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Supplementary Discussion

Role of PV interneurons in mediating 4 Hz oscillations.

In the present manuscript, as well as in a recent paper from our group[1], we optogenetically manipulated dmPFC PV interneurons as a mean (i) to induce artificial 4 Hz oscillations in the dmPFC[1], and (ii) to manipulate the activity of dmPFC neurons in the ascending or descending phases of 4 Hz oscillations (present manuscript). Although, we used the optogenetic manipulation of PV interneurons as a specific tool to alter the activity of dmPFC PNs during 4 Hz oscillations, it is possible that PV interneurons directly participate to the generation of 4 Hz oscillations. However, we do not think that PV interneurons play a key role in mediating 4 Hz oscillations for several reasons. First of all, we observed in the present manuscript and our previous paper[1] that PV interneurons phase locking was rather heterogeneous as phasic modulation was very weak. More particularly, we observed that the amount of spikes fired by PV interneurons did not differ between ascending and descending phase of 4 Hz oscillations (Present manuscript, Extended Data Figure 7e, f). These results indicate that during freezing episodes PV interneurons do not preferentially fire within the descending or ascending phase of the oscillation and that the inhibitory tonus they provide on target neurons is virtually identical for each phase of the 4 Hz cycle. It is therefore highly unlikely that the unspecific 4 Hz phase firing of PV interneurons could be sufficient to influence freezing behaviour. Moreover, a strong argument for the weak participation of PV cells in dmPFC 4 Hz oscillations is the fact that their optogenetic manipulation, whichever the phase, does not modify 4 Hz power (Present manuscript, Extended Data Figure 8). If 4 Hz is generated elsewhere, as these results suggest, it is therefore unlikely that PV interneurons play the role of an amplifier for 4 Hz oscillations. Overall the faint phase locking of PV interneurons during 4 Hz oscillations along with their fast and massive influence on dmPFC PNs and the minimal size of light evoked artifacts conferred PV interneurons the properties of a sound vector for a flexible and fast manipulation of dmPFC pyramidal cells. These aspects are even more relevant in the frame of our fine closed-loop dynamic optogenetic approach.

Inducing fear behaviour through manipulation of 4 Hz oscillations

In the present study and our recent paper[1], we performed two different manipulations of dmPFC PV interneurons that were associated with the induction of freezing behaviour. In the present manuscript, we maintained a freezing state by activating PV interneurons (and thereby inhibiting dmPFC PNs) in the descending phase of 4 Hz oscillations. These results strongly suggest that the temporal segregation between ascending and descending phase neural activity is a key element of freezing expression. The enhancement of this physiological mechanism through descending phase inhibition leads to constant, sustained freezing behaviour. In our previous paper[1], we performed a continuous analog optogenetic induction of 4 Hz oscillations in the dmPFC, which imposes an ascending/descending pattern to dmPFC PNs and therefore mimics the brainstate associated with freezing behaviour. This artificial optogenetic waxing and

waning entrainment of dmPFC PNs is likely to promote two distinct network states in dmPFC microcircuits. First, when inhibition is low in the trough of the stimulation cycle, the microcircuit has a window for neuronal synchronization and behaves as in the ascending phase. Second, when inhibition is high around stimulation cycle peak, the microcircuit is silenced as in the descending phase of 4 Hz. This could explain why 4 Hz analog stimulation is sufficient to induce fear behaviour. Nonetheless mimicking is not mirroring as shows our observation that this artificial stimulation was less potent, or in a few cases inefficient, in inducing freezing compared to a proper US[1]. This could be explained by the fact that our stimulation recruits neurons and circuits not necessarily involved in physiological freezing, compared to the specific recruitment of dedicated peripheral and central neural elements in normal freezing conditions.

Induction of 4 Hz oscillations generate an aversive behavioural state

In our previous paper[1], the induction of 4 Hz oscillations could act as a US and promote long lasting contextual fear memories. There are at least two possible explanations of these results. First the artificial optogenetic induction of dmPFC 4 Hz oscillations might be involved in the formation of associative fear memories. Second, contextual fear memory observed 24 hours following the optogenetic stimulation could be the direct consequence of the association between contextual elements and the aversive state induced by 4 Hz oscillations. In the present manuscript we did not formally test whether the pairing of a neutral context with the optogenetic activation of dmPFC neurons in place of the US could promote the formation of associative fear memories. Nevertheless, we think this would be the case because activating dmPFC neurons in the ascending phase of 4 Hz oscillations in the present manuscript was much more efficient in promoting an aversive state compared to the artificial optogenetic induction of dmPFC 4 Hz oscillations in our previous paper[1].

4 Hz oscillations as a physiological signature of fear memories and orchestrator a phase-specific encoding of fear behaviour

In our recent paper we identified and characterized a sustained brain state (4 Hz oscillations) that accompanies and predict freezing behaviour. Moreover, we observed that persistent 4 Hz oscillations synchronize the firing activity of dmPFC and BLA neurons. Finally, using an optogenetic analog induction of dmPFC 4 Hz oscillations we provided a causal demonstration that dmPFC 4 Hz oscillations drive freezing behaviour[1]. Thus, these results identify dmPFC 4 Hz oscillations as a physiological signature of fear behaviour. In the present paper, we go a step further and identify a phase-specific coding of freezing behaviour. More precisely, we identified a subpopulation of dmPFC neurons participating to neuronal assemblies co-activated in the ascending phase of 4 Hz oscillations. These results indicate that in addition to their role in predicting freezing episodes, 4 Hz oscillations also orchestrate the firing activity of dmPFC neuronal assemblies and thereby provide a temporal code for controlling the onset, offset and duration of freezing episodes. In comparison to our previous findings, the present results demonstrate that the temporal segregation between ascending and descending phase

neural activity is a key element of freezing expression. One strong advantage of phase-specific coding over rate coding mechanism for the expression of fear behaviour is its dynamic nature. With phase-specific coding, dmPFC neurons can rapidly switch between multiple neuronal networks depending on sensory or internal inputs, a mechanism that could strongly promote the rapid shift between different behavioural states. Consistently with this hypothesis, our data revealed that the activation of dmPFC PNs assemblies during the ascending phase of 4 Hz oscillations constrains the initiation, maintenance and termination of freezing episodes and the precise switch between freezing and no freezing states.

1 Karalis, N. *et al.* 4-Hz oscillations synchronize prefrontal-amygdala circuits during fear behavior. *Nature neuroscience* **19**, 605-612, doi:10.1038/nn.4251 (2016).