Aberrant Gamma-Band Oscillations and Prepulse Inhibition in Mice with Vitamin D Deficiency

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Background

Gamma-band oscillations (GBO) are brain rhythms in the frequency range of 30-100 Hz, as measured by electroencephalogram (EEG), and typically around 40 Hz. GBO have been associated with cognitive functions such as attention, perception, and memory. In addition, abnormal GBO have been observed with cognitive disorders such as schizophrenia. Patients with schizophrenia show reduced power of GBO in response to auditory, visual, electrical stimuli, and task. However, the spontaneous (resting) GBO power is stronger in schizophrenic patients. These paradoxical features of schizophrenia have been recently explained by differentiating evoked-GBO from spontaneous-GBO.

Vitamin D deficiency affects multiple brain processes, including cognitive operations. Particularly, low levels of vitamin D can be a risk factor for schizophrenia. The relative risk of schizophrenia shows a fitted sine function of the month of birth, and it has a negative correlation with vitamin D concentration. One of the neurobiological pathomechanisms in schizophrenia is the reduced perineuronal nets (PNN), a component of the extracellular matrix. Vitamin D deficiency may downregulate the integrity of PNN.

Hypothesis

vitamin D and PNN are necessary for normal GBO and related function in freely moving mice. We sought to investigate the impact of vitamin D deficiency and disrupted PNN on the generation of GBO and cognitive functions.

Methods

- We used PV-Cre mice (Stock No. 012358, Jackson Laboratory) to evoke frontal GBO by basal forebrain PV optogenetic stimulation. For the vitamin D deficient (VDD) group, vitamin D deficient chow (Cat. No. TD. 89123, ENVIGO) was provided for 20 weeks.
- We performed optogenetically-evoked GBO, auditory-evoked GBO, prepulse inhibition test, and spontaneous GBO in freely moving mice at 6 and 20 weeks.
- We prepared another experimental group to check the GBO before and after the enzymatic digestion of the PNN and also performed previous four measurements.

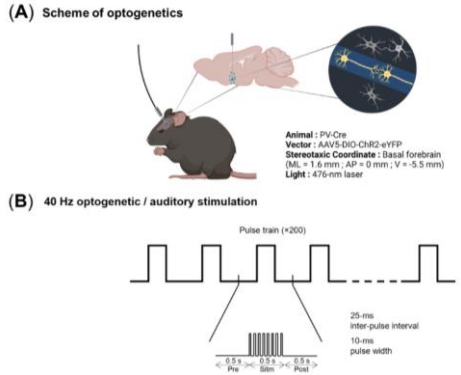

Figure 1. Scheme of the experimental design for measuring frontal gamma-band oscillations. (A) Schematic diagram of the optogenetic stimulation. We injected AAV5-DIO-ChR2-eYFP vector into basal forebrain of PV-Cre mice and illuminate blue light through optic fiber. (B) Stimulation protocol for 40 Hz optogenetically- and auditory-evoked GBO.

Results

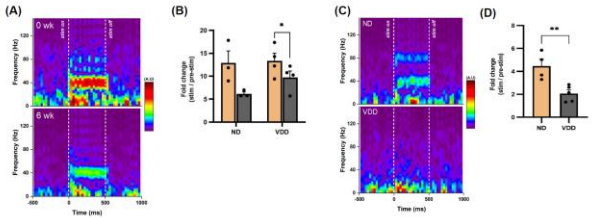

Figure 2. Optogenetically- and auditory-evoked GBO reduced by vitamin D deficiency. (A) Representative optogenetically-evoked time-frequency spectrogram from before and after vitamin D-deficient diet. (B) Fold change during the stimulation period in comparison with pre-stimulation period. (C-D) Auditory evoked GBO. Data are presented as mean ± SEM. * $p < 0.05$ by the paired-t-test.

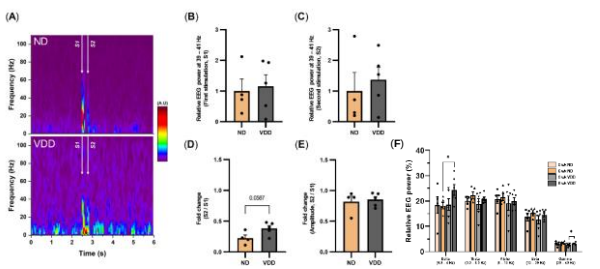

Figure 3. Prepulse inhibition reduced and spontaneous GBO increased by vitamin D Deficiency. (A) Representative time-frequency spectrogram from before and after vitamin D-deficient diet. (B-C) Relative EEG power at 40 ± 1 Hz of stimulation 1 (S1, 2.5 -2.55 s) and stimulation 2 (S2, 2.75 – 2.8 s). (D) Fold change during S2 period in comparision with S1 period. (E) Amplitude means the averaged traces' peak amplitude during the stimulation period. Fold change during S2 period in comparision with S1 period. (F) Frontal brain oscillations were analyzed by separating them in Alpha (8 – 13 Hz), Beta (13 – 30 Hz), Gamma (35 – 45 Hz), Delta (0.5 – 4 Hz), and Theta (5.5 – 8.5 Hz). Each frequency are normalized by total power (0.5 – 1000 Hz). Data are expressed as mean ± SEM. * $p < 0.05$ by Student's t-test and paired t-test.

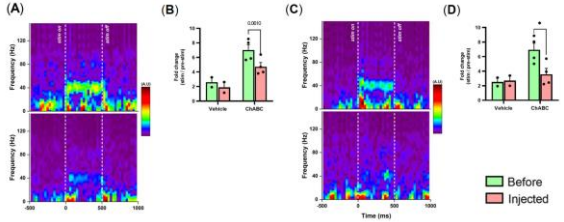

Figure 4. Optogenetically- and auditory-evoked GBO reduced by ChABC injection. (A) Representative optogenetically-evoked time-frequency spectrogram from before and after vitamin D-deficient diet. (B) Fold change during the stimulation period in comparison with pre-stimulation period. (C-D) Auditory evoked GBO. Data are presented as mean n ± SEM. . * $p < 0.05$ and ** $p < 0.001$ by the paired t-test.

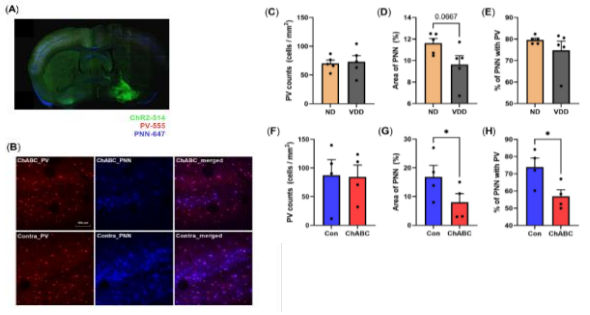

Figure 5. Immunohistochemistry for the quantification of PV and PNN. (A) Representative 10X confocal image of vitamin D-deficient mouse. (B) Representative 30X confocal images of a ChABC-injected mouse. ChABC-injected hemisphere showed a reduction of PNN. (C-E) In comparision between normal and vitamin D deficient group, the number of PV neurons, area of PNN, and colocalization percent of PV and PNN among all PV neurons. (F-H) In comparision between vehicle and ChABC injected group, the number of PV neurons, area of PNN, and colocalization percent of PV and PNN among all PV neurons. Data are presented as mean n ± SEM. . * $p < 0.05$ by the Student's t-test.

Conclusion Our findings may indicate the VDD could be a risk factor exacerbating pre-existing abnormalities in GBO and sensory gating in psychiatric patients, such as those with schizophrenia

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