Supplemental Methods

Humans

Nine patients with a DSM-IV diagnosis of schizophrenia (7 men, 2 women) were recruited from the Iowa Longitudinal Database and 9 sex-, education- and age-matched controls (7 men, 2 women) recruited from the University of Iowa Department of Neurology's Cognitive Neuroscience Registry for Normative Data (Table 1 for patient data). Matched controls had no history of significant psychiatric, neurological or medical illnesses. All participants were determined to have the decisional capacity to provide informed consent, resided within 100 miles of Iowa City and were able to independently travel to the University of Iowa Hospitals and Clinics.

Human Interval timing task

Interval timing was investigated in humans with and without schizophrenia according to methods described at length previously[1]. The interval timing task consisted of 4 blocks of 40 trials (160 trials in total). Trials were presented in pseudorandom order. All trials began when a numerical cue stimulus appeared on the center of the screen indicating the temporal interval the participants were instructed to estimate (3 or 12 s – 3 s trials were excluded in this study). Participants made responses by pressing the space bar on a keyboard using their dominant hand when they estimated the temporal interval had elapsed. Participants received feedback about their response time at the end of each trial. There was a 3-6 s interval between response and feedback. After feedback, participants moved to the next trial by pressing the space bar. The task was self-paced and the participants were asked not to count in their head during the task. Participants performed 4 practice trials prior to the real task. The interval-timing task consisted

of 160 trials with either a 3 or 12 s interval; only data from the 12 s interval was included in this manuscript. All trials began when a numerical cue stimulus appeared on the center of the screen indicating the temporal interval the participants were instructed to estimate (3 or 12 s). Participants made responses by pressing the space bar on a keyboard using their dominant hand when they estimated the temporal interval had elapsed. Participants received feedback about their response time at the end of each trial. There was a uniformly varying, randomly chosen 3- to 6-s interval between response and feedback. After feedback, participants moved to the next trial by pressing the space bar again. The task was self-paced, and the participants were asked not to count in their head during the task.

EEG Recording and analysis

EEG recording and analysis was similar to methods described in detail in prior work[1,6]. EEG was recorded on a Nihon Kohden system with a sampling rate of 500 Hz. EEG was recorded from 21 channels based on the 10-20 system (Fz, Cz, Pz, FP1/2, F3/4, C3/4, P3/4, F7/8, T3/4, T5/6, O1/2, M1/2), as well as left-eye VEOG and ground (forehead). This approach was selected to match our previous EEG datasets that described differences in low-frequency rhythms between patients with Parkinson's disease and controls[1,7]. Impedance of all electrodes was below 5 kΩ. Continuous data were parsed in to 16 s epochs (-2 to 14 seconds following the cue) and re-referenced to the mathematical average of the two mastoid channels, yielding a total of 19 scalp EEG channels. Eye blinks and horizontal eye movements were removed by hand using independent component analysis and EEGLab[8]. Time-frequency measures were computed by multiplying the fast Fourier transformed (FFT) power spectrum of single trial EEG data with the

FFT power spectrum of a set of complex Morlet wavelets (defined as a Gaussian-windowed complex sine wave: $e^{i2\pi tf}\, e^{-t^2/(2\,x\,\sigma^2)}$, where t is time, f is frequency (which increased from 1 to 50 Hz in 50 logarithmically-spaced steps), and σ defines the width (or "cycles") of each frequency band, set according to 4/(2πf)), and taking the inverse FFT. The end result of this process is identical to time-domain signal convolution, and it resulted in estimates of instantaneous power (the magnitude of the analytic signal). Each epoch was then cut in length (-0.5 to +2 s). Power was normalized by conversion to a decibel (dB) scale ($10*\log10(power_t/power_{baseline})$), allowing a direct comparison of effects across frequency bands. The baseline for each frequency consisted of the average power from ⁻0.5 to ⁻0.3 s prior to the onset of the cues. Human time-frequency plots and ERPs were analyzed from electrode Cz in delta (1- 4 Hz) and theta (5-8 Hz) in accordance with well-established prior hypotheses[1,4]. Two additional electrodes were placed 1 cm below the inion and 2 cm lateral to record activity from cerebellar hemispheres.

Rodents

Animals were motivated by regulated access to water, while food was available *ad libitum*. Rats consumed 10-15 mL of water during each behavioral session and additional water (5-10 mL) was provided 1-3 hours after each behavioral session in the home cage. Single housing and a 12 hour light/dark cycle were used; all experiments took place during the light cycle. Rats were maintained at ~90% of their free-access body weight during the course of these experiments and received one day of free access to water per week. All procedures were approved by the Animal Care and Use Committee at the University of Iowa.

Rodent Interval timing task

All rats were trained to perform an interval timing task according to previously published methods[3–5,9]. Animals learned to make operant lever presses to receive liquid rewards. After fixed-ratio training, animals were trained in a 12 s fixed-interval timing task in which rewards were delivered for responses after a 12 s interval. Rewarded presses were signaled by a click and an 'off' house light. Once they were well-trained, a second light was added on the right side of lever to indicate a shorter, 3 s interval (these trials were not included in this study). Each rewarded trial was followed by a 6, 8, 10 or 12 s pseudorandom intertrial interval which concluded with an 'on' house light signaling the beginning of the next trial. Early responses occurring before interval end were not reinforced. The house light was turned on at trial onset and lasted until the onset of the intertrial interval, which began when the rewarded press was made or a time-out occurred after 18 seconds on trials with no responses. Training and infusion sessions were 60 minutes long. Mean response time was defined as the average time the animals pressed the lever on each trial, which is used to estimate animals' internal estimates of time[4]. All behavior took place in operant chambers (MedAssociates, St Albans, VT) equipped with a lever, a drinking tube, and a speaker driven to produce an 8 kHz tone at 72 dB. Behavioral arenas were housed in sound-attenuating chambers (MedAssociates). Water rewards were delivered via a pump (MedAssociates) connected to a metal drinking tube (AnCare) via Tygon tubing.

Rodent surgery

Rats were trained in the interval timing task, assigned to an experimental group, and implanted accordingly. The MFC and LCN of rats trained in the two interval task were implanted

with microwire arrays, 33-gauge infusion cannula (Plastics One), or fiber optics and ChR2 according experimental protocol. The coordinates for the left medial frontal implants were AP: +3.2, ML: ±1.2, DV: -3.5 @ 12° in the lateral plane; for the left ventrolateral thalamus: AP: -2.3, ML: ±1.8, DV: -5.4; and for right lateral cerebellar nuclei AP: -10.8, ML: ±3.6, DV: -6.2. A surgical level of anesthesia was maintained with hourly (or as needed) ketamine supplements (10 mg/kg). The electrode array was inserted while concurrently recording neuronal activity to verify implantation in layer II/III of the MFC or the LCN. The craniotomy was sealed with cyanoacrylate ('SloZap', Pacer Technologies, Rancho Cucamonga, CA) accelerated by 'ZipKicker' (Pacer Technologies), and methyl methacrylate (i.e., dental cement; AM Systems, Port Angeles, WA). Following implantation, animals recovered for one week before being reacclimatized to behavioral and recording procedures.

Rodent perfusions

When experiments were complete, rats were anesthetized, sacrificed by injections of 100 mg/kg sodium pentobarbital, and transcardially perfused with 10% formalin. Brains were post fixed in a solution of 10% formalin and 20% sucrose before being sectioned on a freezing microtome. Brain slices were mounted on gelatin-subbed slides and stained for cell bodies using DAPI. Histological reconstruction was completed using post mortem analysis of electrode and cannula placements and confocal microscopy in each animal. These data were used to determine electrode, infusion cannula, optical cannula, and spread of viral infection according to each experiment.

Focal drug infusions

Focal drug infusions into MFC and LCN were performed according to procedures described previously[4,5,9]. On subsequent days while anesthetized via isoflorane, the MFC was infused with 0.9% saline (Phoenix Scientific, St. Joseph, MO) during control sessions or D1-dopamine receptor antagonist SCH23390 (0.5 µg of 1.0 µg/µL)[4,10]. Infusions were conducted by inserting an injector into the cannula and 0.5 µL of infusion fluid was delivered at a rate of 30 µL/hr (0.5 µL/min) via a syringe infusion pump (KDS Scientific, Holliston, MA). After the injection was complete, the injector was left in place for 2 minutes to allow for diffusion. Infusions were counterbalanced and always separated by 24 hours.

Optogenetic stimulation of the LCN projections to the ventrolateral thalamus

To target the cerebellar output pathways originating in lateral cerebellar nuclei, an AAV viral construct CamKII-ChR2 and CamKII-mCherry was infused in LCN. An optical fiber cannula (200 um core, 0.22NA, Doric Lenses) was implanted in the ventrolateral thalamus to specifically target terminals expressing ChR2 in the LCN-VL pathway. Rats were injected with AAV-ChR2 into LCN, with immediate placement of an optical fiber cannula (200 um core, 0.22NA, Doric Lenses). The injection consisted of 0.5 microliters of ~10^{11} infectious particles per milliliter. A separate group of 6 control animals were injected with AAV- CamKII-mCherry. No randomization or blinding was used to select these animals. During testing, each rat performed the fixed-interval timing task for 1 hr with op togenetic stimulation on half of pseudorandomly chosen trials. In stimulation sessions, 473 nm light was delivered at 10 mW

power on 50% of trials. Task performance and neuronal activity was compared between illuminated and unilluminated trials within each animal on the test day.

Neurophysiological analyses

Neuronal ensemble recordings in the MFC were acquired using a multi-electrode recording system (Plexon, Dallas, TX). Putative single neurons were identified on-line using an oscilloscope and audio monitor. The Plexon off-line sorter was used to analyze the signals after the experiments and to remove artifacts. Spike activity was analyzed for all cells that fired at rates above 0.1 Hz. Statistical summaries were based on all recorded neurons. Principal component analysis (PCA) and waveform shape were used for spike sorting. Single units were identified as having 1) consistent waveform shape, 2) separable clusters in PCA space, and 3) a consistent refractory period of at least 2 ms in interspike interval histograms. Preliminary analysis of neuronal activity and quantitative analysis of basic firing properties were carried out using NeuroExplorer (Nex Technologies, Littleton, MA), and quantitative analyses were performed with custom routines for MATLAB. Peri-event rasters and average histograms were constructed around light on, lever release, lever press, and lick. Microwire electrode arrays were comprised of 16 electrodes. Local field potential (LFPs) were recorded from 4 of these electrodes per rodent. LFP channels were analog filtered between 0.7 and 100 Hz online and recorded in parallel with single unit channels using a wide-band board. In each animal, one electrode without single units was reserved for local referencing. Recordings from this reference electrode did not have single units and had minimal line noise, and this electrode was used for local referencing for both single unit and LFP recordings.

For single-unit recordings, peri-event time histograms were calculated by recording the time of each putative action potential around cue onset with 0.01 s bins. Each occurrence of the cue was considered a trial, and putative action potentials were plotted relative to these events (0-12 s) using a raster plot. Histograms were calculated by taking the average firing rate and smoothing over 1 s using a Gaussian window. Data were tested for normality prior to subsequent analyses.

We defined time-related ramping activity as firing rate that progressed uniformly over the interval. We measured this in two ways: PCA and linear regression. PCA was used to identify dominant patterns of neuronal activity using orthogonal basis functions from peri-event histograms during the 12 s interval[4,5]. All neurons from both areas were included in PCA. The same principal components were projected onto MFC and LCN recordings, and PC weights were compared via a t-test[4]. Secondly, we used regression to define neuronal ramping activity according to the formula:

$$y = at + bt^2 + c$$

Where y is firing rate, t is the time in seconds, and a is the linear slope and b is quadratic slope. Goodness of fit was derived from an ANOVA[11].

Correlation analysis (MATLAB function PARTIALCORR) was used to explore the relationship of spiking activity average response time and other neurons using Pearson's correlation[4]. Only 12 s trials were used for correlation data. Response time was defined by the average time the animals pressed the lever on each trial and can be used to estimate animals' internal estimate of time[4]. If the animal made multiple presses on a single trial, response times were averaged. In this analysis, response time was treated as a continuous variable. Interval

177 epochs were divided into 3 s windows for correlation; as most movements occurred after 6 s,
178 these epochs were excluded from analyses. Trial-by-trial correlations of firing rate were created
179 by performing partial correlation of firing rate over the entire interval on each trial for two
180 neurons, accounting for heavier correlations with average response time. Joint peristimulus trial
181 histograms were calculated at 0.1 and 1 s bins during the interval, and the shift-predicted matrix
182 was subtracted from the raw matrix. In both cases, shuffled correlations were generated by
183 randomly permuting trial order for comparisons. Analysis of fast interactions was performed by
184 canonical cross-correlation, spike-spike coherence, analysis of neuronal synchrony and by
185 performing the above analysis at smaller bin sizes (1 to 100 ms).

186 Time-frequency calculations were computed using custom-written MATLAB routines
187 using identical approaches to EEG data above[4,5,9,12]. To examine the time-frequency component
188 of interactions between individual spikes and the field potential, we applied spike-field
189 coherence analysis using the Neurospec toolbox [4,6,13], in which multivariate Fourier analysis was
190 used to extract phase-locking among spike trains and local field potentials. Phase-locking
191 coherence values varied from 0 to 1, where 0 indicates no coherence, and 1 indicates perfect
192 coherence. To compare across neurons with different coherence distributions, all phase-locking
193 values were divided by the 95% confidence for each interval, so that 1 indicates a $p<0.05$.
194 Correlated neurons were selected as those that had Pearson's correlation coefficient summed at
195 >5 across the entire ensemble. Only neurons with a firing rate > 1 Hz were analyzed for spike-
196 field coherence. All code available from:
197 http://www.healthcare.uiowa.edu/labs/narayanan/resources.html

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Granger causality

Nine MFC LFPs and nine LCN LFPs recorded in 3 rodents were downsampled to 200Hz and a 59-61 Hz Butterworth notch filter was applied to remove line noise. We selected a model order of 500ms and 2000ms sliding time segments (6 segments per 12s interval timing trial) for subsequent analysis to maximize the resolution of low frequency Granger prediction. A model order of 500ms contains >= 1 cycle of a >= 2Hz oscillation, allowing increased frequency specificity of Granger prediction. Therefore, we subtracted single-subject ERPs from time series segments, detrended and z-scored all modeled data. To visualize bandlimited directed connectivity, *armorf.m* derived autoregression coefficient matrices were Fourier transformed and inverted to generate transfer functions for the spectral factorization of autoregression residuals (Fig 3F)[14]. Spectral pairwise Granger prediction was subsequently calculated by inverse Fourier transformation of spectral factorization outputs, resulting in time-frequency domain connectivity estimates[14]. Optimal VAR model order was determined by varying model order across repeated bivariate Granger parameter estimates using the BSMART toolbox function *armorf.m,* then inputting residuals matrices into Bayes information criterion (BIC) tests as described previously[14]. Mean BIC values across segments converged to a stable minimum across orders > 100 ms, indicating minimized regression error (Fig S7A). Time series segments were submitted to KPSS unit root testing using MVGC Toolbox routines to determine single trial KPSS test statistics. Statistical testing of stationarity was performed by compared single trial test statistics to a nonstationarity critical value computed at $p = 0.05$ (Fig S7B)[14]. Significant Granger causality during 12s interval timing was assessed using Granger's F-test routines in MVGC Toolbox at $p = 0.05$. Inputs were F-statistics grand averaged across animals and time segments for directed connectivity between MFC and LCN in experimental and time-shuffled data.

Power analysis

The statistical power for each comparison was calculated for each test in using the matlab function sampsizepwr.m by assuming a *t* or a *chi-square* distribution where appropriate, and assuming comparisons between two independent samples using two-tailed tests. These power analyses were used to estimate human and animal sample sizes.

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Supplementary References

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Supplementary Figures

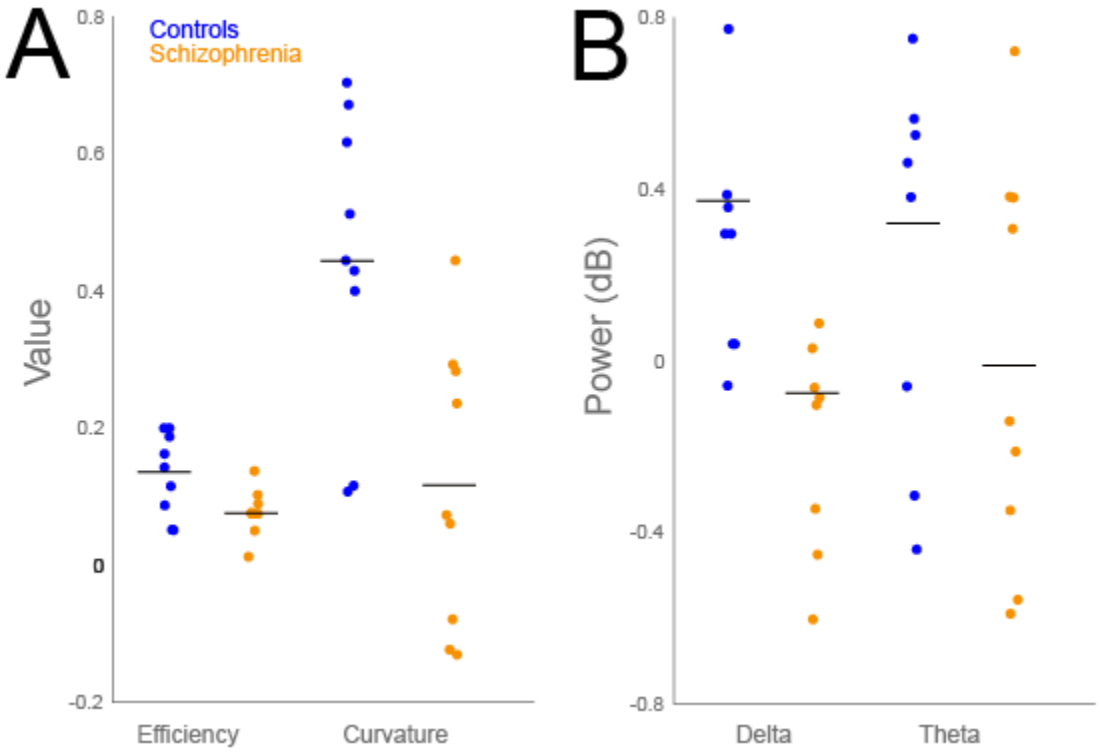

Figure S1: Individual subject data for Figure 1C (A) and Figure 1E (B). Each point is a single subject for control (blue) and schizophrenic patients (orange).

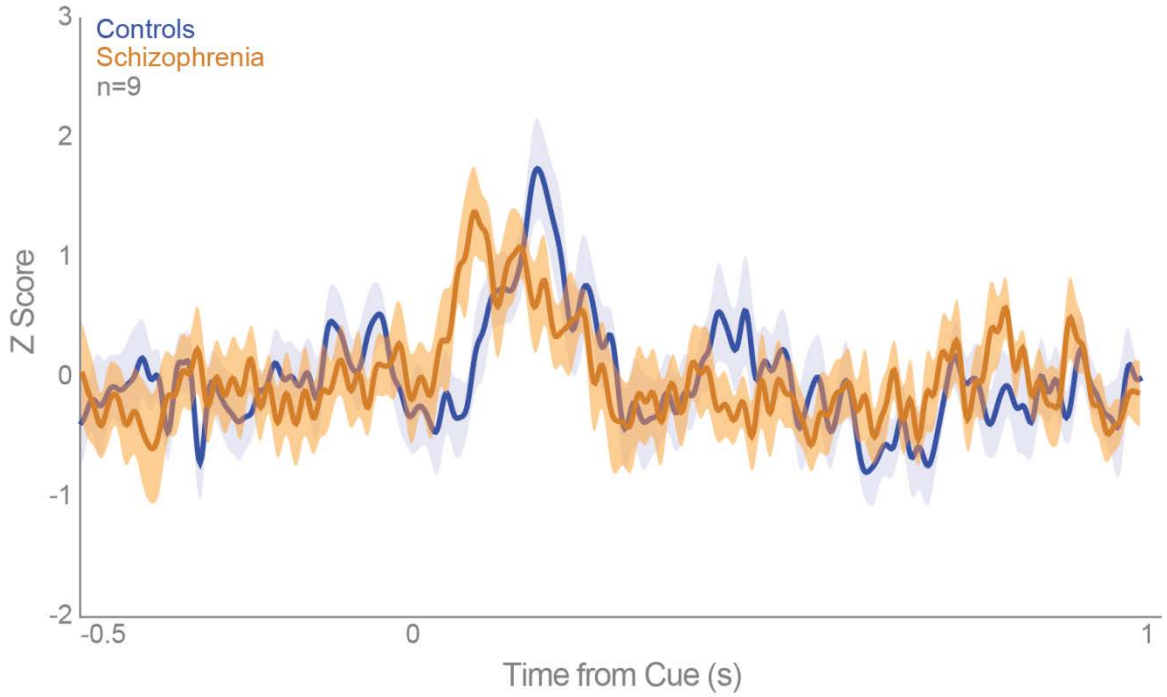

Figure S2: Event-related potentials from patients with schizophrenia (orange) and demographically-matched controls (blue).

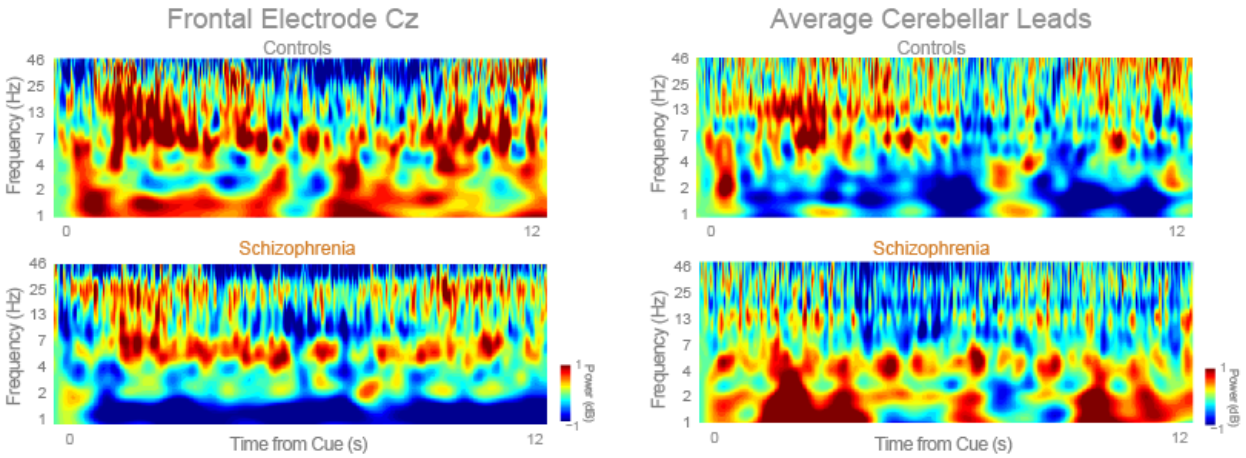

Figure S3: Time-frequency spectral plots reveal that patients with schizophrenia have decreased activity in the MFC throughout the interval in the interval timing task in comparison to healthy controls. Patients with schizophrenia can have low-frequency cerebellar activation in the interval timing task (9 patients vs. 9 controls).

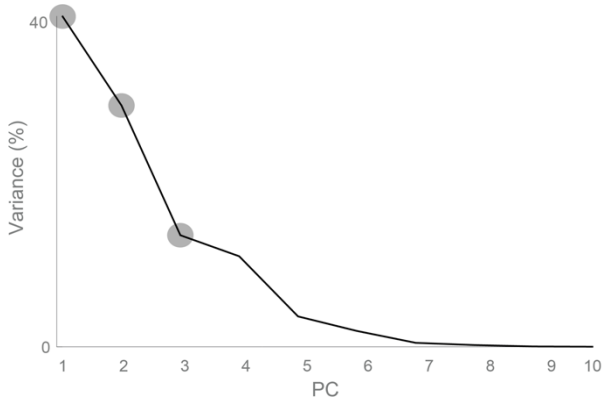

Figure S4: Scree plot of principal component analysis. Ramping activity was the most prominent pattern of neural activity among both MFC and LCN neurons (PC1) explained ~40 % of the variance.

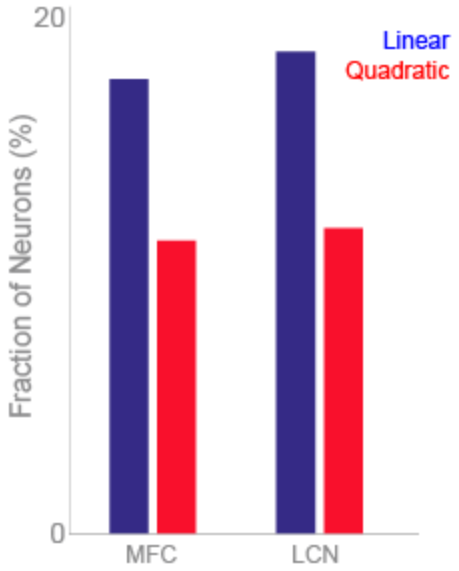

Figure S5: Number of MFC and LCN neurons with linear and quadratic coefficients via regression. For MFC, 17% of neurons had a linear fit, and 11% had a quadratic fit; for LCN 18% had a linear fit and 12% had a quadratic fit.

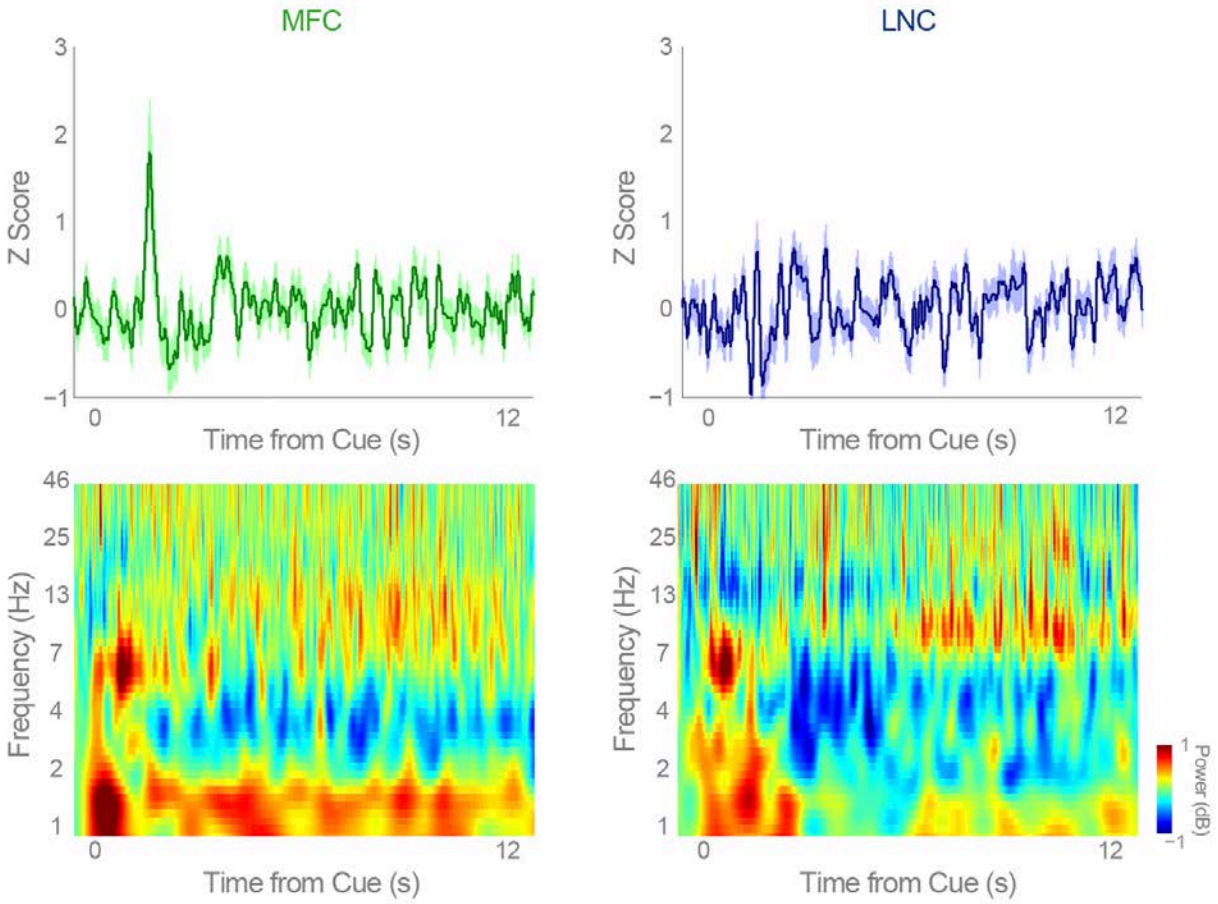

Figure S6: Field potentials and time-frequency plots of activity from the MFC and LCN.

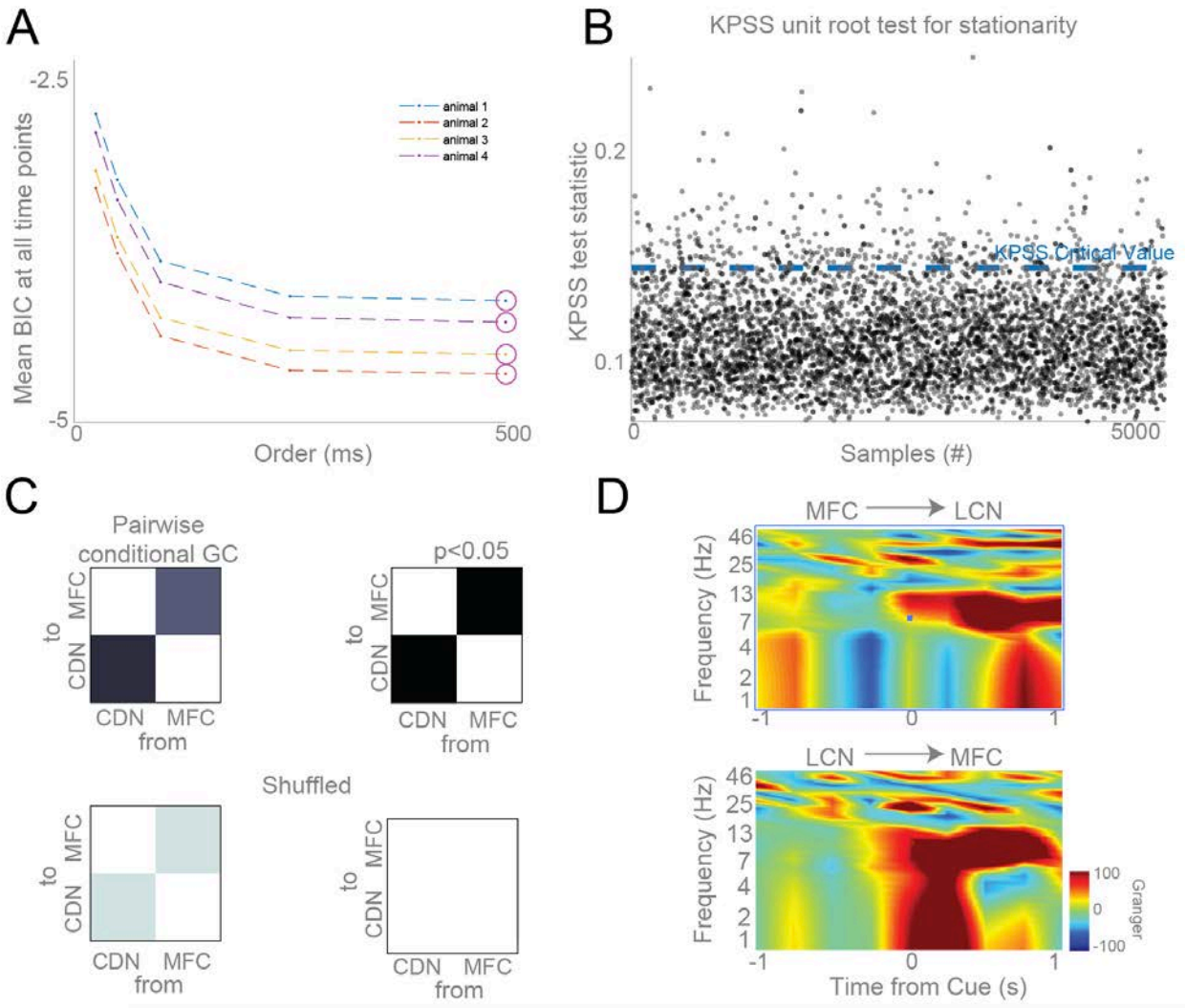

Figure S7: Granger causality analyses. A) Mean Bayesian information criteria values across segments converged to a stable minimum across orders > 100 ms, indicating minimized regression error. We selected a model order of 500ms and 2000ms sliding time segments (6 segments per 12s interval timing trial) for subsequent analysis to maximize the resolution of low frequency Granger prediction. B) Corrected time series segments were submitted to KPSS unit root testing using MVGC Toolbox routines to determine single trial KPSS test statistics to determine stationarity. C) Significant Granger causality during 12s interval timing was assessed using Granger's F-test routines in MVGC Toolbox at p = 0.05. Inputs were F-statistics averaged

across animals and time segments for directed connectivity between MFC and LCN in experimental and time-shuffled data. D) Spectral pairwise Granger prediction indicates that the cerebellar activity is predictive of activity in the medial frontal cortex at the time of trial start [14,15].

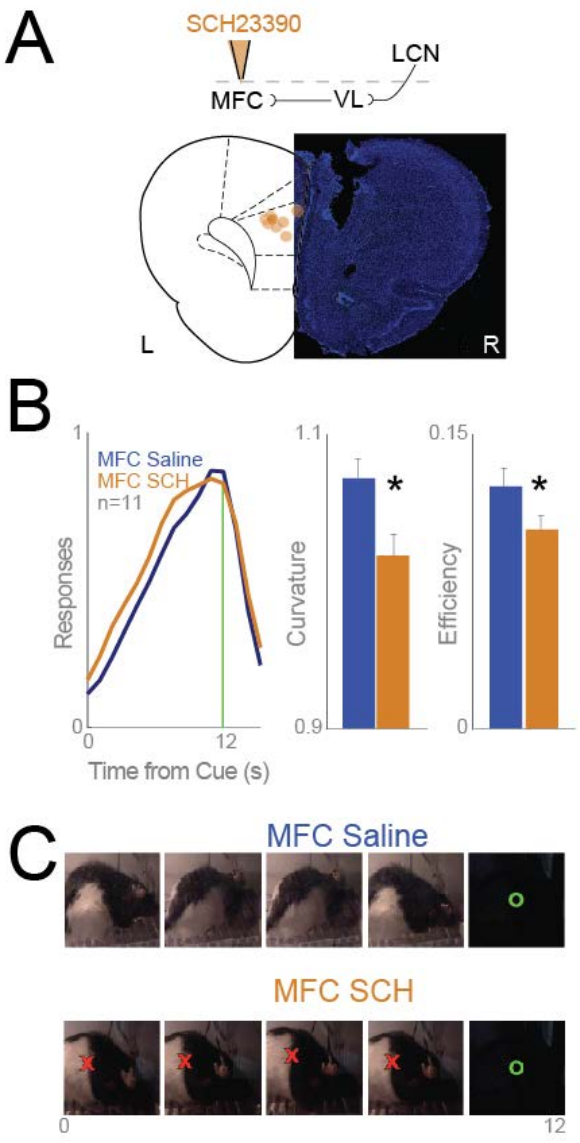

Figure S8: A) We modeled aspects of frontal dysfunction in schizophrenia in rodents by unilaterally infusing the D1DR antagonist SCH23390 (orange) into the left (L). Locations for each animal are represented by light blue dots on lower schematic with a representative brain section at right (blue is DAPI). B) Rodents with unilateral MFC SCH23390 infusions (orange; n=11) had broader estimates of the 12 second interval compared to control sessions, with saline infused into the MFC (blue). Time-response histograms were flatter ($p<0.02$) and animals were less efficient ($p<0.004$) during interval

363 timing with MFC SCH23390. C) MFC SCH23390 did not change other aspects of

364 interval timing performance such as lever pressing or reward acquisition.

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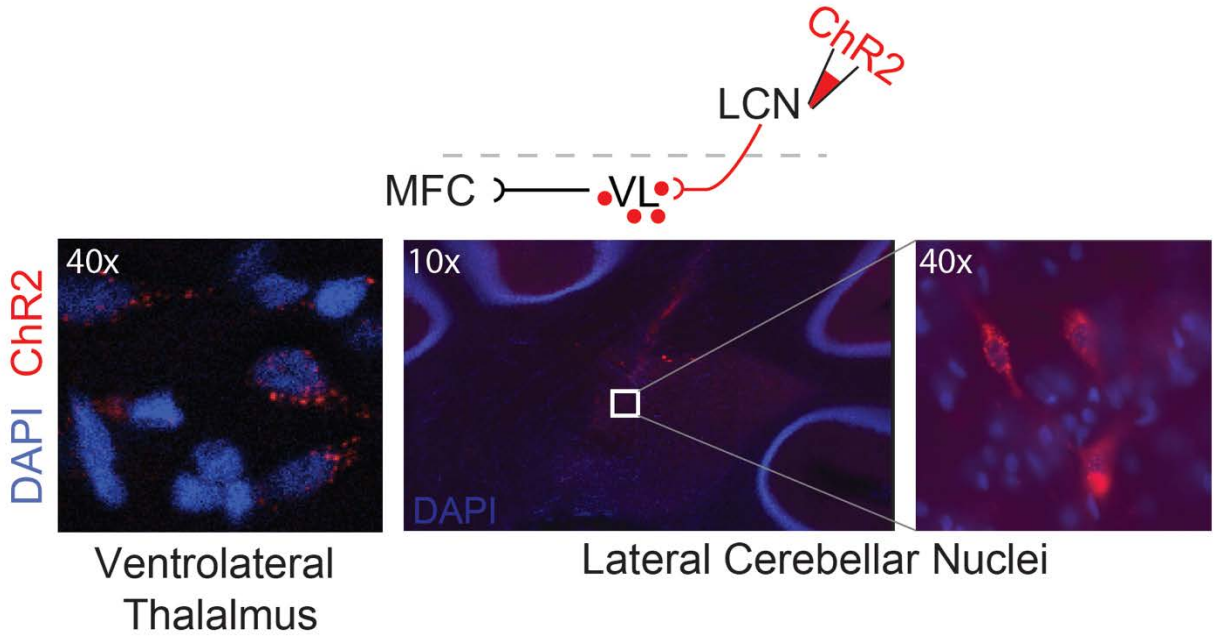

Figure S9: ChR2 infected cells at the injection site in the lateral cerebellar nuclei and in terminal projections in the ventrolateral thalamus. Red is ChR2 and blue is DAPI.

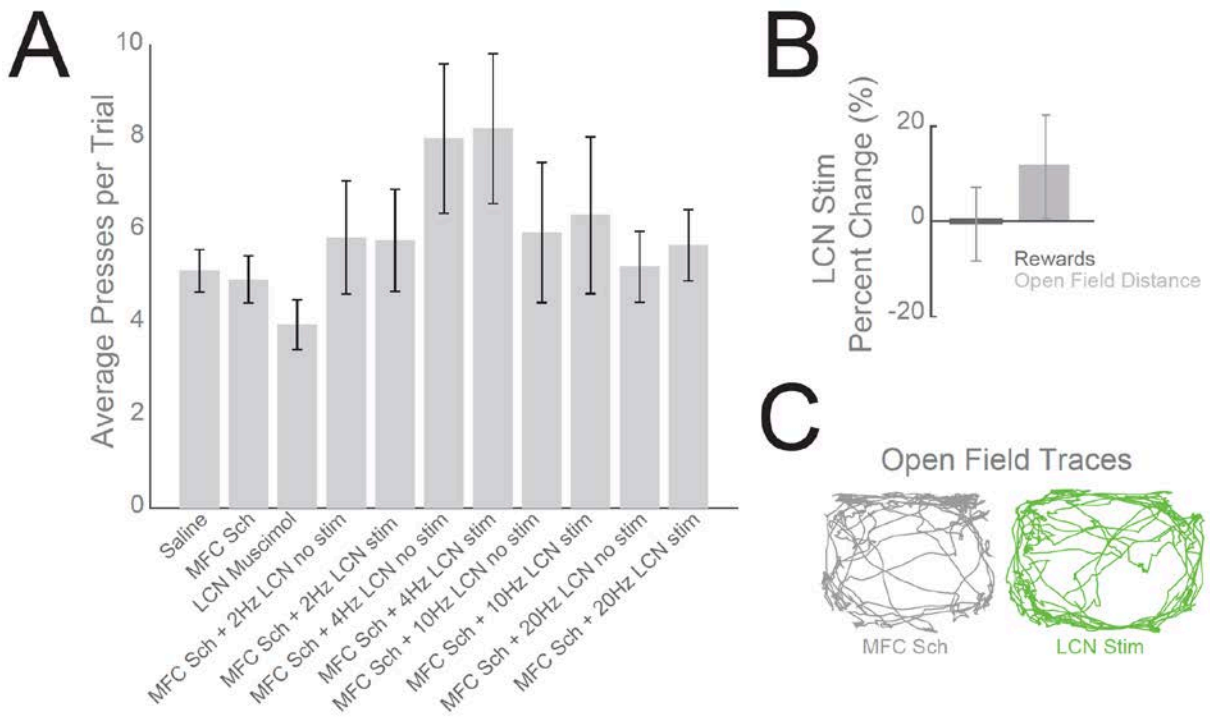

Figure S10: There were no clear motor effects of LCN stimulation on A) the number of presses per trial, B) rewards, or distance traveled in an open field. C) An example of open field distance traveled in an animal with MFC D1 dopamine blockade without (gray) and with cerebellar stimulation (green).

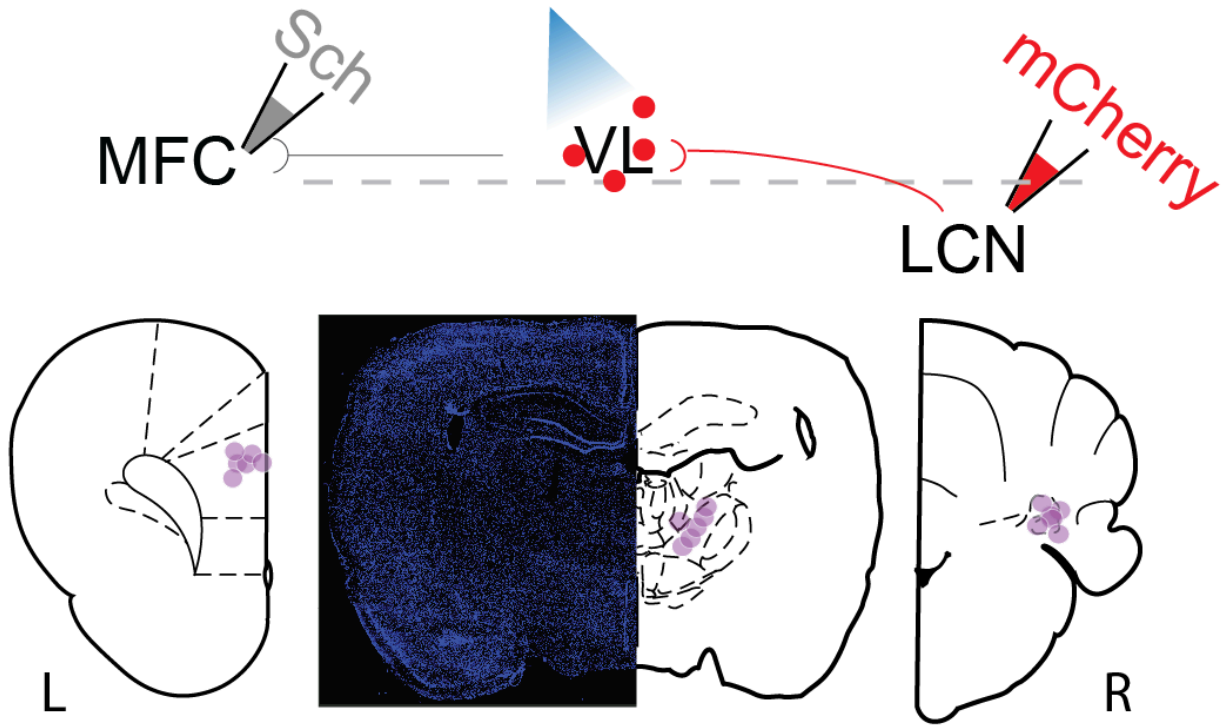

Figure S11: Optogenetic stimulation in 6 control animals did not significantly influence G) presses, H) rewards, I) interval timing efficiency, or J) curvature in time-response histograms.

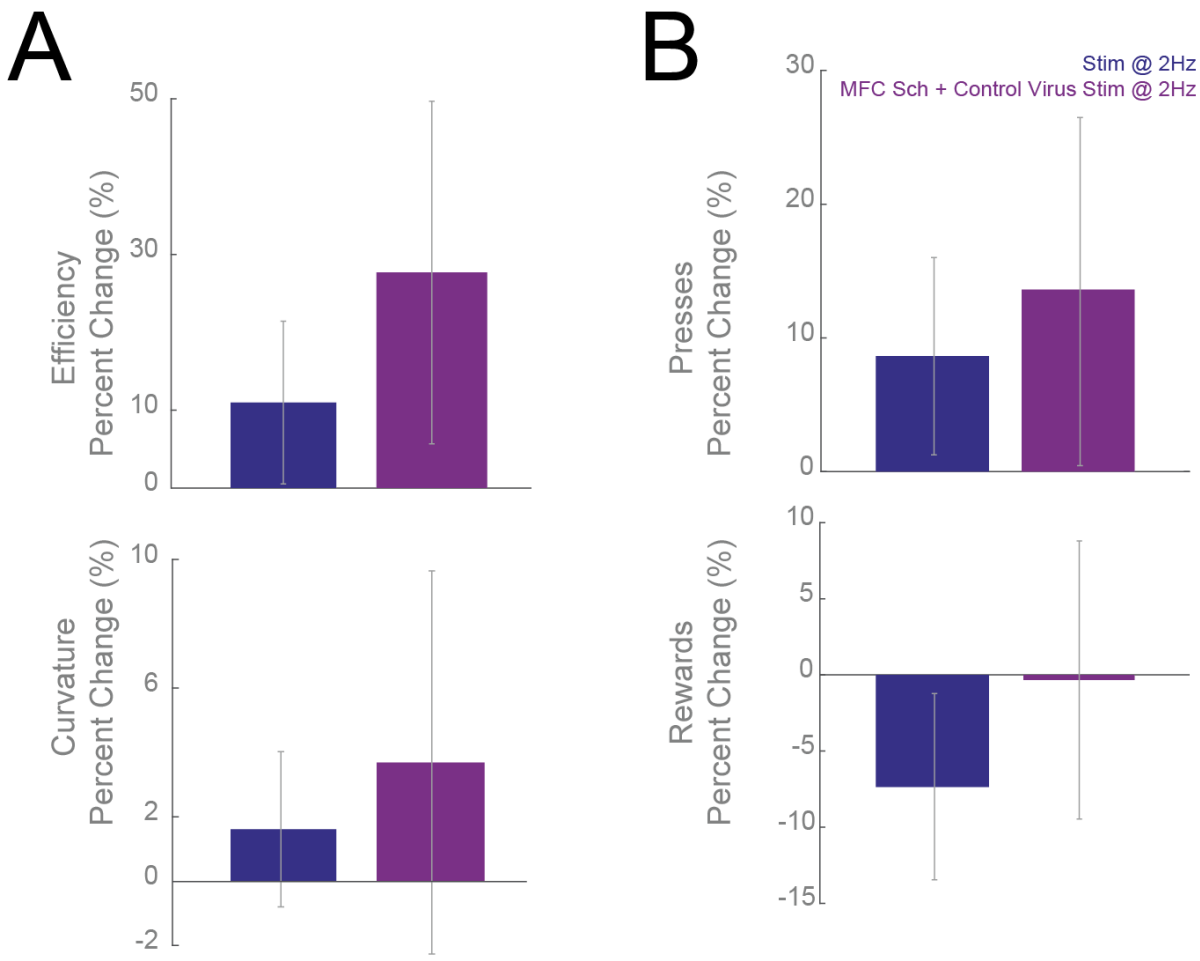

Figure S12: Optogenetic stimulation in 6 control animals did not significantly influence interval timing efficiency, curvature in time-response histograms, presses, or rewards.

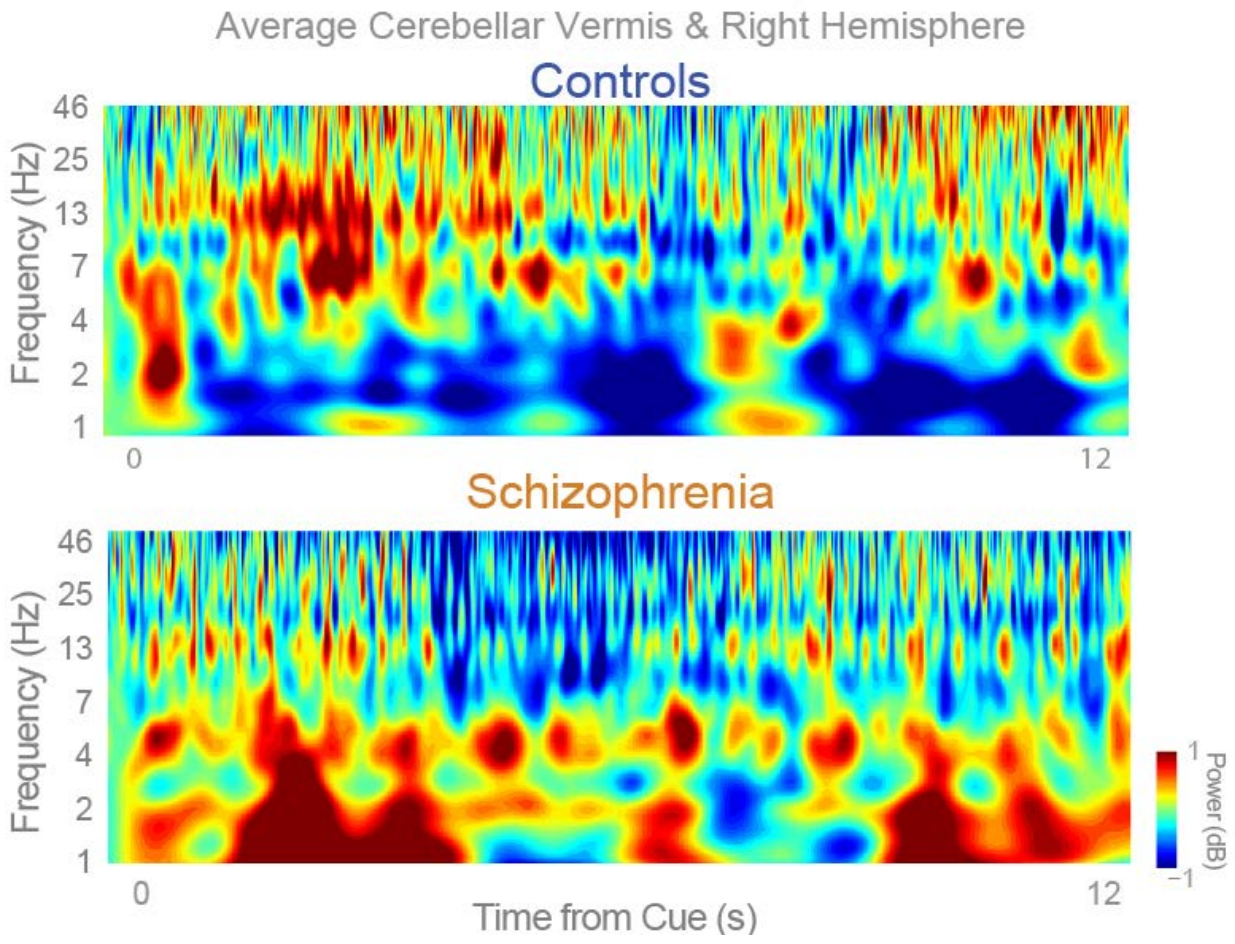

Figure S13: Human EEG activity from cerebellar leads

Supplementary Videos

Video S1: A video of rodent with saline infused in MFC – most lever presses in these conditions are towards the end of the interval.

Video S2: A video of the same rodent in Video S1 with the D1 antagonist SCH23390 infused into the MFC. This animal had a disruption in behavior with many irrelevant responses. See Narayanan et al., 2012; Parker et al., 2013; 2014, and 2015 for more details on this behavior.

Video S3: A video of the same rodent and on the same day on Video S2 on a LCN-VL 2 Hz stimulation trial. This animal appeared to have fewer irrelevant responses and make more responses towards the end of the interval.