Rat prefrontal cortex is not crucial for cognitive control during spatial learning and memory

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ABSTRACT

Cognitive control tasks require using one class of information and ignoring another competing class of information. A central role of the medial prefrontal cortex (mPFC) in cognitive control is well established in the primate literature, and largely accepted in the rodent literature because mPFC damage causes deficits in tasks that may require cognitive control, as inferred, typically from the task design. Here we test the "central-computation" hypothesis that rodent mPFC is essential for the computations required for cognitive control, the results of which other structures inherit. Ibotenic acid lesion of mPFC was effective and altered the coordination of metabolic activity, including between dorsal hippocampus and dorsal subiculum. Nonetheless, the lesion did not impair alternation between task-relevant and task-irrelevant representations of space in dorsal hippocampal CA1 discharge during an active place avoidance task that requires cognitive control. Nor did mPFC lesion impair initial learning, long-term memory, or conflict learning that tests cognitive flexibility. 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 a central computational locus for cognitive control.

INTRODUCTION

Cognitive control is the ability to judiciously use task-relevant information while ignoring salient concurrent information that is task irrelevant. This ability, also known as executive control, is crucial for completing tasks that require manipulation of complex knowledge and, because cognitive control is often impaired in neuropsychiatric disorders, it is a therapeutic target to improve clinical outcomes (Solomon et al., 2008; Lesh et al., 2011). The consensus view is that prefrontal cortex is crucial for cognitive control and other executive functions such that prefrontal cortical activity is central to the underlying computations that operate through prefrontal interactions with diverse brain areas during cognitive control tasks (Miller and Cohen, 2001; Guise and Shapiro, 2017; Marton et al., 2018). Recent work has emphasized a central role for other brain areas such as sensory thalamus (Wimmer et al., 2015) and hippocampus (Kelemen and Fenton, 2010b; Lee et al., 2012) in cognitive control-related interactions between modality-specific information processing neural circuits and control-specialized processing in the prefrontal cortex (Preston and Eichenbaum, 2013; Murray et al., 2017).

Although investigations of hippocampus function have traditionally emphasized spatial and memory-related information processing, recent work has explored the role of the hippocampus in the cognitive control of spatial and mnemonic information using an active place avoidance task (van Dijk and Fenton, 2018; Chung et al., 2021). 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 spaces, one defined by the rotating floor and the other defined by the stationary room. By selectively conditioning

place avoidance responses to either shock at stationary room locations or shock at rotating arena locations, place learning tasks are configured to require cognitive control to judiciously use spatial information from the spatial frame that is conditioned and ignore spatial information from the other, task-irrelevant frame (Fenton et al., 1998). When both a stationary and a rotating shock zone are defined, inactivation of the dorsal hippocampus impairs task performance (Kelemen and Fenton, 2010b). Importantly, the impairment is also observed when only the stationary shock zone is defined on the rotating arena, suggesting cognitive control is needed, not only to identify the locations of shock, but also to ignore the irrelevant arena locations of shock (Cimadevilla et al., 2000; Cimadevilla et al., 2001; Wesierska et al., 2005). Indeed, physiological recordings of place cell activity in hippocampus subfields, as well as head-direction cell recordings from the medial entorhinal cortical input, confirm cognitive control of spatial representations because neural ensemble activity spontaneously alternates between representing room and arena locations depending on the rat or mouse's proximity to the frame-specific location of shock (Kelemen and Fenton, 2010b; Talbot et al., 2018; van Dijk and Fenton, 2018; Park et al., 2019a; Chung et al., 2021). This explicit observation of the dynamic functional grouping of neural activity into alternating neural representations of what is relevant during the place avoidance task is some of the most direct evidence of cognitive control, which is an internal process that is typically only inferred from the task demands and performance.

What, then, is the role of prefrontal cortex in active place avoidance tasks that require cognitive control? The consensus prefrontal hypothesis asserts that the prefrontal cortex is essential for the computations necessary for cognitive control. This "central-

computation" hypothesis centralizes the necessary computations to the prefrontal cortex and predicts that lesion of the prefrontal cortex will be impairing. An alternative "local-computation" hypothesis asserts that the computations needed for cognitive control can be performed locally in neural networks that are specialized for the particular information upon which the task depends and on which cognitive control operates. This hypothesis predicts that prefrontal lesion can be sparing of cognitive control. We tested these mutually exclusive hypotheses by making lesions of the prefrontal cortex in rats then evaluated their behavior and hippocampal physiology during active place avoidance (Fig. 1A).

RESULTS

Histological evaluation of mPFC lesion

The mPFC lesions are summarized in Figure 1. The ibotenic acid-induced lesions were restricted to mPFC and included the cingulate cortex, prelimbic and infralimbic areas (Fig. 1B). All animals included in further analyses had bilateral mPFC lesions (Fig. 1C,D).

mPFC lesion can alter baseline functional relationships amongst related brain areas

To evaluate whether the consequences of the lesion were restricted to mPFC, we measured the activity of cytochrome oxidase (CO) in the brains of a subset of rats (lesion n = 8, sham n = 8). CO is a sensitive metabolic marker for neuronal function (Wong-Riley, 1989; O'Reilly et al., 2019), so to evaluate the consequence of mPFC lesion on brain

function, CO activity was estimated in 14 functionally-related brain regions that included the dorsal and ventral hippocampus, amygdala, retrosplenial cortex and nucleus reunions (Fig. S1). We detected a group difference in the central amygdala with greater CO activity in the lesion rats (Table S1). We then examined the across-subject metabolic coupling between CO activity in pairs of these areas to evaluate whether mPFC lesion might alter extra-mPFC functional relationships. The pattern of interregional correlations appears to differ between the lesion and sham groups, with some interregional correlations appearing weaker in the lesion group (Figs. 1E, S1). Univariate correlations amongst hippocampus, nucleus reunions, amygdala, and dorsal subiculum decreased after mPFC lesion, but these decreases did not survive the $p < 0.0005$ Bonferroni correction for the 91 comparisons (Fig. 1E; See legend or Table S1 for abbreviations: BLA-RE: $z = 1.91$, $p = 0.05$, dCA1-CEA: $z = 3.12$, $p = 0.001$, dDG-dCA3: $z = 2.79$, $p = 0.005$, vCA1-RE: $z = 1.94$, $p = 0.05$, DS-RE: $z = 1.92$, $p = 0.05$, DS-dDG: $z = 1.9$, $p = 0.05$).

To further investigate whether mPFC lesion alters baseline covariations in metabolic function amongst the sample of brain areas, we applied network graph theoretical analyses with the 14 brain regions representing the nodes; the significant univariate interregional correlations ($p < 0.05$) represent the edges (Fig. 1E). After FDR correction (0.01) 15 of the 91 correlations were judged significant in the sham sample but only 7 in the lesion sample (test of proportions: $z = 2.26$, $p = 0.03$). The correlations within the ventral hippocampus were preserved but correlations to and amongst the dorsal hippocampus subfields were lost after mPFC lesion. These CO data indicate that the mPFC lesion caused functional changes beyond mPFC and specifically in hippocampus.

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

To assess the effect of mPFC lesion on behavior, we first examined the effect on spontaneous locomotion during the pretraining sessions (Fig. 2A). There is no effect of lesion on the total distance walked across the two sessions (Fig. 2B; 2-way RM ANOVA $F_{1,16} = 0.04$, $p = 0.85$, $\eta_p^2 = 0.002$). There is an effect of trial ($F_{1,16} = 15.31$, $p = 0.001$, $\eta_p^2 = 0.49$) but there is no effect of the interaction ($F_{1,16} = 0.14$, $p = 0.71$, $\eta_p^2 = 0.01$).

We next examined active place avoidance learning over the initial training period (Fig. 2A; Days 2-3). Both groups learned to reduce their entrances into the shock zone with training (Fig. 2C). There is no effect of group ($F_{1,16} = 0.002$, $p = 0.9$, $\eta_p^2 = 0.00$), but the effects of day ($F_{1,16} = 26.34$, $p = 10^{-4}$, $\eta_p^2 = 0.62$) and trials ($F_{3.22, 51.50} = 36.76$, $p = 10^{-13}$, $\eta_p^2 = 0.70$) are significant. There are no effects of the group interactions (group x day: $F_{1,16} = 0.003$, $p = 0.96$, $\eta_p^2 = 0.00$; group x trial: $F_{3.22, 51.50} = 0.74$, $p = 0.54$, $\eta_p^2 = 0.00$; group x day x trial: $F_{2.26, 36.08} = 0.35$, $p = 0.74$, $\eta_p^2 = 0.02$). The interaction of day x trial is significant ($F_{2.26, 36.08} = 13.75$, $p = 10^{-5}$, $\eta_p^2 = 0.02$).

We also examined the latency to first enter the shock zone on each trial because this estimates between-trial learning without the confound of within-trial learning (Fig. 2D). Both groups increase their latencies to first enter the shock zone. There is no effect of group ($F_{1,16} = 0.1$, $p = 0.76$, $\eta^2 = 0.01$) but clear effects of day ($F_{1,16} = 69.61$, $p = 10^{-7}$, $\eta^2 = 0.81$) and trial ($F_{5.33, 85.33} = 12.15$, $p = 10^{-9}$, $\eta_p^2 = 0.43$). There are also no significant group

interactions (group x day: $F_{1,16} = 0.94$; $p = 0.35$, $\eta_p^2 = 0.06$; group x trial: $F_{5.33, 85.33} = 1.83$, $p = 0.09$, $\eta_p^2 = 0.10$; group x day x trial: $F_{4.78, 76.43} = 0.26$, $p = 0.93$, $\eta_p^2 = 0.02$). The day x trial interaction is significant ($F_{4.78, 76.43} = 1.93$, $p = 0.10$, $\eta_p^2 = 0.11$).

Twenty-four-hour memory retention was tested on day 4. The two groups are indistinguishable in their times to first enter the shock zone ($t_{16} = 1.01$, $p = 0.3$, $d= 0.49$) and the number of entrances ($t_{16}= 0.95$, $p = 0.4$, $d= 0.002$).

We then trained the rats to avoid the shock zone relocated 180° to assess the impact of mPFC lesion on cognitive flexibility in this potentially greater cognitive control challenge. Both groups learn the conflict task, measured by the number of entrances into the new shock zone (Fig. 2C, Days 4-5). There was a non-significant trend for sham rats to show greater savings comparing the first and second days of conflict trials (sham = 11.12± 4.58 entrances, lesion = 4.3 ± 4.09 entrances; $t_{16} = 1.11$; $p = 0.3$, $d = 0.52$) and there was no effect of group ($F_{1,16} = 0.21$, $p = 0.65$, $\eta_p^2= 0.01$), but the effects of day ($F_{1,16} = 8.99$, $p = 0.01$, $\eta_p^2= 0.36$), and trials ($F_{1.89, 30.12} = 18.55$, $p = 10^{-9}$, $\eta_p^2 = 0.54$) are significant. The group interactions are not significant (group x day: $F_{1,16} = 0.55$, $p = 0.47$, $\eta_p^2= 0.36$; group x trial: $F_{1.88, 30.12} = 1.38$, $p = 0.27$, $\eta_p^2= 0.08$; day x trial: $F_{2.30, 36.87} = 2.31$, $p = 0.11$ $\eta_p^2= 0.13$; group x day x trial: $F_{2.30, 36.87} = 1.16$, $p = 0.33$, $\eta_p^2= 0.07$). The latency to first enter the new shock zone also increases for both groups (Fig. 2D, Days 4-5). The effect of group is not significant ($F_{1,16} = 0.21$, $p = 0.65$, $\eta_p^2= 0.01$) but the effects of day ($F_{1,16} = 20.46$, $p = 10^{-3}$, $\eta_p^2= 0.01$) and trials ($F_{4.84, 154.93} = 10.94$, $p = 10^{-8}$, $\eta_p^2= 0.002$) are significant. There are also no significant interactions (group x day: $F_{1,16} = 0.03$, $p = 0.86$,

η_p^2= 0.002; group x trial: $F_{4.02, 64.38}$= 0.38, p = 0.54, η_p^2= 0.05; day x trial: $F_{4.05, 64.81}$ = 1.29, p = 0.28, η_p^2= 0.08; group x day x trial: $F_{4.05, 64.81}$ = 0.5, p = 0.76, η_p^2= 0.03). We find no evidence that mPFC lesion causes a deficit in the active place avoidance task.

mPFC lesion does not alter cognitive control of hippocampal neural representations

By definition, cognitive control requires the subject to process and use one class of information purposefully, at the expense of other competing information, which we have previously demonstrated in the population dynamics of dorsal hippocampus spatial discharge of rats and mice as they navigate on a rotating arena (Kelemen and Fenton, 2010b; Kelemen and Fenton, 2013; Kelemen and Fenton, 2016; van Dijk and Fenton, 2018; Chung et al., 2021). We thus examined spatial frame ensemble preference (SFEP), the representational dynamics of hippocampus CA1 discharge on the rotating arena during pretraining, as well as place avoidance of the initial and conflicting shock zone locations. The discharge properties of individual CA1 principal cells did not differ between the sham and mPFC lesion groups (Table 1). CA1 ensemble discharge alternated between preferentially representing room locations and arena locations (Fig. 2E; runs tests for all recordings were significant; z's ≥ -12.3, p's ≤ 10^{-33}). The representational dynamics were expressed during pretraining on the rotating arena such that the SFEP in hippocampus discharge was equally likely to signal the current room location or alternatively, the current arena location, in both the sham and lesion rats (Fig. 2F). Importantly, during avoidance of the initial shock location, SFEP favored the arena frame because the rat avoided the room frame location of shock (Fig. 2F), as previously

demonstrated (van Dijk and Fenton, 2018; Chung et al., 2021). Relocating the shock zone 180° for conflict training changed SFEP in the lesion group so that it was equally probable to be room- or arena-preferring, consistent with needing to decide between avoiding the current and previous shock location throughout the environment (Dvorak et al., 2018); the change was not observed for the sham group (Fig. 2F). While the effect of Group ($F_{1,12}$ = 0.084, p = 0.8) was not significant on the probability of room-preferring hippocampal representations, the effects of Session ($F_{2,12}$ = 9.75, p = 0.003) and the Group X Session effect were significant ($F_{2,12}$ = 4.87, p = 0.03; post-hoc tests confirmed Sham = Lesion during pretraining and retention of the initial shock location and Sham < Lesion during conflict). The ability to manipulate SFEP of hippocampal representational dynamics by the presence and location of shock location directly demonstrates cognitive control at the level of the hippocampus, and that despite an influence of mPFC lesion on cognitive control of spatial representations, the lesion does not impair this ability.

DISCUSSION

Although permanent lesion of mPFC altered baseline metabolic coupling of hippocampus-related brain areas (Fig. 1) and altered hippocampal representational dynamics during expression of cognitive flexibility in an active place avoidance (Fig. 1F), we find no evidence that the lesion impaired cognitive control assessed either behaviorally or by hippocampal representational dynamics (Fig. 2). We confirmed the extent of the lesion and, using CO imaging, independently confirmed the lesion caused changes beyond the directly damaged area. We also observed reduced bursting in the discharge of the

underlying thalamus in the lesion group (Table S2). A metabolic increase was observed in the amygdala and changes in the covariation of metabolic activity were detected in the interregional correlations, especially reduced correlations within ventral and dorsal hippocampus and between them and other structures; presumably these changes are compensatory in response to loss of innervation caused by the lesion. 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. Parenthetically, we stress that the present findings do not rule out a role for mPFC and hippocampal interactions in other cognitive processes (Adhikari et al., 2010; Sigurdsson et al., 2010; Spellman et al., 2015; Guise and Shapiro, 2017; Negrón-Oyarzo et al., 2018), especially those that rely on ventral hippocampus and perhaps the direct excitatory mPFC-ventral hippocampal and direct mPFC-dorsal hippocampus inhibitory connections (Hoover and Vertes, 2007; Malik et al., 2022), as such questions are beyond the scope of the experimental design. Because numerous manipulations of dorsal hippocampus, including bilateral inactivation, and unilateral inactivation, impair learning, consolidation and retrieval of the conditioned place avoidance (Cimadevilla et al., 2000; Cimadevilla et al., 2001; Jezek et al., 2002; Kubik and Fenton, 2005; Wesierska et al., 2005), the present data instead favor the local-computation hypothesis. Accordingly, we directly confirmed that mPFC lesion does not reduce the goal-directed representational switching of dorsal hippocampus (Fig. 2E,F) that is a *sine qua non* for cognitive control of spatial information (Kelemen and Fenton, 2010b, 2016; Dvorak et al., 2018; Talbot et al., 2018; van Dijk and Fenton, 2018; Chung et al., 2021), despite detecting an effect of the lesion. More targeted hippocampal manipulations like X-irradiation and genetic ablation of adult neurogenesis

(Burghardt et al., 2012), and optogenetic inactivation of granule cells (Kheirbek et al., 2013; van Dijk and Fenton, 2018) spare learning to avoid the initial location of shock in the active place avoidance task, but impair conflict learning to avoid the relocated location of the shock, unlike lesion of mPFC, as observed in the present study (Fig. 2C,D). Global manipulations such as genetic deletion of calbindin to promote aging (Moreno et al., 2011), as well as less specific manipulations, such as transient global cerebral ischemia to cause CA1 lesion (Popp et al., 2011), also impair active place avoidance learning, whereas mPFC lesion does not. It is certainly possible that acute manipulations of mPFC, such as optogenetic, chemogenetic or pharmacological inactivation, would have led to different results, suggesting that 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. Nonetheless, 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 (Kesner et al., 1989), rather than the allocentric spatial information upon which the present active place avoidance task variant relies, but this very fact would also require rejecting, or at least severely limiting the central-computation hypothesis.

Taken together these data are difficult to explain on the central-computation hypothesis that the mPFC is essential for cognitive control, but 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 that the circuit is specialized to process.

METHODS

All methods complied with the Public Health and Service Policy on Humane Care and Use of Laboratory Animals and were approved by the Author'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. Twelve rats were assigned to the lesion group and 11 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.

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) but only a subset were stained for cytochrome oxidase (lesion n = 8) because the tissue from one lesion and one sham rat was 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; his brain was still processed for cytochrome oxidase activity. One rat was excluded from the behavioral experiments due to equipment issues during training, and one rat had tissue damage during the sham surgery that appeared as a lesion. 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.

Electrophysiology procedures

The following procedures have been previously described in detail (Park et al., 2019b). 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 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 like the rats that received active place avoidance training. 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 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.

Electrophysiology data analysis

Single units were automatically sorted using Kilosort2 (Pachitariu et al., 2016). 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 3-10 ms divided by the number of spikes with ISI 200 -300 ms.

Hippocampus single units were classified as complex-spike or theta cells as in prior work (Ranck, 1973; Fenton et al., 2008; Kelemen and Fenton, 2010a). 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 (Fox and Ranck, 1975), 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 (Fig. 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 current location *x* on the rotating arena (Olypher et al., 2003). $I_{pos}(t)$ 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 ensemble activity was room (or arena) preferring (van Dijk and Fenton, 2018; Chung et al., 2021).

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, 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 Canvas 5.02.

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 (Shumake et al., 2000) were included. Cytochrome oxidase staining was performed according to (O'Reilly et al., 2009). 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 (NIH) and optical densities read from the standard slides and 14 brain regions (Figure 1) and included: dygranular 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 (O'Reilly et al., 2016). 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. 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.

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FIGURES AND TABLES

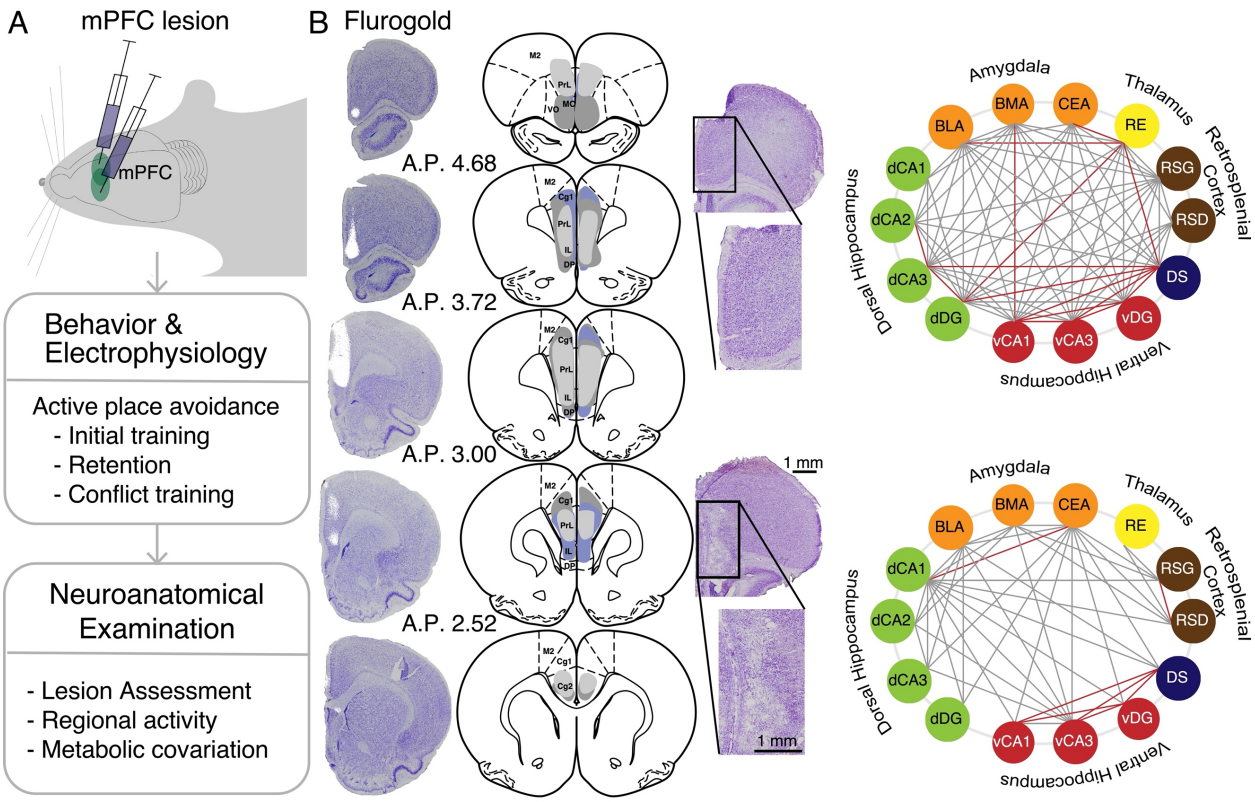

Figure 1. Assessing the mPFC lesions. A) Rats with mPFC or sham lesions were tested for cognitive ability using the two-frame active place avoidance task. Training to an initial shock zone (Initial training) estimated cognitive control, the ability to ignore the irrelevant cues associated with the rotating arena while using the relevant stationary room cues that indicate the shock zone location. A retention trial was used to assess memory of the shock zone after two days of training, and cognitive flexibility was assessed by relocating the shock zone by 180° (conflict training). Dorsal CA1 ensembles were recorded during active place avoidance. mPFC lesions were histologically verified and the impact of the lesion on brain networks was examined by histological assessment of cytochrome oxidase activity, a metabolic marker of neuronal activity. Metabolic covariation between

brain regions was estimated by calculating the interregional Pearson correlations of cytochrome oxidase activity. **B**) To determine the appropriate coordinates for injection of ibotenic acid, we first injected fluorogold and examined the extent of fluorescent labeling. **C**) mPFC and sham lesions were evaluated in Nissl stained histological sections and mapped onto a standard brain from the Rat Stereotaxic Coordinates Atlas. **D**) Representative mPFC from a sham and a lesion rat. PrL(prelimbic), IL(infralimbic), Cg (cingulate cortex). **E**) Graph theoretical analysis of resting-state metabolic functional connectivity was used to visualize differences between the sham and lesion groups. Each line in a network represents 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.

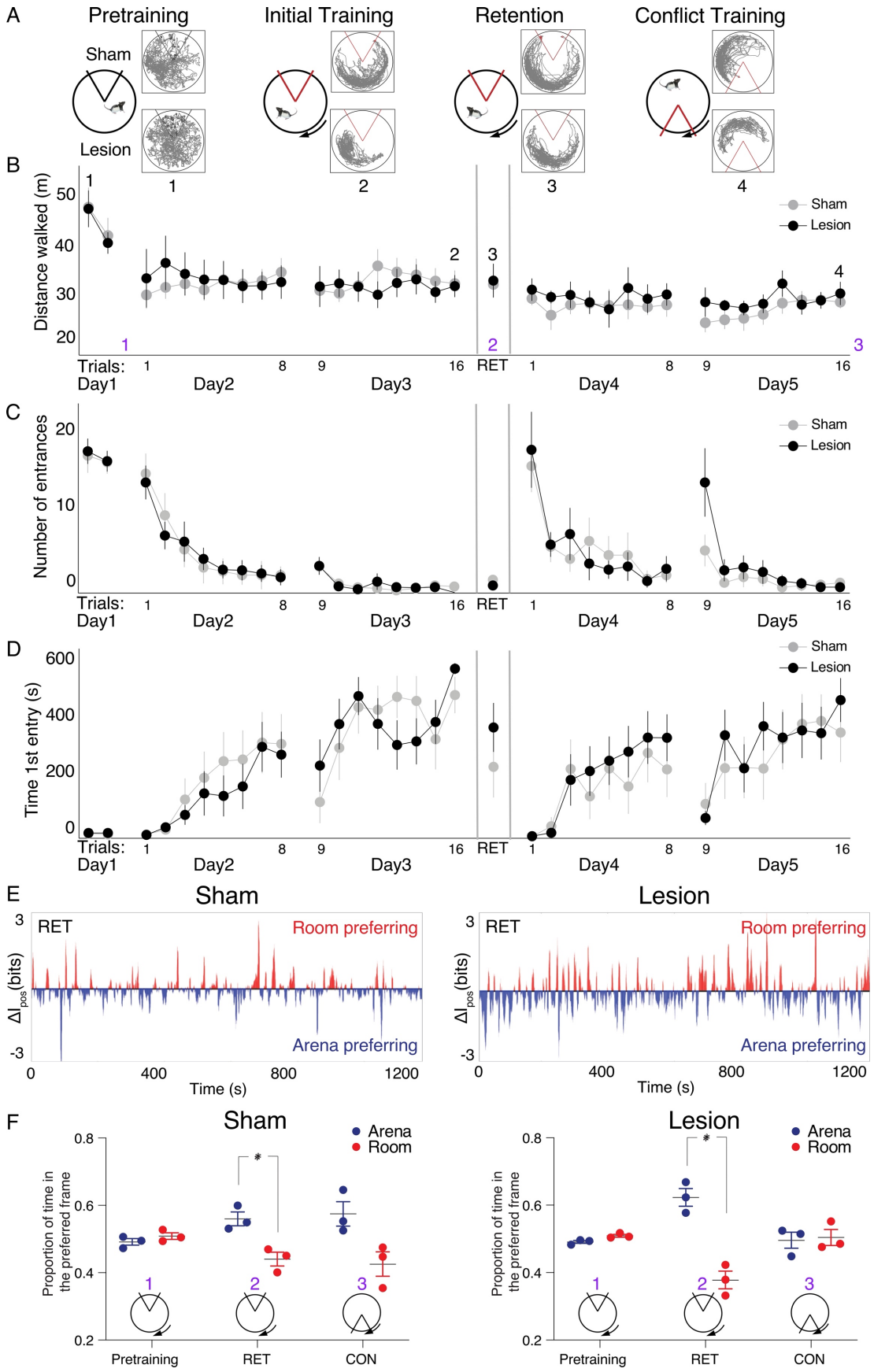
A
Pretraining
Initial Training
Retention
Conflict Training
Sham
Lesion
1
2
3
4
B
Distance walked (m)
Sham
Lesion
Trials:
Day1
Day2
Day3
RET
Day4
Day5
C
Number of entrances
D
Time 1st entry (s)
E
Sham
Lesion
RET
Room preferring
Arena preferring
ΔI_pos (bits)
Time (s)
F
Proportion of time in the preferred frame
Arena
Room
Pretraining
RET
CON

Figure 2. No detectable impact of mPFC lesion on cognitive control and flexibility measured during the active place avoidance task. A) Rats were trained in the two-frame active place avoidance task over the course of five days. Pretraining: On day 1, the rats were introduced to the stationary arena through two habituation trials. Initial Training: On days 2 and 3 the rats were trained to avoid a 60° sector of the arena in which they would receive a mild foot shock. Each day the rats received eight training trials. Retention: Rats were tested in a single trial to the initial shock zone on day 4. Afterwards the rats were given Conflict Training for two days (days 4 and 5), in which rats were given eight trials per day to learn to avoid a new shock zone located 180° from the initial shock location. All trials were 10 min with a 10 min inter-trial interval. Sham and mPFC lesion rats did not differ in **B**) locomotor activity, **C**) learning to avoid the initial or relocated shock zone locations, or **D**) memory for the shock zone. Neither did the sham (n=3) and mPFC (n=3) lesion rats differ in expressing cognitive control of spatial frame specific representations of location as shown by **E**) individual ensemble examples during the retention session after training to avoid the initial shock zone location and **F**) group statistics of spatial frame ensemble preference (SFEP), demonstrating cognitive control in both groups. t tests indicate that during initial training, SFEP is biased to the arena frame in both the sham ($t_4 = 4.16$, $p = 0.01$) and lesion rats ($t_4 = 6.62$, $p = 0.003$).

CA1	Sham lesion (101 cells)	mPFC Lesion (85 cells)	t(d.f); p values
Firing rate (AP/s)	3.29 ± 0.27	3.68± 0.33	0.91(184); 0.36
Burst ratio	1.53 ± 0.25	2.02 ± 0.28	1.29(174); 0.19

Table 1. Electrophysiological properties of CA1 principal cells