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

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eLife Assessment

This **important** study includes **convincing** evidence to show that behavioral measures and hippocampal representations when animals use task-relevant information and ignore irrelevant information do not depend on the medial prefrontal cortex. The results are expected to be of interest to those studying neural mechanisms of cognitive control and functions of associational brain regions.

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Abstract

Cognitive control tasks require using one class of information while ignoring competing classes of information. The 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. In prior work, we used an active place avoidance task where a rat or mouse on a rotating arena is required to avoid the stationary task-relevant locations of a mild shock and ignore the rotating task-irrelevant locations of those shocks. The task is impaired by hippocampal manipulations, and the discharge of hippocampal place cell populations judiciously alternates between representing stationary locations near the shock zone and rotating locations far from the shock zone, demonstrating cognitive control concurrently in behavior and the hippocampal representation of spatial information. Here we test whether rat mPFC lesion impairs the active place avoidance task to evaluate two competing hypotheses, a "central-computation" hypothesis that the mPFC is essential for the computations required for cognitive control and an alternative "local-computation" hypothesis that other brain areas can perform the computations required for cognitive control, independent of mPFC. Ibotenic acid lesion of the mPFC was effective, damaging the cingulate, prelimbic and infralimbic cortices. The lesion also altered the normal coordination of metabolic activity across remaining structures. The lesion did not impair learning to avoid the initial location of shock or long-term place avoidance memory, but impaired avoidance after the shock was relocated. The lesion also did not impair the

alternation between task-relevant and task-irrelevant hippocampal representations of place information. These findings support the local-computation hypothesis that computations required for cognitive control can occur locally in brain networks independently of the mPFC.

Introduction

Cognitive control is a psychological construct that refers to a set of processes that allow information processing and purposeful behavior to vary and adjust according to the demands of a subject's current goals. Without cognitive control, information processing and behavior would be rigid and inflexible when task demands and goals change [1]. Because cognitive control is not limited to any one task or task domain, and because it engages various processes that include forms of attention, memory processes, behavioral inhibition and the like, it has been a challenge to establish the neuronal basis of cognitive control. What would the neuronal network activity look like in a part of the brain that was implementing cognitive control? From our perspective, until we connect neuronal network function to cognitive control, the construct's value will be limited for understanding brain function and treating the diverse types of dysfunction that manifest as impaired cognitive control. Towards this goal, it has been important to identify regions of the brain that might be crucial for cognitive control. This has largely been approached by investigating brain function while subjects perform tasks designed to require cognitive control because performance depends on the ability to judiciously use task-relevant information while ignoring salient concurrent information that is currently irrelevant for the task.

Studies of human brain function identified subdivisions of the prefrontal cortex (PFC) that are engaged by tasks that are thought to require cognitive control, such as the Wisconsin Card Sort and Stroop tasks. As noted above, it is important to identify the neuronal network activity that the construct of cognitive control assumes must be operating. Recordings of neuronal activity from the PFC of non-human primates as they perform tasks that are thought to require cognitive control has been a particularly important development towards the identification of such neural network activity. Indeed, the consensus view is that 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 [2–4]. An influential theory states: "We assume that the PFC serves a specific function in cognitive control: the active maintenance of patterns of activity that represent goals and the means to achieve them. They provide bias signals throughout much of the rest of the brain, affecting not only visual processes but also other sensory modalities, as well as systems responsible for response execution, memory retrieval, emotional evaluation, etc. The aggregate effect of these bias signals is to guide the flow of neural activity along pathways that establish the proper mappings between inputs, internal states, and outputs needed to perform a given task. This is especially important whenever stimuli are ambiguous (i.e. they activate more than one input representation), or when multiple responses are possible and the task-appropriate response must compete with stronger alternatives. From this perspective, the constellation of PFC biases—which resolves competition, guides activity along appropriate pathways, and establishes the mappings needed to perform the task—can be viewed as the neural implementation of attentional templates, rules, or goals, depending on the target of their biasing influence." [2, page 171] Like biased competition, in which winner-take-all network representations compete to explain selective visual attention [5–9] the theory is especially valuable and powerful because it predicts what neuronal signals should look like when cognitive control is operating. This enables experimentalists to operationally identify cognitive control using neuronal network behavior so that hypotheses can be rigorously evaluated. Accordingly, cognitive control would be at work when there is sustained neuronal network representations of task-relevant information that suppresses or gates representations of salient task-irrelevant information in accord with purposeful judicious behavior. This is not to say that the control signal itself would have this property, although a so-called "transmissive" type of control signal would

include task-relevant context and stimulus information, whereas a "modulatory" type of control signal would only carry the control signal that biases expression of the task-relevant context and stimulus information [10, 11].

The hippocampal role in the cognitive control of spatial and mnemonic information has been investigated using an active place avoidance task [12]. 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 [13]. Avoiding shock at a stationary room location requires using the task-relevant spatial information and ignoring spatial information from the other, task-irrelevant arena frame. Dorsal hippocampus dysfunction impairs this avoidance without impairing the place avoidance when the two frames are not dissociated by stopping the rotation or attenuating the arena cues with shallow water [14–17]. During active place avoidance task variants that require cognitive control, place cell discharge in hippocampus subfields, and head-direction cell discharge in the medial entorhinal cortical input to hippocampus 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 [12, 13, 18–20].

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 [21, 22]. Recent work indicating that the hippocampus [18] and sensory thalamus [23] 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 [22, 24, 25]. 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**). We reasoned that if the central-computation hypothesis was valid, active place avoidance behavior would be impaired by mPFC lesion along with discoordination of the alternating dynamics between concurrent hippocampal network representations of task-relevant room-frame and task-irrelevant arena-frame locations of the rotating arena. Alternatively, if the local-computation hypothesis was valid, then mPFC lesions would spare the active place avoidance behavior and the hippocampal place representation dynamics. We used cytochrome oxidase, a metabolic marker of baseline neuronal activity, to confirm the mPFC lesions were effective and that there are non-local network consequences despite the local lesion. We first evaluated cytochrome oxidase activity in regions known to be associated with performance in the active place avoidance task, or regions with known connectivity to the mPFC. We then evaluated covariance of activity amongst the regions in an effort to detect network consequences of the lesion.

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**; S2). 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**; $F_{1,16} = 0.04$, $p = 0.85$, $\eta^2 = 0.002$).

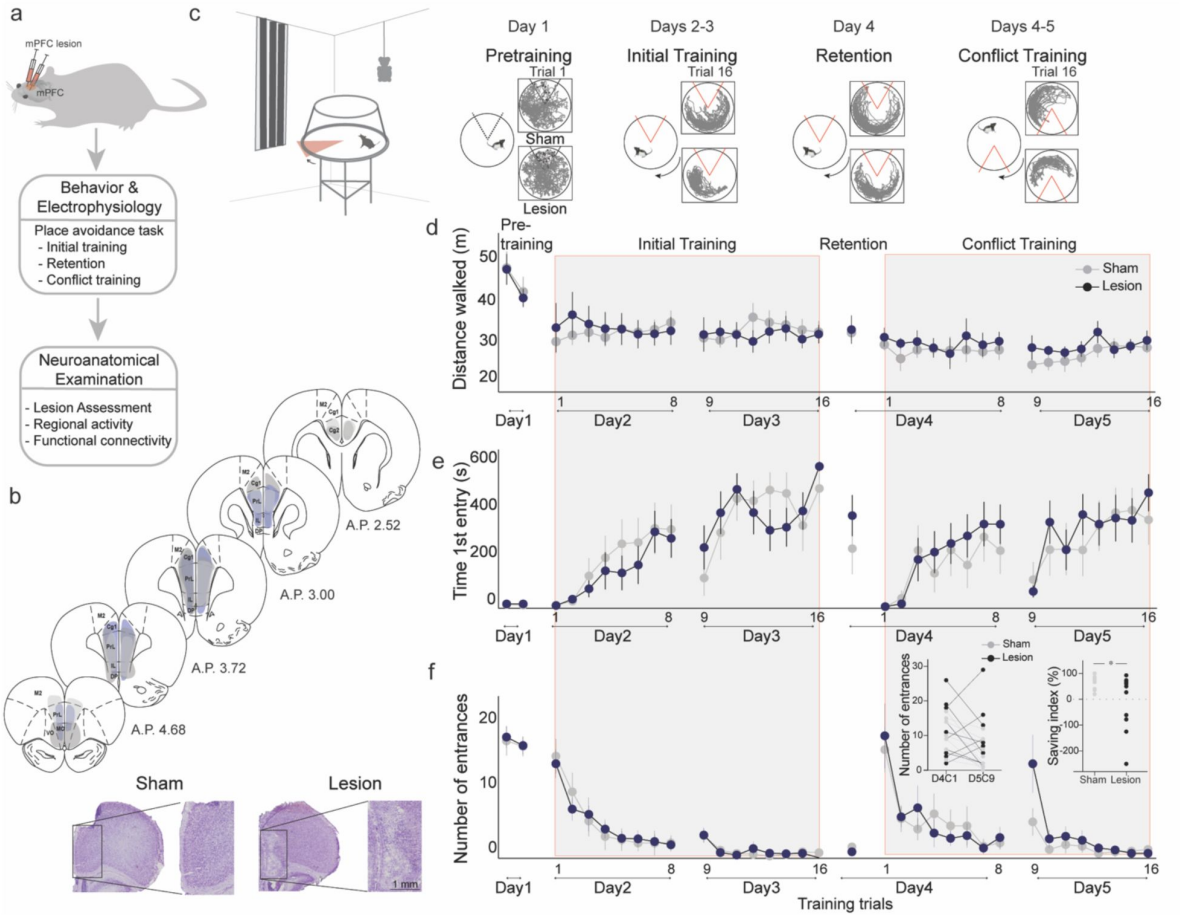

Figure 1.

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 (intermediate with dorsal anterior lesion), dark grey (intermediate with ventral anterior lesion – includes medial orbital cortex), and purple (largest lesion)). The smallest lesion spanned A-P 3.24-2.52. PrL (prelimbic), IL (infralimbic), Cg (cingulate cortex), M2 (secondary motor cortex). Representative mPFC from a sham and a lesion rat (bottom). **(C)** Tracked room-frame positions 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. **(F)** The left inset compares the number of entrances during the first 5 minutes of the first (D2T1) and second days of initial training (D3T9). The right inset compares the first 5 minutes of 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. Savings was not related to lesion size $r = 0.009$, $p = 0.98$. *$p < 0.05$.

There is a main effect of trial ($F_{1,16}$ = 15.31, p = 0.001, η^2 = 0.09) but there is no effect of the group x trial interaction ($F_{1,16}$ = 0.14, p =0.71, $\eta^2 = 10^{-3}$). Similarly, locomotion does not differ between the groups or across trials, nor is there a significant group x trial interaction during Initial Training on days 2-3 (df = 3.17/50.77) or during Conflict Training on days 4-5 (df = 4.97/79.58; $F'_S \leq 1.96$, $p \geq 0.09$, $\eta^2 \leq 0.03$).

We then examined the acquisition of active place avoidance memory during Initial Training using the time to first enter the shock zone, the clearest estimate of between-trial memory. Both groups learn to increase the latency to first enter the shock zone (**Fig. 1E**). 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, $\eta^2 = 10^{-3}$; 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, $\eta^2 = 10^{-3}$). Neither was there a difference in avoidance learning between the groups measured by the number of entrances into the shock zone (**Fig. 1F**). The effect of group is not significant ($F_{1,16}$ = 0.002, p = 0.96, $\eta^2 = 10^{-5}$) 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, $\eta^2 = 10^{-5}$; group x trial: $F_{3.22, 11.50}$= 0.74, p = 0.54, $\eta^2 = 10^{-3}$; group x day x trial: $F_{4.05, 64.81}$ = 0.35, p = 0.74, $1^2 = 10^{-3}$). 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 also indistinguishable on Day 4 during which Retention of 24-h memory is assessed by 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).

To increase the cognitive challenge, we were motivated by evidence that mPFC manipulation can affect cognitive flexibility as assessed by reversal learning tasks [26, 27]. Accordingly, we trained the rats to avoid the shock zone after relocating it 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 [28]. Both groups learn the conflict task variant, measured by the number of entrances. There is no effect of group ($F_{1,16}$ = 0.21, p = 0.65, $\eta^2 = 10^{-3}$), 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, $p = 10^{-9}$, η^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, η^2= 0.02; day x trial: $F_{2.30, 36.87}$ = 2.13, p = 0.11, η^2= 0.02, group x day x trial: $F_{2.30, 36.87}$ = 1.16, p = 0.33, $\eta^2 = 10^{-3}$). Also, the time to enter the new shock zone, which was low in both groups, consistent with poor between-day memory for the novel location of shock (**Fig. 1**, Days 4-5 Conflict Training). There is no effect of group ($F_{1,16}$ = 0.21, p = 0.65, $\eta^2 = 10^{-3}$), but the effects of day ($F_{1,16}$ = 20.46, p = 0.01, η^2 = 0.04), and trials ($F_{1.89, 30.12}$ = 11.52, $p = 10^{-}$, η^2 = 0.13) are significant. The group interactions are not significant (group x day: $F_{1,16}$ = 0.03, p = 0.86, $\eta^2 = 10^{-3}$; group x trial: $F_{1.88, 30.12}$ = 0.78, p = 0.54, η^2= 0.01; group x day x trial: $F_{2.30, 36.87}$ = 0.47, p = 0.76, $\eta^2 = 10^{-2}$). Also, the interaction between day and trial is not significant (day x trial: $F_{2.30, 36.87}$ = 1.29, p = 0.28 η^2= 0.02). We more closely examined conflict learning by analyzing 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 during the first 5 minutes on the first trial of each day to estimate savings, measured as each animal's difference in performance) during initial training (**Fig. 1F** left inset) and during conflict training (**Fig. 1F** middle inset). During initial training, both sham (paired t_7 = 4.5, p = 0.003, d = 1.2) and lesion (paired t_9 = 4.7, p = 0.001, d = 1.5) rats improved. During conflict training, sham rats improved across the pair of first conflict trials of each day (**Fig. 1F** middle inset; paired t_7 = 3.8, p = 0.006, d = 1.4) but not lesion rats (paired t_9 = 0.87, p = 0.4, d = 0.27). Indeed, while all sham rats improved across the first two conflict trials of each day only 6/10 lesion rats improved (test of proportions z = 2.3, p = 0.03). We also quantified the improvement for each subject during conflict as a savings index (**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). This difference on the second day of conflict training was observed despite equivalent performance across eight trials on the first day. We

therefore 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. However, the lesion is sufficient to impair conflict learning after a 24-h delay.

mPFC lesion alters functional relationships amongst related brain areas

Cytochrome oxidase (CO), a sensitive metabolic marker for neuronal function [29], 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**, S3b; 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 appears 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

When the discharge of principal cells of the hippocampus is location-specific to so-called place fields, the cells are called place cells. Place fields are disrupted by molecular manipulations that disrupt spatial learning and memory and changes in place cell firing tend to track environmental changes like geometry and other spatial features that often accompany spatial learning and memory challenges [review 30]. In contrast, spatial learning and memory tasks that depend crucially on hippocampus and hippocampal synaptic plasticity, like active place avoidance or task changes, such as those introduced by the conflict task variant, cannot be assessed by simple changes in place fields and their quality [12, 13, 19, 31–36]. This is in part because dynamic cognitive variables, like adjustments of attention and cognitive control, operate during learning and memory tasks. The dynamics of such internal cognitive variables modulate place cell firing, creating extra-positional signals [12, 18, 19, 37–39]. The presence of these extra-positional signals can be detected as momentary discharge deviations from the expectations of firing-field based spatial discharge [40]. On the timescale of the few seconds it takes to walk across a firing field, these noise-like deviations can be measured as overdispersion [41, 42]. We can also measure the momentary positional information in the collective neuronal population discharge dynamics, which during active place avoidance alternates between encoding locations in the room and locations in the arena [18, 43]. The hippocampal and entorhinal cortex neuronal populations, each fluctuate between representing space in these two distinct spatial frames every few seconds [20, 31, 44]. These representational dynamics are purposeful and can be summarized as a spatial frame ensemble preference (SFEP) that is biased to room-frame locations near the room-defined shock zone and is biased to arena-frame locations far from the shock zone [12, 13].

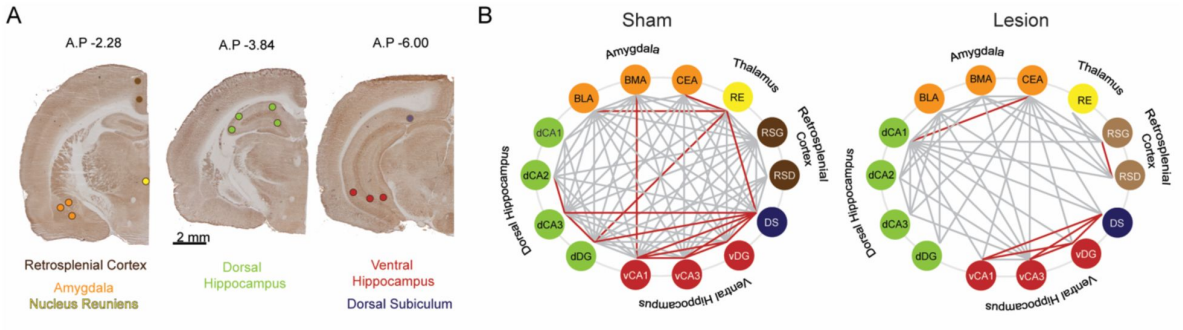

Figure 2.

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.

In light of these cognitive dynamics in neuronal activity representations of position, and the ability to decode task information from neuronal population discharge, it is powerful to characterize cognitive control by investigating neuronal population discharge. Cognitive control requires the subject to process and use a class of information purposefully, at the expense of other competing information. Accordingly, we previously demonstrated such a cognitive control signal in the population dynamics of dorsal hippocampus spatial discharge representations of room and arena positions as rats and mice navigate on a rotating arena [12, 13, 18, 39]. Consequently, we examined the 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 in these three trials because the shock was off.

We first examined basic discharge properties of individual CA1 principal cells, which do not differ between the sham and mPFC lesion groups (**Fig 3B**, Table S2). Nor does mPFC lesion alter thalamic firing rates, but it reduces the likelihood of bursting activity (Table S3) complementing the CO evidence that the mPFC lesion affected activity in areas beyond the lesion sites.

We then examined overdispersion of place cell firing, which is known to be reduced by prefrontal inactivation [45]. In contrast, overdispersion is expected to increase with cognitive control and other extra-positional processes that increase discharge non-stationarity [38]. We find that during pretraining, overdispersion of hippocampal discharge 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 the spatial frame ensemble preference (SFEP), the electrophysiological signature of cognitive control of place representations, 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 ≥ 12.3, p's ≤ 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 [12, 13]. 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**) [46]. 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 of place representations 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**, S3), 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 place avoidance task (**Fig.1**, 3, 4), despite the lesion being sufficient to cause a

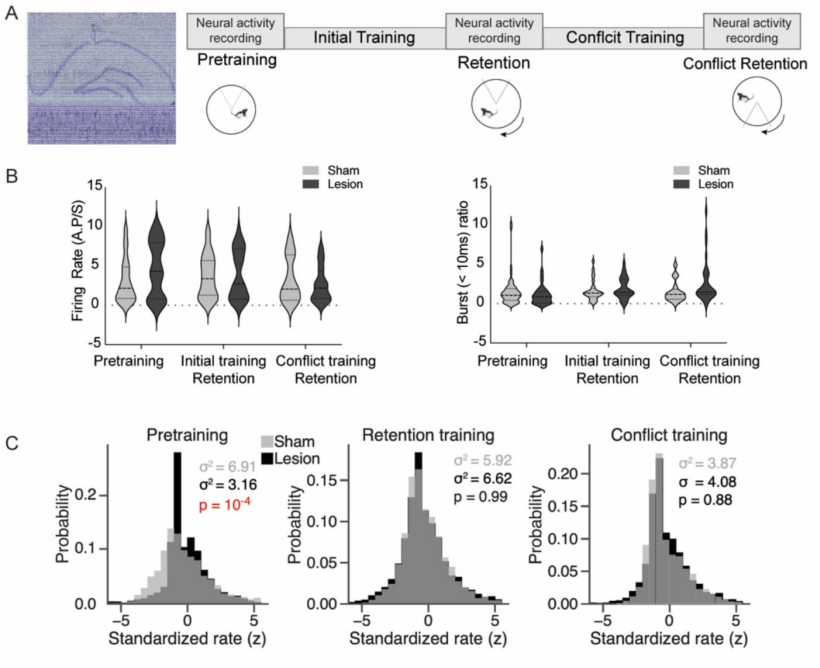

Figure 3.

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: $F_{2776,3397} = 2.19$, $p = 5.8\times10^{-4}$).

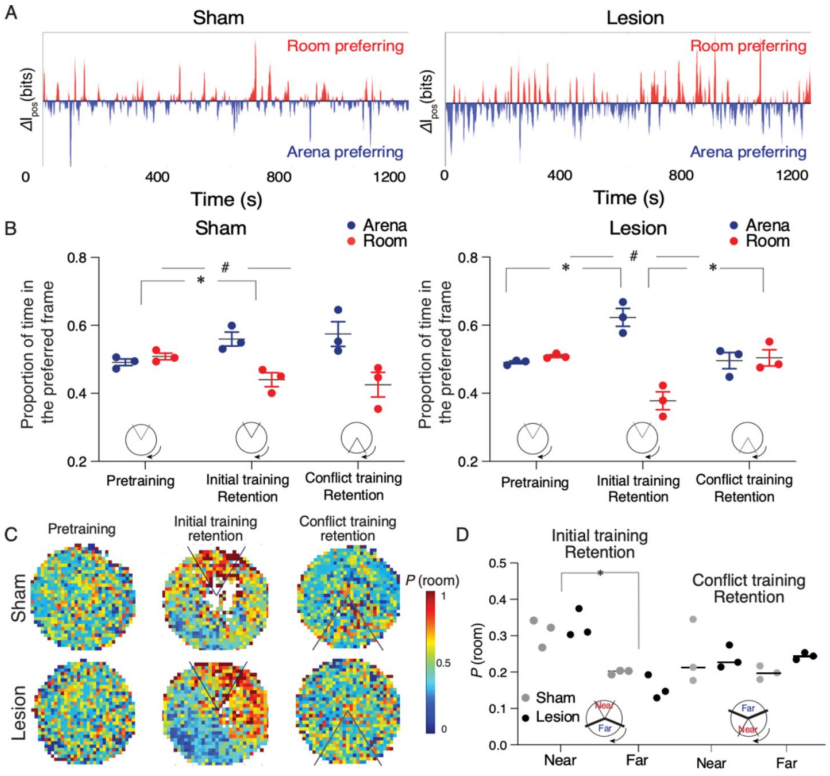

Figure 4.

Sham and mPFC 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 pretraining, initial, and conflict retention sessions with no shock. **(D)** Summary of average probability of room-preferring SFEP discharge in half the arena near and far from the shock zone during the initial and conflict retention sessions. Two-way Group x Location ANOVA (Sham: n=3, Lesion: n=3) during retention of the initial shock zone location: Group: $F_{1,4} = 0.17$ $p = 0.68$, $\eta^2 = 10^{-3}$; Location: $F_{1,4} = 54.69$, $p = 0.001$, $\eta^2 = 0.84$; Group x Location $F_{1,4} = 2.04$, $p = 0.2$, $\eta^2 = 10^{-2}$; during retention of the conflict shock zone location: Group: $F_{1,4} = 0.71$ $p = 0.45$, $\eta^2 = 0.05$; Location: F1,4 = 0.42, p = 0.55, h2 = 0.056; Group x Location F1,4 = 0.67, p = 0.45, h2 = 0.08.

cognitive flexibility deficit when the shock zone was relocated for the conflict training trials (**Fig. 1F** insets). We observed increased overdispersion in lesion rats but only during pretraining; place cell tuning was not disturbed, only the discharge reliability was reduced in the absence of the place task. We also observed reduced bursting in the discharge of the underlying posterior and lateral posterior thalamus in the lesion group compared to sham (Table S3). 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 [47, 48] and loses the metabolic correlation with the nucleus reuniens, which is thought to be a relay between the mPFC and the dorsal hippocampus [49, 50].

We have previously demonstrated cognitive control in the active place avoidance task variant we used (**Fig. 1**) because the rats must ignore local rotating place cues to avoid the stationary shock zone. Even when the arena does not rotate, rats distinctly learn to avoid the location of shock according to distal visual room cues and local olfactory arena cues, such that the distinct place memories can be independently manipulated using probe trials [51, 52]. When the arena rotates, as in the present studies, neural manipulations that impair the place avoidance are no longer impairing when the irrelevant arena cues are hidden by shallow water [16, 17, 53, 54].

Furthermore, persistent hippocampal neural circuit changes caused by active place avoidance training are not detected when shallow water hides the irrelevant arena cues to reduce the cognitive control demand [12, 33, 35]. While these findings unequivocally demonstrate the salience of relevant stationary room cues to use for avoiding shock and irrelevant arena cues to ignore during active place avoidance, the most compelling evidence of cognitive control comes from recording hippocampal ensemble discharge. Hippocampal ensemble discharge purposefully represents current position using stationary room information when the subject is close to the stationary shock zone and alternatively represents rotating arena information when the mouse is far from the stationary shock zone [**Fig. 4**; 12]. Despite this evidence from task design, behavioral observations, and direct electrophysiological representational switching as required to directly demonstrate cognitive control, one might still argue that it is logically possible that the active place avoidance task does not require cognitive control and this is why the mPFC lesion did not impair place avoidance of the initial shock zone. We consider such reasoning to be unproductive because it presumes that only tasks that require an intact mPFC can be cognitive control tasks. We nonetheless acknowledge that, for some, we have not provided sufficient evidence that the active place avoidance requires cognitive control.

We assert that the evidence presented here is compelling, and that these findings require rejecting the central-computation hypothesis, which states 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 [3, 55–58]. For example, mPFC may be crucial for processes that rely on the ventral hippocampus and perhaps the direct excitatory mPFC-ventral hippocampus and/or the direct mPFC-dorsal hippocampus excitatory [59] and inhibitory connections [47, 48]. Nonetheless, the present data favor the local-computation hypothesis because bilateral and unilateral inactivation, as well as numerous other manipulations of the dorsal hippocampus, impair learning, consolidation, and retrieval of the conditioned active place avoidance [14–16, 60], 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 [12, 13, 18, 19, 39, 46], despite detecting other behavioral and electrophysiological effects of the lesion. We emphasize that observed representational switching is neurobiological evidence of cognitive control; it is not itself cognitive control, although it could be part of transmissive control. Rather, the observed representational switching indicates that cognitive control persists after mPFC lesion; indeed, what a control signal itself should resemble is uncertain, even in primates [10, 11]. 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.

Although muscimol inactivation of mPFC did not impair active place avoidance [61], as observed in the present study, it is possible that acute manipulations of the mPFC, such as optogenetic, chemogenetic or other 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 [62], 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 [63, 64]. 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 [13, 46, 65], but not a form of cognitive control that has been demonstrated in the representational discharge of hippocampal cells [13].

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.

Materials and 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 under protocol 12-1383, 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.

Behavioral analysis followed by cytochrome oxidase activity

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 resulted in a large skin lesion and required euthanasia. Because the abrupt change to single housing is stressful and can affect cognitive behavior, the control rat was also excluded 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 ±0.6, DV - 5.0, relative to the skull surface, Site 2: AP +3.5, ML ± 0.6, DV - 5.2, relative to skull surface. Ibotenic acid (0.06 M in PBS) was used to create an excitotoxic lesion following published work [66]. A 0.2 µl volume was injected at each site with a micro-infusion pump at a flow rate 0.2 µL/min through a stainless-steel cannula (0.25 mm outer diameter); the cannula was left in place for 4 min (for a total of 5 min per injection site) before being slowly withdrawn. 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 task

A commercial rotating arena and tracking software (Tracker, Bio-Signal Group, Acton, MA) was used for the active place avoidance task, which has been described previously [34, 36, 67]. Briefly, the rats were placed on a stainless steel 82-cm diameter arena that could rotate about its center at 1 rpm under computer control. The arena was in the center of a 3m x 3m space that was surrounded by black curtains with a distinctive orienting cue (striped fabric). The arena floor was made of parallel stainless-steel rods organized into 5 electrical poles. A constant current source could be triggered by the software to deliver shock that was scrambled across the 5 poles to ensure it was unlikely that shock could be avoided by a fortuitous posture or by feces shorting the bars. A 50-cm high transparent PETG wall ensured the rats remined on the arena and allowed them to see into the room as the arena rotated. The position of the rat in the spatial frame of the room was tracked 30-times a second at 3.2 mm resolution by analysis of the video image from an overhead camera. An infrared LED that rotated with the arena was tracked similarly from the video image, and the rat's arena-frame position on the arena surface was computed relative to the arena LED [51, 68]. The room-frame and arena-frame position time series along with the delivery of shock were stored in data files for off-line automated software analysis of end-point measures (TrackAnalysis, Bio-Signal Group, Acton, MA). Three end-point measures are reported. i) The distance walked measures how much the rat walked on the arena surface. ii) The time to first enter the shock zone increased with training; it estimates the between-session active place avoidance memory by estimating how well the rat can remember the previous room-frame locations of shock. iii) The number of entrances into the shock zone decreased with training; it estimates place learning and can decrease due to between-session memory as well as within-session memory of the locations of shock.

Active place avoidance training

After recovery from surgery, the rats were brought to the laboratory in their home cages that were placed to a rack to the side of the experimental room. The rats were each handled for 5 min per day for 5 days. Behavioral training consisted of 10-min trials with an intertrial interval of 10 minutes during which the rat was returned to it's home cage in the experimental room. 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 the 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 and was the same for all rats. 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:

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

Electrophysiology procedures

The following procedures have been previously described in detail [20]. Briefly, rats were anesthetized (pentobarbital 50 mg/kg) and received mPFC or sham lesions as described above. The Neuropixel recording system was used [69]. A Neuropixel electrode array was implanted in the brain to traverse Bregma coordinates 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 posterior and lateral posterior 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 using SpikeGLX. 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 [70]. 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 [18, 71, 72]. 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[73], 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, recorded from the posterior and lateral posterior thalamic nucleus, which lies directly beneath the hippocampus, fired at high rates and were less likely to discharge in bursts (Table S3).

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. *Ipos(room)* estimates the information about the current location *x* in the room, whereas *Ipos(arena)* separately estimates the information about current location *x* on the rotating arena[43]. $I_{pos}(t)$ 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 $|EI_{pos}(t)|$ is abbreviated I_{pos}. A unit's spatial-frame preference is estimated at each Δt moment as the difference:

$\Delta I_{pos} = I_{pos(room)} - I_{pos(arena)}$, 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 [12, 13]. 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.

Place cell overdispersion measures discharge non-stationarity by estimating how much neuronal discharge deviates from what is expected if the discharge only signals position [41]. Increased overdispersion indicates an extra-positional signal like attention or cognitive control is modulating discharge up and down in addition to position, whereas decreased overdispersion indicates such modulation is dampened, possibly to sustained attention [38]. Overdispersion of hippocampal discharge was computed as previously described [38]. 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 [74]) were included. Cytochrome oxidase staining was performed according to [75]. 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 [76] 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 [67]. 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. Contours of the lesion area were drawn using Adobe Illustrator in a layer overlaid on the tissue images. The images were matched to the appropriate section of the Paxinos and Watson rat brain atlas [77]. The filled contours were converted to a.tiff file for quantification of the lesion area (pixels) using ImageJ (see Fig. S2). Rats with verified bilateral lesions that spanned at least 3 sections were included in the data analysis; one rat was excluded from analysis because the lesion was only evident in one hemisphere.

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. Pairs of binomial proportions were compared by a test for proportions (z). 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, in addition to exact p values. These values were computed using SPSS Statistics 28 and Microsoft Excel.

Additional information

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.

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Additional files

Supporting Information

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Reviewer #1 (Public review):

Summary:

The authors examine the role of the medial prefrontal cortex (mPFC) in cognitive control, i.e. the ability to use task-relevant information and ignore irrelevant information, in the rat. According to the central-computation hypothesis, cognitive control in the brain is centralized in the mPFC and according to the local hypothesis, cognitive control is performed in task-related local neural circuits. Using the place avoidance task which involves cognitive control, it is predicted that if mPFC lesions affect learning, this would support the central computation hypothesis whereas no effect of lesions would rather support the local hypothesis. The authors thus examine the effect of mPFC lesions in learning and retention of the place avoidance task. They also look at functional interconnectivity within a large network of areas that could be activated during the task by using cytochrome oxydase, a metabolic marker. In addition, electrophysiological unit recordings of CA1 hippocampal cells are made in a subset of (mPFC-lesioned or intact) animals to evaluate overdispersion, a firing property that reflects cognitive control in the hippocampus. The results indicate that mPFC lesions disrupted correlations of activity between functionally-related regions. Behaviorally, lesions did not impair place avoidance learning and retention (though flexibility was altered during conflict training). In addition, hippocampal place cell overdispersion was decreased in lesioned rats only in the absence of cognitive control challenge (pretraining). Cognitive control seen in hippocampal place cell activity (alternation of frame-specific firing) was not affected by the lesion. Overall, the absence of effects of mPFC lesions on cognitive control in the task or in hippocampal place cells firing support the local hypothesis.

Strengths:

Straightforward hypothesis: clarification of the involvement of the mPFC in the brain is expected and achieved. Appropriate use of fully mastered methods (active place avoidance task, electrophysiological unit recordings, measure of metabolic marker cytochrome oxidase) and rigorous analysis of the data. The conclusion is strongly supported by the data.

Weaknesses:

No notable weaknesses in the conception, making of the study and data analysis.

Comments on revisions:

The authors have satisfactorily addressed all my comments in the revised version.

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Reviewer #2 (Public review):

Park et al. set out to test two competing hypotheses about the role of the medial prefrontal cortex (PFC) in cognitive control, the ability to use task-relevant cues and ignore task-irrelevant cues to guide behavior. The "central computation" hypothesis assumes that cognitive control relies on computations performed by the PFC, which then interacts with other brain regions to accomplish the task. Alternatively, the "local computation" hypothesis suggests that computations necessary for cognitive control are carried out by other brain regions that have been shown to be essential for cognitive control tasks, such as the dorsal hippocampus and the thalamus. If the central computation hypothesis is correct, PFC lesions should disrupt cognitive control. Alternatively, if the local computation hypothesis is correct, cognitive control would be spared after PFC lesions. The task used to assess cognitive control is the active place avoidance task in which rats must avoid a sector of a rotating arena using the stationary room cues and ignoring the local olfactory cues on the rotating platform. Performance on this task has previously been shown to be disrupted by hippocampal lesions and hippocampal ensembles dynamically represent the room and arena depending on the animal's proximity to the shock zone. They found no group (lesion vs. sham) differences in the three behavioral parameters tested: distance traveled, latency to enter the shock zone, and number of shock zone entries for both the standard task and the "conflict" task in which the shock zone was rotated by 180 degrees. The only significant difference was the savings index; the lesion group entered the new shock zone more often than the sham group during the first 5 minutes of the second conflict session. This deficit was interpreted as a cognitive flexibility deficit rather than a cognitive control failure. Next, the authors compared cytochrome oxidase activity between sham and lesion groups in 14 brain regions and found that only the amygdala shows significant elevation in the lesion vs. sham group. Pairwise correlation analysis revealed a striking difference between groups, with many correlations between regions lost in the lesion group (between reuniens and hippocampus, reuniens and amygdala and a correlation between dorsal CA1 and central amygdala that appeared in the lesion group and were absent in the sham group. Finally, the authors assessed dorsal hippocampal representations of the spatial frame (arena vs. room) and found no differences between lesion and sham groups. The only difference in hippocampal activity was reduced overdispersion in the lesion group compared to the sham group on the pretraining session only and this difference disappeared after the task began. Collectively, the authors interpret their findings as supporting the local computation hypothesis; computations necessary for cognitive control occur in brain regions other than the PFC.

Strengths:

The data were collected in a rigorous way with experimental blinding and appropriate statistical analyses.

Multiple approaches were used to assess differences between lesion and sham groups, including behavior, metabolic activity in multiple brain regions, and hippocampal single unit recording.

Weaknesses:

Only male rats were used with no justification provided for excluding females from the sample.

The conceptual framework used to interpret the findings was to present two competing hypotheses with mutually exclusive predictions about the impact of PFC lesions on cognitive control. The authors then use mainly null findings as evidence in support of the local computation hypothesis. They acknowledge that some people may question the notion that the active place avoidance task indeed requires cognitive control, but then call the argument "circular" because PFC has to be involved in cognitive control. This assertion does not address the possibility that the active place avoidance task simply does not require cognitive control.

The authors did not link the CO activity with the behavioral parameters even though the CO imaging was done on a subset of the animals that ran the behavioral task nor do they make any attempt to interpret these findings in light of the two competing hypotheses posed in the introduction. Moreover, the discussion is lacking any mechanistic interpretations of the findings. For example, there are no attempts to explain why amygdala activity and its correlation with dCA1 activity might be higher in the PFC lesioned group.

Publishing null results is important to avoid wasting animals, time, and money. This study's results will have a significant impact on how the field views the role of the PFC in cognitive control. Whether or not some people reject the notion that the active place avoidance task measures cognitive control, the findings are solid and can serve as a starting point for generating hypotheses about how brain networks change when deprived of PFC input.

https://doi.org/10.7554/eLife.104475.2.sa2

Reviewer #3 (Public review):

Summary:

This study by Park and colleagues investigated how the medial prefrontal cortex (mPFC) influences behavior and hippocampal place cell activity during a two-frame active place avoidance task in rats. Rats learned to avoid the location of mild shock within a rotating arena, with the shock zone being defined relative to distal cues in the room. Permanent chemical lesions of the mPFC did not impair the ability to avoid the shock zone by using the distal cues and ignoring proximal cues in the arena. In parallel, hippocampal place cells alternated between two spatial tuning patterns, one anchored to the distal cues and the other to the proximal cues, and this alteration was not affected by the mPFC lesion. Based on these findings, the authors argue that the mPFC is not essential for differentiating between task-relevant and irrelevant information.

Strengths:

This study was built on substantial work by the Fenton lab that validated their two-frame active place avoidance task and provided sound theoretical and analytical foundations. Additionally, the effectiveness of mPFC lesions was validated by several measures, enabling the authors to base their argument on the lack of lesion effects on behavior and place cell dynamics.

Weaknesses:

The authors define cognitive control as "the ability to judiciously use task-relevant information while ignoring salient concurrent information that is currently irrelevant for the task." (Lines 77-78). This definition is much simpler than the one by Miller and Cohen: "the ability to orchestrate thought and action in accordance with internal goals (Ref. 1)" and by Robbins: "processes necessary for optimal scheduling of complex sequence of behaviour." (Dalley et al., 2004, PMID: 15555683). Differentiating between task-relevant and irrelevant information is required in various behavioral tasks, such as differential learning, reversal learning, and set-shifting tasks. Previous rodent behavioral studies have shown that the integrity of the mPFC is necessary for set-shifting but not for differential or reversal learning (e.g., Enomoto et al., 2011, PMID: 21146155; Cho et al., 2015, PMID: 25754826). In the present task design, the initial training is a form of differential learning between proximal and distal cues, and the conflict training is akin to reversal learning. Therefore, the lack of lesion effects is somewhat expected. It would be interesting to test whether mPFC lesions impair set-shifting in their paradigm (e.g., the shock zone initially defined by distal cues and later by proximal cues). If the mPFC lesions do not impair this ability and associated hippocampal place dynamics, it will provide strong support for the authors' local-computation hypothesis.

Comments on revisions:

The authors fully addressed my comments. I do not have any additional suggestions.

https://doi.org/10.7554/eLife.104475.2.sa1

Author response:

The following is the authors' response to the original reviews

> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *The authors examine the role of the medial prefrontal cortex (mPFC) in cognitive control, i.e. the ability to use task-relevant information and ignore irrelevant information, in the rat. According to the central-computation hypothesis, cognitive control in the brain is centralized in the mPFC and according to the local hypothesis, cognitive control is performed in task-related local neural circuits. Using the place avoidance task which involves cognitive control, it is predicted that if mPFC lesions affect learning, this would support the central computation hypothesis whereas no effect of lesions would rather support the local hypothesis. The authors thus examine the effect of mPFC lesions in learning and retention of the place avoidance task. They also look at functional interconnectivity within a large network of areas that could be activated during the task by using cytochrome oxidase, a metabolic marker. In addition, electrophysiological unit recordings of CA1 hippocampal cells are made in a subset of (lesioned or intact) animals to evaluate overdispersion, a firing property that reflects cognitive control in the hippocampus. The results indicate that mPFC lesions do not impair place avoidance learning and retention (though flexibility is altered during conflict training), do not affect cognitive control seen in hippocampal place cell activity (alternation of frame-specific firing), a measure of location-specific firing variability, in pretraining. It nevertheless has some effect on functional interconnections. The results overall support the local hypothesis.*
>
> *Strengths:*
>
> *Straightforward hypothesis: clarification of the involvement of the mPFC in the brain is expected and achieved. Appropriate use of fully mastered methods (behavioral task,*

> *electrophysiological recordings, measure of metabolic marker cytochrome oxidase) and rigorous analysis of the data. The conclusion is strongly supported by the data.*
>
> *Weaknesses:*
>
> *No notable weaknesses in the conception, making of the study, and data analysis. The introduction does not mention important aspects of the work, i.e. cytochrome oxidase measure and electrophysiological recordings. The study is actually richer than expected from the introduction.*

The revised Introduction now includes:

"We used cytochrome oxidase, a metabolic marker of baseline neuronal activity, to confirm the mPFC lesions were effective and that there are non-local network consequences despite the local lesion. We first evaluated cytochrome oxidase activity in regions known to be associated with performance in the active place avoidance task, or regions with known connectivity to the mPFC. We then evaluated covariance of activity amongst the regions in an effort to detect network consequences of the lesion."

> ***Reviewer #2 (Public review):***
>
> *Park et al. set out to test two competing hypotheses about the role of the medial prefrontal cortex (PFC) in cognitive control, the ability to use task-relevant cues and ignore taskirrelevant cues to guide behavior. The "central computation" hypothesis assumes that cognitive control relies on computations performed by the PFC, which then interacts with other brain regions to accomplish the task. Alternatively, the "local computation" hypothesis suggests that computations necessary for cognitive control are carried out by other brain regions that have been shown to be essential for cognitive control tasks, such as the dorsal hippocampus and the thalamus. If the central computation hypothesis is correct, PFC lesions should disrupt cognitive control. Alternatively, if the local computation hypothesis is correct, cognitive control would be spared after PFC lesions. The task used to assess cognitive control is the active place avoidance task in which rats must avoid a section of a rotating arena using the stationary room cues and ignoring the local olfactory cues on the rotating platform. Performance on this task has previously been shown to be disrupted by hippocampal lesions and hippocampal ensembles dynamically represent the room and arena depending on the animal's proximity to the shock zone. They found no group (lesion vs. sham) differences in the three behavioral parameters tested: distance traveled, latency to enter the shock zone, and number of shock zone entries for both the standard task and the "conflict" task in which the shock zone was rotated by 180 degrees. The only significant difference was the savings index; the lesion group entered the new shock zone more often than the sham group during the first 5 minutes of the second conflict session. This deficit was interpreted as a cognitive flexibility deficit rather than a cognitive control failure. Next, the authors compared cytochrome oxidase activity between sham and lesion groups in 14 brain regions and found that only the amygdala showed significant elevation in the lesion vs. sham group. Pairwise correlation analysis revealed a striking difference between groups, with many correlations between regions lost in the lesion group (between reuniens and hippocampus, reuniens and amygdala and a correlation between dorsal CA1 and central amygdala that appeared in the lesion group and were absent in the sham group. Finally, the authors assessed dorsal hippocampal representations of the spatial frame (arena vs. room) and found no differences between lesion and sham groups. The only difference in hippocampal activity was reduced overdispersion in the lesion group compared to the sham group on the pretraining session only and this difference disappeared after the task began. Collectively, the*

> *authors interpret their findings as supporting the local computation hypothesis; computations necessary for cognitive control occur in brain regions other than the PFC.*
>
> *Strengths:*
>
> *(1) The data were collected in a rigorous way with experimental blinding and appropriate statistical analyses.*
>
> *(2) Multiple approaches were used to assess differences between lesion and sham groups, including behavior, metabolic activity in multiple brain regions, and hippocampal singleunit recording.*
>
> *Weaknesses:*
>
> *(1) Only male rats were used with no justification provided for excluding females from the sample.*

This is a weakness we acknowledge. The experiments were performed at a time when we did not have female rats in the lab.

> *(2) The conceptual framework used to interpret the findings was to present two competing hypotheses with mutually exclusive predictions about the impact of PFC lesions on cognitive control. The authors then use mainly null findings as evidence in support of the local computation hypothesis. They acknowledge that some people may question the notion that the active place avoidance task indeed requires cognitive control, but then call the argument "circular" because PFC has to be involved in cognitive control. This assertion does not address the possibility that the active place avoidance task simply does not require cognitive control.*

We beg to differ that the possibility was not addressed. Prior to making the assertion, the manuscript describes the evidence that the active place avoidance task requires cognitive control. The evidence is multifold, and includes task design, behavior, and electrophysiology; we argue that this is more evidence than has been provided for other tasks that are asserted to require cognitive control. Specifically line 417 states:

“We have previously demonstrated cognitive control in the active place avoidance task variant we used (Fig. 1) because the rats must ignore local rotating place cues to avoid the stationary shock zone. Even when the arena does not rotate, rats distinctly learn to avoid the location of shock according to distal visual room cues and local olfactory arena cues, such that the distinct place memories can be independently manipulated using probe trials [49, 50]. When the arena rotates as in the present studies, neural manipulations that impair the place avoidance are no longer impairing when the irrelevant arena cues are hidden by shallow water [14, 15, 51, 52]. Furthermore, persistent hippocampal neural circuit changes caused by active place avoidance training are not detected when shallow water hides the irrelevant arena cues to reduce the cognitive control demand [10, 31, 33]. While these findings unequivocally demonstrate the salience of relevant stationary room cues to use for avoiding shock and irrelevant arena cues to ignore during active place avoidance, the most compelling evidence of cognitive control comes from recording hippocampal ensemble discharge. Hippocampal ensemble discharge purposefully represents current position using stationary room information when the subject is close to the stationary shock zone and alternatively represents rotating arena information when the mouse is far from the stationary shock zone [Fig. 4; 10].”

Line 436, however, acknowledges a fact that will always be true: no matter what anyone opines - until there are universally agreed upon objective criteria, it is logically possible that active place avoidance does not require cognitive control. The revision states: Despite this

evidence from task design, behavioral observations, and direct electrophysiological representational switching as required to directly demonstrate cognitive control, one might still argue that it is logically possible that the active place avoidance task does not require cognitive control and this is why the mPFC lesion did not impair place avoidance of the initial shock zone. We consider such reasoning to be unproductive because it presumes that only tasks that require an intact mPFC can be cognitive control tasks. We nonetheless acknowledge that for some, we have not provided sufficient evidence that the active place avoidance requires cognitive control.

"We assert the evidence is compelling, and 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."

> *(3) The authors did not link the CO activity with the behavioral parameters even though the CO imaging was done on a subset of the animals that ran the behavioral task nor did they make any attempt to interpret these findings in light of the two competing hypotheses posed in the introduction. Moreover, the discussion lacks any mechanistic interpretations of the findings. For example, there are no attempts to explain why amygdala activity and its correlation with dCA1 activity might be higher in the PFC lesioned group.*

The CO study was performed to assess the effects of the lesion, as stated on line 262 "Cytochrome oxidase (CO), a sensitive metabolic marker for neuronal function [27], was used to evaluate whether lesion effects were restricted to the mPFC." Furthermore, as a matter of fact, line 411 states "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 [45, 46] and loses the metabolic correlation with the nucleus reuniens, which is thought to be a relay between the mPFC and the dorsal hippocampus [47, 48]."

These CO measures assess baseline metabolic function and so it would be inappropriate to correlate them with the measures of behavior. Because the lesion and control groups do not differ on most measures of behavior, a relationship to CO measures is not expected. Importantly, even if there were differences in correlations between CO activity and behavioral measures, what could they mean? The study was designed to distinguish between two hypotheses, not to determine what CO differences could mean for behavior. As such, it is not at all clear how metabolic consequences of the lesion relate to the two hypotheses being evaluated, and so we consider it inappropriate to speculate. We did examine, and now include, the correlation between lesion size and conflict behavior. The Fig. 1 legend states "Savings was not related to lesion size $r = 0.009$, $p = 0.98$. *$p < 0.05$."

> *(4) Publishing null results is important to avoid wasting animals, time, and money. This study's results will have a significant impact on how the field views the role of the PFC in cognitive control. Whether or not some people reject the notion that the active place avoidance task measures cognitive control, the findings are solid and can serve as a starting point for generating hypotheses about how brain networks change when deprived of PFC input.*

We thank the reviewer for the acknowledgement.

> ***Reviewer #3 (Public review):***
>
> *Summary:*

> *This study by Park and colleagues investigated how the medial prefrontal cortex (mPFC) influences behavior and hippocampal place cell activity during a two-frame active place avoidance task in rats. Rats learned to avoid the location of mild shock within a rotating arena, with the shock zone being defined relative to distal cues in the room. Permanent chemical lesions of the mPFC did not impair the ability to avoid the shock zone by using distal cues and ignoring proximal cues in the arena. In parallel, hippocampal place cells alternated between two spatial tuning patterns, one anchored to the distal cues and the other to the proximal cues, and this alteration was not affected by the mPFC lesion. Based on these findings, the authors argue that the mPFC is not essential for differentiating between task-relevant and irrelevant information.*
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>
> *Weaknesses:*
>
> *The authors define cognitive control as "the ability to judiciously use task-relevant information while ignoring salient concurrent information that is currently irrelevant for the task." (Lines 77-78). This definition is much simpler than the one by Miller and Cohen: "the ability to orchestrate thought and action in accordance with internal goals (Ref. 1)" and by Robbins: "processes necessary for optimal scheduling of complex sequence of behaviour." (Dalley et al., 2004, PMID: 15555683). Differentiating between task-relevant and irrelevant information is required in various behavioral tasks, such as differential learning, reversal learning, and set-shifting tasks. Previous rodent behavioral studies have shown that the integrity of the mPFC is necessary for set-shifting but not for differential or reversal learning (e.g., Enomoto et al., 2011, PMID: 21146155; Cho et al., 2015, PMID: 25754826). In the present task design, the initial training is a form of differential learning between proximal and distal cues, and the conflict training is akin to reversal learning. Therefore, the lack of lesion effects is somewhat expected. It would be interesting to test whether mPFC lesions impair set-shifting in their paradigm (e.g., the shock zone initially defined by distal cues and later by proximal cues). If the mPFC lesions do not impair this ability and associated hippocampal place dynamics, it will provide strong support for the authors' local computation hypothesis.*

Thank you for these comments. In addressing them we have provided a significant revision to the manuscript's Introduction. While authors like those cited by the reviewer have defined cognitive control, those definitions are difficult to test rigorously, as it is almost a matter of opinion whether a subject is displaying "the ability to orchestrate thought and action in accordance with internal goals" or whether they are using "processes necessary for optimal scheduling of complex sequence of behaviour." What would such definitions of cognitive control predict about neuronal activity? We have deliberately used a simple, operational definition of cognitive control because it is physiologically testable. In the revision, starting at line 93, we have provided an excerpt from Miller and Cohen (2001) with discussion. The importance of that work is that it provides explicit neuronal criteria and a means to operationally define cognitive control. As stated on Line 118 "Accordingly, cognitive control would be at work when there is sustained neuronal network representations of task-relevant information that suppresses or gates representations of salient task-irrelevant information in accord with purposeful judicious behavior."

We used a R+A- task variant in which there is a stationary room-frame shock zone and task irrelevant arena-frame information. A strict correspondence to shift-shifting task design cannot be accomplished with active place avoidance because an A+R- task that requires avoiding an arena-frame shock zone in the absence of a room-frame shock zone can be accomplished trivially if the subject chooses to not move when it is in a place with no shock. However, the R+A+ task variant is readily learned, in which there is both a room-frame and an arena-frame shock zone (see cited work below). This task variant requires the subject to judiciously shift between avoiding the room-frame shock zone using stationary room information and avoiding the arena-frame shock zone using rotating arena information. This R+A+ task variant might meet the reviewer's criteria for cognitive control. We have recorded hippocampal and entorhinal ensemble activity during the R+A+ task variant and it is very similar to the activity during the R+A- task we used. Nonetheless, future work will investigate the efect of mPFC lesion on the R+A+ task variant.

Cited work:

Fenton AA, Wesierska M, Kaminsky Y, Bures J (1998), Both here and there: simultaneous expression of autonomous spatial memories in rats. Proc Natl Acad Sci U S A 95:11493-11498.
Kelemen E, Fenton AA (2010), Dynamic grouping of hippocampal neural activity during cognitive control of two spatial frames. PLoS Biol 8:e1000403.

Burghardt NS, Park EH, Hen R, Fenton AA (2012), Adult-born hippocampal neurons promote cognitive flexibility in mice. Hippocampus 22:1795-1808.

Park EH, Keeley S, Savin C, Ranck JB, Jr., Fenton AA (2019), How the Internally Organized Direction Sense Is Used to Navigate. Neuron 101:1-9.

> ***Recommendations for the authors:***
>
> ***Reviewer #1 (Recommendations for the authors):***
>
> *(1) Incorporate the cytochrome oxidase and hippocampal recordings (rationale and hypothesis) in the introduction, explaining how these aspects are relevant to the general question.*

We have done this as requested. See lines 159-173 of the revised introduction.

> *(2) Figure 1C. On Day 4-5 (conflict training) in which the shock zone was relocated 180 deg from the initial location, the behavioral tracks did not show any presence of the rat in this sector (in particular for the lesion example). Figure 4 nevertheless indicates that entrances have been made (which was expected since rats have to know that the shock zone was relocated).*

Thanks for pointing this out. The tracks are from the end of the sessions. The labels have been changed to specify which trials the tracks are from.

> *(3) Figure 1C. The caption is huge as it contains the statistical analyses details. I would prefer to have these details in the text and keep the caption at a "reasonable" length. At the end of the caption (l. 190-191), it would be less confusing the keep the numbering of the training days: replace D1T1 with D2T1 and D2T9 with D3T9).*

The statistical details have been relocated to the main text and the numbering updated, as suggested, thank you.

> *(4) It was not inconsiderable to show that mPFC lesion had some effects in the present task if it were only to validate the effectiveness of the lesion. This brain area has been shown to be important for planning, cognitive flexibility, etc. Indeed the authors found that the saving index was greater in sham than in mPFC rats (overdispersion in hippocampal firing was also reduced in pretraining) and interpreted this result as impaired flexibility. Would an alternative explanation be a memory deficit? I nevertheless expected that impaired flexibility in mPFC rats would be expressed in conflict trials in the form of more entrances in the zone that was initially not associated with shock (at least in the first trials of Day 4). But it appears to not be the case.*

A memory deficit is unlikely to explain the difference between the groups on the first trial of Day 5. Memory in the lesion rats was tested multiple times, specifically at the start of each trial (time to first entrance), including on the 24-h retention test, and no deficits were observed. Performance on Day 9 trial 1 is worse in the lesion group than in the controls, but it is not parsimonious to attribute this to a simple memory deficit since 24-h memory was good and similar between lesion and control rats on days 3 and 4, and memory on Day 5 was equally poor in both the lesion and control rats, as measured by time to first entrance.

> *(5) Material and methods. The injected volume of ibotenic acid should be mentioned.*

The volume 0.2 µl was added. See line 531.

> *(6) The rationale for doing the conflict training session should be indicated somewhere.*

The rationale was provided. See lines 204-208.

> ***Reviewer #2 (Recommendations for the authors):***
>
> *(1) Line 132: The text states that all sham rats improved and only 6/10 lesion rats improved is followed by a t-test, which tests the difference between means; it does not compare proportions. Also, what criterion was used to determine if an improvement was seen or not?*

The statistical comparison is provided (now lines 230: test of proportions $z = 2.3$, $p = 0.03$). Improvement was simply numerically fewer entrances.

> *(2) Line 138: This is a very long and confusing sentence. Consider revising for clarity.*

The sentence (now line 234) was revised.

> *(3) Figure 1B only includes data from 3 animals. Most published studies show the whole dataset by presenting the largest and smallest lesions.*

Supplemental Figure S2 was added with all the lesions depicted and quantified.

> *(4) Figure 1C suggestion to make the schematic shock zone line up with the shock zone shown for the tracking data.*

Graphically, it looks better as drawn as it uses to perspective to depict a three-dimensional structure.

> *(5) Methods: Clarify if the shock zone location was the same across all rats.*

Line 570 states that the shock zone was the same for all rats.

> *(6) Line 158: "Behavioral tracks" is not clear. Suggest more precise wording.*

Reworded to “Tracked room-frame positions” (now line 249)

> *(7) Line 166: "effect of trial" - should this be the main effect of trial?; "interaction" - should this be "group x trial" interaction?*

Reworded (now line 181).

> *(8) Line 167: "or their interaction" is awkward in the context of the sentence.*

Reworded (now line 182).

> *(9) Line 182: Avoid talking about "trends" as if they are almost significant unless the authors suspect that they did not have sufficient statistical power to detect differences. In that case, a power analysis should be provided.*

Removed.

> *(10) Line 190: "left:...right..." is hard to follow, especially with acronyms like D1T1. Consider revising for clarity.*

Revised (now lines 246-248).

> *(11) Line 195: "effectiveness of the PFC to impair" is unnecessarily verbose.*

Reworded (now lines 255-257).

> *(12) Savings results: There is a lot of variability in the lesion group. It would be interesting to know if the extent of the lesion correlates with savings.*

Savings was not related to lesion. See line 259.

> *(13) Line 300: The thalamic recording results are not reported in the results section (other than appearing in the table). Moreover, there is no detail about which thalamic nucleus these recordings are from.*

Lines 411 and 614 provides these details.

> *(14) Line 312: "no longer impair" contains a grammatical error.*

Corrected (now line 422)

> *(15) Line 325: "was not impairing" contains a grammatical error.*

Corrected (now line 437).

> *(16) Line 327: The sentence ending with "...opinion of others" seems unnecessarily confrontational.*

Previous reviewers at other journals have maintained this position, we therefore included such a strong statement in our initial submission. However, we now revised this statement to avoid appearing confrontational.

> *(17) Line 329: Sentence is awkward. Consider revising.*

Revised (now line 443).

> *(18) Line 384: The authors should disclose if there was an objective metric for determining the adequacy of the lesion.*

The lesion assessment and quantification is better explained in the Methods under "Cytochrome oxidase activity and Nissl staining," (lines 708-714).

> *(19) Line 385: The authors should clarify how they got from 15 rats (Line 376) to 10.*

This information is provided in the methods.

> *(20) Line 390: It is not clear why skin irritation in the cage mate would prevent the rat from being tested.*

This has been explained in the Methods under "Behavioral analysis followed by cytochrome oxidase activity" (lines 515-518).

> *(21) Methods section: The authors should describe how the tracking data were acquired. Overhead camera? Tracker based on luminance or body position? What software program was used? What was the sampling rate?*

This is now better explained in the Methods under "Active place avoidance task) (lines 538551).

> *(22) Methods section: Include how fast the arena was rotating and other details about the task such as where rats were placed during the ITI.*

Better explained in the Methods under "Active place avoidance task".

> *(23) Line 439: The recording system used (hardware & software) should be stated.*

This is now included in the Methods (line 538).

> *(24) Line 435: Though overdispersion calculation is described thoroughly, there is nothing in the paper that tells me what overdispersion means.*

What the measure means is now described in the Methods under "Electrophysiology data analysis" (lines 646-650).

> *(25) Line 561: The test used to assess effect sizes should be stated.*

Effect sizes corresponding to the statistical tests are provided.

> ***Reviewer #3 (Recommendations for the authors):***
>
> *(1) At the end of the conflict training, rats with mPFC lesions learned to avoid the new shock zone (Figure 1F, Block 16), but their place cells did not show room-preferring*

> *activity near the shock zone (Figure 4B). This observation questions whether spatial frame-specific representation is relevant for active avoidance. Can the authors clarify this point?*

This is a dynamic behavior and the hippocampal dynamics match, changing with a dynamic that is a few seconds, as we have shown in several published papers. The lack of a preference averaged over 20 minutes when the rats are avoiding both the current and former shock zones during the conflict session is pretty much what would be expected from such a coarse measurement. The important measure is the spatially-resolved measure of room versus arena preference. Figure 4B shows that in the lesion rats there is less of a frame preference during conflict, generally (consistent with poorer flexibility). However, Figure 4D quantifies the frame preference near and far from the shock zone and accordingly, there is no difference between the groups.

> *(2) Related to the point above, the author might consider including panels in Figures 4C and D to show the neural activity during the pretraining and conflict training retention period. I assume p(room) will be comparable between the Near and Far segment in both sessions, but the p(room) may be higher in the Conflict training session than the Pretraining session. This would show that the mPFC lesion impairs suppressing the place cell activity encoding the old shock location.*

Thanks for the suggestion. While we don't think we can draw any strong conclusions from this analysis we are fine to show it. The issue is that during conflict, the rats have two perfectly reasonable representations of where there was shock, the initial location that was turned off to make the conflict, and the most recent conflict location of shock. Importantly, these recordings are during conflict retention after we turned off the shock for the retention recording (for the second time in the rat's experience). Turning off the shock allows us to exactly match the physical conditions of pretraining, initial retention and conflict retention, which was the experimental design's goal. However, the experiential history of the rats prior to initial retention and conflict retention cannot match, because during initial retention the rats had never experienced a changed shock zone whereas, by conflict retention, they had experienced multiple changes. Importantly, we have previously shown that mouse hippocampal ensembles represent both initial and conflict shock locations, as the animals consider their options during conflict trials (see Dvorak et al 2018, PLoS Biol 16:e2003354). Consequently, we cannot make any strong predictions about whether or not hippocampal activity during conflict retention should be room-frame preferring selectively in the vicinity of the current shock zone. As I am sure the reviewer appreciates from their own introspection, mental representations are mercifully not obliged to dictate behavior. In fact, that is what is interesting and controversial about cognitive control – it is a dynamic internal process and the innovation of our work lies in demonstrating that one cannot only rely on behavior to assess this process. Nonetheless, we did this analysis and now present it in the revised Fig. 4. During pretraining both lesion and sham groups express no particular spatially-modulated preference for either the room or the arena frame, as expected. During initial training both groups express a room-frame preference in the vicinity of the shock zone, as we initially reported. By inspection, during conflict, the sham rats express a preference for room-frame activity in the vicinity of the most recent shock zone location; this preference is weaker than what is expressed during initial retention. The lesion rats do not show this preference. These impressions are quantified in revised Fig. 4D; the comparisons within the conflict retention sessions did not reach statistical significance. We leave it to the reader to interpret what that means. Thanks for the nudge.

> *(3) The significant group difference in place cell overdispersion during the pretraining phase (Figure 3C) is interesting, but some readers would appreciate additional sentences*

> *on its functional implication. Does it mean the spatial tuning of place cells was disrupted by the mPFC lesion?*

Only the reliability of spatial firing was altered, not the spatial tuning.

> *(4) Although the method section described how to calculate overdispersion and SFEP, some concise, intuitive descriptions of these measures in the result section would help readers understand these results.*

Overdispersion is better explained. See lines 646-650.

> *(5) I recommend adding a figure of the task performance of the rats used in the electrophysiological recording experiment and a table summarizing the number of cells recorded per animal.*

We have included Table S2 with the cell counts and a summary of the performance for each of the rat in the electrophysiological recording experiment.

> *(6) Readers would appreciate additional information on task apparatus, such as the size, appearance, and rotating speed of the arena, as well as stationary cues available in the room.*

This is now provided in the Methods under “Active place avoidance task”.

> *(7) Lines 425-416: "On the fourth day of the behavioral training, the rats had a single trial with the shock on to test retention of the training." Shouldn't it be "shock off"?*

No the shock was on to prevent extinction learning and to increase the challenge for conflict learning.

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