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Materials and Methods

Subjects

Male Long-Evans rats weighing 200-225 grams (approximately 6-8 weeks) were obtained from Charles River. Rats were maintained on a standard 12 hour light-dark cycle and given food and water *ad libitum*. Rats were initially housed two per cage. Animals implanted with tetrode microdrives or fixed wire arrays were housed individually after implantation to minimize damage to recording hardware. Animals implanted with only fiber optics or cannulas continued to be housed two per cage after implantation. All procedures conformed to guidelines established by the National Institutes of Health and have been approved by the Stanford Institutional Animal Care and Use Committee.

Automated Forced Swim Test (FST)

A 9-inch diameter tank of water (Tap Plastics, Mountain View, CA) was surrounded by a 10-inch diameter coil constructed from 10 pounds of 26 gauge enamel-coated copper wire (Cal Coil Magnetics, El Monte, CA). A 2g rare-earth magnet (Applied Magnets, Plano, TX) was placed on the rat's back foot with a comfortably snug rubber band. Magnet placement was performed immediately before behavioral testing and was tolerated well by awake rats. During the FST, movement of the magnet within the coil of wire during swimming was found to induce a robust current in the coil. Coil voltage was bandpass filtered between 1 and 300 Hz, digitized at 2 kHz, and recorded for later analysis using a Digital Lynx data acquisition system (Neuralynx, Bozeman, MT). Data was simultaneously collected from a reference coil, and referencing was performed offline to reduce line noise. The same system was used to measure locomotor activity in a familiar cage by placing the induction coil directly underneath the cage; a similar method has been used previously to measure Parkinsonian tremor in rats[1]. Coil data and video data were collected for all experiments. Manual scoring for validation of the automatic method was performed blind to experimental condition and automatically scored immobility/kick frequency. During manual scoring visual observations were taken every 5 seconds, and immobile epochs were defined as either an absence of movement or the minimum necessary movement required to stay afloat.

Tetrode microdrive and fixed wire array placement

For the data shown in Figure 2, rats reached a minimum of 400 g before surgery. For the data shown in Supplementary Figures 6-9, rats were bilaterally injected with virus in the mPFC (described below) at 8-10 weeks, and virus was allowed to express for a minimum of four months before electrode implantation (rats typically reached weights > 400 g). Rats were initially anesthetized with 5% isoflurane. The scalp was shaved and rats were placed in a stereotaxic frame with non-rupturing ear bars. A heating pad was used to prevent hypothermia. Isoflurane was delivered at 1-3% throughout surgery; this level was adjusted to maintain a constant surgical plane. Ophthalmic ointment was used to protect the eyes. Buprenorphine (0.05 mg/kg, subcutaneous) and enrofloxacin (5 mg/kg, subcutaneous) were given before the start of surgery. A mixture of 0.5% lidocaine and 0.25% bupivicaine (100 μL) was injected subdermally along the incision line. The scalp was disinfected with betadine and alcohol. A midline incision exposed the skull, which was thoroughly cleaned. 8-11 skull screws were implanted at the periphery of the exposed skull to ensure stable recordings, a 2-mm craniotomy was drilled over the right mPFC, and the dura mater was carefully resected. A 4-tetrode microdrive (Neuralynx, Bozeman, MT) with tetrodes wound from 13 μm nichrome (Kanthal Palm Coast, Palm Coast, FL) or a 24-electrode fixed wire array with 50 μm stainless steel electrodes (NB Labs, Denison, TX) was implanted over the craniotomy (AP: 2.7 to 3.3 ML: 0.8 DV: 4.0). For the data shown in Supplementary Figures 6-9, a fiber optic targeting the DRN was also implanted at this time (described below). Dental acrylic was used to secure the electrodes and fiber to the skull and screws. The acrylic was shaped to make a thin neck between the skull screws and the electrode interface board in order to facilitate waterproofing. The skin was sutured closed and the rats were given carprofen (5 mg/kg, subcutaneous) and lactated ringer's solution (2.5 mL, subcutaneous) and recovered under a heat lamp. After implantation tetrodes were adjusted daily.

Freely moving neurophysiology

Rats were briefly anesthetized with isoflurane. The headstage and tether (Neuralynx, Bozeman, MT) were connected to the microdrive or fixed wire array and secured with thread to prevent detaching during "wet dog" shakes. To protect the electronics from water damage a latex tube was secured around the headstage attachment point with tightly wound rubber bands. At this time the magnet was attached to the back foot for behavioral readout. The total time under isoflurane anesthesia was limited to less than 10 minutes, and rats were allowed to recover for at least 1 hour prior to the start of recordings. For the recordings in Supplementary Figures 6-9, the fiber optic cable was attached immediately before the FST in order to minimize breakage from twisting during rotation, and light power was checked immediately after recordings to confirm that the fiber optic was intact.

Neural data was acquired with a 64 channel Digital Lynx data acquisition system (Neuralynx, Bozeman, MT). Spiking channels were first referenced to an electrode exhibiting no spiking activity to reduce behavioral noise. The signal was then bandpass filtered between 600 and 6000 Hz and digitized at 32 kHz. Induction coil data and video data were also recorded during all epochs in order to validate the use of the induction coil method for both the FST and familiar cage activity. Data was recorded for a variable number of epochs depending on the experiment. For Figure 2 we recorded 15 minutes of data pre-FST in a familiar cage, 15 minutes during the FST, and 15 minutes in a familiar cage post-FST. For Supplementary Figures 6-9 we recorded 15 minutes pre-FST in a familiar cage, 20 minutes during the FST with stimulation (five two-minute no-stimulation epochs interleaved with five two-minute stimulation epochs), 15 minutes in a familiar cage post-FST, and 20 minutes in a familiar cage post-FST with stimulation (five two-minute no-stimulation epochs interleaved with five two-minute stimulation epochs). Rats were handled gently during transfer between the familiar cage and the swim tank to minimize neural drift. After recording was completed the waterproofing was removed and the rat was placed on a towel under a heat lamp for 10 minutes to dry. Before sacrifice for histology rats were deeply anesthetized and current passed through all electrodes (50 μA for 30 seconds) to make electrolytic lesions for anatomical localization. Data obtained using this method in freely moving rats is shown in Figures 1 and 2, and Supplementary Figures 1-3 and 6-9.

Virus construction and packaging

Recombinant AAV vectors were serotyped with AAV5 coat proteins and packaged by the viral vector core at the University of North Carolina. Viral titers were 2 x 10^{12} particles / mL for AAV5 CaMKIIα::hChR2(H134R)-EYFP, 3 x 10^{12} particles / mL for AAV5 CaMKIIα::EYFP, 4 x 10^{12} particles / mL for AAV5 CaMKIIα::eNpHR3.0-EYFP, 4 x 10^{12} particles / mL for AAV5 hSyn::hChR2(H134R)-EYFP, and 2 x 10^{12} particles / mL for AAV5 hSyn::EYFP. Maps are available online at www.optogenetics.org.

Stereotaxic virus injection and optical fiber implantation

Rats were prepared for surgery and given analgesics and fluids as described above. A midline incision exposed the skull, and craniotomies were made bilaterally above the mPFC. Virus was injected with a 10 μL syringe and a 33 gauge beveled needle with the bevel facing anteriorly at 150 nL/min using an injection pump. Two 1 μL injections were delivered to each hemisphere at AP 2.2 mm, ML 0.5, DV 5.2 and AP 2.2, ML 0.5, DV 4.2 for a total of 4 μL per rat. After each injection the

needle was left in place for 7 minutes and then slowly withdrawn. The skin was sutured closed. For the hSyn::ChR2 DRN rats a similar protocol was followed, but only one injection of 1 μL was delivered to the DRN from the right at an angle of 20 degrees with the bevel facing medially. Coordinates for DRN injections were AP -7.8 mm, ML 0.0, DV: 6.7. In projection-targeting experiments virus was allowed to express for a minimum of 4 months in order to allow time for sufficient opsin accumulation in the axons, while cell body stimulation experiments were conducted at 8 weeks.

At least 10 days before behavioral testing a fiber optic with an external metal ferrule (200 μm diameter, 0.22 NA, Doric Lenses, Québec, Canada) or a 22GA cannula (Plastics One, Roanoke, VA) was implanted over the target structure of interest, as described previously[2]. Coordinates for mPFC implantation were AP 2.7, ML 0.5, DV 3.8. DRN fibers were implanted at a 30º angle from the right to avoid both the central sinus and the cerebral aqueduct, and the coordinates for the tip of the fiber were AP -7.8, ML 0.5, DV 5.9. When cannulas were used in the DRN for the glutamate antagonist experiments they were also implanted at a 30º angle from the right, and the coordinates at the tip were AP: -7.8, ML: 1.0, DV: 4.67. Internal cannulas for drug infusion extended 2 mm past the tip of the cannula (to the center of the DRN), while the inserted fiber extended 1 mm past the tip of the cannula in order to provide illumination to the whole DRN. LHb fiber optics were implanted bilaterally at a 10º angle from midline on each side at AP: -3.6, ML: +/-0.8, DV: 4.6. Rats were prepared for surgery and given analgesics and fluids as described above. A midline incision was made, the skull was thoroughly cleaned, and a craniotomy was made over the target structure of interest. Four to six skull screws were attached, and the fiber optic was lowered. A thin layer of metabond (Parkell, Inc., Edgewood, NY) was used to firmly attach the hardware to the skull, which was followed by a thicker layer of dental acrylic for structural support.

Light delivery

During behavioral testing an external optical fiber (200 μm diameter, 0.22 NA, Doric Lenses, Québec, Canada) was coupled to the implanted fiber optic with a zirconia sleeve. If cannulas were used, as for the glutamate antagonist experiments, a fiber for insertion through the cannula was constructed from the same optical fiber coupled to an internal cannula (Plastics One, Roanoke, VA). An optical commutator allowed for unrestricted rotation (Doric Lenses, Québec, Canada)[3]. Optical stimulation was provided with a 100 mW 473 nm or 594 nm diode pumped solid state laser (OEM Laser Systems, Inc., Salt Lake City, UT) and controlled by a Master-8 stimulus generator (A.M.P.I., Jerusalem, Israel). Light pulses were recorded with a Digital Lynx data acquisition system (Neuralynx, Bozeman, CA) simultaneously with behavioral and neural data. Pulse trains with 5 ms

long 473 nm light pulses at 20 Hz were used for all ChR2 experiments, while continuous 5 mW 594 nm light was used for all eNpHR3.0 experiments. The mPFC cell body stimulation experiments used 3 mW light (24 mW/mm^2 at the fiber tip); higher light power induced seizures. The mPFC-DRN axonal stimulation experiments used 10-20 mW light (79-159 mW/mm^2 at the fiber tip). Greater light power was required during the DRN axonal stimulation experiments because of the lower EYFP fluorescence at this site, a predictor of lower opsin expression. The mPFC-LHb and DRN cell body experiments used 10 mW light (79 mW/mm^2 at the fiber tip).

In Vivo Pharmacology

A glutamate antagonist cocktail of either 22 mM NBQX and 38 mM AP5 or 8 mM NBQX and 13 mM AP5 was used; both concentrations were effective at blocking the stimulation-induced behavioral effect, and therefore the data from both concentrations was pooled. Ten minutes before the onset of behavioral testing, 0.4 µL of this solution was infused through an internal cannula into the center of the DRN. Solution was infused at a rate of 0.1 µL/min through a 28GA internal cannula (Plastics One, Roanoke, VA) extending 2 mm past the end of the implanted cannula. A 10µL NanoFil syringe (WPI, Sarasota, FL) was used in a syringe pump (Harvard Apparatus, Holliston, MA). The internal cannula was left in place for 2 minutes after infusion, and then gently withdrawn and replaced with a fiber optic extending 1 mm past the end of the implanted cannula.

Forced Swim Test

We utilized the Porsolt Forced Swim Test for these experiments[4]. The swim tank was filled with 25°C water to a height of 40 cm. The induction coil was placed around the tank at the level of the feet, and a small magnet was attached to the rat's back foot, as described above. The rats were placed in the FST for 15 minutes on the first day during the light part of the light/dark cycle for pre-exposure. They were then dried with a towel and placed under a heat lamp for 10 minutes to warm before returning to the home cage. Data was collected during a second 15-20 minute test performed 24 hours later. An external fiber optic was suspended above the FST tank and attached to the implanted fiber optic with a zirconia sheath. During experiments with light stimulation, stimulation alternated between on and off in two minute epochs, starting with no stimulation, using the parameters described above for a total of 20 minutes. Induction coil data, video data, and laser pulse time data were collected for all experiments. The FST tank water was changed between each animal.

Open Field Test

An external fiber optic was suspended above the open field and attached to the implanted fiber optic with a zirconia sheath. Rats were placed in the center of a white, dimly lit open field chamber (105 x 105 cm) and allowed to freely explore the environment. Light stimulation alternated between on and off in three minute epochs, starting with no stimulation, for a total of 15 minutes. A video camera was placed directly above the open field, and locomotor activity was detected and analyzed with Viewer[2] software (BiObserve, Fort Lee, NJ). Laser pulse time data was collected and synchronized to behavioral data.

Anesthetized in vivo recordings

To confirm function expression of opsin, simultaneous dual site recording and optical stimulation of the mPFC and the DRN was performed as described previously[3] in anesthetized rats transduced in the mPFC with the AAV5 CaMKIIα::ChR2-EYFP construct. Data obtained with this method are shown in Supplementary Figures 4 and 5. Rats were deeply anesthetized with isoflurane before the start of recording. A midline incision was made and the skin reflected. 2-3 mm diameter craniotomies were made above the mPFC (vertical penetration) and the DRN (30° penetration). A 1 Mohm epoxy-coated tungsten electrode (A-M Systems, Sequim, WA) coupled to a 200 μm 0.37 NA optical fiber (Thorlabs Inc., Newton, NJ) was stereotaxically lowered until a unit was isolated starting at AP 2.7, ML 0.5, DV 3.6 for the mPFC recordings, and AP -7.8, ML 0.5, DV 6.0 (30° penetration) for the DRN recordings. Recorded signals were bandpass filtered between 0.3 and 10 kHz, amplified 10000x (A-M Systems), digitized at 30 kHz (Molecular Devices, Sunnyvale, CA) and recorded with Clampex software (Molecular Devices). Optical stimulation was provided with a 100 mW 473 nm diode pumped solid state laser (OEM Laser Systems, Inc., Salt Lake City, UT). Clampex software was used for both recording neural data and controlling laser output. Light powers between 1 mW (8 mW/mm^2 at the fiber tip) and 20 mW (159 mW/mm^2) were used. At the end of all experiments current was passed through the electrode (50 μA for 30 seconds) to make an electrolytic lesion for anatomical localization.

Histology, immunohistochemistry, and confocal imaging

Rats were deeply anesthetized with Beuthanasia-D and transcardially perfused with ice-cold 4% paraformaldehyde (PFA) in PBS (pH 7.4). Brains were fixed in PFA overnight and then transferred to 30% sucrose in PBS to equilibrate for at least 3 days. 40 μm coronal sections were cut on a freezing microtome and stored in cryoprotectant at 4°C. Sections were washed with PBS and incubated for 30 minutes in PBS++ (PBS with 0.3% Triton X-100 and 3% normal donkey serum (NDS)). Sections were incubated with primary antibody overnight in PBS++ at 4°C. Primary antibodies used were rabbit anti-5-HT 1:1000 (ImmunoStar 20080, Hudson, WI) and rabbit anti-GABA 1:500 (Sigma A2052, St. Louis, MO). Sections were then washed in PBS and incubated with secondary antibody conjugated to Cy3 or Cy5 for three hours at room temperature in PBS++ (1:500, Jackson Laboratories, West Grove, PA). Sections were washed in PBS and incubated in 4′,6-diamidino-2-phenylindole (DAPI, 1:50000) for 10 minutes, then washed again and mounted on slides with PVA-DABCO. Images were acquired using a Leica TCS SP5 confocal scanning laser microscope with a 10X air objective or a 20X or 40X oil immersion objective.

Data Analysis

Spikes were imported into Offline Sorter software (Plexon Inc, Dallas, TX) and sorted offline using waveform features (peak and valley heights) and principal components. Analyses of neural data and behavioral data were done using custom written Matlab (Mathworks, Natick, MA) scripts and the Neuroexplorer data analysis package (Nex Technologies, Littleton, MA). Statistical significance was defined as $p <= 0.05$ for all analyses.

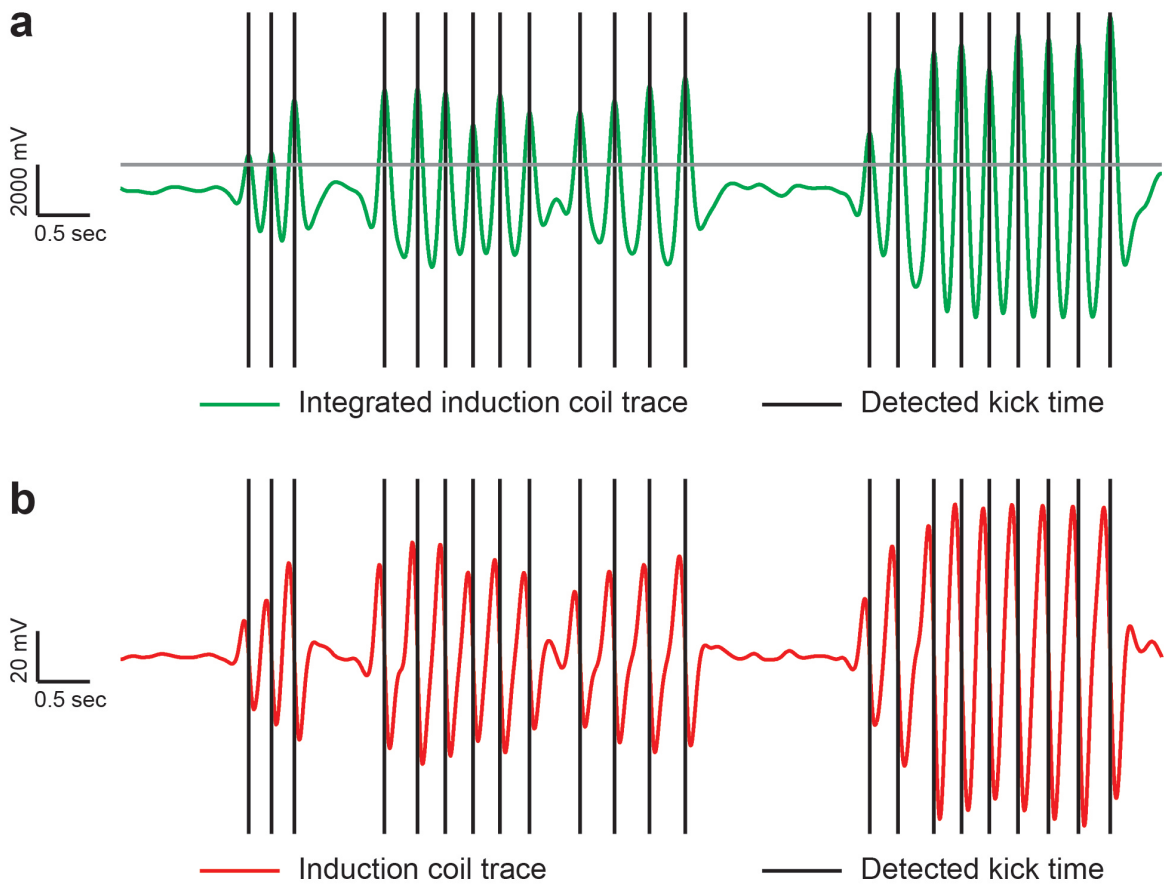
Induction coil data was referenced and zero-phase filtered offline at 1-6 Hz, which preserved the shape of individual kicks while reducing high frequency line noise. The referenced and filtered coil data was then integrated and thresholded (at 10% of the maximum deviation) and the peaks corresponding to individual kicks were detected (Supplementary Figure 1). Kicks made during struggling corresponded to large deflections in the recorded signal and could easily be separated from periods of passive floating. Instantaneous kick frequency was defined as the average number of kicks per second in 10 second bins. Automatically scored immobility was determined by scoring epochs with a gap between kicks greater than one second as immobile. The same analysis was performed on induction coil data collected during familiar cage recordings. In this case, the induction coil data was filtered at 1-20 Hz, thresholded at 4% of the maximum deviation, and was not integrated because the unipolar nature of the waveform. Automatically scored behavioral data was regressed against manually scored behavioral data to determine the correspondence between scoring methods. R-square and F statistics and p values were derived from this regression analysis.

Determination of statistically significant differences in neural firing rate between different behavioral epochs was done using the Mann-Whitney U test. Neurons were first tested for differences in firing rate between the pre-FST epoch and the FST epoch. For this analysis neural and behavioral data was binned in 10-second intervals. Neurons were then tested for differences in firing rate between mobile and immobile states during the FST. For this analysis, mobile and immobile behavioral epochs were divided into two different "continuous" data streams and then statistically tested as above using 10-second bins. The selectivity index used in Figure 2 was defined as the difference in firing rate between conditions divided by the sum. Criteria for identifying putative fast spiking inhibitory neurons were a mean firing rate over 20 Hz and a narrow waveform[5].

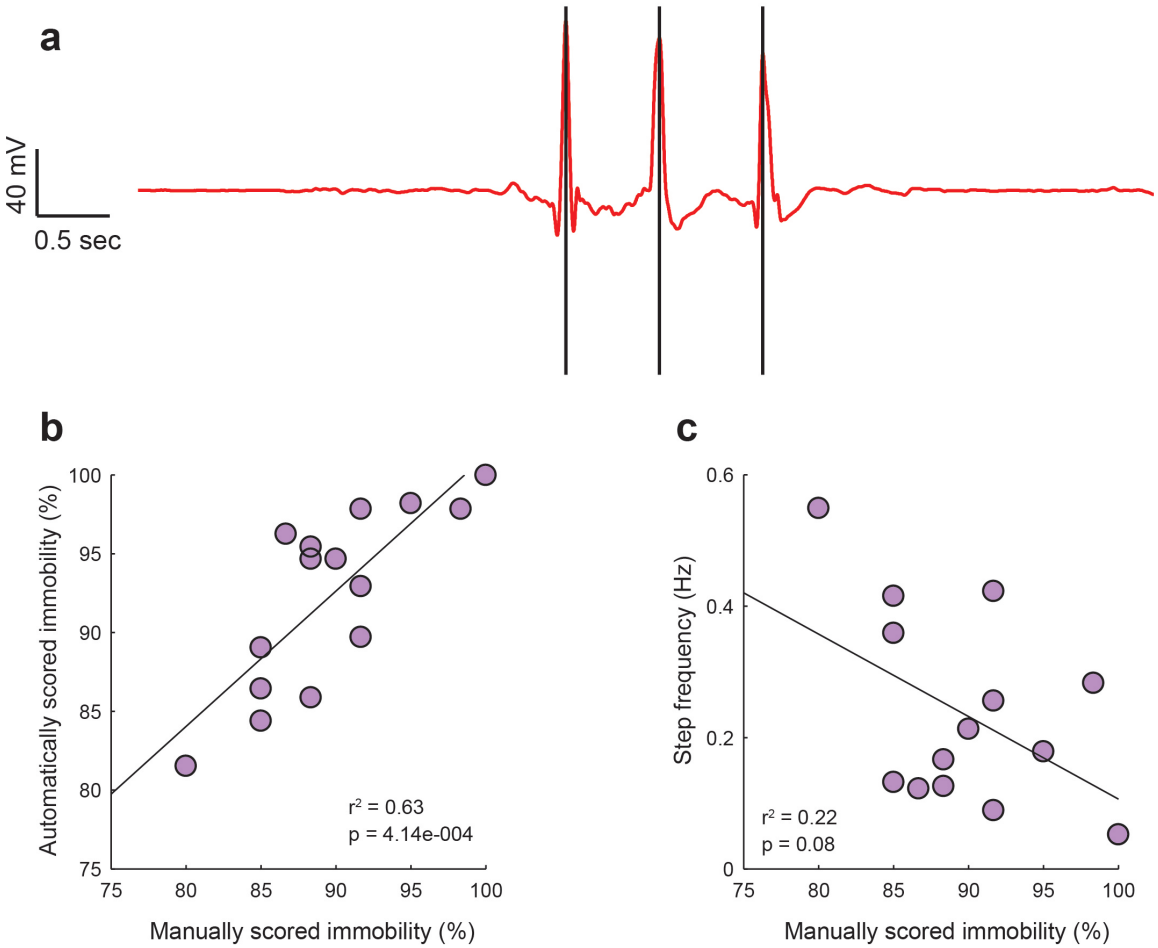
Statistical significance of the behavioral data in Figures 3 and 4 was determined using the Wilcoxon signed-rank test. Data was first exponentially detrended. This was necessary because a decrease in struggling with time upon exposure to the forced swim test environment is well known and has been previously described[67]. Similarly, a decrease in locomotor activity with time upon exposure to the open field test is usually reported[8].

The instantaneous average firing rate depicted in Supplementary Figure 6 was calculated in 10-second bins, and statistical significance for individual neurons was again calculated using the Mann-Whitney U test. The distribution of mobility-immobility differences was tested for changes in variance between stimulated and non-stimulated conditions using the F test for equal variances. Differences in slope were tested with analysis of covariance (ANCOVA).

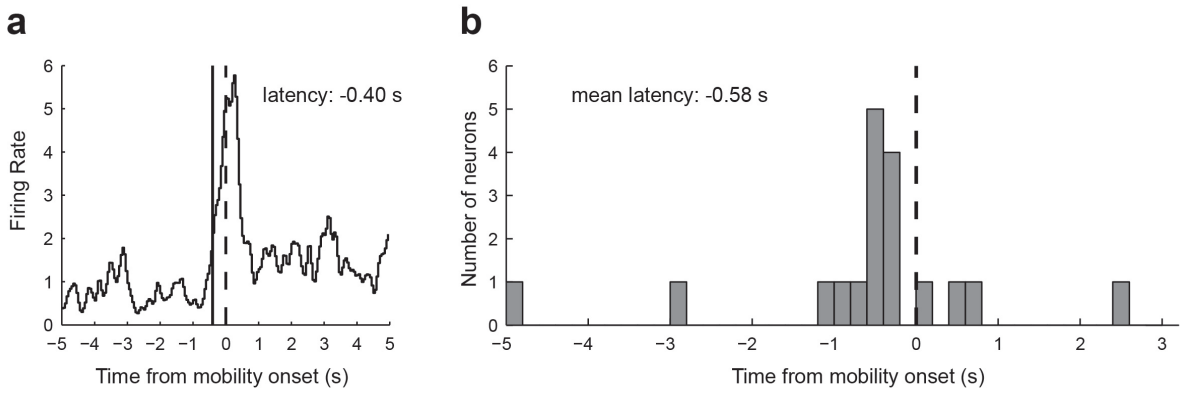
For the latency analysis in Supplementary Figure 3 a "baseline" firing rate was first established for each neuron using the -15 to -5 seconds before the onset of mobility. A PSTH was made from the data in this time period with 50 ms bins, smoothed with a 150 ms gaussian kernel, and the mean and standard deviation were calculated across bins. A second PSTH was then made for the 10 seconds surrounding the onset of mobility (-5 to +5), and also used 50 ms bins and was smoothed with the same kernel. The latency to activation was defined as the first bin of the first sequence during this epoch of at least 10 bins where all firing rates were above the mean plus 2.58 times the standard deviation. Relatively large bins were used for this analysis due to the typically low firing rates of mPFC neurons and the low number of immobile-to-mobile transitions.



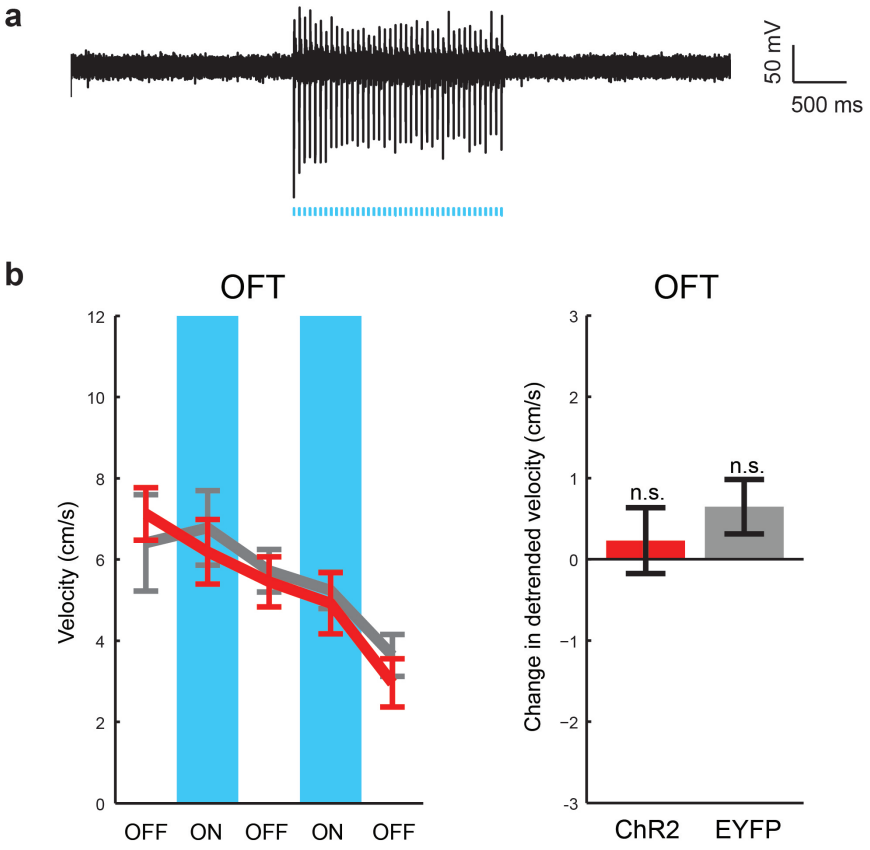
Supplementary Figure 1: Detection of individual kicks in the Forced Swim Test. **a)** The induction coil trace was first zero-phase filtered (1-6 Hz) and then integrated to yield a peak at the midpoint of each kick. The integrated trace was then thresholded (10% of the maximum deviation) and the peaks were detected. The threshold is shown in gray. **b)** The filtered coil trace before integration. Kick times correspond to the midpoint of each kick.



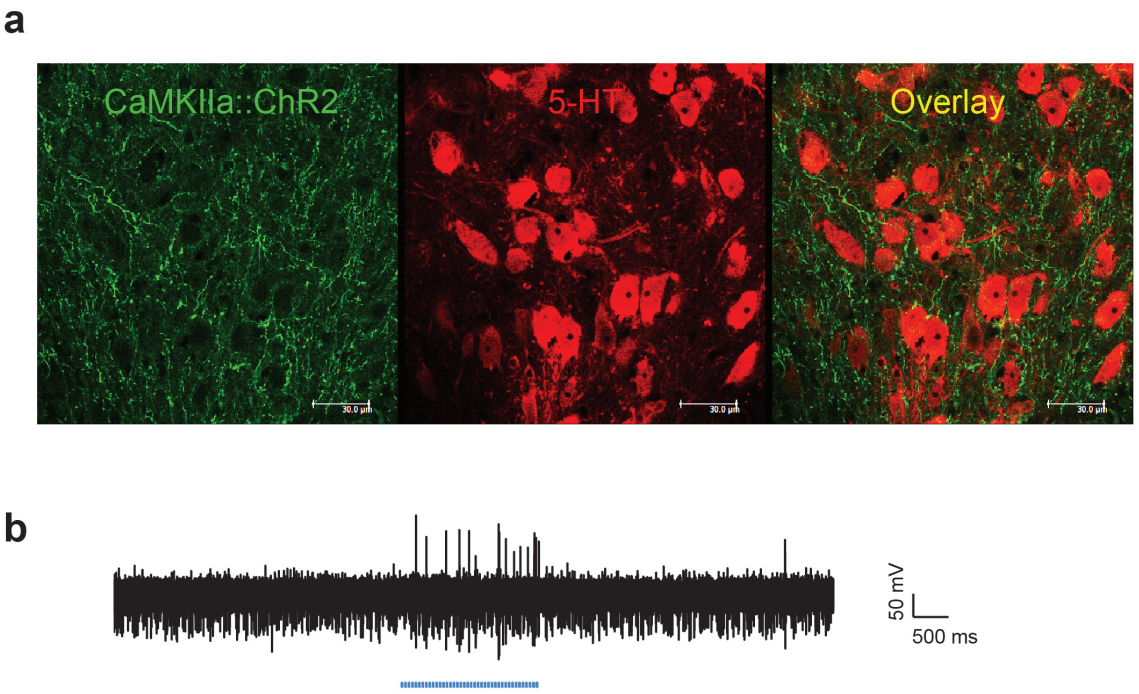
Supplementary Figure 2: The magnetic induction method can be used to detect immobility in a cage. **a)** The induction coil trace was zero-phase filtered (1-20 Hz), thresholded (4% of the maximum deviation), and the peaks were detected. The cage coil trace was not integrated before peak detection because of the unipolar waveform associated with steps. **b)** Automatically scored cage immobility corresponds well to manually scored cage immobility. **c)** Average step frequency corresponds well to manually scored immobility.



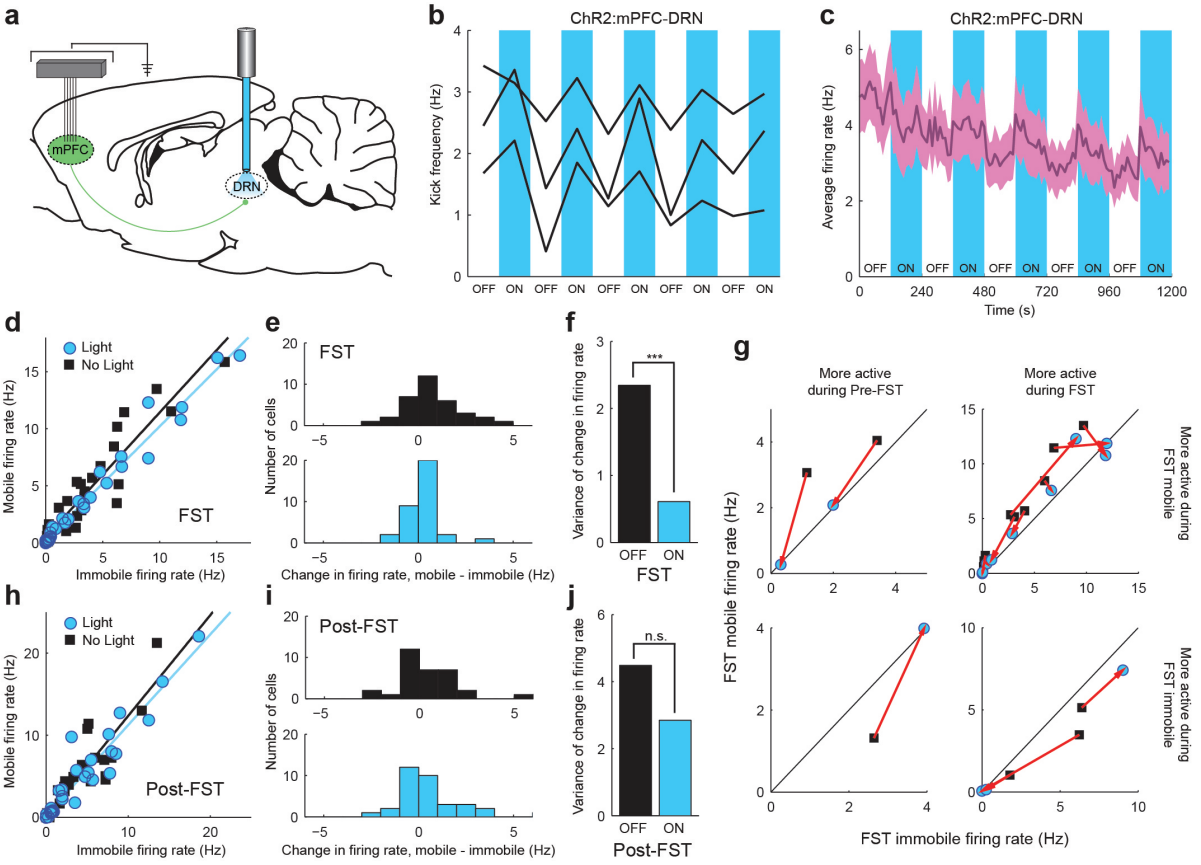
Supplementary Figure 3: mPFC neurons activated during mobile epochs show elevated firing preceding the onset of mobility epochs. a) Peristimulus time histogram (PSTH) for an example mPFC neuron recorded during the FST. The latency to activation (see Supplementary Methods) for this neuron preceded the onset of mobility by 0.4 s. Solid vertical line indicates latency. **b)** Latency distribution for the population of mPFC neurons activated during mobile epochs.



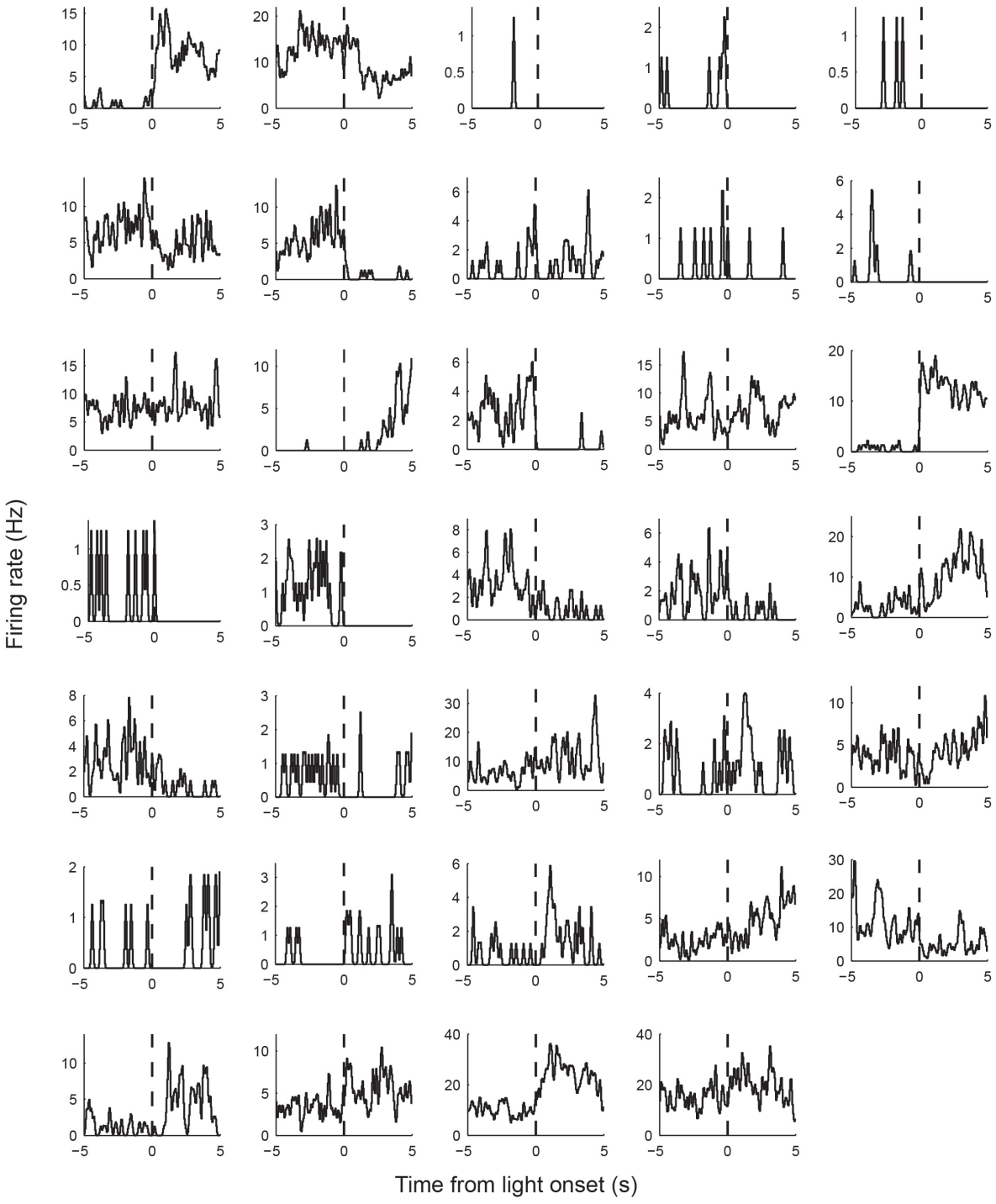
Supplementary Figure 4: mPFC functional ChR2 expression and open field test a) AAV5 CaMKIIα::ChR2-EYFP was infused bilaterally in the mPFC. An optrode recording in an anesthetized rat detected spiking activity in the mPFC induced by local cell body illumination. **b)** Open field test. AAV5 CaMKIIα::ChR2-EYFP or AAV5 CaMKIIα::EYFP was infused bilaterally in the mPFC. Light stimulation of the mPFC did not affect velocity in either ChR2-EYFP rats (Wilcoxon signed-rank test, p=0.50, n=10) or EYFP rats (Wilcoxon signed-rank test, p=0.09, n=8). Red line indicates the ChR2-EYFP group average. Gray line indicates the EYFP group average. Blue bars indicate light on. Significance calculations were performed on detrended data.



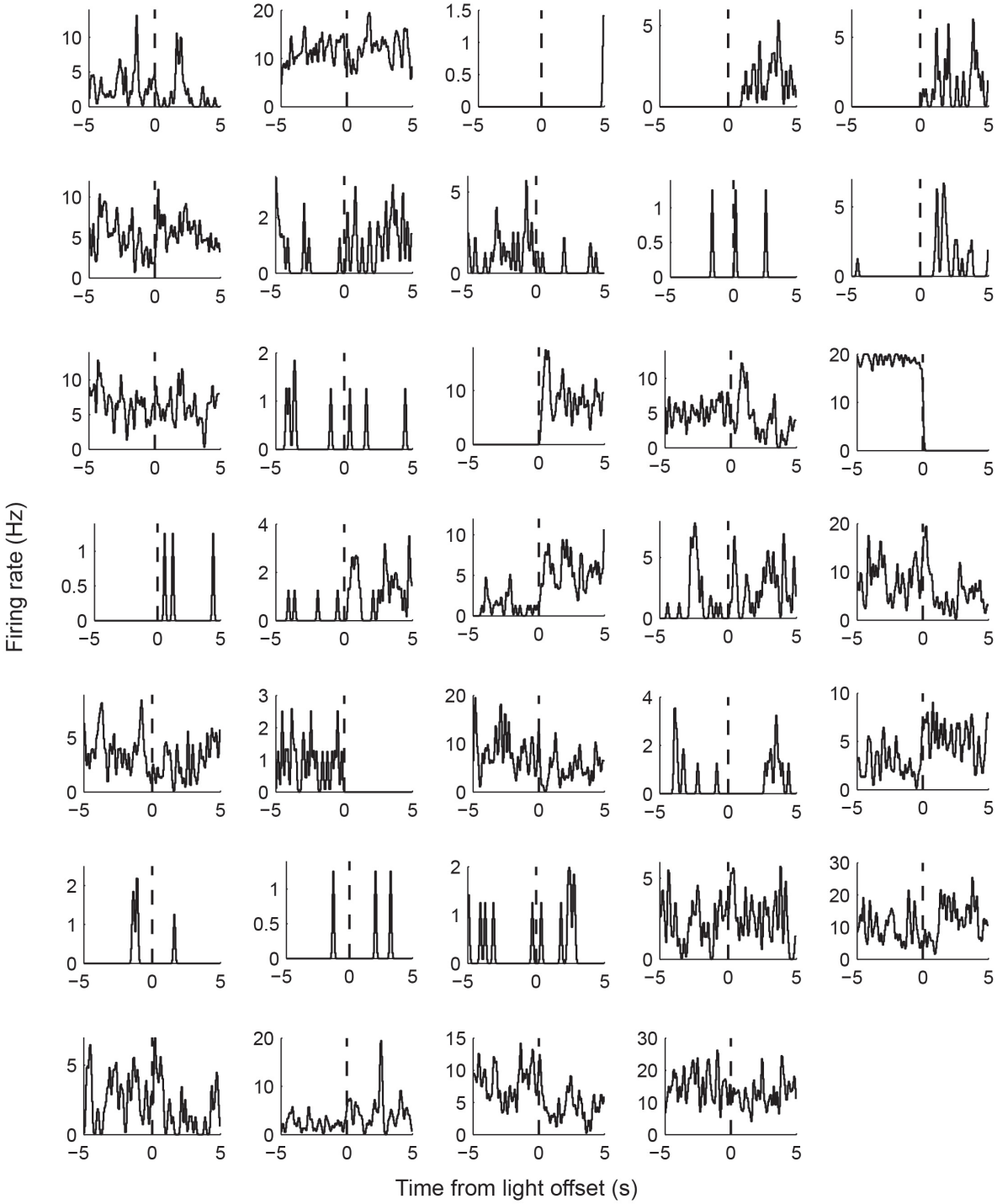
Supplementary Figure 5: DRN histology and optrode recording. **a)** AAV5 CaMKIIα::ChR2-EYFP was infused bilaterally in the mPFC. EYFP fluorescence in mPFC axons in the DRN is shown in green and immunostaining for 5-HT is shown in red. **b)** AAV5 CaMKIIα::ChR2-EYFP was infused bilaterally in the mPFC. To confirm functional opsin expression in DRN-projecting mPFC axons, an anesthetized optrode recording was performed in the DRN. Local spiking activity in the DRN induced by illumination of mPFC axons in the DRN is shown. Spikes were not elicited with every light pulse. 12 overlaid traces are shown.



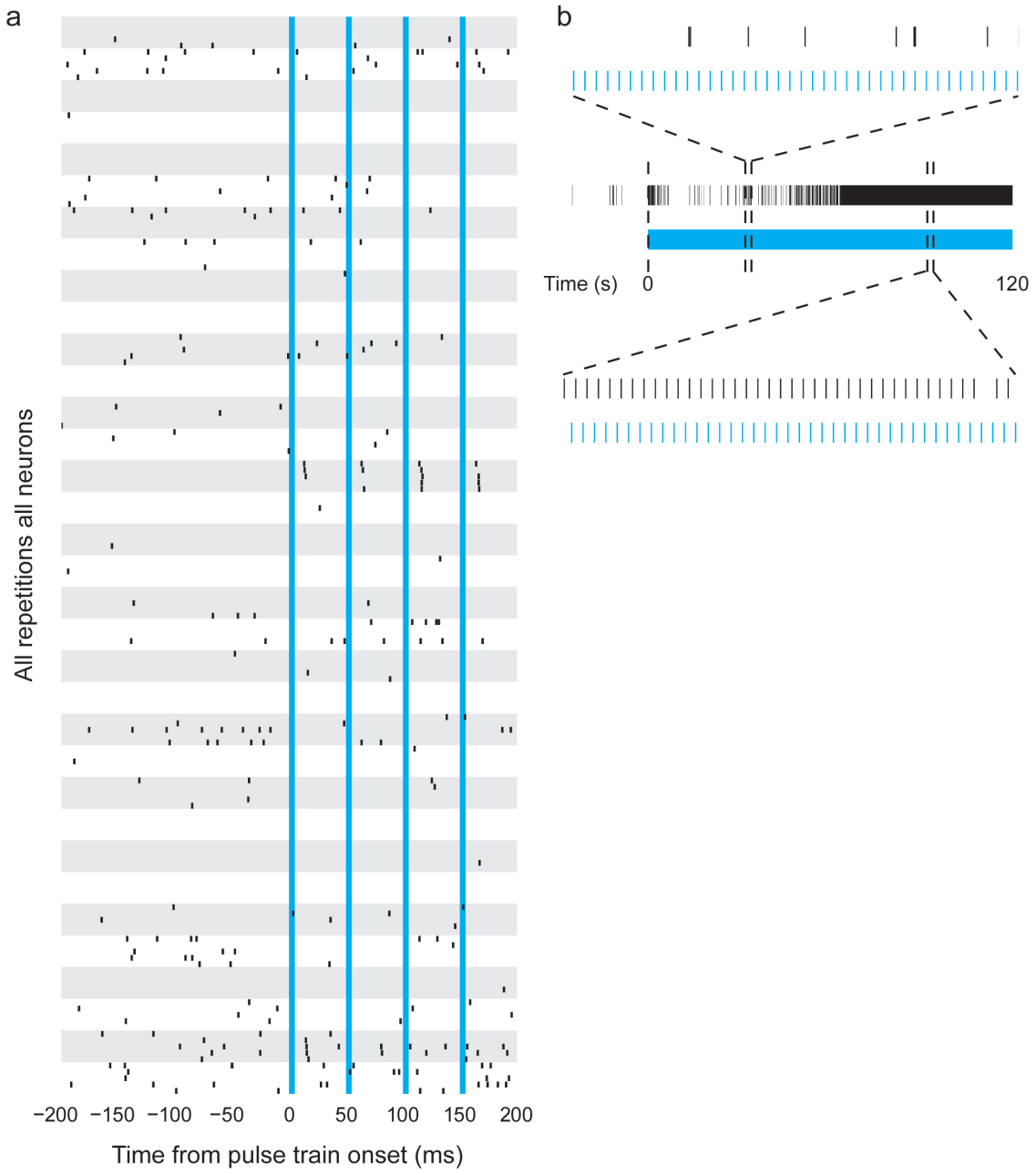
Supplementary Figure 6: Optogenetic stimulation of DRN-projecting mPFC neurons decreases mPFC encoding of mobility. **a)** AAV5 CaMKIIα::ChR2-EYFP was infused bilaterally into the mPFC, and a fiber optic was implanted over the DRN. A 24-electrode fixed-wire array was implanted over the mPFC. **b)** Injected and implanted rats (n=3) showed a robust increase in FST mobility during stimulation. **c)** Light stimulation influenced firing rate in almost all recorded mPFC neurons (31/34, 91%, Mann-Whitney U test). The average firing rate across the population increased slightly during stimulation epochs, although more neurons were significantly inhibited (22/31, 71%) than excited (9/31, 29%) by stimulation. **d)** Illumination of mPFC axons in the DRN decreased mPFC encoding of mobility state during the FST. Each point represents one neuron. Black squares depict mobile vs. immobile firing rate of neurons without light stimulation, while blue circles depict mobile vs. immobile firing rates with light stimulation. Light stimulation causes these points to cluster tightly around the best-fit line. During epochs without light stimulation, 62% (21/34) of neurons were significantly modulated by mobility state, but when these same neurons were tested during light stimulation, the significantly selective proportion fell to 21% (7/34). There was not a significant change in slope with light stimulation (ANCOVA, p=0.20). **e-f)** Histograms of the change in firing rate between mobile and immobile states. Top: no light stimulation. Bottom: light stimulation. Illumination decreases the variance in this distribution (F test for equal variance, p=2.11e-4), indicating decreased encoding of mobility state in these neurons. **g)** All four quadrants of neurons (see Fig. 2g) show a decrease in encoding of mobility state with light stimulation. **h-j)** Stimulation does not have a significant effect on mobility state encoding during the post-FST epoch. Neurons are the same as those shown in Supplementary Figures 7, 8, and 9.



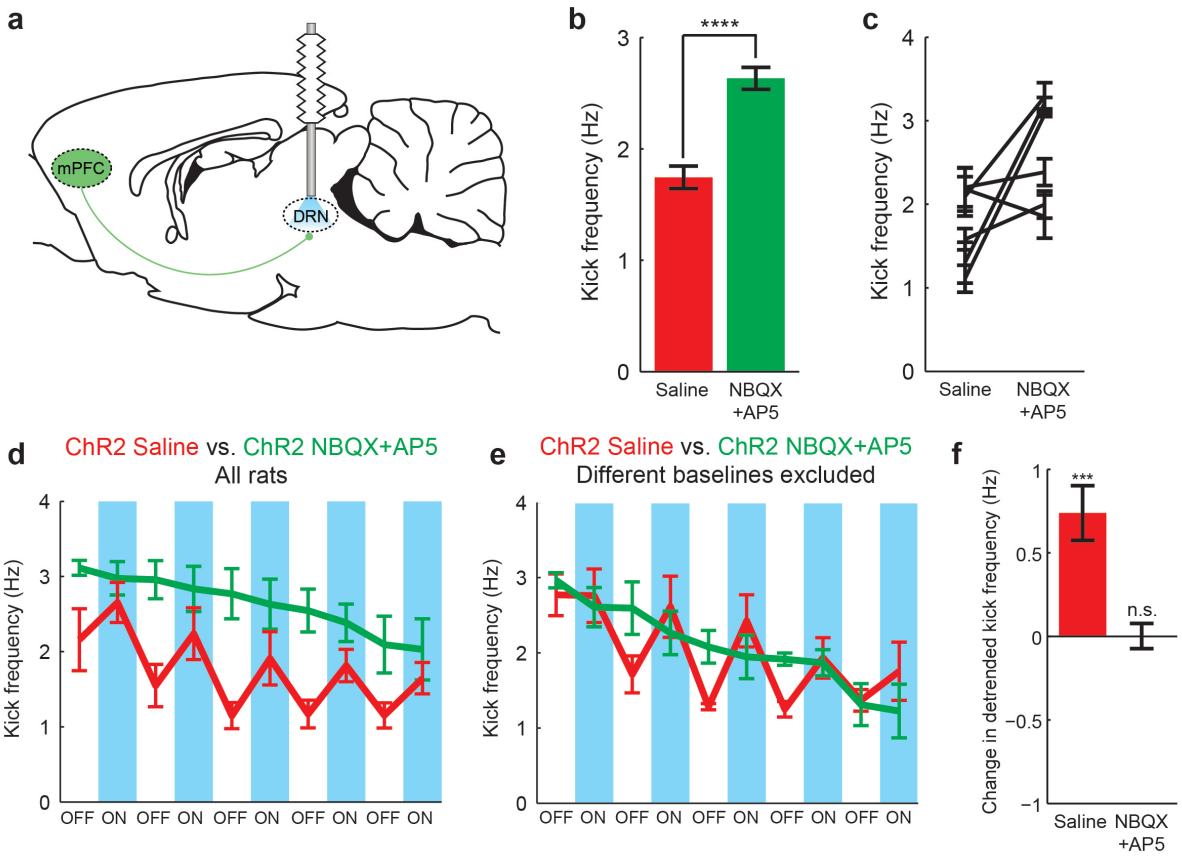
Supplementary Figure 7: Light onset-triggered mPFC PSTHs. Peristimulus time histograms of 34 mPFC neurons recorded during optical stimulation of mPFC axons in the DRN during the FST. Histograms are aligned to the start of two-minute light-on stimulation epochs. Neurons are the same as those shown in Supplementary Figures 6, 8, and 9.



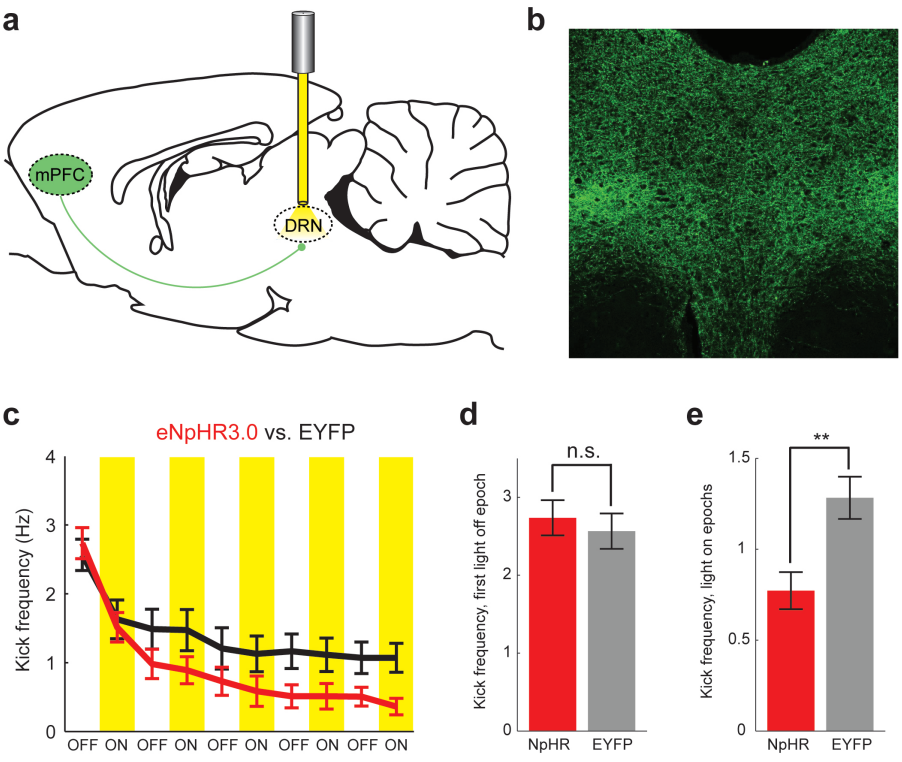
Supplementary Figure 8: Light offset-triggered mPFC PSTHs. Peristimulus time histograms of 34 mPFC neurons recorded during optical stimulation of mPFC axons in the DRN during the FST. Histograms are aligned to the end of two-minute light-on stimulation epochs. Neurons are the same as those shown in Supplementary Figures 6, 7, and 9.



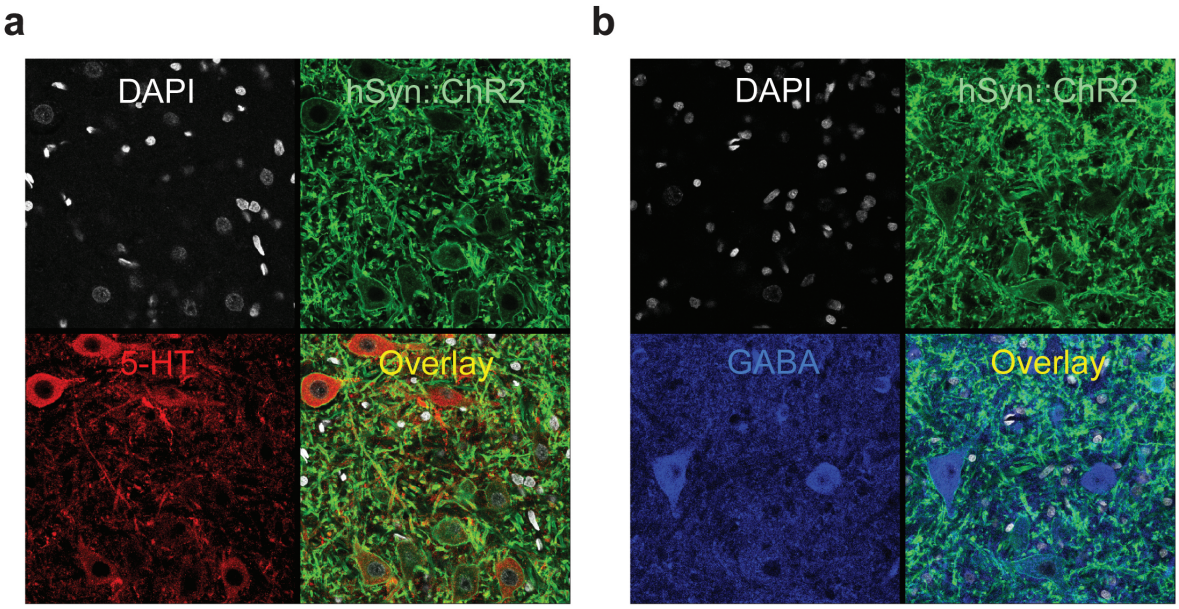
Supplementary Figure 9: Perievent rasters for all mPFC neurons recorded during optical stimulation of the mPFC-DRN projection. a) All spikes for all recorded neurons aligned to the start of stimulation. Each shaded horizontal bar indicates 1 neuron with 5 stimulus onset repetitions. Black ticks indicate spikes; vertical blue bars indicate light pulses. **b)** 33/34 neurons did not exhibit a low-latency, low-jitter response to stimulation as shown in part a, but 1/34 neurons (neuron 15) did. However, stimulation-driven spiking in this neuron was unreliable during the first minute of stimulation, and this neuron did not pass the collision test, ruling out the possibility of direct antidromic activation. Blue ticks indicate light pulses, black ticks indicate action potentials. Neurons are the same as those shown in Supplementary Figures 6, 7, and 8.



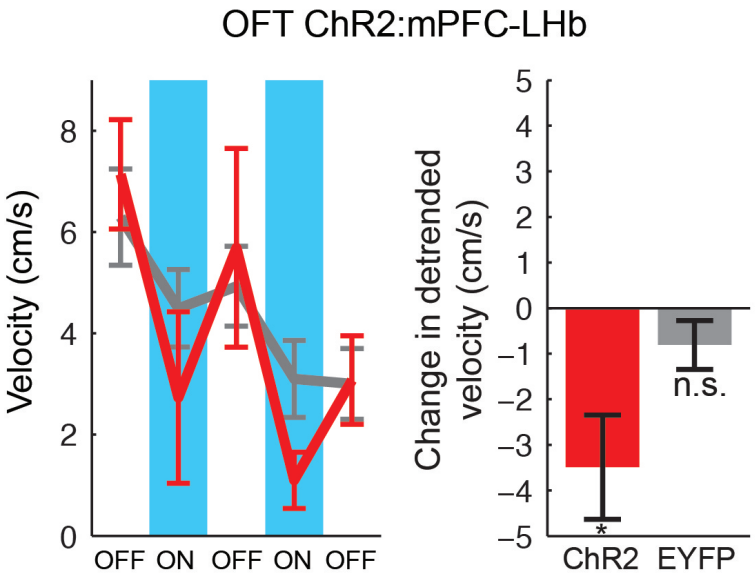
Supplementary Figure 10: Glutamate antagonists in the DRN block the effect of mPFC-DRN stimulation. a) Rats were infused bilaterally in the mPFC with AAV5 CaMKIIα::ChR2-EYFP and implanted with a cannula targeting the DRN. Six rats were tested twice in the FST (counterbalanced), each time receiving either an infusion of saline or a cocktail of the glutamate antagonists NBQX and AP5 10 minutes before testing. **b)** On average, rats receiving intra-DRN NBQX+AP5 were significantly more mobile in the FST (p=3.73e-8, Mann-Whitney U test) than rats receiving saline. **c)** 3/6 rats did not show a within-rat significant (p>0.05, Mann-Whitney U test) change in mobility, and were used for the analysis in panels e and f. Each line represents one rat. **d)** Application of blue light to ChR2-expressing axons in the DRN induced a robust behavioral activation during the FST when saline was infused (red line, n=6); this activation was blocked by infusion of NBQX + AP5 in the DRN (green line, n=6). All rats were included in this panel, therefore the drug-induced increase in mobility is also evident. Blue bars indicate stimulation epochs. **e-f)** When rats with differences in mobility between saline and NBQX+AP5 were excluded, rats infused with saline continued to exhibit behavioral activation upon light exposure (Wilcoxon signed-rank test, p=3.05e-4), and NBQX+AP5 continued to block the stimulation-induced increase in FST mobility (Wilcoxon signed-rank test, p=0.93).



Supplementary Figure 11: Optogenetic inhibition of DRN-projecting mPFC axons. **a)** Rats were infused bilaterally in the mPFC with either AAV5 CaMKIIα::eNpHR3.0-EYFP or AAV5 CaMKIIα::EYFP and implanted with a fiber optic targeting the DRN. **b)** EYFP fluorescence in the DRN. **c)** Forced swim test behavioral data from eNpHR3.0-expressing (n=9, red line) and EYFP-expressing (n=10, black line) rats. Yellow bars indicate light on. Each epoch is 2 minutes. **d)** Kick frequency during the first light-off epoch was not distinguishable between conditions (p=0.55, Mann-Whitney U test). **e)** Consistent with the time course suggested in (c), kick frequency pooled over all light-on epochs or all post-first-light-exposure epochs was significantly different between the eNpHR3.0 and EYFP groups (p=0.0013 and p=0. 7.76e-7 respectively, Mann-Whitney U test).



Supplementary Figure 12: AAV5 hSyn::ChR2-EYFP is expressed in both 5-HT and GABA DRN neurons. a) AAV5 hSyn::ChR2-EYFP was infused in the DRN. EYFP fluorescence in DRN neuronal cell bodies is shown in green, immunostaining for 5-HT is shown in red, and DAPI staining for nuclei is shown in white. **b)** EYFP fluorescence in DRN cell bodies is shown in green, immunostaining for GABA is shown in blue, and DAPI staining for nuclei is shown in white.



Supplementary Figure 13: Stimulation of the mPFC-LHb projection affected locomotor activity in the open field test. AAV5 CaMKIIα::ChR2-EYFP or AAV5 CaMKIIα::EYFP was infused in the mPFC and bilateral fiber optics were implanted over the LHb. Light stimulation of the mPFC-LHb projection significantly affected velocity in ChR2-EYFP rats (Wilcoxon signed-rank test, p=0.039, n=4) but not EYFP rats (Wilcoxon signed-rank test, p=0.157, n=9). Red line indicates the ChR2-EYFP group average. Gray line indicates the EYFP group average. Blue bars indicate light on. Significance calculations were performed on detrended data.

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