Implicit timing and the cerebellum: implications for the understanding of schizophrenia

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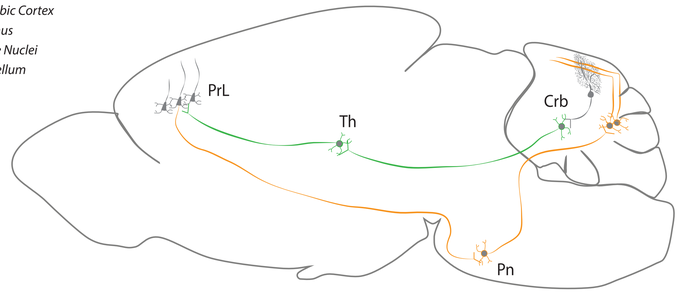
INTRODUCTION

Time perception has been shown to be altered in schizophrenic patients and first degree relatives. The subjective present is the time window (30-50 ms) in which two events are consciously considered to be simultaneous. Lalanne, Giersch and coworkers[1] have shown that both controls and schizophrenic patients are able to implicitly (uncounscously) perceive asynchronicities for intervals below the subjective present, up to 8-17 ms. Nevertheless, patients fail to properly sequence these two events in time. Cerebellar patients show similar impairments in action sequencing.

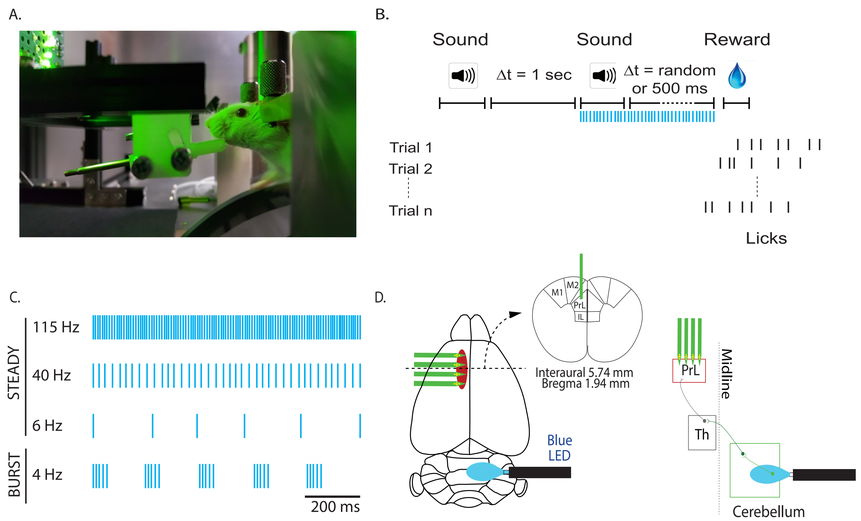
Schizophrenic symptoms are underpinned by communication impairments between brain areas, notably between the cerebellum and the prefrontal cortex[2]. Theta burst stimulation via TMS has been shown to ameliorate part of the negative symptoms in schizophrenia patients[3].

PrL = PreLimbic Cortex
Th = Thalamus
Pn = Pontine Nuclei
Crb = Cerebellum



The goal of the present study is firstly, to unveil the role of the cerebello-thalamo-prefrontal loop in the fine tuning of sequencing of events in the millisecond time scale, when sequences of events are perceived implicitly. In the future, we aim at describing the impairment of this loop in a model of schizophrenia in rodents.

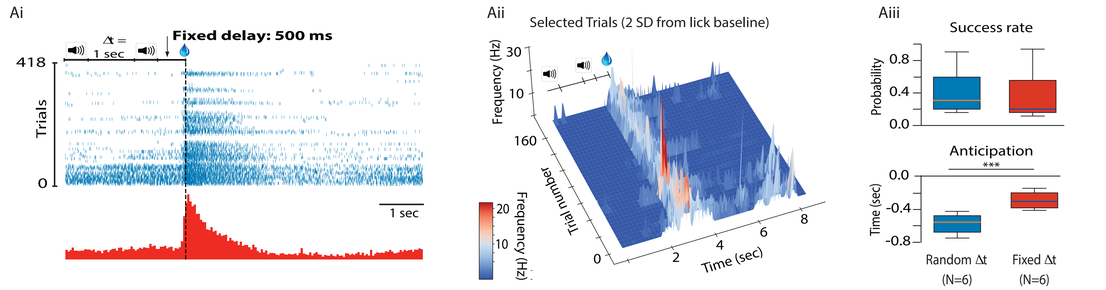
METHODS



(A) Picture of the head-restrain apparatus. The headbar is surgically implanted ~2 weeks before the beginning of the training. After the surgery, mice are habituated to a water deprivation protocol. When the mice are fully stabilized to the water deprivation, the training on the wheel starts. (B) Behavioural task and expected output. Two conditioning acoustic cues are played in a sequence, after which a liquid reward is given following a delay that can be either fixed (500 ms) or random (250 - 1500 ms). The expected output below shows the lick events as vertical bars, each row is corresponding to a different behavioural trial. Vertical blue lines: example of the timing of the optogenetic stimulation. (C) Protocols of optogenetic stimulation. Some protocols are longer than 1s but have been cut for representation purposes. (D) Schematic rapresentation of the experimental condition. L7-ChR2-eYFP[4] mice are used. A blue LED is shined on the cerebellar cortex (lobule V and VI) to influence the cerebello-thalamo-frontal pathway, by activating Purkinje cells (right panel). A silicone probe with 16 channels is inserted in the prelimbic area (PrL) which is part of the mPFC. The activity of the PrL is recorded during the behavioural task.

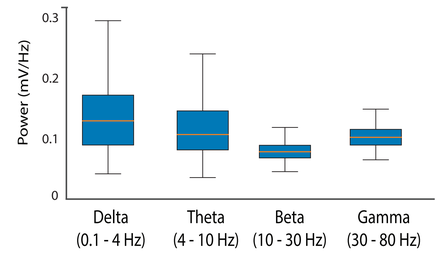
RESULTS

Figure A: Anticipation of the reward during the behavioural task



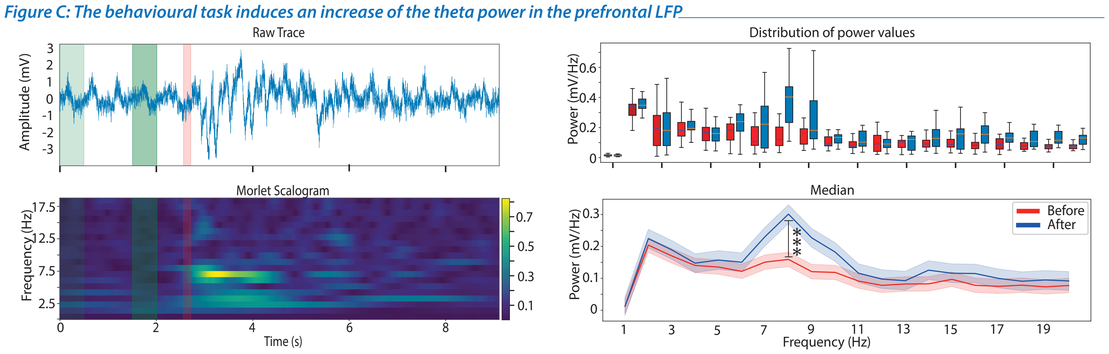
*(Ai) Raster plot of the lick events trial by trial (Top). PSTH of all the events. (Bottom) Dotted line represents the delivery of the reward. (Aii) 3D rapresentation of the lick events, after the selection of the trials. The selection was performed by rejecting the trials below a specific threshold: lick frequency during the reward > 2SD above the baseline lick frequency, established in the first 2s of the trial. (Aiii) Success rate is quantified as the number of trials above the threshold over the total number of trials in the session (Top). Anticipation is quantified as the first lick event in the selected trials. *** (p-value = 0.000013), Mann-Whitney U Test.*

Figure B: Presence of the major wavelenght bands in th PrL



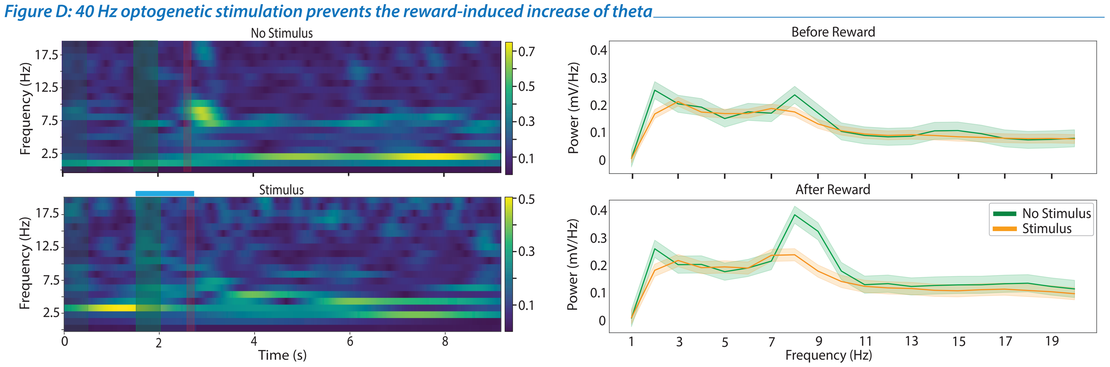
Characterization of the major neuronal oscillations present in the recoded area (PrL). The bandwidth limits have been taken from Buzsáki[5] for coherence with the litterature. The power values have been extracted from the total lenght of the episodes and averaged across multiple episodes. Small and infrequent burst may have been outweighted by the baseline.

Figure C: The behavioural task induces an increase of the theta power in the prefrontal LFP



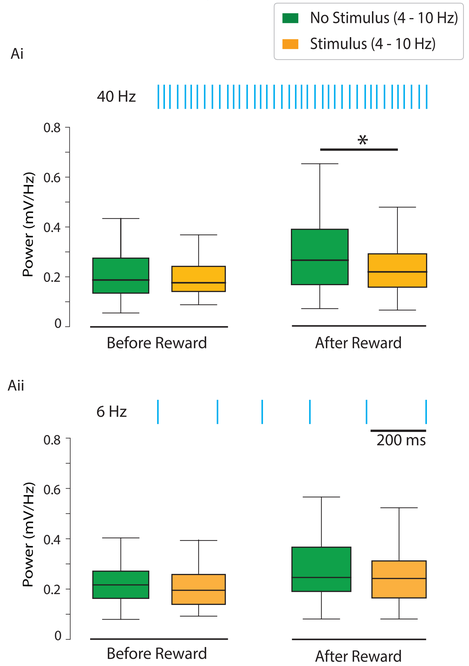
*Average raw trace of the signal recorded from the 4 sites of a single tetrode. Green bands represent the acoustic conditioning cues. Red band indicates the delivery of the reward (Top left). Scalogram (time-frequency analysis with morlet wavelet) of the above represented raw trace. The range of frequencies analyzed is 0.1-20 Hz (Bottom left). Top right: Distribution of the power values extracted from the scalogram for the range of frequencies 0.1-20 Hz. In red are shown the values before the delivery of the reward (interval of 500 ms before). In blue are shown the values of the 500 ms after the delivery of the reward, starting from the appearance of the water (Top right). Distribution of the median values of the power for each frequency analyzed. Shaded area represents the MAD (Median Absolute Deviation) (Bottom right). *** (p-value = 0.0008), Wilcoxon signed-rank test (performed on the theta frequency band, 4-10 Hz).*

Figure D: 40 Hz optogenetic stimulation prevents the reward-induced increase of theta



Morlet scalogram of a control episode, with no optogenetic stimulation of the cerebellum (Top left) and of an episode with 40 Hz optogenetic stimulation between 1.5s and 2.5s (indicated by blue line below the figure) . Green bands represent the acoustic conditioning cues. Red band indicates the delivery of the reward (Bottom left). Distribution of the median values of the power for each frequency analyzed, in the 500 ms before the delivery of the reward (Top right) and in the 500 ms after the delivery of the reward (Bottom right). Shaded area represents the MAD. In green: control (no stimulus) recordings. In yellow: recordings with 40 Hz optogenetic stimulation.

Figure E: 40 Hz and not 6 Hz optogenetic stimulation prevents the reward-induced theta rhytm



*Power values obtained from the Morlet scalogram, for the theta band frequencies (4-10 Hz). (Ai) Optogenetic stimulation of 40 Hz, protocol shown above. The light is shined for 1s before the delivery of the reward (from 1.5s to 2.5s, reward delivered at 2.55s). In green: Control (no stimulus) recordings. In yellow: recording with 40 Hz stimulation. * (p-value = 0.045), Mann-Whitney U Test. N = 5 animals. (Aii) Optogenetic stimulation of 6 Hz, protocol shown above.*

CONCLUSIONS & PERSPECTIVES

- The behavioural task with random delay between the second acoustic cue and the reward, produces a significantly higher anticipation. We speculate that the hazard function could play a role in the attentional level of the mice and thus improve the learning process.
- The behavioural task is inducing a significant increase in the theta band in the prefrontal cortex, time-locked with the release of the reward. Further analyses must be done to investigate wheter this theta activation is significantly reduced in the electrophysiological recordings of failed behavioural trials (when the licking behaviour is not time-locked to the task).
- Only one (40 Hz) of the four tested frequencies of stimulation, significantly prevents the increase of power in the theta band. Further analyses will focus on the relation between this change in the LFP and possible changes in the behaviour, wheter the stimulation is able to alter or suppress the licking behaviour.

References

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3 Tatlidede et al. (2010). Safety and proof of principle study of cerebellar vermal theta burst stimulation in refractory schizophrenia. Schizophr. Res., 124(1-3):91-100

4 Chaumont et al. (2013). Clusters of cerebellar Purkinje cells control their afferent climbing fiber discharge. PNAS, 110(40):16223-8

5 Buzsáki and Draghun (2004). Neuronal oscillations in cortical networks. Science, 304, 1926/1929