Cognitive control of behavior and hippocampal information processing without medial prefrontal cortex

Eun Hye Park[a], Kally C. O'Reilly Sparks[a,#], Griffin Grubbs[a], David Taborga[a], Kyndall Nicholas[a], Armaan S Ahmed[a], Natalie Ruiz-Pérez[a], Natalie Kim[a], Simon Segura-Carrillo[a,b], André A. Fenton[a,c,#]

[a]Center for Neural Science, New York University, New York, NY 10003

[b]Tandon School of Engineering, New York University, New York, NY 10003

[c]Neuroscience Institute at the New York University Langone Medical Center, New York, NY 10016

[#]Current address: Child and Adolescent Psychiatry, New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY 10032

Author Contributions: EP, KCO, and AAF designed the research, EP, KCO, GG, DT, KN, ASA, and NR performed the research, EP, NK, SS-C, and KCO analyzed the data and AAF, KCO, and EP wrote the paper.

Inclusion and Ethics: This research was conducted with respect for the animal subjects, the individual researchers and their unique perspectives, and our collective effort to learn and generate individually useful knowledge.

Acknowledgements: Supported by NIH grant R01NS105472

[#]Correspondence should be addressed to:
André A. Fenton
Center for Neural Science
New York University
4 Washington Place
New York, NY 10003

SUMMARY

Cognitive control tasks require using one class of information and ignoring competing classes of information. A central role for the medial prefrontal cortex (mPFC) in cognitive control is well established because mPFC damage impairs tasks that may require cognitive control, as inferred, typically from the task design. We tested the “central-computation” hypothesis that rat mPFC is essential for cognitive control. Ibotenic acid lesion of the mPFC was effective and altered the coordination of metabolic activity in remaining structures. The lesion did not impair an active place avoidance task that requires cognitive control, nor did it impair concurrent alternation between task-relevant and task-irrelevant hippocampal CA1 representations of space. Conflict avoidance learning that tests cognitive flexibility was impaired, but not initial learning or long-term memory. These findings support an alternative “local-computation” hypothesis: the computations required for cognitive control can occur locally in brain networks independently of the mPFC, as demonstrated.

INTRODUCTION

Cognitive control is the ability to judiciously use task-relevant information while ignoring salient concurrent information that is currently irrelevant for the task. The consensus view is that the prefrontal cortex (PFC) is crucial for cognitive control such that PFC activity is central to the underlying computations that operate through prefrontal interactions with diverse brain areas during cognitive control tasks[1-3]. The hippocampal role in the cognitive control of spatial and mnemonic information has been investigated using an active place avoidance task[4]. The task conditions rodents to avoid the location of a mild shock and uses continuous rotation of the behavioral arena to dissociate the environment into two spatial frames, one defined by the rotating arena and the other defined by the stationary room[5]. Avoiding shock at a stationary room location requires using the task-relevant spatial information and ignoring spatial information from the other, task-irrelevant frame. Dorsal hippocampus dysfunction impairs this avoidance[6-9]. During active place avoidance task variants, place cell discharge in hippocampus subfields, and head-direction cell discharge in the medial entorhinal cortical input demonstrate cognitive control of spatial representations; neural ensemble activity alternates between representing room and arena locations depending on the subject's proximity to the frame-specific location of shock[4,5,10-12].

A standard "central-computation" hypothesis centralizes the computations that are necessary for cognitive control to the PFC and predicts that lesion of the PFC will impair active place avoidance[13,14]. Recent work indicating that the hippocampus[10] and sensory thalamus[15] are also necessary for cognitive control can be reconciled with the central-computation hypothesis by assuming there are necessary interactions between modality-specific information processing in hippocampus or thalamus and the primary, control-specialized processing in the PFC[14,16,17]. Alternatively, the role of non-PFC brain structures in cognitive control could be explained by a "local-computation" hypothesis; the computations needed for cognitive control can be performed locally in neural networks specialized for the particular information upon which the task depends

and on which cognitive control operates. The local-computation hypothesis predicts that PFC lesion can spare cognitive control. We tested these mutually exclusive hypotheses by making medial prefrontal cortex (mPFC) lesions in rats and evaluating their behavior and hippocampal physiology during active place avoidance (Fig. 1A).

RESULTS

mPFC lesion does not impair cognitive control in the active place avoidance task

Ibotenic acid, but not vehicle, targeted to mPFC caused lesions that included the cingulate cortex, prelimbic and infralimbic areas (Fig. 1B). To assess the effect of mPFC lesion on behavior, we first examined locomotion during the pretraining sessions (Fig. 1C). There is no effect of lesion on the total distance walked across the session (Fig.1D). We next examined active place avoidance learning during initial training (Days 2-3). Both groups learn to reduce their entrances into the shock zone with training (Fig. 1E). There is no behavioral difference between groups by measuring the number of entrances or time to first enter the shock zone, the clearest estimate of memory (Fig. 1F). The two groups are also indistinguishable on Day 4 during which 24-h memory is assessed by their times to first enter the shock zone and the number of entrances (Fig. 1, Day 4 Retention).

We then trained the rats to avoid the shock zone relocated 180° to assess the impact of mPFC lesion on cognitive flexibility with the additional challenge to distinguish between the current and previously learned location of shock, under the cognitive control challenge[18]. Both groups learn the conflict task variant, measured by the number of entrances and the time to enter the new shock zone (Fig. 1, Days 4-5 Conflict Training). There is a non-significant trend for greater savings in sham rats, comparing the entrances on the first and second days of conflict trials (sham = 11.12 ± 4.58 entrances, lesion = 4.3 ± 4.09 entrances; $t_{16} = 1.11$; $p = 0.3$, $d = 0.52$). Consequently, we more closely examined the initial experience of the new shock zone location when the rats are

first confronted with the change in the location of shock. We compared the number of entrances on the first trial of each day to estimate savings during initial training (Fig. 1F left inset) and during conflict training (Fig. 1F middle inset). During initial training, both sham (t_7 = 4.5, p = 0.003, d = 1.2) and lesion (t_9 = 4.7, p = 0.001, d = 1.5) rats improved. During conflict training, while sham rats all improved across the first two conflict trials of each day (t_7 = 3.8, p = 0.006, d = 1.4) only 6/10 lesion rats improved (t_9 = 0.87, p = 0.4, d = 0.27). We also quantified the improvement for each subject during conflict as a savings index using the first 5 minutes of the first conflict trials of each day (Fig. 1F right inset). The sham rats improved, avoiding more than the lesion rats in the first 5 minutes of the second conflict day compared to the first conflict day (t_{16} = 2.4, one-tailed p = 0.03, d = 0.85), despite equivalent performance across eight trials on the first day. Although, we find no evidence that mPFC lesion causes a deficit in active place avoidance learning to avoid the initial location of shock, nor do we observe a deficit in memory retention up to 24-h for the initial shock location, both of which require cognitive control, the lesion is sufficient to impair conflict learning after a 24-h delay.

mPFC lesion can alter functional relationships amongst related brain areas

Cytochrome oxidase (CO), a sensitive metabolic marker for neuronal function[19], was used to evaluate whether lesion effects were restricted to the mPFC. In a subset of rats (lesion n = 8, sham n = 8), CO activity was evaluated in 14 functionally-related brain regions (Fig. 2A). CO activity in the central amygdala, but not elsewhere, is altered by the lesion, with activity increased in lesion rats (Table S1). Pair-wise interregional correlations of CO activity were evaluated to assess whether extra-mPFC functional relationships are altered after mPFC lesion. After FDR correction (0.01) 15 of the 91 correlations are significant in the sham sample but only 7 in the lesion sample (Fig. 2B, S2b; test of proportions: z = 2.26, p = 0.03). The correlations within the ventral hippocampus are preserved after mPFC lesion, but some correlations are lost within the dorsal hippocampal formation (Fig. 2B). Correlations between the nucleus reuniens and both the

dorsal and ventral hippocampus are also lost after mPFC lesion. Correlations between the basolateral amygdala and nucleus reuniens and between the basomedial amygdala and ventral hippocampus are lost after mPFC lesion, and a correlation between dorsal CA1 and the central nucleus of the amygdala appear after mPFC lesion. These data indicate that the mPFC lesion causes functional changes beyond mPFC.

mPFC lesion does not alter cognitive control of hippocampal neural representations

Cognitive control requires the subject to process and use a class of information purposefully, at the expense of other competing information, which we previously demonstrated in the population dynamics of dorsal hippocampus spatial discharge as rats and mice navigate on a rotating arena[4,5,10,20]. Thus, we examined spatial frame ensemble preference (SFEP), the representational dynamics of hippocampus CA1 discharge on the rotating arena during pretraining, as well as retention of the conditioned place avoidance of the initial and conflicting shock zone locations (Fig. 3A). Note that the physical conditions were identical because the shock was off in these three trials.

We first examined basic discharge properties of individual CA1 principal cells, which do not differ between the sham and mPFC lesion groups (Fig 3B). We then examined overdispersion of place cell firing, which is known to be reduced by prefrontal inactivation[21]. In contrast, overdispersion is expected to increase with cognitive control and other extra-positional processes that increase discharge non-stationarity[22]. We find that during pretraining, overdispersion is reduced by a factor of two in lesion compared to sham rats, consistent with an effective lesion (Fig. 3C left). Might this also indicate reduced cognitive control in the lesion rats? Remarkably, during conditioned place avoidance the overdispersion in lesion rats increases to the level observed in sham rats, consistent with the increased demand for cognitive control (Fig. 3C middle and right). These

findings confirm the effectiveness of the prefrontal lesion and also provide electrophysiological evidence consistent with intact cognitive control in the lesion rats.

We next examined SFEP, the electrophysiological signature of cognitive control, during conditioned place avoidance. CA1 ensemble discharge alternates between preferentially representing room locations and arena locations (Fig. 4A; runs tests for all recordings were significant; z's $\geq$ 12.3, p's $\leq 10^{-33}$). During pretraining, SFEP is equally likely to signal the current room location or alternatively, the current arena location, in both the sham and lesion rats (Fig. 4A). Importantly, during avoidance of the initial shock location, SFEP favors the arena frame because the rat avoids the room frame location of shock (Fig. 4B) and SFEP is room-preferring near the shock zone and arena-preferring far from the shock zone (Fig. 4C,D), as previously demonstrated[4,5]. Relocating the shock zone 180° for conflict training changes SFEP, but only in the lesion group such that it is equally probable to be room- or arena-preferring, consistent with needing to decide between avoiding the current and previous shock location throughout the environment (Fig. 4B)[23]. The ability to manipulate SFEP of hippocampal representational dynamics by the presence and location of shock directly demonstrates cognitive control at the level of the hippocampus. Despite an influence of mPFC lesion on cognitive control and the non-stationarity of hippocampal representations of space in hippocampal discharge, the lesion does not impair cognitive control in the place avoidance task.

DISCUSSION

Although permanent lesion of the mPFC alters baseline metabolic coupling of mPFC- and hippocampus-related brain areas (Fig.2, S2), as well as alters hippocampal representational dynamics during expression of cognitive flexibility in an active place avoidance task (Fig.3), we find no behavioral or electrophysiological evidence that the lesion impairs cognitive control in the

basic active avoidance task (Fig.1, 3, 4), despite the lesion being sufficient to cause a cognitive flexibility deficit when the shock zone was relocated for the conflict training trials (Fig. 1F insets). We also observed reduced bursting in the discharge of the underlying thalamus in the lesion group compared to sham (Table S2). Thus, CO imaging and electrophysiological evidence identify changes in the brain beyond the directly damaged mPFC area. In particular, the dorsal hippocampus loses the inhibitory input from mPFC[24,25] and loses the metabolic correlation with the nucleus reuniens, which is thought to be a relay between the mPFC and the dorsal hippocampus[26,27].

Together, these findings require rejecting the central-computation hypothesis that the mPFC is essential for the neural computations that are necessary for all cognitive control tasks. The present findings do not rule out a role for mPFC and hippocampal interactions in other cognitive processes[2,28-31], especially those that rely on the ventral hippocampus and perhaps the direct excitatory mPFC-ventral hippocampus and direct mPFC-dorsal hippocampus inhibitory connections[24,25]. Nonetheless, the present data favor the local-computation hypothesis because numerous manipulations of the dorsal hippocampus, including bilateral and unilateral inactivation, impair learning, consolidation, and retrieval of the conditioned place avoidance[6-8,32], and optogenetic silencing of dentate gyrus impairs conflict learning. Indeed, we directly confirmed that mPFC lesion does not reduce the goal-directed representational switching of dorsal hippocampus (Fig.4) that is a *sine qua non* for cognitive control of spatial information[4,5,10,11,20,23], despite detecting other behavioral and electrophysiological effects of the lesion. It is especially important that mPFC lesion impaired conflict avoidance of the relocated shock zone even after eight trials during which both sham and lesion animals learned and performed at a behavioral asymptote (Fig. 1F). We suggest this identifies a key role for the mPFC in cognitive flexibility, specifically the ability to judiciously select between different memories of the location of shock, and that this is

demonstrably distinct from judiciously selecting between room- and arena-based classes of spatial information.

It is possible that acute manipulations of the mPFC, such as optogenetic, chemogenetic or pharmacological inactivation would lead to different results. In which case, post-lesion reorganization of neural circuits is sufficient to compensate for the loss of the mPFC functions that support cognitive control as required by the active place avoidance task. However, this would not change the conclusion to reject the central-computation hypothesis. It is also possible that the role of the mPFC is best evaluated in tasks that rely on egocentric spatial information[33], rather than the allocentric spatial information upon which the present active place avoidance task variant relies, but this possibility would also require rejecting, or at least severely limiting, the central-computation hypothesis. Finally, we observed impaired performance characterized by cognitive flexibility in the conflict task variant, demonstrating not only that the lesion was effective, but a dissociation of the neural substrates needed for different components of cognitive control, as has been previously reported[34,35]. The mPFC has a crucial role in judiciously selecting between distinct memories of the room-frame shock location, which arguably is a form of memory-guided cognitive control that also depends on hippocampus[5,23,36], but not one that has been demonstrated in the representational discharge of hippocampal cells[5].

Taken together, the findings are consistent with the local-computation hypothesis that diverse (but probably not all) brain circuits can perform the computations needed for cognitive control of the information and/or behavior for which that circuit is specialized to process.

FIGURES AND TABLES

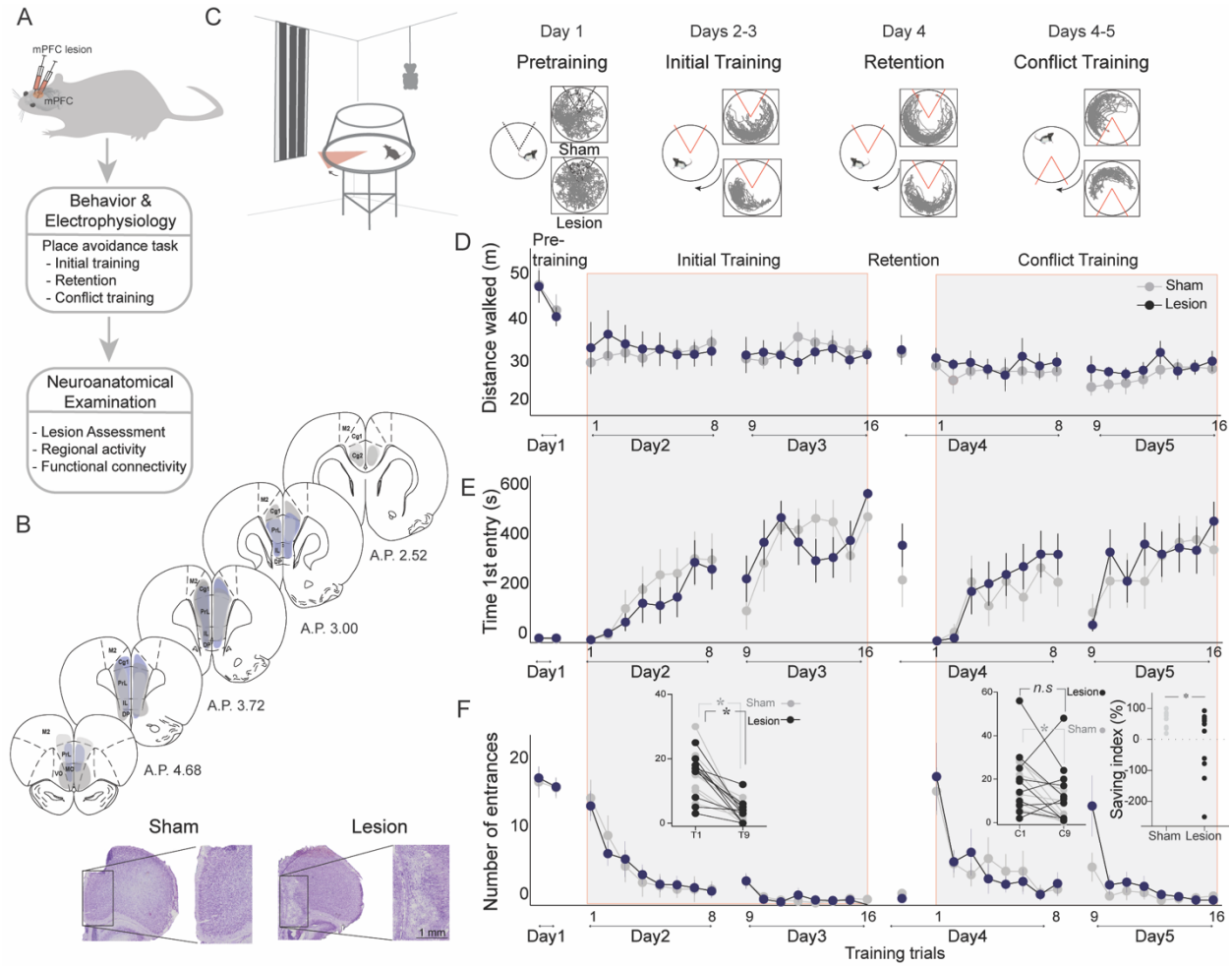

Figure 1. mPFC lesion does not impair initial active place avoidance learning, but impairs cognitive flexibility in the conflict task variant (A) Workflow to assess the impact of mPFC or sham lesions on spatial cognitive control. (**B)** Assessment and impact of the mPFC lesion in three representative subjects (light grey, dark grey, and purple). PrL (prelimbic), IL (infralimbic), Cg (cingulate cortex), M2 (secondary motor cortex). Representative mPFC from a sham and a lesion rat (bottom). **(C)** Behavioral tracks from two example rats across active place avoidance training. The shock zone is indicated as a 60° sector, gray (shock off) and red (shock on), and arena rotation by the curved arrow. Day 1 - Pretraining: free exploration with shock off; Days 2&3 - Initial Training: Eight daily trials to avoid the shock zone. Day 4 - Retention: One trial with shock on. Days 4&5 - Conflict Training: Eight daily trials to avoid the shock zone relocated 180° from the

initial location. Sham and mPFC lesion rats did not differ in **(D)** locomotor activity, **(E)** avoidance memory, or **(F)** place learning.

Locomotor activity: During pretraining, there is no effect of lesion on the total distance walked across the two trials ($F_{1,16}$ = 0.04, p = 0. 85, η^2 = 0.002). There is an effect of trial ($F_{1,16}$ = 15.31, p = 0.001, η^2 = 0.09) but there is no effect of the interaction ($F_{1,16}$ = 0.14, p =0.71, η^2 = 10^{-3}). Locomotion does not differ between the groups, across trials, or their interaction during Initial Training (df = 3.17/50.77), or Conflict Training (df = 4.97/79.58; $F'_S \leq 1.96$, $p \geq 0.09$, $\eta^2 \leq 0.03$).

Avoidance Memory: (Time to first enter the shock zone) The latency to first enter the new shock zone increases for both groups in Initial training. There is no effect of group ($F_{1,16}$ = 0.1, p = 0.76, η^2 = 0.001) but clear effects of day ($F_{1,16}$ = 69.61, p = 10^{-7}, η^2 = 0.17) and trial ($F_{5.33, 85.33}$ = 12.15, p = 10^{-9}, η^2 = 0.12). There are also no significant interactions (group x day: $F_{1,16}$ = 0.94; p = 0.35, η^2 = 10^{-3}; group x trial: $F_{5.33, 85.33}$ = 1.83, p = 0.11, η^2 = 0.02; day x trial: $F_{4.78, 76.43}$ = 1.93, p = 0.10, η^2 = 0.03); group x day x trial: $F_{4.78, 76.43}$ = 0.26, p = 0.93, η^2 = 10^{-3}). Place Learning (Number of entrances): The effect of group is not significant ($F_{1,16}$ = 0.002, p = 0.96, η^2 = 10^{-5}) but the effects of day ($F_{1,16}$ = 26.34, p = 10^{-3}, η^2 = 0.19) and trials ($F_{3.22, 51.50}$ = 36.76, p = 10^{-8}, η^2 = 0.1) are significant. There are no significant interactions (group x day: $F_{1,16}$ = 0.003, p = 0.96, η^2 = 10^{-5}; group x trial: $F_{3.22, 11.50}$= 0.74, p = 0.54, η^2= 10^{-3} ; group x day x trial: $F_{4.05, 64.81}$ = 0.35, p = 0.74, η^2 = 10^{-3}). But the interaction between day and trial is significant (day x trial: $F_{2.26, 36.08}$= 13.75, p = 10^{-5}, η^2 = 0.11). The two groups are indistinguishable on Day 4 during which 24-h memory is assessed by their times to first enter the shock zone (t_{17} = 1.01, p = 0.3, d = 0.49) and the number of entrances (t_{17}= 0.95, p = 0.4, d = 0.002). There is a non-significant trend for greater savings in sham rats, comparing the first and second days of conflict trials (sham = 11.12 ± 2.89 entrances, lesion = 3.9 ± 4.50 entrances; t_{17} = 1.34; p = 0.20, d = 0.56). In the conflict learning, there is no effect of group ($F_{1,16}$ = 0.21, p = 0.65, η^2 = 10^{-3}), but the effects of day ($F_{1,16}$ = 8.99, p = 0.01, η^2 =

0.05), and trials ($F_{1.89, 30.12} = 18.55$, $p = 10^{-9}$, $\eta^2 = 0.28$) are significant. The group interactions are not significant (group x day: $F_{1,16} = 0.55$, $p = 0.47$, $\eta^2 = 10^{-3}$; group x trial: $F_{1.88, 30.12} = 1.38$, $p = 0.27$, $\eta^2 = 0.02$; group x day x trial: $F_{2.30, 36.87} = 1.16$, $p = 0.33$, $\eta^2 = 10^{-3}$). Also, the interaction between day and trial is not significant (day x trial: $F_{2.30, 36.87} = 2.31$, $p = 0.11$ $\eta^2 = 0.019$). The insets compare number of entrances during the first 5 minutes of left: the first (D1T1) and second days of initial training (D2T9) as well as right: the first (D4C1) and second (D5C9) days of conflict trials. The percent difference in number of entrances between D5C9 and D4C1 of conflict training was computed as a savings index for statistical comparison. Sham rats demonstrate greater savings than lesion rats ($t_{16} = 2.4$, one-tailed *$p = 0.03$, $d = 0.85$), demonstrating effectiveness of the mPFC lesion to impair cognitive flexibility.

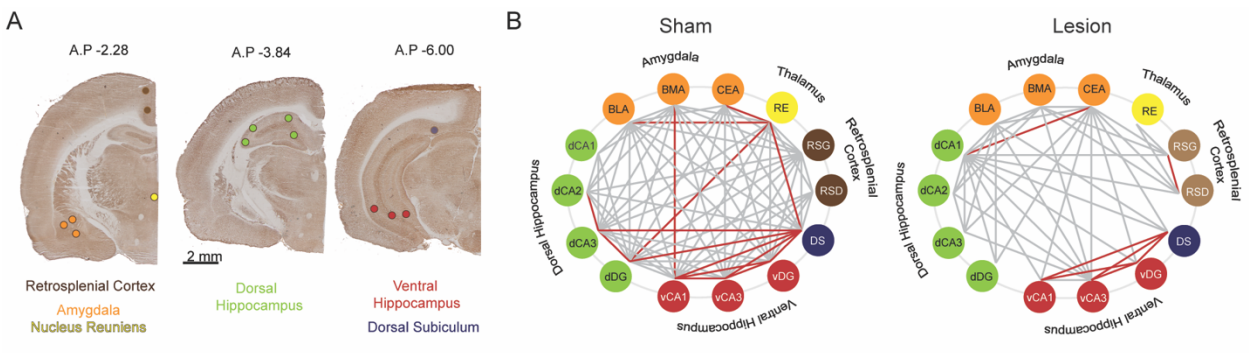

Figure 2. Cytochrome oxidase analysis demonstrates widespread metabolic consequences of mPFC lesion. (A) Representative cytochrome oxidase staining and locations of the optical density readings. Cytochrome oxidase activity was measured in the dysgranular and granular retrosplenial cortices (RSD and RSG, respectively), the nucleus reuniens (RE), the central nucleus of the amygdala (CEA), basomedial and basolateral amygdala (BMA, and BLA, respectively), the dorsal hippocampus CA1, CA2, CA3 and dentate gyrus areas (dCA1, dCA2, dCA3, dDG, respectively) the ventral hippocampus CA1, CA3 and dentate gyrus areas (vCA1, vCA3, vDG, respectively) and the dorsal subiculum (DS) marked as colored circle areas. **(B)** Interarea covariations of CO activity by graph theory analysis. Each line indicates a significant

correlation ($p < 0.05$) between the two brain regions ("nodes") it connects; only the red lines survived False Discovery Rate (FDR < 0.01) correction. Sham: n = 8; Lesion: n = 8.

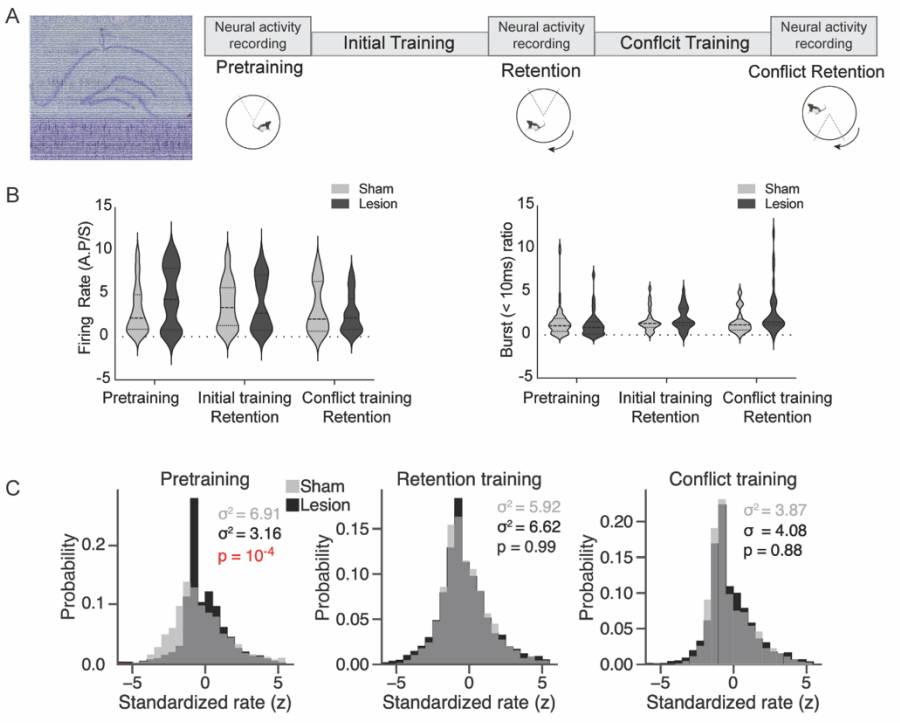

Figure 3. mPFC lesion does not change basic discharge properties, but decreases hippocampus place cell overdispersion only in the absence of the cognitive control challenge. (A) (left) Representative histology with overlaid recording traces from the Neuropixel probe. (right) Recording schedule workflow during the cognitive control task. **(B)** There is no difference between sham and mPFC lesion rats in firing rate (Sham: 3.29 ± 0.27, Lesion: 3.68 ± 0.33, $t_{184} = 0.91$, $p = 0.36$) and burst ratio in hippocampal neurons (Sham: 1.53 ± 0.25, Lesion: 2.02 ± 0.28, $t_{174} = 1.29$, $p = 0.19$). **(C)** Distribution of standardized place cell discharge (z scores) computed during every 5-second episode in which the rat passed through place cell firing fields in the data set. Different numbers of passes qualified for evaluation during the pretraining (Sham = 2777, Lesion = 3398), retention (Sham = 4039, Lesion = 3366), and conflict (Sham = 1748,

Lesion = 2336) recordings. The variance of the histograms characterizes overdispersion, statistically evaluated by their ratio as an F-test (pretraining: $F_{2776,3397} = 2.19$, $p = 5.8 \times 10^{-4}$).

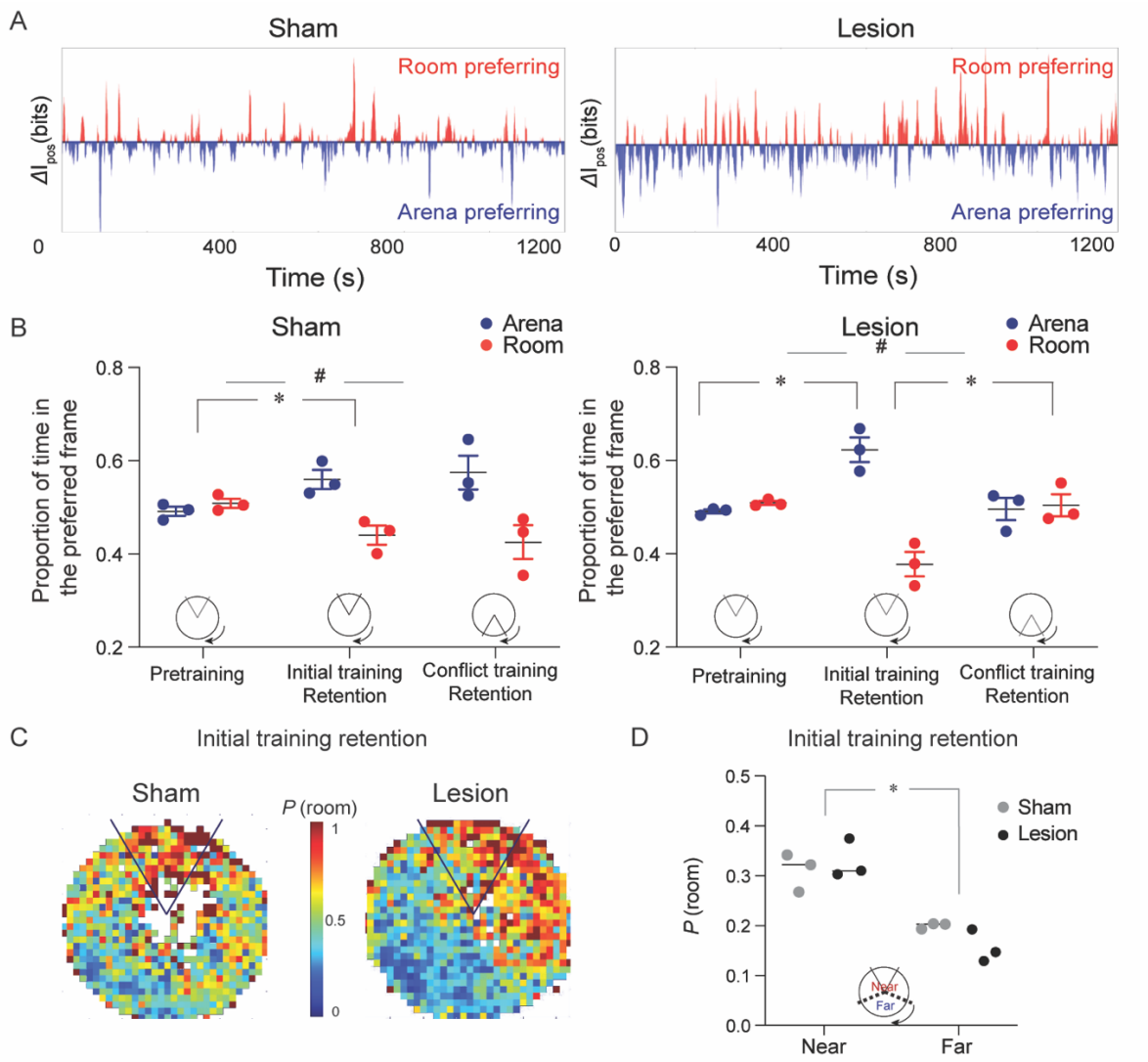

Figure 4. Sham (n=3) and mPFC (n=3) lesion rats do not differ in expressing cognitive control of spatial frame-specific representations of location. (**A**) Individual ensemble examples during the Day 4 retention session, and **(B)** group statistics of spatial frame ensemble preference (SFEP), demonstrating cognitive control in both groups. During retention, SFEP is

biased to the arena frame in both the sham (t_4 = 4.16, $^{\#}p$ = 0.01) and lesion rats (t_4 = 6.62, $^{\#}p$ = 0.003). *p < 0.05 post-hoc differences. **(C)** Group spatial probability distribution of SFEP for room frame preference during the retention session with no shock. **(D)** Summary of average probability of room-preferring SFEP discharge in half the arena near and far from the shock zone. Two-way Group X Location ANOVA: Group: $F_{1,4}$ = 0.17 p = 0.68, $\eta^2 = 10^{-3}$; Location: $F_{1,4}$ = 54.69, p = 0.001, η^2 = 0.84; Group X Location ($F_{1,4}$ = 2.04, p = 0.2 , $\eta^2 = 10^{-2}$).

STAR METHODS

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, André A. Fenton.

Methods

All methods complied with the Public Health and Service Policy on Humane Care and Use of Laboratory Animals and were approved by NYU's University Animal Welfare Committee, which follow National Institutes of Health guidelines.

Subjects

Thirty adult, male, Long-Evans rats were purchased from Charles River to arrive at New York University at approximately 40 days old. The rats were given at least one week to acclimate to the facility and were housed two per cage on a 12:12 light:dark cycle with free access to food and water. Fifteen rats were assigned to the lesion group and 14 to the sham group. One animal was used to confirm the coordinates, by injecting fluorogold (Fluorochrome, Denver, CO, 2.5% w/v in distilled water) at the same rate and for the same duration as for the lesions. Three of the sham and three of the mPFC lesions rats were used for the electrophysiology recordings. The behavior of these rats was not included in the behavioral assessment due to the requirement for extended training sessions to record neuronal activity.

<u>Behavioral analysis followed by cytochrome oxidase activity</u>: One rat in the lesion group was excluded because the lesion was inadequate and one had to be removed from the study due to a skin irritation, resulting in 10 rats in the lesion group. All of these rats underwent behavioral assessment (lesion n=10), eight of which were stained for cytochrome oxidase. The tissue from

one lesion and one sham rat could not be used for CO because the tissues were lost to thawing and tissue from one rat from each group was not processed. For the control group, one rat was excluded from performing behavioral experiments because his lesioned cage mate had a skin irritation that prevented the lesion rat from behavioral testing; this control brain was still processed for cytochrome oxidase activity. One rat was excluded from the behavioral experiments due to equipment malfunction during training, and one rat had tissue damage during the sham surgery that appeared as a lesion, so this rat was excluded from the study. Thus, the sham group had eight rats for behavioral assessment and eight for cytochrome oxidase assessment. Our final group numbers for the behavioral assessment were therefore sham group n = 8, lesion group n = 10, and our final group numbers for cytochrome oxidase activity were sham group n = 8 and lesion group n = 8.

Lesion Surgery

Between the ages of 48-56 days, the rat received prefrontal cortex or sham lesion under sodium pentobarbital (50 mg/kg, i.p.) anesthesia. Bilateral lesion targeted two sites in each hemisphere at the following stereotaxic coordinates: from Bregma, Site 1: A.P +2.5, ML $\pm$0.6, DV - 5.0, relative to the skull surface, Site 2: AP +3.5, ML $\pm$ 0.6, DV - 5.2, relative to skull surface. Ibotenic acid (0.06 M in PBS) was used to create an excitotoxic lesion. It was injected with a micro-infusion pump at a flow rate 0.2 μL/min through a stainless-steel cannula (0.25 mm outer diameter). Rats in the sham group underwent the same surgery procedure, but only PBS was injected. The rats were given one week to recover.

Active place avoidance training

After recovery from surgery, the rats were handled for 5 min per day for 5 days. On the first day of behavior training, animals had a pretraining session that consisted of two trials during which

the rats were allowed to explore the stationary arena to habituate to the environment. The following two days, the rats underwent eight training trials per day on a rotating arena, learning to avoid entering a 60° sector shock zone in which the rats received a mild foot shock (500ms, 60Hz, 0.4mA). This shock zone was defined by the stationary cues in the room. On the fourth day of the behavioral training, the rats had a single trial with the shock on to test retention of the training. The rats were then trained on days 4 and 5 in a conflict training session in which the rats received eight trials per day with the shock zone located 180° from the initial training position. All trials were 10 min with 10 min intertrial intervals. The total distance walked during the session measured locomotion, the time to first enter the shock zone location measured avoidance memory, and the number of entrances into the shock zone was used to measure place learning. Savings in place learning on the conflict trials when the shock zone was relocated 180° was assessed for each rat by a savings index, which compares the number of entrances during the first 5 minutes of the first and second day of conflict training, Conflict Trials 1 and 9 respectively. The difference is normalized by the number of entrances on the first conflict trial and reported as a percentage:

$$Savings\ Index = \frac{\left(Number\ of\ Entrances_{conflict1} - Number\ of\ Entrances_{conflict9}\right)}{Number\ of\ Entrances_{conflict1}} X100$$

Electrophysiology procedures

The following procedures have been previously described in detail[12]. Briefly, rats were anesthetized (pentobarbital 50 mg/kg) and received mPFC or sham lesions as described above. A Neuropixel electrode array was implanted in the brain to traverse AP -3.8 mm, ML 2.5 mm for recording dorsal hippocampus and the tip targeted DV 9.3 mm, which allowed recording from the underlying thalamus. The Neuropixel was stabilized and protected by mounting it in a custom 3-D printed appliance. The appliance and five bone screws were cemented to the skull (Unifast, GC America Inc., IL). The rats were allowed at least 1 week to recover before further manipulation.

The rats received active place avoidance training as described above. In addition, to permit neural ensemble recordings, after the last pretraining, training, and conflict training sessions, shock was turned off and the session was extended for 10 - 20 min during which CA1 neural ensemble discharge was recorded. Signals were filtered between 300 Hz and 10 kHz and sampled at 30 kHz for single unit recording. Because of these extended sessions, the behavior from these animals was not included in our behavioral assessment.

Electrophysiology data analysis

Single units were automatically sorted using Kilosort 2[37]. Units were only studied if the estimated contamination rate was < 20% with spikes from other neurons. This was estimated from refractory period violations relative to expected. Units with non-characteristic or noisy waveforms were also excluded. We computed a "burst ratio" to characterize burstiness of discharge as: number of spikes with ISI ≤ 30 ms divided by number of spikes with 100 ms ≥ ISI ≤ 130 ms.

Hippocampus single units were classified as complex-spike or theta cells as in prior work[10,38,39]. Complex-spike cells appear to be pyramidal cells, having long-duration waveforms (> 250 μs), low discharge rate (< 5 AP/s) and a tendency to fire in bursts. Theta cells are likely local interneurons[40], and had short-duration waveforms (< 250 μs), high discharge rate (> 2 AP/s), and did not tend to fire in bursts. Only hippocampal units classified as pyramidal cells were studied. Thalamic units fired at high rates and were less likely to discharge in bursts (Table S2).

The representational dynamics of CA1 discharge alternating between preferentially signaling the current location in the stationary room or the current location on the rotating arena was investigated to directly evaluate cognitive control during place avoidance behavior. Spatial-frame-

specific momentary positional information (I_{pos}) is used to estimate the location-specific information from the activity of a cell during a brief interval (Δt = 133 ms).

$I_{pos}(t) = p_{i|x} log_2 \left(\frac{p_{i|x}}{p_i}\right)$, $p_{i|x}$ is the probability of observing i activity at location x, and p_i is the overall probability of observing i activity. $I_{pos(room)}$ estimates the information about the current location *x* in the room, whereas $I_{pos(arena)}$ separately estimates the information about current location *x* on the rotating arena[41]. $I_{pos}(t)$ can be positive or negative, but the absolute value is large whenever the cell's activity at the current location is distinct or ''surprising'' compared to the location-independent probability of observing the same activity. The value $\left|I_{pos}(t)\right|$ is abbreviated I_{pos}. A unit's spatial-frame preference is estimated at each Δt moment as the difference:

ΔI_{pos} = $I_{pos(room)}$ - $I_{pos(arena)}$, where positive values indicate a momentary preference for signaling the room location, and negative values indicate a preference for signaling the arena location. ΔI_{pos} timeseries were evaluated for significance compared to chance fluctuations around the mean using the z value from a runs test. The spatial frame ensemble preference (SFEP) at each Δt was computed as the average ΔI_{pos} over all cells. This ensemble ΔI_{pos} timeseries (assessed for significance by runs test) was averaged over all times and locations to estimate an overall SFEP as the proportion of time the ensemble activity was room (or arena) preferring[4,5]. To evaluate if SFEP was purposeful, the arena was divided into approximately two halves based on where individual rats avoided during a retention test with shock off. The "near" half was the largest sector that includes at least 50% of the recording time and surrounds the least visited 20° sector of the room frame (within the shock zone). The "far" half is the remaining sector. The probability of observing room-preferring ΔI_{pos} was computed at each location to make a representational preference map and the average probability in the near and far halves were compared by statistical evaluation.

Overdispersion of hippocampal discharge was computed as previously described[22]. Briefly, The spike and position timeseries is divided into 5-s episodes and the expected firing (*exp*) is computed assuming an inhomogeneous Poisson process. The standardized rate is computed as the normalized difference between the observed (obs) and expected firing: $z = \frac{obs-exp}{\sqrt{exp}}$. Episodes with *exp* = 0 are undefined, and only episodes in which *exp* is greater than the cell's mean firing were analyzed because this criterion selects episodes during which the rat passes through the central region of the firing field. The variance of the distribution of *z* values quantifies overdispersion, which can be compared between conditions using a F test.

Tissue processing and histochemistry

Verification of lesion coordinates

Immediately following surgery to inject fluorogold, the rat was transcardially perfused with 0.9% saline and 10% formalin, and the brain extracted and postfixed in 10% formalin for 24h at 4°C. The brain was then cryoprotected in 30% sucrose (w/v in 1x phosphate buffered saline) and stored at 4°C until cut on a cryostat (40 μm). The sections were mounted onto gelatin coated slides and scanned using an Olympus VS120 microscope (fluorescence, 10x). The slides were then placed in e-pure water (2 times, 1 min each) before being dehydrated (50, 70, 80, 95, 100, 100% ethanol, 1 min each) and defatted in 50:50 chloroform ethanol for 20 min. The slides were then rehydrated (100, 100, 90, 80, 70, 50% ethanol, 1 min each) before being Nissl stained with Cresyl violet (1 min), rinsed in e-pure water, and dehydrated (50% ethanol, 1 min, 70% ethanol until the white matter was white, 95% ethanol with acetic acid until desired intensity, 100, 100% ethanol, 2 min each). The tissues were then cleared in xylenes (3 times, 5 min each) before being coverslipped. The slides were then scanned again (light microscope, 10x). Images were manipulated using Adobe Photoshop CS6 to perform auto-contrast on each image and to remove background from

the images. The fluorescent images were then all thresholded in a single operation to remove the background and the result was superimposed on the corresponding Nissl-stained sections using Adobe Illustrator.

Cytochrome oxidase activity and Nissl staining

On the day following the completion of behavior training, the rats were anesthetized with isoflurane, immediately decapitated, and the brains were extracted. The brains were rapidly frozen in isopentane on dry ice and stored at -80 °C. Sets of brains consisting of two to four animals per group were cut simultaneously on a cryostat (40 μm), and sorted into the three series, one of which was Nissl stained and one used for cytochrome oxidase histochemistry. The series used for Nissl staining was stored at room temperature and the other two series stored at -80°C until processed for cytochrome oxidase histochemistry. To control for variability across batches of histochemical staining, 20, 40, 60 and 80 μm sections of fresh rat brain tissue homogenate (prepared as in[42]) were included. Cytochrome oxidase staining was performed according to[43]. Stained slides were scanned with an Olympus VS 120 light microscope (2 x) and the optical densities measured from captured images. Images were converted to 8-bit gray scale using ImageJ[44] and optical densities read from the standard slides and 14 brain regions (Figure 1). These brain regions included the dysgranular and granular retrosplenial cortices (RSD and RSG, respectively), the nucleus reuniens (RE), the central nucleus of the amygdala (CEA), basomedial and basolateral amgydala (BMA, and BLA, respectively), the dorsal hippocampal CA1, CA2, CA3 and dentate gyrus areas (dCA1, dCA2, dCA3, dDG, respectively) the ventral hippocampal CA1, CA3 and dentate gyrus areas (vCA1,vCA3, vDG, respectively) and the dorsal subiculum (DS). The optical densities were measured using ImageJ (NIH) and cytochrome oxidase activity was normalized as in[45]. Optical densities were measured while blind to the group identity. Three to six

optical density readings were taken for each brain region, from both hemispheres and averaged for each individual subject.

To confirm the lesion site, one series of sections was stained with Cresyl violet. The slides were placed in the e-pure water (2 times, 1 min each) and dehydrated in a series of ethanol baths (50, 70, 80, 90, 100, 100%, 1 min each) prior to clearing the fats in a 1:1 mixture of ethanol:chloroform for 20 min. The slides were then rehydrated (100, 100, 90, 80, 70, 50% ethanol, 1 min each), rinsed in e-pure water, and placed in the Cresyl violet for 10 min. The slides were again dehydrated and cleared in xylenes (3 times, 5 min each) before being coverslipped. Images were captured with an Olympus VS120 light microscope at 10x. Only rats with verified bilateral lesions were included in the data analysis.

Statistical analysis

Cytochrome oxidase activity: Group averages of the optical densities were calculated for each brain regions and expressed as mean ± SEM relative cytochrome oxidase activity/ μm of tissue. Interregional metabolic covariation was examined by calculating Pearson correlations between each brain region. The statistical significance of interregional cytochrome oxidase activity correlations was determined by a 0.01 False Discovery Rate. To determine if the interregional correlations were significantly different between groups, we transformed the r value of the correlation to Fisher's z-scores. Significant correlations ($p < 0.05$) were used to generate graph theoretical networks using the Brain Connectivity Toolbox in MATLAB (https://sites.google.com/site/bctnet/).

Behavior and electrophysiology: Place avoidance task performance and SFEP electrophysiology measures were compared using multivariate analysis of variance. Repeated measure analyses were performed using the Greenhouse-Geisser correction. One-factor group comparisons were

performed by t test. Runs tests were used to evaluate the likelihood of observing the ensemble ΔI_{pos} timeseries by chance. Statistical significance was set at 0.05 for all comparisons. Test statistics, degrees of freedom and effect sizes are provided throughout, in addition to exact p values.

REFERENCES

1 Miller, E. K. & Cohen, J. D. An integrative theory of prefrontal cortex function. *Annu Rev Neurosci* **24**, 167-202 (2001). https://doi.org/10.1146/annurev.neuro.24.1.167
24/1/167 [pii]

2 Guise, K. G. & Shapiro, M. L. Medial Prefrontal Cortex Reduces Memory Interference by Modifying Hippocampal Encoding. *Neuron* **94**, 183-192 e188 (2017). https://doi.org/10.1016/j.neuron.2017.03.011

3 Marton, T. F., Seifikar, H., Luongo, F. J., Lee, A. T. & Sohal, V. S. Roles of Prefrontal Cortex and Mediodorsal Thalamus in Task Engagement and Behavioral Flexibility. *The Journal of Neuroscience* **38**, 2569 (2018). https://doi.org/10.1523/JNEUROSCI.1728-17.2018

4 Chung, A. *et al.* Cognitive control persistently enhances hippocampal information processing. *Nature* **600**, 484-488 (2021). https://doi.org/10.1038/s41586-021-04070-5

5 van Dijk, M. T. & Fenton, A. A. On How the Dentate Gyrus Contributes to Memory Discrimination. *Neuron* **98**, 832-845 (2018). https://doi.org/10.1016/j.neuron.2018.04.018

6 Cimadevilla, J. M., Fenton, A. A. & Bures, J. Functional inactivation of dorsal hippocampus impairs active place avoidance in rats. *Neurosci Lett* **285**, 53-56 (2000). https://doi.org/S0304-3940(00)01019-3 [pii]

7 Cimadevilla, J. M., Wesierska, M., Fenton, A. A. & Bures, J. Inactivating one hippocampus impairs avoidance of a stable room-defined place during dissociation of arena cues from room cues by rotation of the arena. *Proc Natl Acad Sci U S A* **98**, 3531-3536 (2001). https://doi.org/10.1073/pnas.051628398
98/6/3531 [pii]

8 Wesierska, M., Dockery, C. & Fenton, A. A. Beyond memory, navigation, and inhibition: behavioral evidence for hippocampus-dependent cognitive coordination in the rat. *J Neurosci* **25**, 2413-2419 (2005). https://doi.org/25/9/2413 [pii]
10.1523/JNEUROSCI.3962-04.2005

9 Lee, H. *et al.* Early cognitive experience prevents adult deficits in a neurodevelopmental schizophrenia model. *Neuron* **75**, 714-724 (2012). https://doi.org/S0896-6273(12)00578-8 [pii]
10.1016/j.neuron.2012.06.016

10 Kelemen, E. & Fenton, A. A. Dynamic grouping of hippocampal neural activity during cognitive control of two spatial frames. *PLoS Biol* **8**, e1000403 (2010). https://doi.org/10.1371/journal.pbio.1000403

11 Talbot, Z. N. *et al.* Normal CA1 Place Fields but Discoordinated Network Discharge in a Fmr1-Null Mouse Model of Fragile X Syndrome. *Neuron* **97**, 684-697 (2018). https://doi.org/10.1016/j.neuron.2017.12.043

12 Park, E. H., Keeley, S., Savin, C., Ranck, J. B., Jr. & Fenton, A. A. How the Internally Organized Direction Sense Is Used to Navigate. *Neuron* (2019). https://doi.org/10.1016/j.neuron.2018.11.019

13 Friedman, N. P. & Robbins, T. W. The role of prefrontal cortex in cognitive control and executive function. *Neuropsychopharmacology* **47**, 72-89 (2022). https://doi.org/10.1038/s41386-021-01132-0

14 Kamigaki, T. Prefrontal circuit organization for executive control. *Neuroscience Research* **140**, 23-36 (2019). https://doi.org/https://doi.org/10.1016/j.neures.2018.08.017

15 Wimmer, R. D. *et al.* Thalamic control of sensory selection in divided attention. *Nature* **526**, 705-709 (2015). https://doi.org/10.1038/nature15398

16 Murray, J. D. *et al.* Stable population coding for working memory coexists with heterogeneous neural dynamics in prefrontal cortex. *Proc Natl Acad Sci U S A* **114**, 394-399 (2017). https://doi.org/10.1073/pnas.1619449114
17 Preston, Alison R. & Eichenbaum, H. Interplay of Hippocampus and Prefrontal Cortex in Memory. *Current Biology* **23**, R764-R773 (2013). https://doi.org/https://doi.org/10.1016/j.cub.2013.05.041
18 Rich, E. L. & Shapiro, M. L. Prelimbic/Infralimbic Inactivation Impairs Memory for Multiple Task Switches, But Not Flexible Selection of Familiar Tasks. *The Journal of Neuroscience* **27**, 4747 (2007). https://doi.org/10.1523/JNEUROSCI.0369-07.2007
19 Wong-Riley, M. T. Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. *Trends Neurosci* **12**, 94-101 (1989).
20 Kelemen, E. & Fenton, A. A. Coordinating different representations in the hippocampus. *Neurobiol Learn Mem* **129**, 50-59 (2016). https://doi.org/10.1016/j.nlm.2015.12.011
21 Hok, V., Chah, E., Save, E. & Poucet, B. Prefrontal cortex focally modulates hippocampal place cell firing patterns. *J Neurosci* **33**, 3443-3451 (2013). https://doi.org/10.1523/JNEUROSCI.3427-12.2013
22 Fenton, A. A. *et al.* Attention-like modulation of hippocampus place cell discharge. *J Neurosci* **30**, 4613-4625 (2010). https://doi.org/30/13/4613 [pii]
10.1523/JNEUROSCI.5576-09.2010
23 Dvorak, D., Radwan, B., Sparks, F. T., Talbot, Z. N. & Fenton, A. A. Control of recollection by slow gamma dominating mid-frequency gamma in hippocampus CA1. *PLoS Biol* **16**, e2003354 (2018). https://doi.org/10.1371/journal.pbio.2003354
24 Hoover, W. B. & Vertes, R. P. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Struct Funct* **212**, 149-179 (2007). https://doi.org/10.1007/s00429-007-0150-4
25 Malik, R., Li, Y., Schamiloglu, S. & Sohal, V. S. Top-down control of hippocampal signal-to-noise by prefrontal long-range inhibition. *Cell* **185**, 1602-1617 e1617 (2022). https://doi.org/10.1016/j.cell.2022.04.001
26 Griffin, A. L. Role of the thalamic nucleus reuniens in mediating interactions between the hippocampus and medial prefrontal cortex during spatial working memory. *Frontiers in Systems Neuroscience* **9** (2015). https://doi.org/10.3389/fnsys.2015.00029
27 Vertes, R. P., Hoover, W. B., Do Valle, A. C., Sherman, A. & Rodriguez, J. J. Efferent projections of reuniens and rhomboid nuclei of the thalamus in the rat. *J Comp Neurol* **499**, 768-796 (2006). https://doi.org/10.1002/cne.21135
28 Negrón-Oyarzo, I. *et al.* Coordinated prefrontal-hippocampal activity and navigation strategy-related prefrontal firing during spatial memory formation. *P Natl Acad Sci USA* **115**, 7123-7128 (2018). https://doi.org/10.1073/pnas.1720117115
29 Spellman, T. *et al.* Hippocampal-prefrontal input supports spatial encoding in working memory. *Nature* **522**, 309-314 (2015). https://doi.org/10.1038/nature14445
30 Sigurdsson, T., Stark, K. L., Karayiorgou, M., Gogos, J. A. & Gordon, J. A. Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia. *Nature* **464**, 763-767 (2010). https://doi.org/nature08855 [pii]
10.1038/nature08855
31 Adhikari, A., Topiwala, M. A. & Gordon, J. A. Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety. *Neuron* **65**, 257-269 (2010). https://doi.org/S0896-6273(09)00979-9 [pii]
10.1016/j.neuron.2009.12.002
32 Jezek, K., Wesierska, M. & Fenton, A. A. Hippocampus-dependent retrieval and hippocampus-independent extinction of place avoidance navigation, and stress-induced out-of-context

activation of a memory revealed by reversible lesion experiments in rats. *Physiol Res* **51 Suppl 1**, S35-47 (2002).

33 Kesner, R. P., Farnsworth, G. & DiMattia, B. V. Double dissociation of egocentric and allocentric space following medial prefrontal and parietal cortex lesions in the rat. *Behavioral neuroscience* **103**, 956-961 (1989). https://doi.org/10.1037//0735-7044.103.5.956

34 Keeler, J. F. & Robbins, T. W. Translating cognition from animals to humans. *Biochem Pharmacol* **81**, 1356-1366 (2011). https://doi.org/S0006-2952(11)00015-3 [pii]
10.1016/j.bcp.2010.12.028

35 Dias, R., Robbins, T. W. & Roberts, A. C. Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: restriction to novel situations and independence from "on-line" processing. *J Neurosci* **17**, 9285-9297 (1997).

36 Burghardt, N. S., Park, E. H., Hen, R. & Fenton, A. A. Adult-born hippocampal neurons promote cognitive flexibility in mice. *Hippocampus* **22**, 1795-1808 (2012). https://doi.org/10.1002/hipo.22013

37 Pachitariu, M., Steinmetz, N., Kadir, S., Carandini, M. & Harris, K. D. Kilosort: realtime spike-sorting for extracellular electrophysiology with hundreds of channels. *bioRxiv 061481 (2016)* (2016). https://doi.org/10.1101/061481

38 Fenton, A. A. *et al.* Unmasking the CA1 ensemble place code by exposures to small and large environments: more place cells and multiple, irregularly-arranged, and expanded place fields in the larger space. *J Neurosci* **28**, 11250-11262 (2008).

39 Ranck, J. B., Jr. Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. I. Behavioral correlates and firing repertoires. *Exp Neurol* **41**, 461-531 (1973).

40 Fox, S. E. & Ranck, J. B., Jr. Localization and anatomical identification of theta and complex spike cells in dorsal hippocampal formation of rats. *Exp Neurol* **49**, 299-313 (1975). https://doi.org/0014-4886(75)90213-7 [pii]

41 Olypher, A. V., Lansky, P., Muller, R. U. & Fenton, A. A. Quantifying location-specific information in the discharge of rat hippocampal place cells. *J Neurosci Methods* **127**, 123-135 (2003). https://doi.org/S0165027003001237 [pii]

42 Shumake, J., Poremba, A., Edwards, E. & Gonzalez-Lima, F. Congenital helpless rats as a genetic model for cortex metabolism in depression. *Neuroreport* **11**, 3793-3798 (2000). https://doi.org/10.1097/00001756-200011270-00040

43 O'Reilly, K. C., Shumake, J., Bailey, S. J., Gonzalez-Lima, F. & Lane, M. A. Chronic 13-cis-retinoic acid administration disrupts network interactions between the raphe nuclei and the hippocampal system in young adult mice. *Eur J Pharmacol* **605**, 68-77 (2009). https://doi.org/10.1016/j.ejphar.2008.12.037

44 Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**, 671-675 (2012). https://doi.org/10.1038/nmeth.2089

45 O'Reilly, K. C., Perica, M. I. & Fenton, A. A. Memory deficits with intact cognitive control in the methylazoxymethanol acetate (MAM) exposure model of neurodevelopmental insult. *Neurobiol Learn Mem* **134 Pt B**, 294-303 (2016). https://doi.org/10.1016/j.nlm.2016.07.034