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Delta Frequency Optogenetic Stimulation of the Thalamic Nucleus Reuniens Is Sufficient to Produce Working Memory Deficits: Relevance to Schizophrenia

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ABSTRACT

BACKGROUND: Low-frequency (delta/theta) oscillations in the thalamocortical system are elevated in schizophrenia during wakefulness and are also induced in the *N*-methyl-D-asparate receptor hypofunction rat model. To determine whether abnormal delta oscillations might produce functional deficits, we used optogenetic methods in awake rats. We illuminated channelrhodopsin-2 in the thalamic nucleus reuniens (RE) at delta frequency and measured the effect on working memory (WM) performance (the RE is involved in WM, a process affected in schizophrenia [SZ]).

METHODS: We injected RE with adeno-associated virus to transduce cells with channelrhodopsin-2. An optical fiber was implanted just dorsal to the hippocampus in order to illuminate RE axon terminals.

RESULTS: During optogenetic delta frequency stimulation, rats displayed a strong WM deficit. On the following day, performance was normal if illumination was omitted.

CONCLUSIONS: The optogenetic experiments show that delta frequency stimulation of a thalamic nucleus is sufficient to produce deficits in WM. This result supports the hypothesis that delta frequency bursting in particular thalamic nuclei has a causal role in producing WM deficits in SZ. The action potentials in these bursts may "jam" communication through the thalamus, thereby interfering with behaviors dependent on WM. Studies in thalamic slices using the *N*-methyl-D-asparate receptor hypofunction model show that delta frequency bursting is dependent on T-type Ca^{2+} channels, a result that we confirmed here in vivo. These channels, which are strongly implicated in SZ by genome-wide association studies, may thus be a therapeutic target for treatment of SZ.

Keywords: Channelrhodopsin, Delta, Optogenetics, Reuniens, Schizophrenia, Thalamus

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Low-frequency cortical oscillations in the delta/theta range have abnormally high power in schizophrenia (SZ) patients in the awake (resting) state, a finding confirmed by electroencephalogram and magnetoencephalography meta-analyses (1,2). Delta oscillations occur globally during slow-wave sleep, but abnormal delta in SZ occurs in subregions of the thalamocortical system (medial prefrontal cortex and temporal lobes) (3). Elevated delta is present in unmedicated, first-episode patients (4–9) and thus cannot be attributed to drug treatment. Although there are some discrepancies, many studies have correlated the magnitude of delta elevation with both positive and negative symptoms of SZ (6,10–13). Importantly, elevated delta correlates closely with the manifestation of the disease itself; increased delta power is observed in SZ patients but not in healthy "at risk" subjects such as first-degree relatives (14–17). Even in twins discordant for SZ, increased delta power is not observed in the healthy twin (18,19). This correlation with the disease stands in contrast to the gamma oscillation abnormality in SZ, which is present in relatives that do not have the disease (16,20,21). Thus, the gamma abnormality appears to be a predisposition for developing the disease (22), whereas the delta abnormality could be a direct cause.

A further connection between abnormal delta and SZ comes from studies using the *N*-methyl-D-asparate (NMDA) hypofunction model of SZ (23–26), a model based on the observation that normal subjects develop both positive and negative symptoms of SZ in response to NMDAR antagonist (27–30). *N*-methyl-D-asparate receptor (NMDAR) antagonist produces delta oscillations in both animal models and humans (31–36), again raising the possibility that these oscillations might have a role in producing symptoms of SZ.

In this study, we used an animal model to evaluate the hypothesis that abnormal delta oscillations can have a causal role in producing functional deficits, as previously suggested (37,38). Although elevated delta correlates with SZ symptoms, as noted above, correlation is not proof. Ketamine and other NMDAR antagonists at high but sub-anesthetic doses induce delta oscillations in animals and humans (31–34,36), and this is accompanied by aspects of

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psychosis (27–30,39,40). However, NMDAR antagonists also cause other changes, notably alteration in gamma oscillations (41–44). The role of delta in causing behavioral deficits therefore cannot be firmly established by these experiments. Thus, the fundamental question of whether abnormal delta oscillations can have a role in producing functional abnormalities remains unanswered.

To address this question, we used optogenetics, a method that allows specific activity patterns to be induced in a defined set of cells. Using this method, we tested whether delta frequency stimulation of the nucleus reuniens (RE) of the thalamus interferes with working memory, a function that has deficits in SZ (45). We targeted channelrhodopsin to RE because this thalamic nucleus interconnects the medial prefrontal cortex and hippocampus, both of which are required for working and contextual memory (46–50), and because lesioning of RE interferes with working memory (46,51–54).

A second goal of the current study relates to the cellular and molecular mechanisms that underlie abnormal delta oscillations. Use of the NMDA hypofunction model has provided mechanistic insight into this mechanism. The source of these oscillations appears to be the thalamus because NMDAR antagonist injected into the rat thalamus can evoke delta oscillations in the thalamus, which then are communicated to the cortex (31) and hippocampus (36). Experiments using a slice preparation of the thalamic reticular nucleus (TRN), an inhibitory pacemaker of the thalamus, have revealed that NMDAR antagonists block the NR2C channels prevalent in this structure (35). These channels generate a basal inward current due to ambient glutamate and can contribute to resting potential [NR2C channels, unlike NR2A and NR2B, are not blocked by Mg^{2+} at resting voltage (55,56)]. The blockade of this basal inward current causes hyperpolarization of the cells and thereby removes the inactivation from T-type Ca^{2+} channels (57). The T-type Ca^{2+} channels then generate Ca^{2+} spikes that trigger bursts of Na^{+} spikes at delta frequency. This bursting can produce delta frequency modulation of synaptic targets in the relay nuclei of the thalamus, and these cells, in turn, can drive delta modulation of their targets in the hippocampus (36) and cortex (31). We previously showed that thalamic delta oscillations induced by NMDAR antagonists can be reduced by antagonists of T-type Ca^{2+} channels and D2 receptors, but these results were obtained in a slice preparation (35). In the current paper, we sought to extend these results by testing whether these antagonists are also effective in vivo.

METHODS AND MATERIALS

All experimental protocols were approved by the institutional animal care and use committees at Massachusetts Institute of Technology, Cambridge, Massachusetts, and Brandeis University, Waltham, Massachusetts.

Subjects

Male Long-Evans rats (Charles River, Wilmington, Massachusetts) were housed under a 12-h light/12-h dark cycle in a temperature- and humidity-controlled environment with free access to food and water.

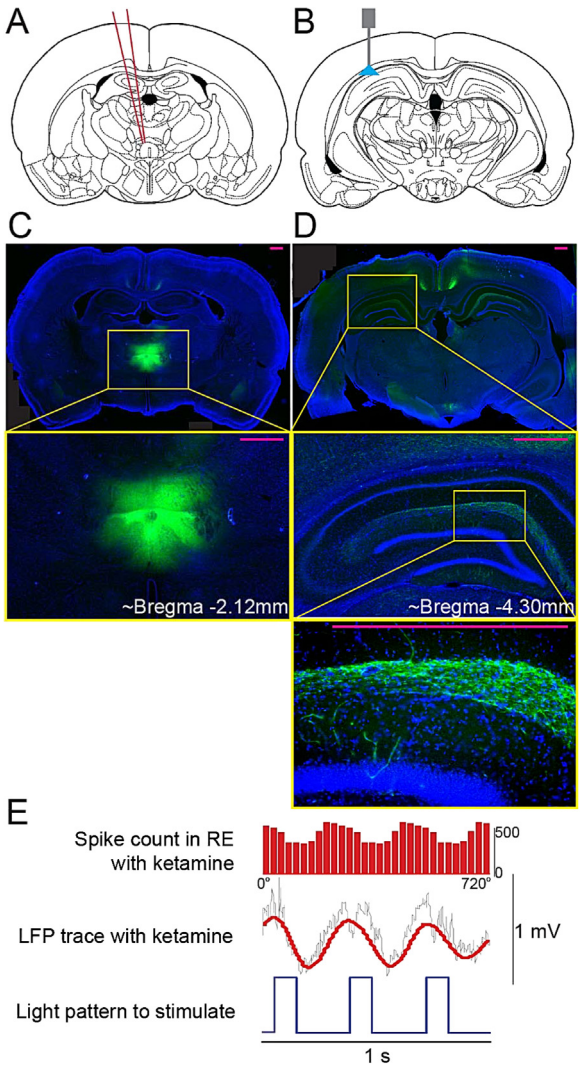

Figure 1. Strategy of experiments to test whether delta frequency optogenetic stimulation of nucleus reuniens (RE) interferes with working memory (74). **(A)** Schematic showing the site of the yellow fluorescent protein fused adeno-associated virus (YFP-AAV) injection in the midline thalamus, including the RE of the thalamus. **(B)** Schematic shows the site of the fiber optic tip placed just above CA1, where it can selectively excite the RE axons in that region. **(C)** Localization of AAV-mediated expression in the RE visualized by YFP immunostaining (shown in green; blue is DAPI nuclear staining). The virally induced expression spreads 1 to 2 mm. The RE is located just above the third ventricle. **(D)** Green shows YFP immunostaining of RE axons in CA1. **(E)** Delta frequency activity in RE induced by systemic injection of ketamine. Top trace shows spike phase relative to delta oscillations in the local field potential of RE. Middle trace shows local field potential in RE (green, raw; red, filtered). Top and middle traces as previously published (36) show a pattern of delta frequency light stimulation (~3 Hz) used in the optogenetic experiments. Tissue shown was perfused 5 weeks after surgery to represent the expression level of injected virus at the time that animal behavior training would begin ($n = 2$); calibration bars, 500 μm. LFP, local field potential.

Surgery for Optogenetic Experiments

For optogenetic experiments (Figures 1–3), anesthesia was induced by intraperitoneal injection of ketamine (25 mg/kg), xylazine (3 mg/kg), and atropine (.027 mg/kg), followed by