = 9$).

C. Food port entries during test, averaged across the two contexts.

D. Food port entries (\pm SEM) across context-outcome training, averaged across the two contexts for vHPC-GFP and vHPC-hM4Di rats ([see Table S1](#)): CNO at degradation training (CNO degrad.): GFP ($n = 15$), hM4Di ($n = 14$), and CNO at test (CNO test): GFP ($n = 14$), hM4Di ($n = 15$).

E. Food port entries during test averaged across the two contexts for vHPC-GFP and vHPC-hM4Di rats. *simple effect of devaluation.

F. Food port entries (\pm SEM) across context-outcome training, averaged across the two contexts for dHPC-GFP and dHPC-hM4Di rats ([see Table S1](#)): CNO at training (CNO degrad.): GFP ($n = 10$), hM4Di ($n = 9$), and CNO at test (CNO test): GFP ($n = 9$), hM4Di ($n = 8$).

G. Rate of food port entries during test, averaged across the two contexts, for dHPC-GFP and dHPC-hM4Di rats. *simple effect of devaluation

Figure 7. Inhibition of the vHPC terminals in mPFC impairs sensitivity to instrumental contingency degradation and context-outcome learning.

A. Strategy used to inhibit ventral hippocampus (vHPC) input to medial prefrontal cortex (mPFC). Rats were injected with the GFP or hM4Di virus in vHPC and a bilateral cannula was implanted above mPFC. CNO or artificial cerebrospinal fluid (aCSF) was microinjected in mPFC. Rats were trained in contingency degradation (GFP: $n = 8$; hM4Di: $n = 7$) followed by context-outcome devaluation (GFP: $n = 9$; hM4Di: $n = 7$). One GFP rat was excluded from contingency degradation for performing > 2 standard deviations from the mean.

- B.** Representative image of vHPC terminals in the mPFC. Scale: 2 mm. Inset: magnification of vHPC efferent fibres in mPFC (scale: 150 μ m). The infusion cannula projected 1.5 mm from the tip of the guide (see Figure S1D).
- C.** Representative image of viral expression (AAV8-CamKII-hM4Di-mCherry) in vHPC (see Figure S1E). Scale: 2.5 mm. Inset: magnification of the area of interest, transfected neurons appear in red (scale: 250 μ m).
- D.** Lever pressing rate (\pm SEM) across instrumental training, averaged across the two levers.
- E.** Lever pressing rate (\pm SEM) across degradation training, presented as performance on the Non-degraded and Degraded levers. Food port entries are shown in [Figure S3D](#).
- F.** Lever pressing rate during the test. *simple effect of degradation.
- G.** Rate of food port entries (\pm SEM) across context-outcome training, averaged across the two contexts. *main effect of group
- H.** Rate of food port entries during test, averaged across the two contexts. *simple effect of devaluation.
- I.** Rate of food port entries during test, expressed as % baseline of responding on final training day, averaged across the two contexts. *simple effect of devaluation.

STAR METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit Anti-RFP	MBL International	Cat#: PM005; RRID:AB_591279
Biotin-SP-AffiniPure Goat Anti-Rabbit IgG	Jackson ImmunoResearch Labs	Cat#: 111-065-003; RRID: AB_2337959
Alexa Fluor 594 Streptavidin	Jackson ImmunoResearch Labs	Cat#: 016-580-084
Bacterial and virus strains		
AAV8-CaMKII-hM4D(Gi)-mCherry	Addgene	RRID:Addgene_50477
AAV8-CaMKII-EGFP	Addgene	RRID:Addgene_50469
Chemicals, peptides, and recombinant proteins		
Clozapine N-Oxide	Enzo Life Sciences	Cat#: BML-NS105-0025
Deposited data		
Source data	This paper	Zenodo: 10.5281/zenodo.8220640
Experimental models: Organisms/strains		
Rat: Long Evans	Janvier, France	
Software and algorithms		
PSY open access statistical program https://www.unsw.edu.au/science/our-schools/psychology/our-research/research-tools/psy-statistical-program	<i>Kevin Bird, Dusan Hadzi-Pavlovic, and Andrew Issac © School of Psychology, University of New South Wales.</i>	
Other		
45 mg grain-based dustless precision pellets	Bio-Serv, NJ	Cat#: F0165
45 mg sugar reward precision pellets	TestDiet	Cat#: 5TUT

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Dr. Shauna Parkes (shauna.parkes@u-bordeaux.fr).

Materials availability

This study did not generate any new reagents.

Data and code availability

All source data is publicly available at Zenodo as of the date of publication. DOI is listed in the key resources table.

Any additional information required to reanalyse the data reported in this paper is available from the lead contact on request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Subjects

Subjects were 178 experimentally naïve, male Long-Evans rats aged 3-4 months (Janvier, France). Rats were housed in pairs in plastic boxes located in a climate controlled room maintained on a 12 h light/dark cycle (lights on at 07:00). All behaviour occurred during the light phase of the cycle. Rats were handled daily for five days before the behavioural procedures and were put on food restriction two days before behaviour to maintain them at approximately 90% of their *ad libitum* feeding weight. All experimental procedures were conducted in agreement with French (council directive 2013-118, February 1, 2013) and European (directive 2010-63, September 22, 2010, European Community) legislations and received project approval from the local Ethics Committee.

METHOD DETAILS

Viral vectors and ligand

In experiments with chemogenetic inhibition, an adeno-associated viral vector carrying the inhibitory hM4Di designer receptor exclusively activated by designer drugs^{22,90} was obtained from Viral Vector Production Unit (Universitat Autònoma de Barcelona, Spain) using a plasmid obtained from Addgene (pAAV-CaMKIIa-hM4D(Gi)-mCherry; Addgene plasmid # 50477; <http://n2t.net/addgene:50477>;

RRID:Addgene_50477; gift from Bryan Roth). The vector used was AAV8-CaMKII-hM4D(Gi)-mCherry (1.46×10^{12} gc/ μ l). In addition, a control vector lacking the hM4Di receptor was obtained from Addgene (AAV8-CaMKII-EGFP; 2.1×10^{12} gc/ μ l Addgene viral prep # 50469-AAV8; <http://n2t.net/addgene:50469>; RRID:Addgene_50469; plasmid was a gift from Bryan Roth).

The exogenous ligand, clozapine-N-oxide (CNO; Enzo Life Sciences) was dissolved in 0.9% saline containing 0.5% of dimethyl sulfoxide (DMSO; Sigma) to obtain a final concentration of 1mg/ml. CNO or vehicle (0.9% saline containing 0.5% DMSO) was injected intraperitoneally (i.p., 1 mg/kg) 30 min before behaviour. For intracerebral CNO infusions, CNO was dissolved in aCSF to obtain a final concentration of 1 mM. CNO or aCSF was intracranially infused over 1 min (0.25 μ l in each hemisphere) 10 min before behaviour^{91–94}.

Surgeries

Rats were anaesthetized using Isoflurane (5% induction; 1-2% maintenance) and mounted on a stereotaxic apparatus (Kopf). The incision site was subcutaneously injected with 0.2ml of local anaesthetic (ropivacaine) and then disinfected using betadine. During surgery, a heating pad was placed under the rat to maintain body temperature and rats were rehydrated with subcutaneous injections of warm saline (0.9%, 10 ml/kg/hour).

For lesion experiments, half of the rats received intra-cerebral injections of N-Methyl-D-aspartic acid (NMDA; 20 μ g/ μ l diluted in 0.1M PBS; group Lesion) and the remaining rats received intra-cerebral injections of 0.1M PBS (group Sham). NMDA or PBS was bilaterally pressure injected using a glass micropipette (Picospritzer II, General Valve Corporation) over 5 minutes to deliver 0.075 μ l in each injection site (three sites per hemisphere). The coordinates to target the ventral hippocampus

were: AP - 5.4, ML \pm 5.3, DV - 7.5; AP - 5.4, ML \pm 5.8, DV - 7.2; and AP - 6.0, ML \pm 4.6, DV - 8.2 (in mm from Bregma; Paxinos and Watson, 2014).

For chemogenetic experiments, the viral vector was injected using a 10 μ l Hamilton syringe connected to a microinjector (UMP3 UltraMicroPump II with Micro4 Controller, World Precision Instruments). For each experiment, half of the rats received the AAV8-CaMKII-hM4D(Gi)-mCherry vector (group hM4Di) and the other half received the control construct (group GFP). 0.8 μ l of AAV was injected in either the ventral hippocampus (vHPC) or the dorsal hippocampus (dHPC) at a rate of 0.2 μ l/min at two sites in each hemisphere, i.e., 1.6 μ l per hemisphere. The co-ordinates for vHPC were the same as those used for NMDA injections. The co-ordinates for dHPC were: AP -3.5, ML \pm 1.4, DV -3.0 and AP -3.8, ML \pm 3.0, DV -2.5 (mm from bregma).

To target vHPC terminals in the medial prefrontal cortex (mPFC), the viral vector (hM4Di or GFP) was injected bilaterally in the vHPC as previously described and rats were implanted with a stainless steel bilateral 26-gauge cannula (5 mm, Plastics One) above the mPFC (AP +3.0, ML \pm 0.5, DV -2.5). The cannula was cemented to the skull and a dummy cannula was kept in the guide at all times.

At the end of surgery, rats were subcutaneously injected with a nonsteroidal anti-inflammatory drug (meloxicam, 2 mg/ml/kg). For 1-2 h post-surgery, rats were individually housed in a warm cage with facilitated access to food and water and were continuously monitored. Finally, all rats were given at least 5 days of post-operative care and were allowed a minimum of 7 days (lesion experiments), 4 weeks (chemogenetic experiments with i.p. CNO injection) or 6 weeks (chemogenetic experiment with intracerebral CNO infusion) to recover before behavioral procedures began.

Intracerebral infusions

During intracerebral infusions, the dummy cannula was removed and all rats (both GFP and hM4Di groups) were infused with CNO (1 mM) or aCSF (test phase) via a 33-gauge bilateral injection cannula. The number and type (CNO or vehicle) was identical for all rats. The internal cannula was connected to a 10 μ L glass syringe attached to an infusion pump (Pump 11 Elite, Harvard Apparatus) and projected 1.5 mm from the tip of the guide cannula. A total volume of 0.25 μ L was delivered per hemisphere at a rate of 0.25 μ L/min. The internal cannula remained in place for a further 1 min after the infusion.

Behavioural procedures and analysis

A timeline of behavioural procedures for each cohort of rats is shown in Table S1.

Apparatus

Instrumental training and testing took place in 8 operant chambers (40 cm width x 30 cm depth x 35 cm height, Imetronic, France) enclosed in sound and light resistant shells. Each chamber was equipped with two pellet dispensers that delivered grain or sugar pellets into a single, common food port when activated. The chambers contained two retractable levers that could be inserted to the left and right of the food port, and a house light illuminated the chamber. Experimental events were controlled and recorded by a computer located in the room. Outcome devaluation and consumption tests occurred in individual polycarbonate feeding cages located in a different room to the operant chambers.

The Pavlovian context-outcome devaluation experiments occurred in two distinct sets of cages located in different rooms (Context A and Context B). These cages were different to those used for instrumental training and testing. Context A was composed of two grey plastic side walls and two transparent Plexiglass walls

(front and back). The back wall was covered with vertical black and white stripes. The floor consisted of stainless steel bars. Drops of 10% lemon scent (Arôme citron, Vahiné, France) were added to the bedding below the cage floor each day. Context B was located in a different experimental room and was composed of two grey plastic side walls and two transparent Plexiglass walls (front and back). The floor consisted of solid Plexiglass with a black and white checkered print and no odour was added.

Instrumental conditioning

Rats were first trained over 3 days to enter the recessed food port to retrieve grain pellets (45 mg; BioServ; 3.35 kcal/g) and sugar pellets (45mg; Test Diet; 3.4 kcal/g) that were automatically delivered on a variable 60 s interval. During each daily 40 min session, rats received 20 pellets of each in a random order. The levers were withdrawn during these sessions. For the next 6 days, rats were given two successive instrumental training sessions per day in which one lever (left or right) was presented and responding on the lever earned a specific food outcome (grain or sugar pellets). Lever-outcome associations were fully counterbalanced between rats and the order (left or right lever first) was counterbalanced between rats and across days. Each session lasted 20 min and rats could earn a maximum of 40 pellets per session (40 grain and 40 sugar pellets maximum per day). For the first 2 days, rats were trained under continuous reinforcement (CRF), then under an RR5 schedule (on average, rewards were delivered after 5 presses) for 2 days, and under an RR10 schedule (on average, reward delivered after 10 presses) for the last 2 days.

Instrumental outcome-specific devaluation

Rats received instrumental training as detailed above and, 24 h later, they were given 1 h access to one of the rewards in polycarbonate feeding cages. The identity of the reward (grain or sugar pellet) was fully counterbalanced. Rats were

habituated to the polycarbonate cages for 1 h the day before the devaluation session. Immediately after devaluation, rats were given an unrewarded choice test in the same operant chambers used for training. During this 10 min test, both levers were presented simultaneously but no rewards were delivered (see Figure 1C). Finally, immediately after the unrewarded test, rats were given a choice consumption test. They were placed back in the feeding cages and were given 10 min access to both food pellets to test the effectiveness of the specific satiety-induced devaluation.

Instrumental contingency degradation

Rats received instrumental training as described above. Then, the contingency for one of the action→outcome (A→O) associations was maintained on the RR10 schedule (non-degraded) but the other A→O association was degraded, such that the pellets previously associated with this lever were randomly delivered throughout the session (Figure 2A). The duration of the degraded session was determined based on individual performance so that the rate of pellets delivered per minute matched the rate of pellets earned during the last day of instrumental training^{96,97}. The degraded A→O association was counterbalanced between rats. For 4 days (chemogenetic experiments) or 6 days (lesion experiment), rats were given two training sessions per day, one for each association (degraded and non-degraded). The order of the sessions (degraded or non-degraded) was fully counterbalanced between rats and across days and the sessions were separated by approximately 10 min. Twenty-four hours after the final degradation training day, rats performed an unrewarded choice test in the same operant chambers used for training. During this 10 min test, both levers were presented simultaneously but no rewards were delivered.

Context exposure and instrumental contingency degradation

Rats received instrumental training (see above) and then half of the rats were given 2.5 h exposure to the training context once per day for 4 days (group Exposed). These rats were placed in the training cages and the house light was turned on. No levers or outcomes were presented during these sessions. The rats that were not exposed to the context (group Non-exposed) were handled and weighed but remained in the colony room. Next, all rats were given 4 days of contingency degradation, which was identical to that previously described except that rats in group Exposed received 30 min of context exposure immediately prior to the first training session of the day. These rats were placed into the cages for 30 min and were then removed. Twenty minutes later, rats in group Exposed and Non-exposed were then placed in the cages for their first contingency degradation session of the day. As explained above, the first session of the day was either the Non-degraded session or the Degraded session and this was counterbalanced across the 4 days of training. Rats in group Exposed thus had 12 h of additional exposure to the context in the absence of the instrumental levers and outcomes prior to the test. Twenty-four hours after the final contingency degradation session, all rats were returned to the operant cages and were given an unrewarded choice test during which both levers were presented simultaneously but no rewards were delivered.

Pavlovian context-outcome devaluation

Rats received 4 days (lesion experiment) or 5 days (chemogenetic experiments) of training with two sessions per day (one in each context), separated by approximately 2 h (lesion experiment) or 10 min (chemogenetic experiment). During each session of 20 min, rats were placed in a context (A or B) and 20 outcomes were delivered (grain or sugar pellets) on a variable interval 60 s schedule.

No levers were presented. Twenty-four hours after the final training session, rats underwent outcome specific devaluation via sensory-specific satiety in polycarbonate feeding cages located in a third room, as previously described. Immediately after devaluation, rats were given one unrewarded test in Context A and one in Context B (see Figure 6A)⁷. The two tests were separated by approximately 10 min. During these tests, food port entries were recorded but no rewards were delivered. The order of the tests and the identity of the devalued outcome was counterbalanced between rats and across groups. Finally, rats were placed back in the polycarbonate feeding cages and were given a consumption test of specific satiety. Forty-eight hours later, rats were given a second outcome devaluation test with the other outcome devalued; if rats previously received grain pellets during satiation, they now received sugar pellets and vice versa.

Open field

At the end of all behaviour, rats were returned to ad libitum food access and the underwent testing to assess the effect of lesions or chemogenetic inhibition on general exploration in an open field environment⁹⁸. In chemogenetic experiments, rats were injected with CNO or vehicle 30 min (i.p. injections) or 10 min (microinjections) prior to the open field. The open field apparatus was located in a different room to all operant chambers. Each rat was individually placed at the same position in a circular open field filled with bedding (1 m diameter, 50 lux at the centre) for 10 min. The 25-cm high wall was made of clear Perspex coated with blue paper. The overall path length travelled by rats was recorded (in centimetres) using a video-tracking system (Viewpoint, Lyon, France).

Tissue processing and immunofluorescence labelling

After behavioral testing, rats received an i.p. injection of lidocaine (1 ml/kg) followed by a lethal i.p. injection of pentobarbital (Euthasol; 1ml/300g; diluted in saline). For lesion experiments, rats were then perfused transcardially with 150 ml of NaCl 0.9% followed by 300 ml of 10% formalin. Brains were stored in 10% formalin for 24 h and were then sectioned coronally at 50 µm using a VT1200S Vibratome (Leica microsystems). Every fourth section was mounted onto gelatin-coated slides, stained with thionine, and cover-slipped with Eukitt (Sigma-Aldrich).

In chemogenetic experiments, rats were perfused transcardially with 4% paraformaldehyde in 0.1M phosphate buffer. Brains were removed and post-fixed in 4% paraformaldehyde overnight. Subsequently, 40 µm coronal sections were cut using a VT1200S Vibratome (Leica Microsystems). Every fourth section was collected to form a series.

To visualize vHPC fibres in the mPFC, immunofluorescence was performed for mCherry. Free-floating sections were prepared by rinsing in 0.1M phosphate buffered saline with 0.3% Triton X-100 (PBST) for 4*5 min, incubated in 0.5% H₂O₂ diluted in 0.1M PBST, rinsed in 0.1M PBST for 4*5 min, blocked (1 h, PBST 0.1M, 4% normal goat serum) and placed in 1:1000 rabbit anti-RFP (red fluorescent protein; PM005 CliniSciences; RRID:AB_591279) diluted in 0.1M PBST at room temperature overnight. Sections were rinsed, incubated in 1:500 Biotin-SP AffiniPure Goat Anti-Rabbit IgG (Jackson ImmunoResearch Labs; 111-065-003; RRID: AB_2337959) diluted in 0.1M PBST for 2 h at room temperature, rinsed again, and then placed in 1:400 Alexa Fluor® 594 Streptavidin (Jackson ImmunoResearch Labs; 016-580-084) diluted in 0.1M PBS. Sections were then rinsed and incubated in 1:5000 bisBenzimide H 33258 (Sigma-Aldrich; 14530) diluted in 0.1M PBS for 15 min.

Finally, sections were rinsed, mounted, and cover-slipped with Fluoromount-G (SouthernBiotech).

The location and size of the lesion or viral expression was determined under a microscope by a trained observer, unaware of the subjects' group designations, using the boundaries defined by the atlas of Paxinos and Watson (2014).

QUANTIFICATION AND STATISTICAL ANALYSIS

All tasks used mixed methods designs with between-subject factors (lesion versus sham; hM4Di versus GFP; CNO versus vehicle) and within-subject factors (session; devalued versus non-devalued; degraded versus non-degraded). The dependent variables were rate of lever presses or food port entries, amount of food consumed (in grams), or latency to first food port entry. Data were analysed using sets of between and within orthogonal contrasts controlling the per contrast error rate at $\alpha = 0.05$ (Harris, 1994; Hays, 1963). Simple effects analyses were conducted to establish the source of significant interactions. Statistical analyses were performed using PSY Statistical Program (<https://www.unsw.edu.au/science/our-schools/psychology/our-research/research-tools/psy-statistical-program>; Kevin Bird, Dusan Hadzi-Pavlovic, and Andrew Issac © School of Psychology, University of New South Wales) and graphs were created using GraphPad Prism. Statistical significance was set at $p \leq 0.05$. Data are presented as mean \pm SEM and individual data points are supplied on histograms depicting test data. Rats performing > 2 standard deviations from the mean were excluded from statistical analyses. Two rats were excluded based on this criterion.

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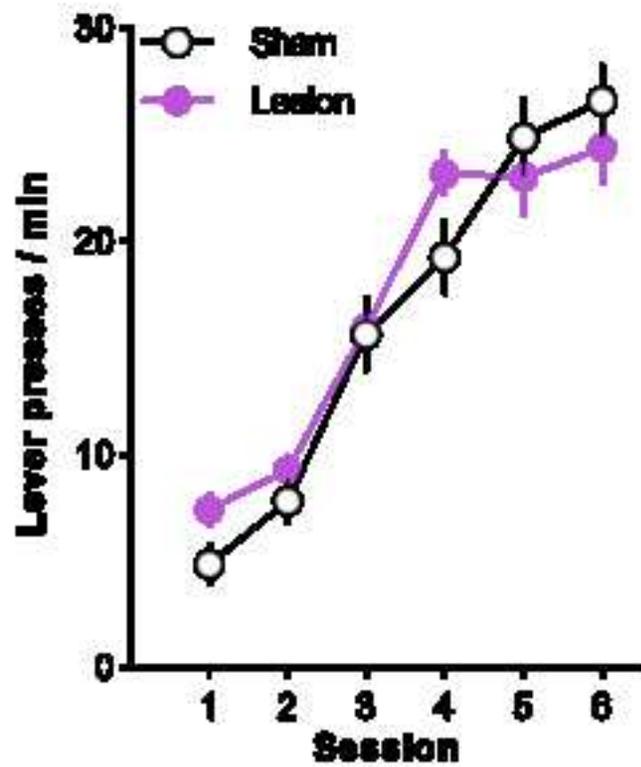
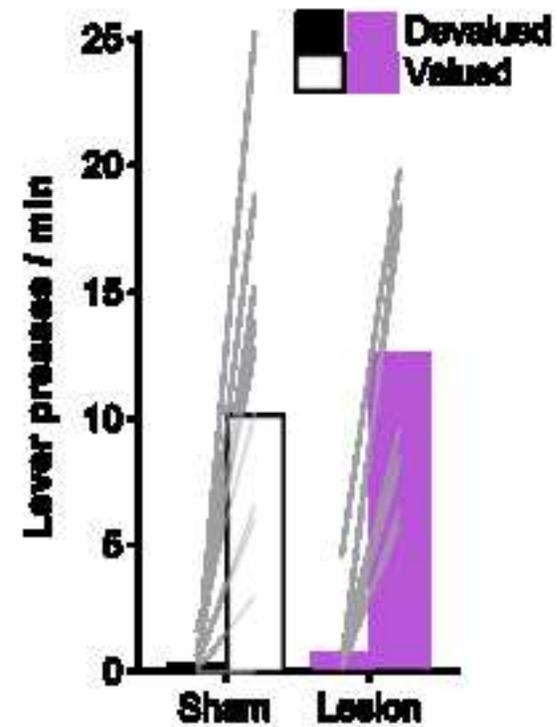
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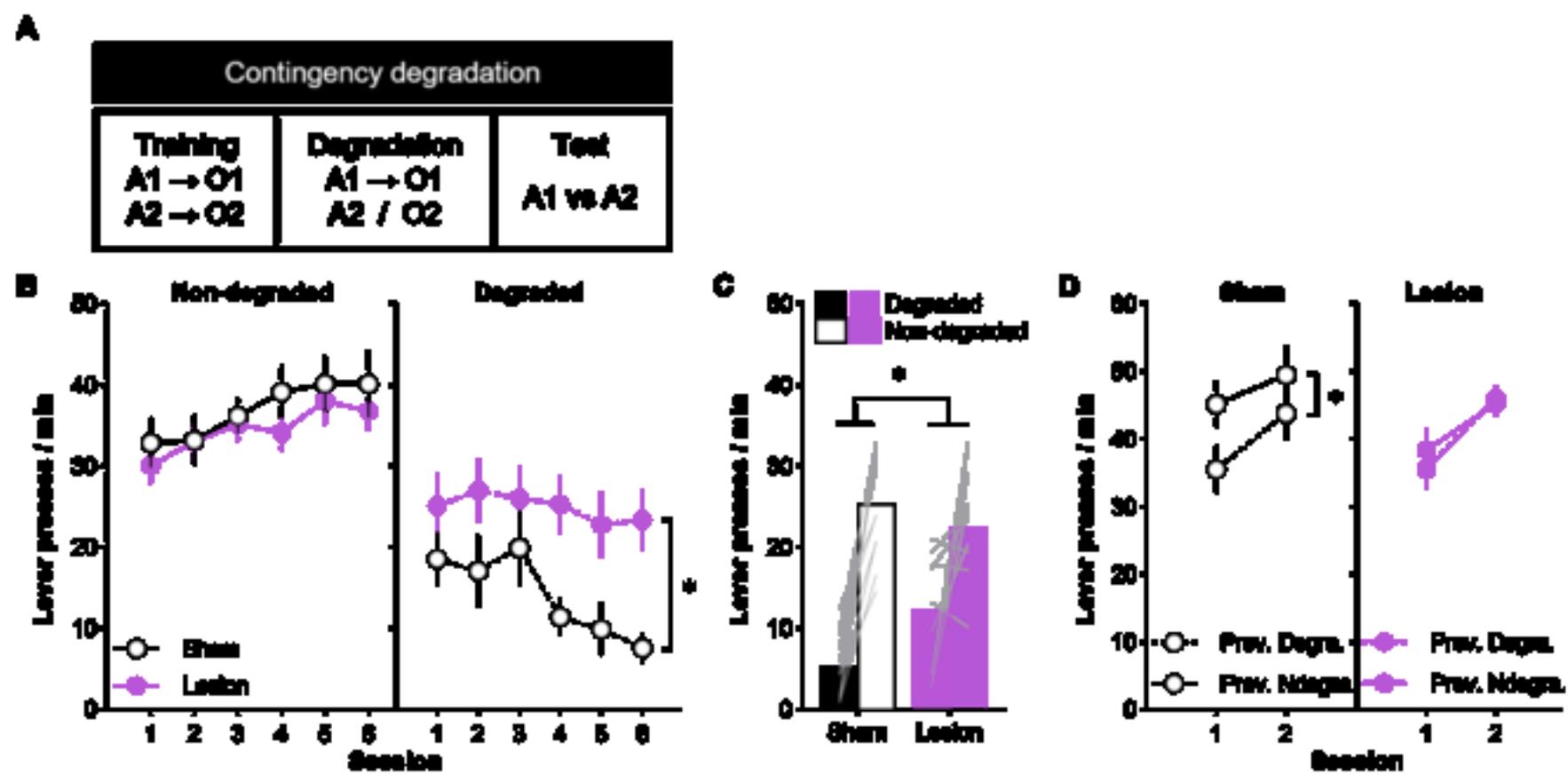
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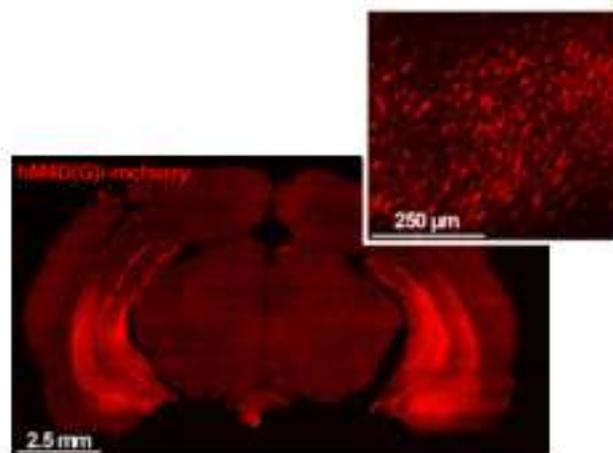
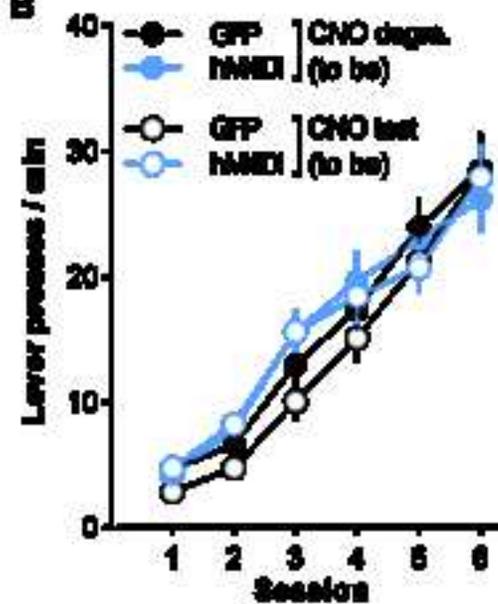
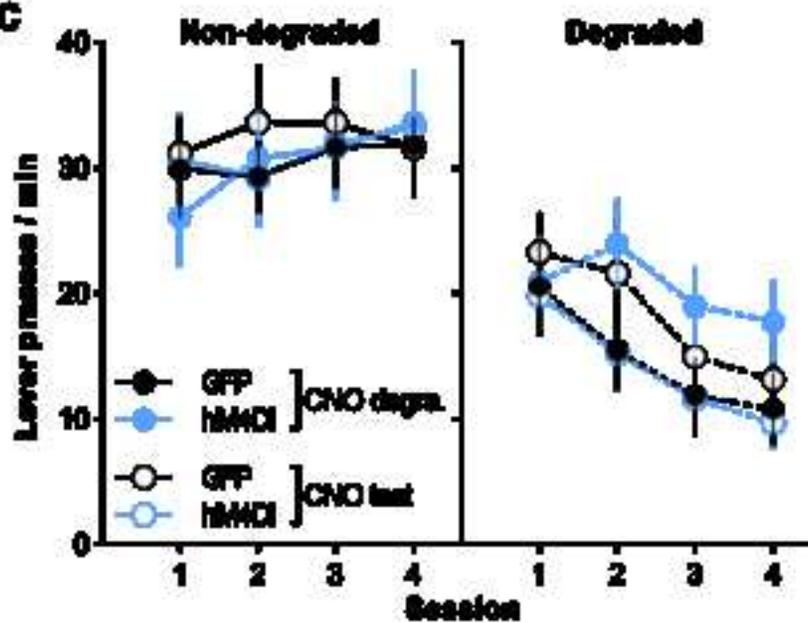
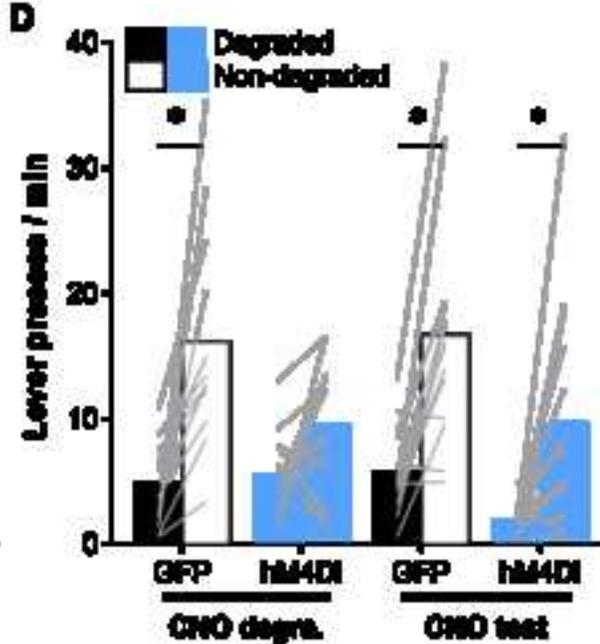
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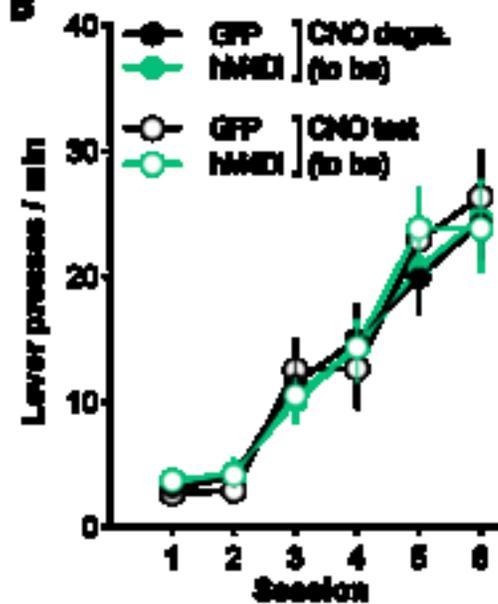
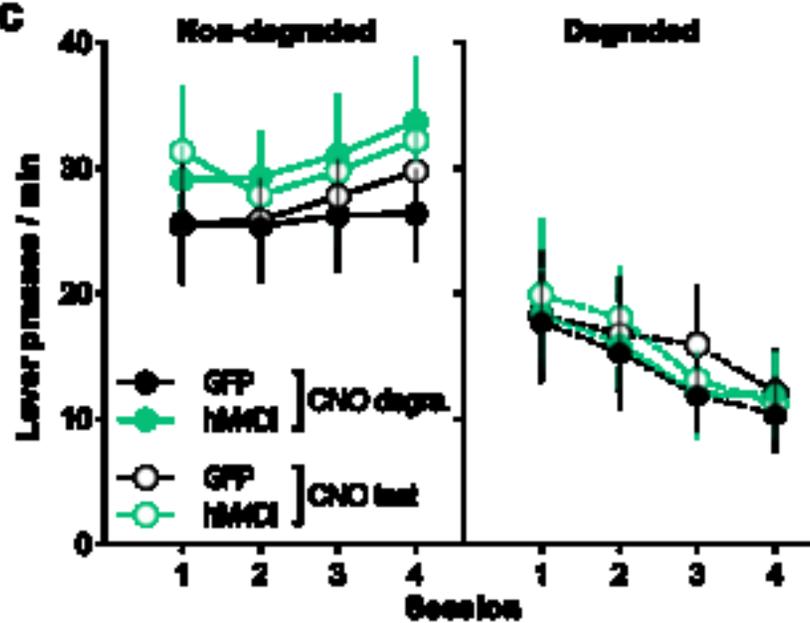
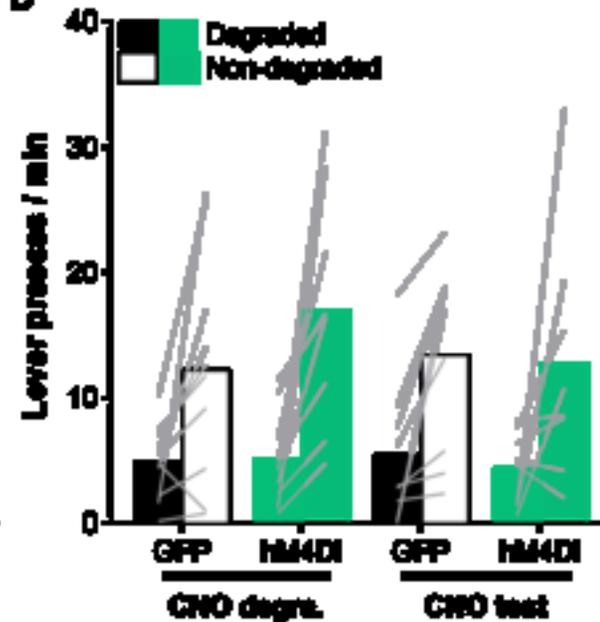
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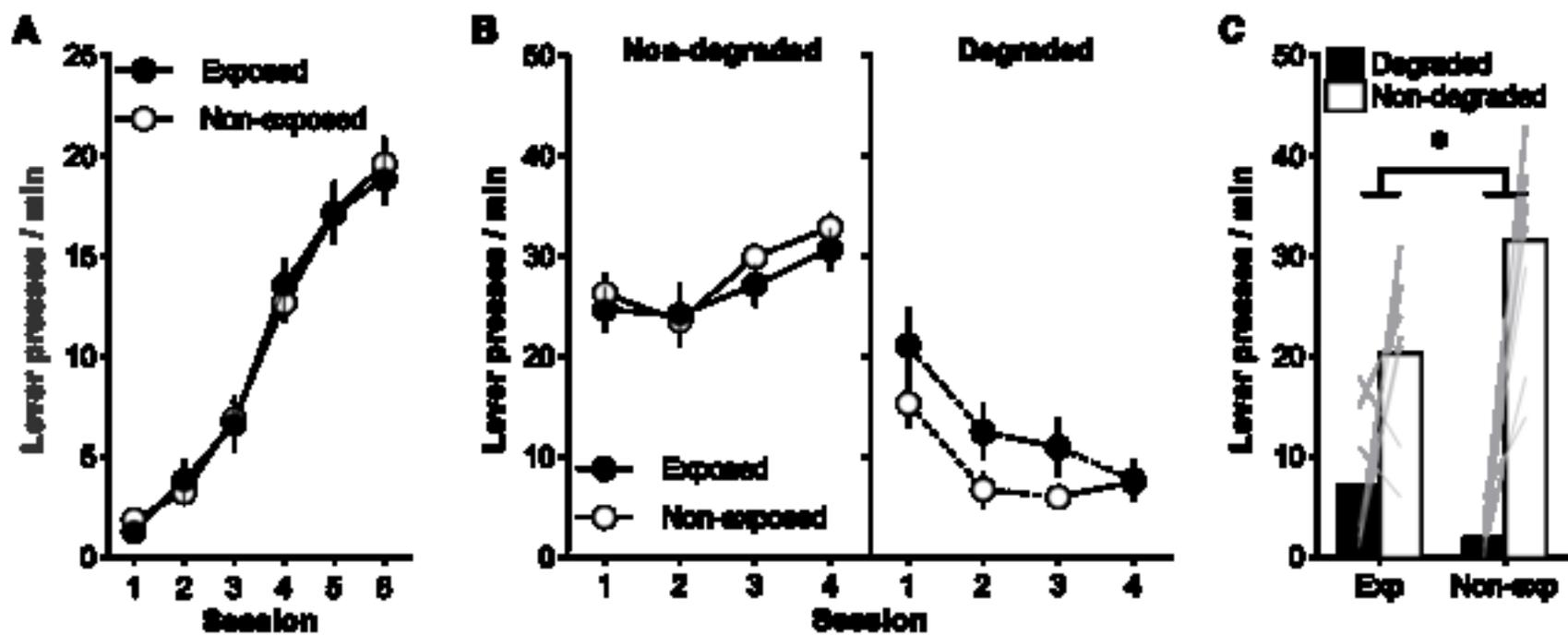
Outcome devaluation		
Training A1 → O1 A2 → O2	Devaluation O1 or O2	Test A1 vs A2

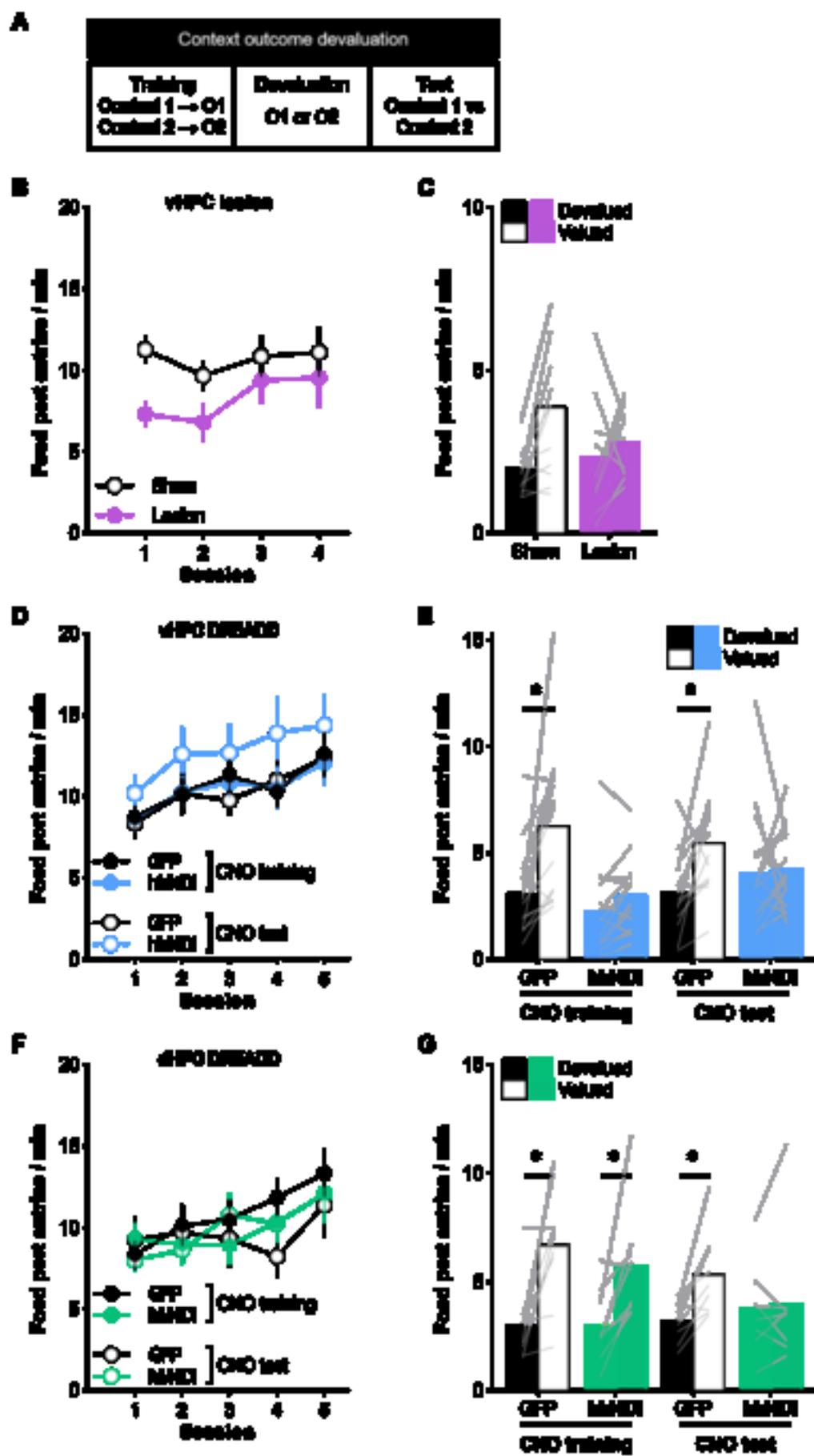
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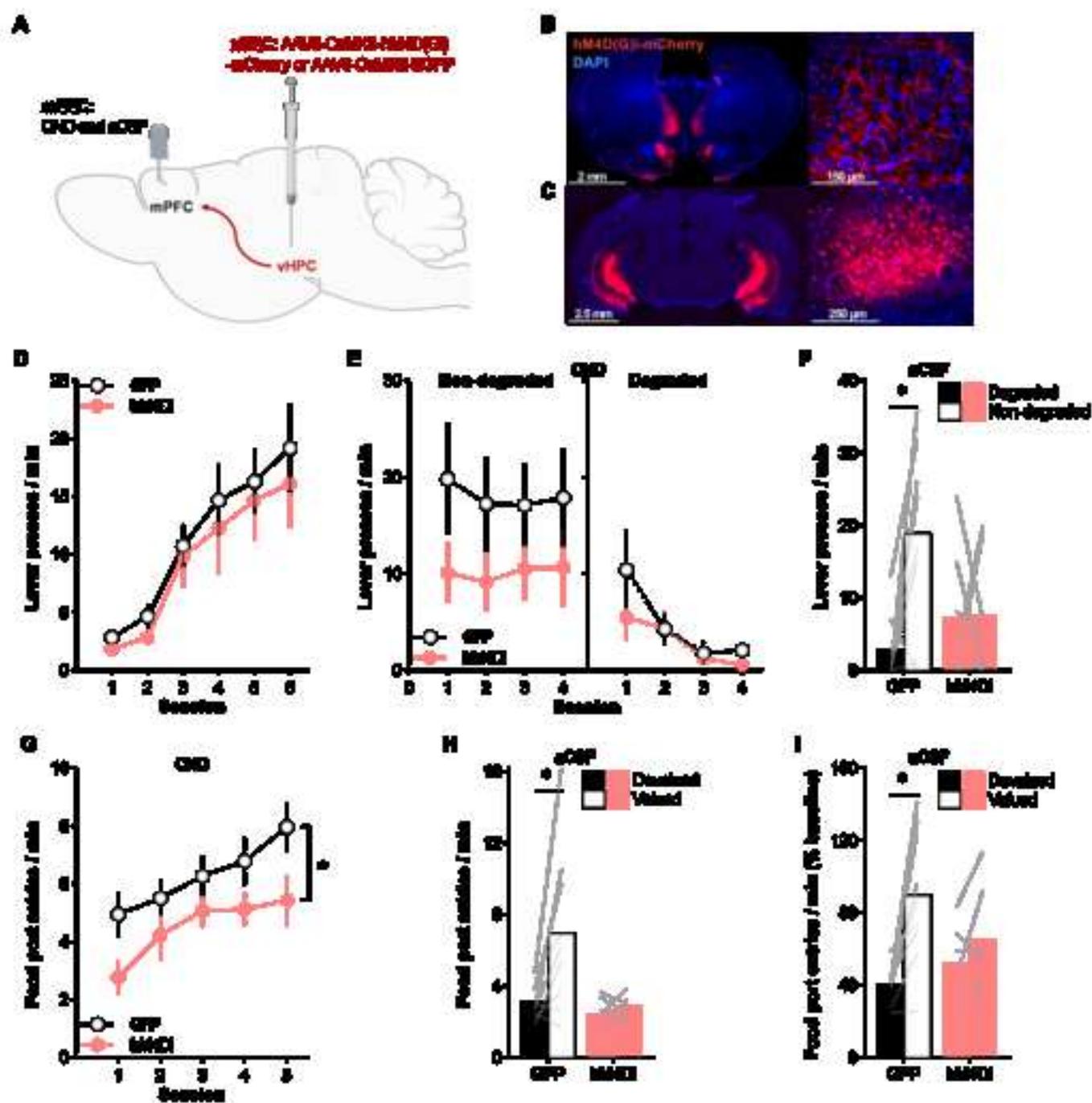


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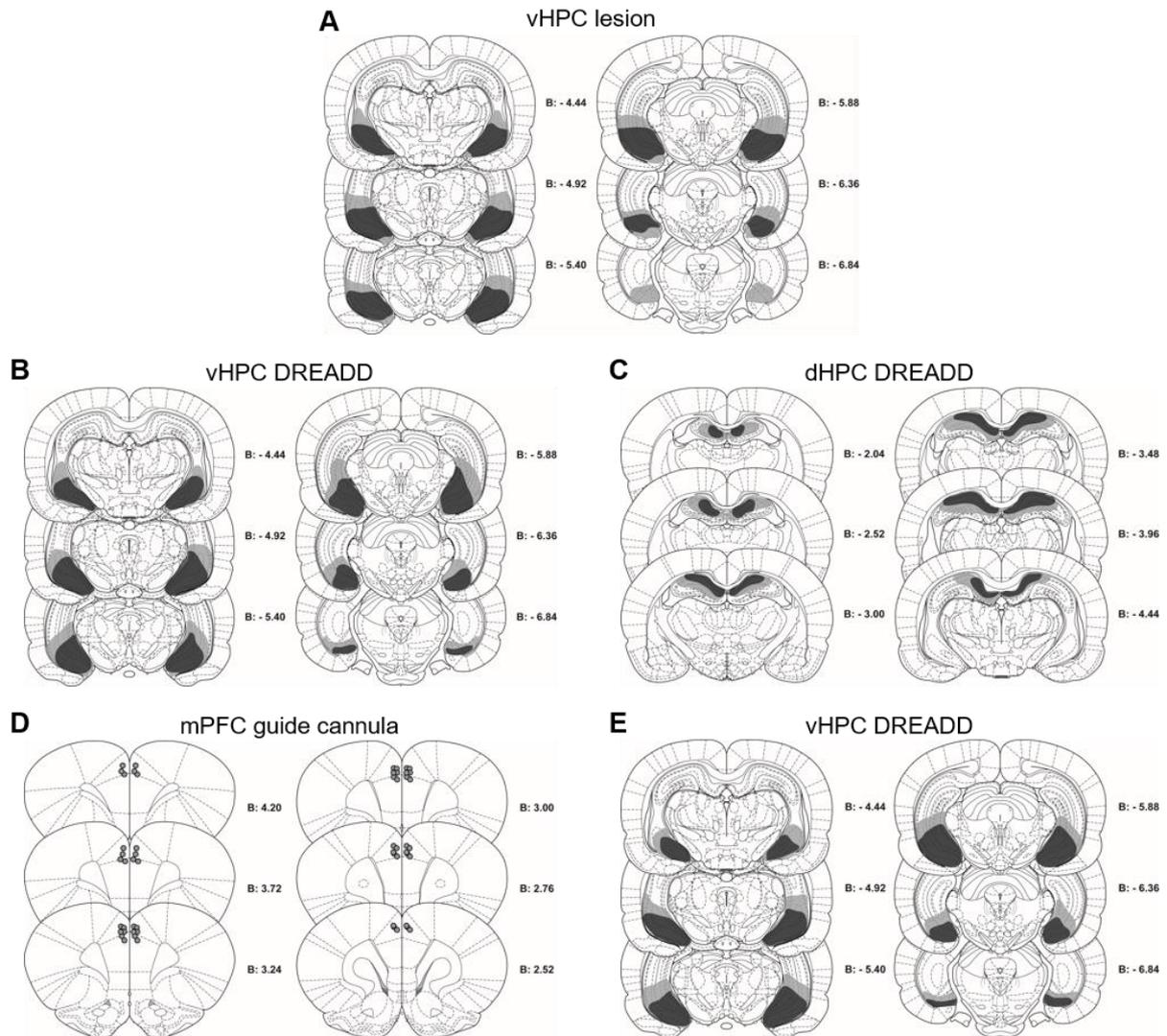


Figure S1. Histological placements showing largest and smallest lesion or viral expression and guide cannula tips for all rats, adapted from Paxinos and Watson¹ (Related to Figures 1-4, 6-7).

A. Schematics showing the largest (grey) and smallest (black) ventral hippocampus (vHPC) lesion from bregma (B): -4.44 to -6.84 (see Figures 1, 2, 6B-C).

B. Schematics showing the largest (grey) and smallest (black) DREADD viral expression in vHPC from B: -4.44 to -6.84 (see Figures 3, 6D-E).

C. Schematics showing the largest (grey) and smallest (black) DREADD viral expression in dorsal hippocampus (dHPC) from B: -2.04 to -4.44 (see Figures 4, 6F-G).

D. Schematic representation of the tip of the guide cannula for each rat in the medial prefrontal cortex (mPFC) from B: +4.20 to +2.52. The infusion cannula projected 1.5 mm from the tip of the guide (see Figure 7).

E. Schematics showing the largest (grey) and smallest (black) DREADD viral expression in vHPC from B: -4.44 to -6.84 (see Figure 7).

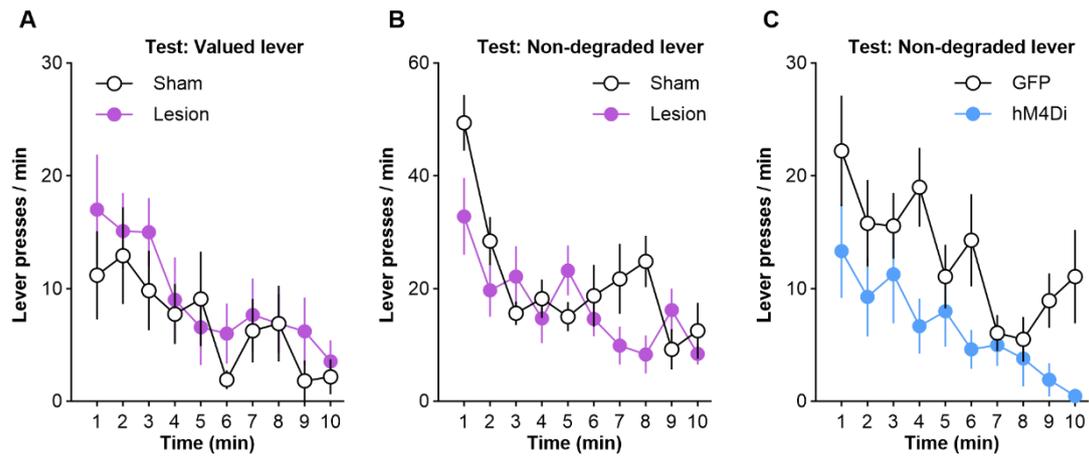


Figure S2. Lesions or chemogenetic inhibition of ventral hippocampus do not affect the rate of extinction during test (Related to Figures 1D, 2C, and 3A).

A. Presses per minute (\pm SEM) on the lever associated with the valued outcome across the unrewarded test session for rats with sham ($n = 11$) and ventral hippocampus (vHPC; $n = 9$) lesions (see Figure 1D). The rate of extinction on the valued lever did not differ between groups (effect of session: $F_{1,18} = 15.65$, $p < 0.01$; no effect of group or interaction: largest $F_{1,18} = 1.74$, $p = 0.20$).

B. Presses per minute (\pm SEM) on the non-degraded lever across the unrewarded test session for sham ($n = 11$) and vHPC lesion ($n = 9$) rats (see Figure 2C). The rate of extinction on the non-degraded lever did not differ between groups (effect of session: $F_{1,18} = 41.76$, $p < 0.01$; effect of group that approached significance: $F_{1,18} = 4.19$, $p = 0.06$; no group \times session interaction: $F_{1,18} = 0.38$, $p = 0.55$).

C. Presses per minute (\pm SEM) on the non-degraded lever across the unrewarded test session for vHPC-GFP ($n = 14$) and vHPC-hM4Di ($n = 15$) rats receiving CNO during test (see Figure 3A). While group vHPC-hM4Di responded less than group vHPC-GFP (effect of group: $F_{1,27} = 8.96$, $p < 0.01$), the rate of extinction did not differ between groups (effect of session: $F_{1,27} = 20.30$, $p < 0.01$; no group \times session interaction: $F_{1,27} = 0.05$, $p = 0.83$).

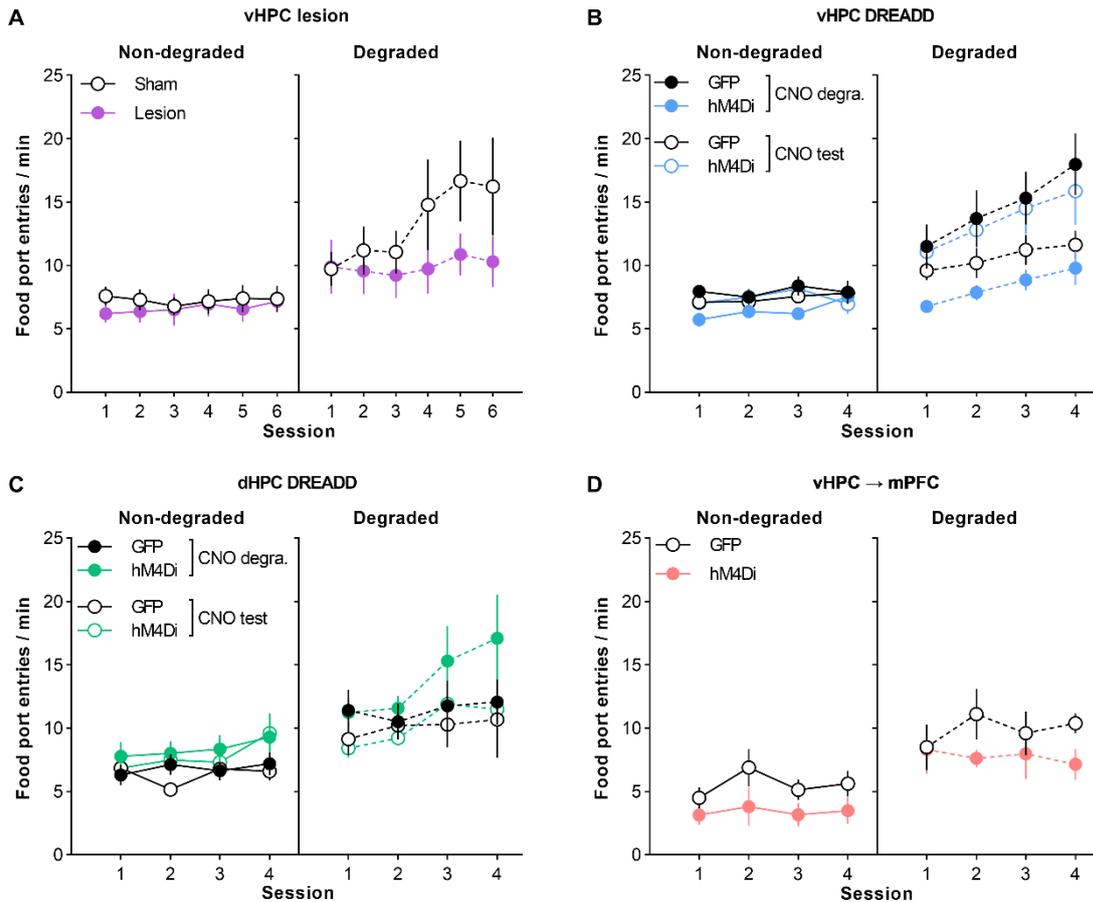


Figure S3. Food port entries during instrumental contingency degradation (Related to Figures 2B, 3C, 4C, 7E).

A. Food port entries (\pm SEM) across degradation training sessions, presented as entries during the Non-degraded (left) and Degraded sessions (right) for rats with sham ($n = 11$) and ventral hippocampus (vHPC; $n = 9$) lesions (see Figure 2B). Both Sham and Lesion rats entered the food port more during the degraded sessions than the non-degraded sessions (effect of degradation: $F_{1,18} = 14.56$, $p < 0.01$; no effect of group or interaction: largest $F_{1,18} = 1.23$, $p = 0.28$).

B. Food port entries (\pm SEM) across degradation training sessions during the Non-degraded (left) and degraded sessions (right) for vHPC-GFP and vHPC-hM4Di rats (see Figure 3C). All groups entered the food port more in the non-degraded versus the degraded session except for the group with vHPC inhibition (blue filled circles) (effect of degradation: $F_{1,52} = 44.68$; $p < 0.01$; no virus or treatment effect: largest $F_{1,52} = 1.9$; $p = 0.17$; virus x treatment interaction: $F_{1,52} = 9.08$; $p < 0.01$; virus x treatment x degradation interaction: $F_{1,52} = 8.29$; $p < 0.01$; simple effect of degradation for all groups: $F_{1,52}$ values > 5.86 ; $p < 0.01$, except the vHPC-hM4Di group that received CNO during degradation training: $F_{1,52} = 1.93$; $p = 0.17$).

C. Food port entries (\pm SEM) across degradation training sessions during the Non-degraded (left) and degraded session (right) for dHPC-GFP and dHPC-hM4Di rats (see Figure 4C). Inhibition of dorsal hippocampus (dHPC) during degradation training had no effect on food port entries and all groups entered the food port more during

the non-degraded session than the degraded session (effect of degradation: $F_{1,32} = 40.00$; $p < 0.01$, no effect of virus, treatment, or any interaction: largest $F_{1,32} = 2.61$; $p = 0.12$).

D. Food port entries (\pm SEM) across degradation training sessions during the Non-degraded (left) and degraded session (right) for rats injected in vHPC with the GFP ($n = 8$) or hM4Di ($n = 7$) virus and microinjected with CNO in medial prefrontal cortex (mPFC) during degradation training (see Figure 7E). Both groups entered the food port more during the degradation session relative to the non-degraded session (effect of degradation: $F_{1,13} = 27.399$, $p < 0.001$; no effect of group or interaction: largest $F_{1,13} = 2.526$, $p = 0.136$).

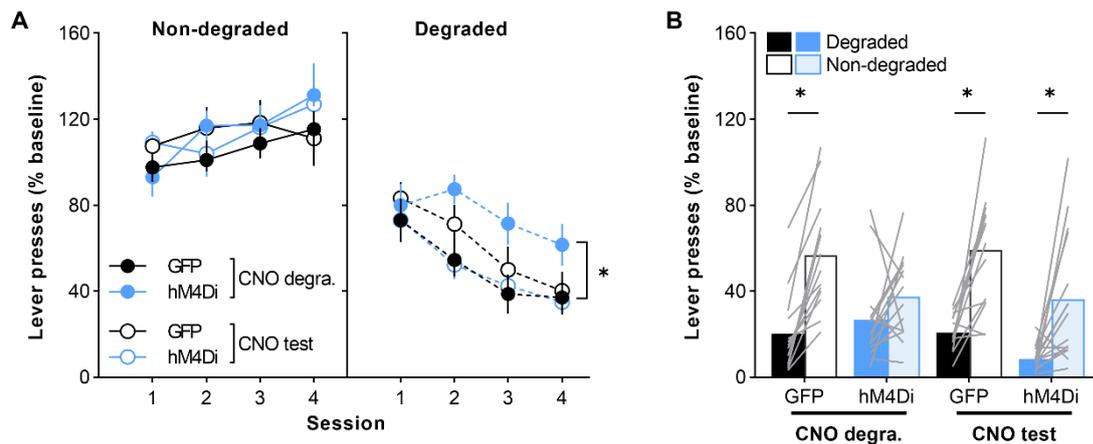


Figure S4. Responding expressed as % baseline for rats with vHPC inhibition (Related to Figure 3C-D).

A. When expressed as a % of responding during the final instrumental training session (baseline), temporary inhibition of vHPC led to impaired performance across degradation training (effect of degradation: $F_{1,52} = 132.72$; $p < 0.01$; no treatment or effect: largest $F_{1,52} = 1.43$; $p = 0.24$; overall virus x treatment interaction: $F_{1,52} = 4.71$; $p = 0.04$). Post-hoc analyses revealed a virus x treatment interaction for the degraded lever ($F_{1,52} = 6.44$; $p = 0.01$) but not for the non-degraded lever ($F_{1,52} = 0.55$; $p = 0.46$). Simple effect analyses conducted on the degraded lever confirmed no difference between vehicle groups ($F_{1,52} = 1.22$; $p = 0.28$) but a significant difference between CNO groups, such that the vHPC-hM4Di group injected with CNO pressed more on the degraded lever than the vHPC-GFP group injected with CNO ($F_{1,52} = 6.06$; $p = 0.02$).

B. Expressing the test data as a % of responding during the final instrumental training session (baseline) led to the same findings as the raw data (see Figure 3D). The vHPC-hM4Di group that received CNO during training failed to respond more on the non-degraded lever relative to the degraded lever (effect of degradation and virus: smallest $F_{1,52} = 7.85$; $p = 0.01$; no treatment effect: $F_{1,52} = 0.90$; $p = 0.35$; overall virus x degradation interaction: $F_{1,52} = 5.84$; $p = 0.02$). Post-hoc analyses indicated a virus x degradation interaction for rats receiving CNO during degradation ($F_{1,52} = 5.64$; $p = 0.02$) but not during test ($F_{1,52} = 1.03$; $p = 0.32$), and simple effects for all groups (all $F_{1,52}$ values > 14.61 ; p values < 0.001) except the vHPC-hM4Di group that received CNO during training ($F_{1,52} = 2.07$; $p = 0.16$).

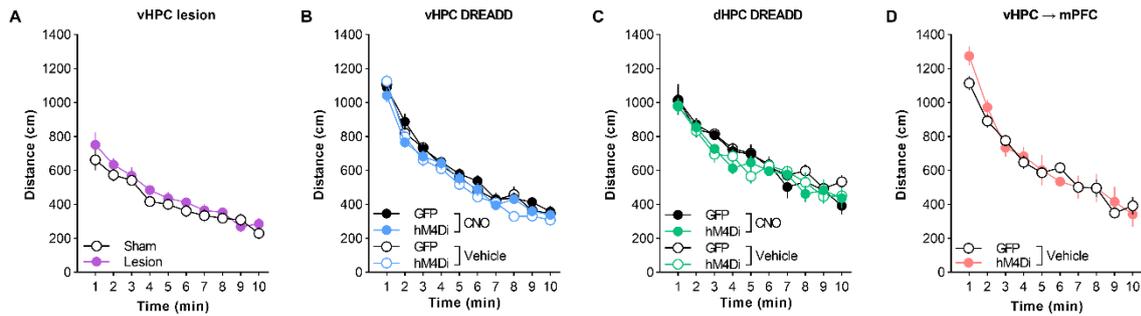


Figure S5. Distance travelled (cm) in an open field for rats with vHPC lesions, vHPC or dHPC chemogenetic inhibition, or inhibition of vHPC terminals in mPFC (Related to Figures 3-4, 6-7).

A. Average distance (\pm SEM) travelled in the open field for rats with sham and ventral hippocampus (vHPC) lesions. The overall level of activity in the open field did not differ between groups and rats exhibited habituation over time (effect of time: $F_{1,13} = 197.359$, $p < 0.001$; no effect of group or interaction: largest $F_{1,13} = 2.100$, $p = 0.171$).

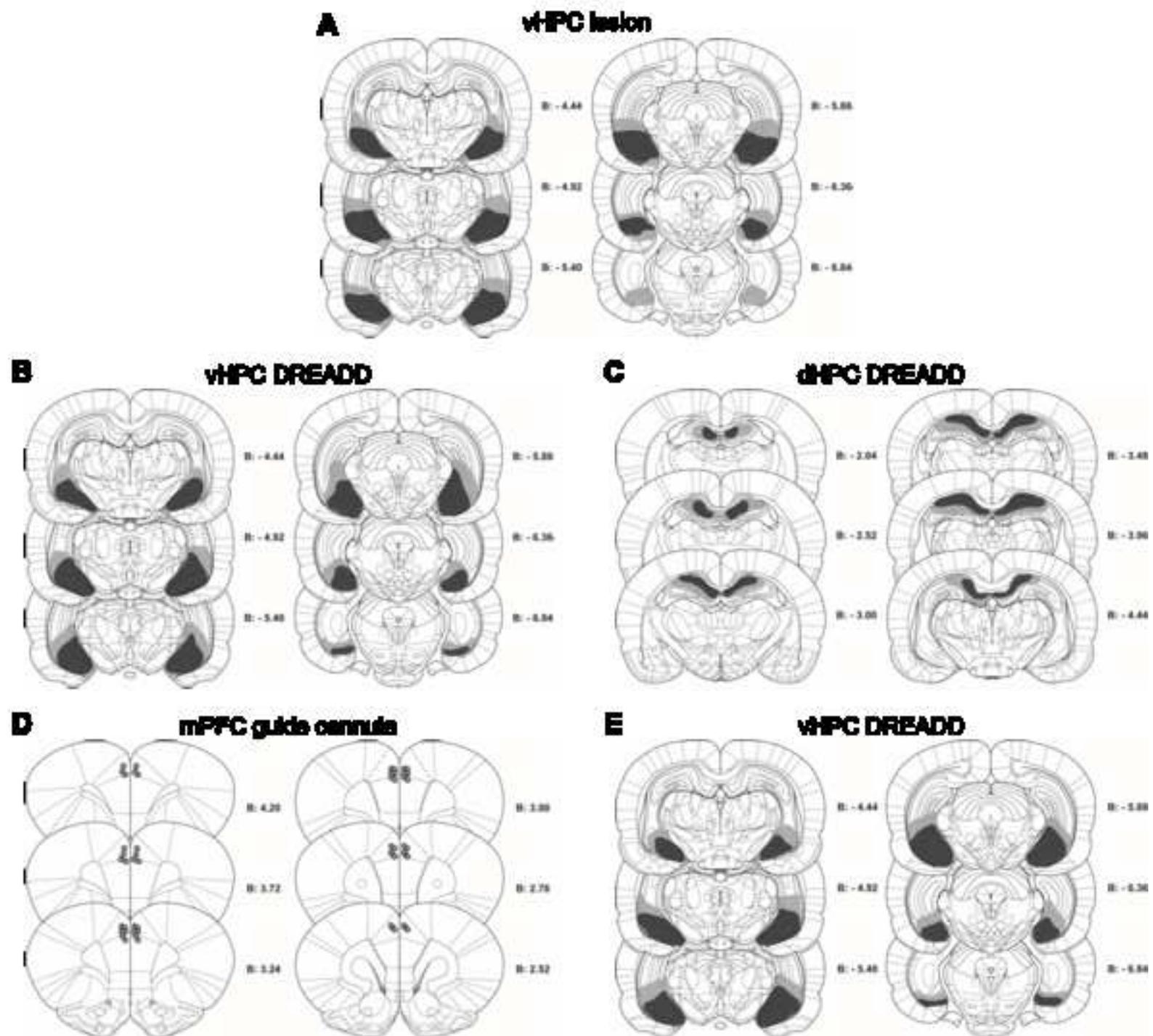
B. Average distance (\pm SEM) travelled for groups vHPC-GFP and vHPC-hM4Di that received an intraperitoneal (i.p.) injection of CNO or vehicle prior to the open field. The distance travelled reduced across time ($F_{1,54} = 835.642$; $p < 0.001$) and did not differ between groups (no effect of virus, treatment, or interactions: largest $F_{1,54} = 3.023$; $p = 0.088$).

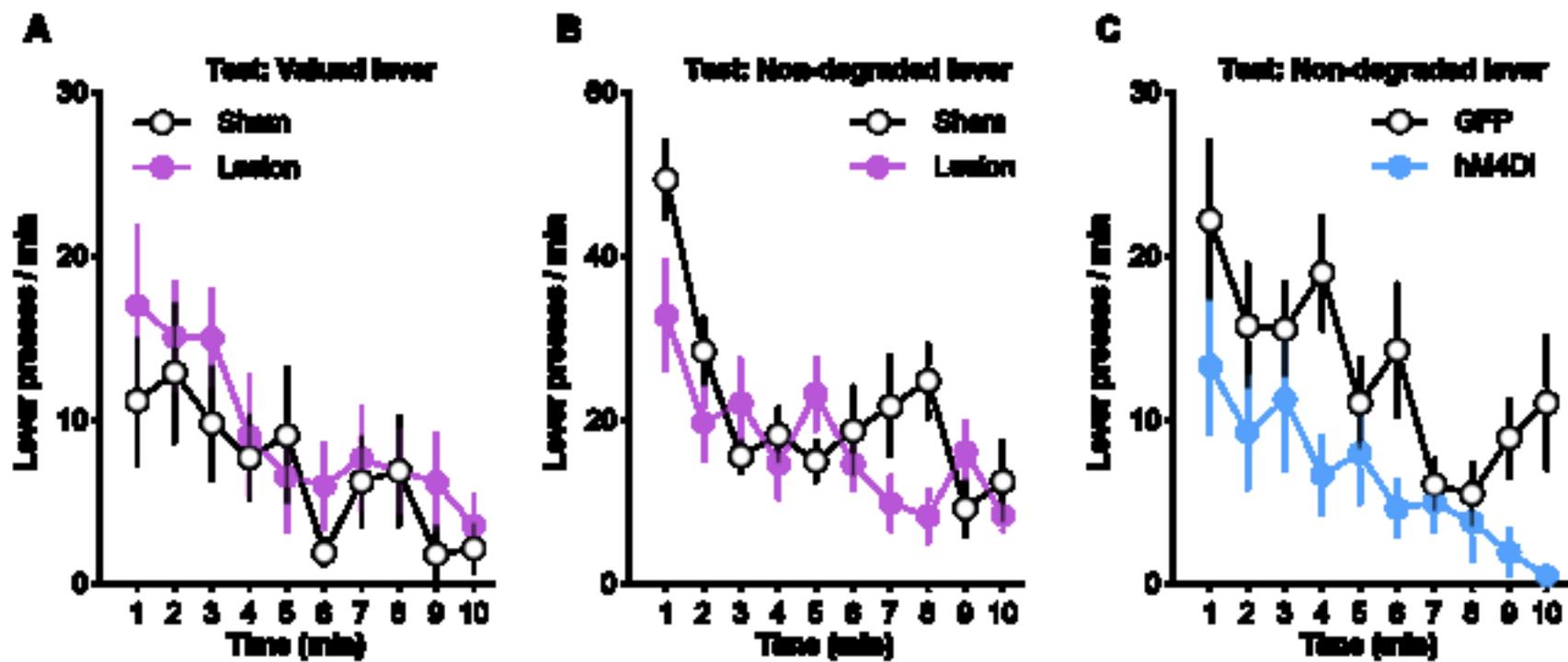
C. Average distance (\pm SEM) travelled for groups dHPC-GFP and dHPC-hM4Di that received i.p. CNO or vehicle prior to the open field. One rat hM4Di rat receiving vehicle was excluded due to a problem with the video-tracking system. The distance travelled reduced across time and did not differ between groups (effect of time: $F_{1,31} = 181.645$; $p < 0.001$; no effect of virus, treatment, or interactions: largest $F_{1,31} = 2.181$; $p = 0.150$).

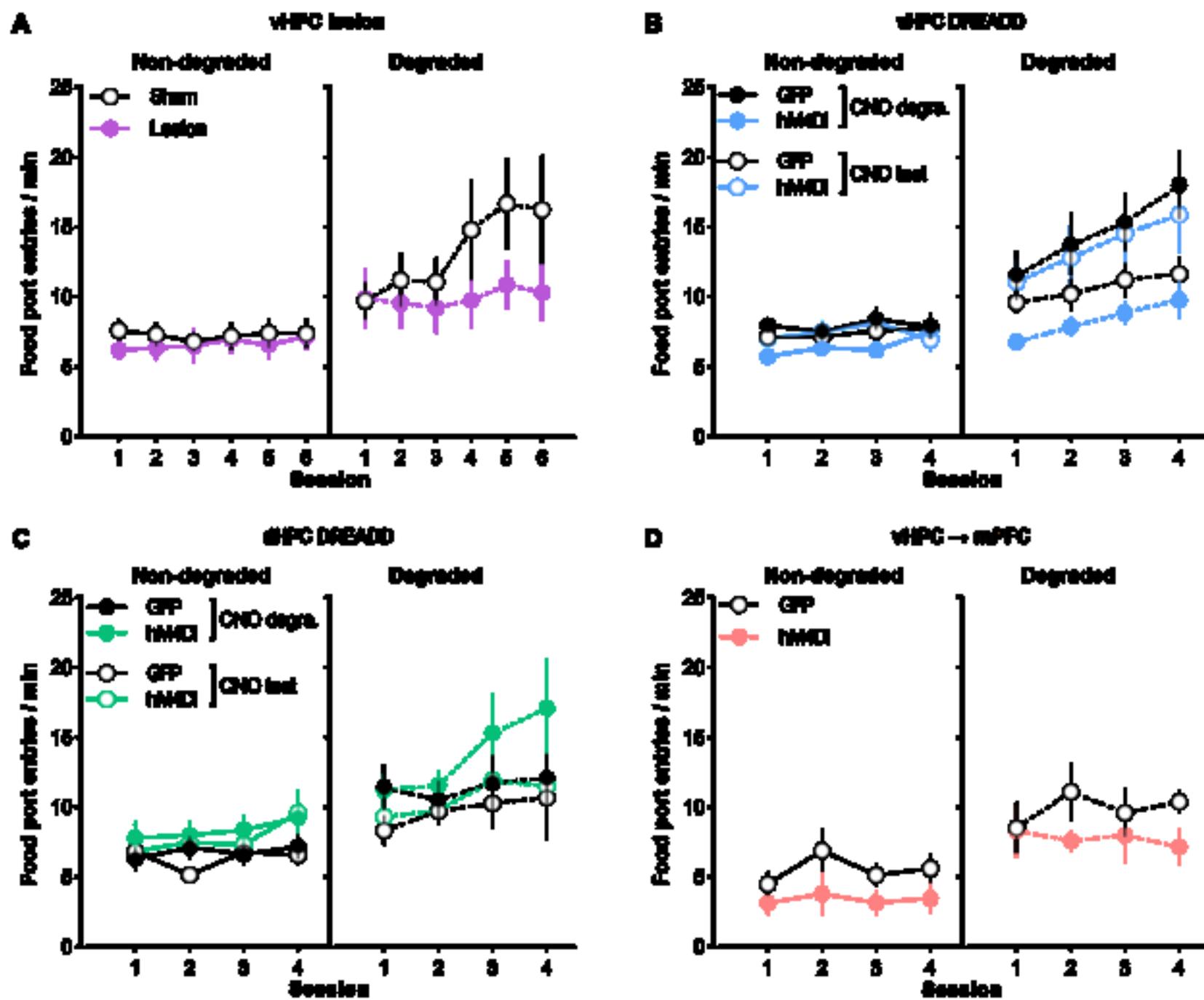
D. Average distance (\pm SEM) travelled for rats that were injected with either the GFP or hM4Di virus in vHPC and received a microinjection of CNO in the medial prefrontal cortex (mPFC) prior to the open field. One rat lost the guide cannula before starting the open field and was therefore excluded. The distance travelled reduced across time and did not differ between groups (effect of time: $F_{1,13} = 147.174$, $p < 0.001$; no effect of group or interaction: largest $F_{1,13} = 0.860$, $p = 0.371$).

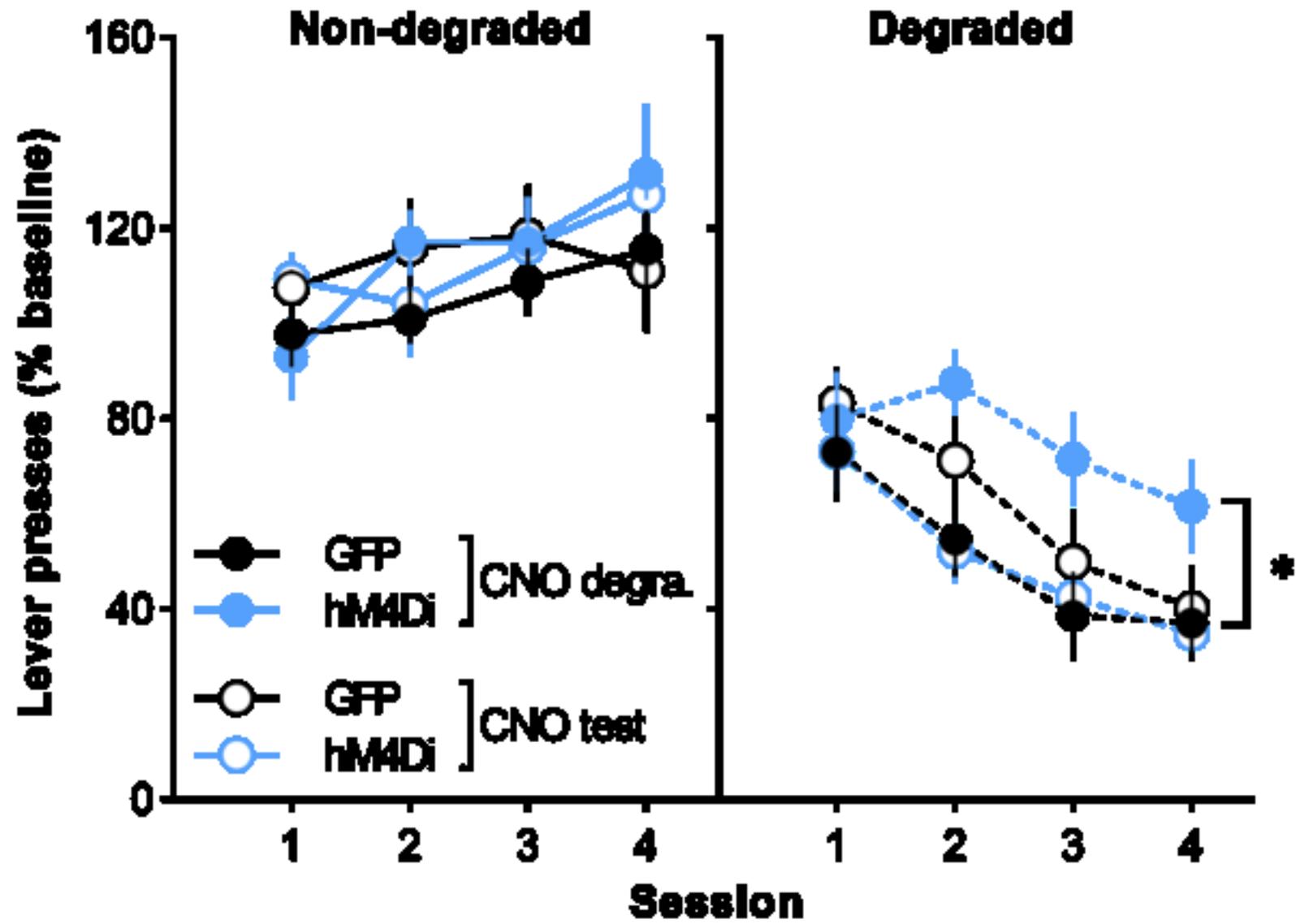
Surgery cohort	Timeline of behavioural procedures			
vHPC lesion (cohort 1)	Instrumental outcome devaluation	Contingency degradation	-	-
vHPC lesion (cohort 2)	-	-	Context outcome devaluation	Open field
vHPC chemogenetic inhibition	-	Contingency degradation	Context outcome devaluation	Open field
dHPC chemogenetic inhibition	-	Contingency degradation	Context outcome devaluation	Open field
vHPC→mPFC chemogenetic inhibition	-	Contingency degradation	Context outcome devaluation	Open field

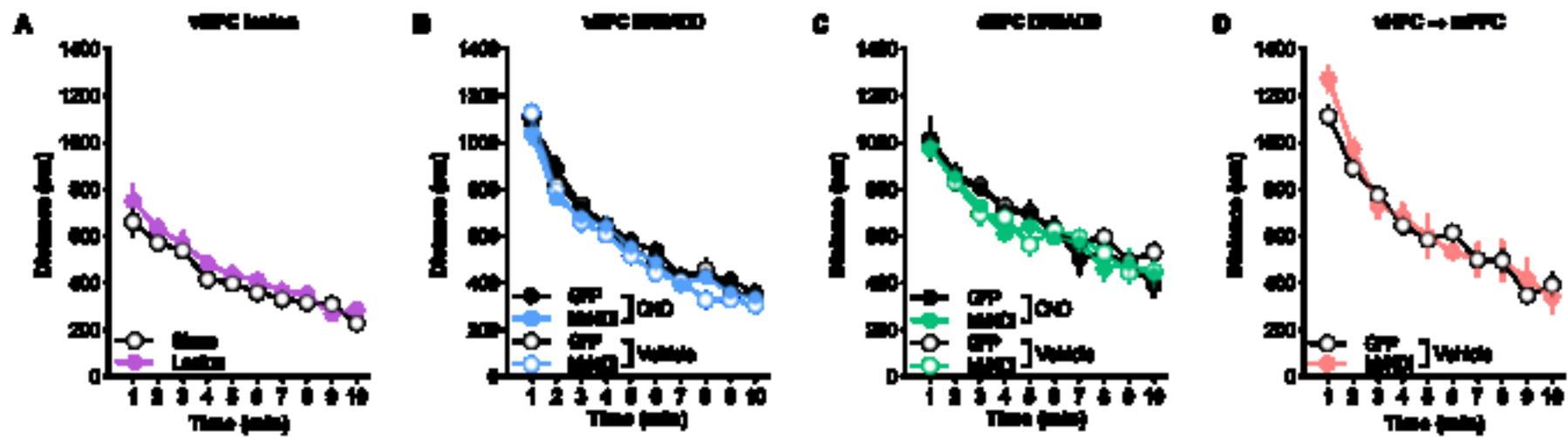
Table S1. Timeline of the behavioural procedures for each surgery cohort. The behavioural procedures used for each surgery cohort are presented in chronological order from left to right. vHPC: ventral hippocampus; dHPC: dorsal hippocampus; mPFC: medial prefrontal cortex; - indicates that the cohort did not receive that particular behavioural procedure.











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