Cell

Supplemental Information

Prefrontal Parvalbumin Neurons in Control of Attention

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Supplemental Experimental Procedures

Animals

All procedures were performed in accordance with the Guidelines of the Stockholm municipal committee for animal experiments. Adult (8-10 weeks old at start of any experiment) PV-Cre (Hippenmeyer et al., 2005) male mice were used in all experiments. The animals were housed under 12:12H light:dark cycles. For animals used in the 3-CSRTT the daily amount of food was restricted to 1 g of food per 10 g body weight until the animals reached 85% of their free-feeding weight at what point training in the 3-CSRTT began. The animals were kept on food restriction throughout all sessions in the 3-CSRTT.

Behavior

In the 3-CSRTT the animals allocate attention to report the location of a visual stimulus in order to receive reward. The 3-CSRTT operant chambers (CeNeS, UK) were equipped with one house light on each of the two sidewalls and nine stimulus (cue) presentation nose poke holes with internal light-emitting diodes and an infrared sensor detecting the insertion of the animals' nose on the front wall, of which three were used. The reward port holding the reward magazine and an infrared sensor detecting the insertion of the animals' nose was situated on the rear wall. The animals were habituated to the experimenter and the operant chambers for 7 days before training. The animals were trained in 6 successive training levels defined by specific criteria (**Figure S1B**) and the criteria of each level had to be met for two consecutive days for progression to the next level. Every animal was trained in all four operant 3-

CSRTT chambers, one chamber per session and one session per day. A session was terminated after 100 trials or after 60 minutes if not 100 trials were executed.

Each trial was initiated and started by the animal's behavior (see further below). In order to increase the attentional load (Robbins, 2002) the visual cue was presented pseudorandomly 3, 4 or 5 seconds after trial start (stimulus onset asynchrony). The pseudorandom delay (delay; time from trial start to cue onset, often referred to as the inter trial interval (ITI) in 5-CSRTT studies) was introduced in training level 5 and used in all trials with recordings or optogenetics. A nose poke into a cue presentation hole within 5 s after cue onset (limited hold) defined the behavioral response (correct; Movie S1 or incorrect; Movie S3) and the reaction time (RT; time from cue onset to first registered nose poke, i.e. the response latency). Correct report of the cue location resulted in immediate access to reward (15% sucrose solution) in the reward port. Time from cue report to first pushing of the reward port in a correct trial defined the reward latency (RL). Failure to report the cue location within the limited hold (5 s for fully trained animal used in recordings and optogenetics) was scored as an omitted trial (omission; Movie S4), and a nose poke response before cue presentation as a premature response (Movie S2). Incorrect, premature and omitted responses resulted in a 5 second illuminated timeout. During this time a new trial could not be initiated.

After correct report of cue location the animal rapidly turns around, pushes the reward port with the nose and consumes reward (**Movie S1**). In the commonly used 5-CSRTT protocol (Bari et al., 2008) a nose poke into the reward port also leads to initiation of a new trial. In essence, reward port nose poke starts the delay and automatically triggers onset of the cue after a defined time interval (often 5 s). In a correct trial the animal thus must consume reward, turn around and successfully

allocate sufficient attention to the cue presentation holes within 5 s in order to be able to correctly report the cue location. In an error trial the animal awaits the termination of the illuminated 5 s timeout (**Movie S2-S4**), pushes the reward port to initiate a new trial and turns to the cue presentation holes to attend to the upcoming cue. In summary, in the commonly used protocol pushing of the reward port results in temporal differences in the behavior during the delay depending on if the previous trial was rewarded or not. To circumvent these discrepancies and to streamline the behavior during the delay (i.e. assure that the behavior and neural activity recorded during the delay reflects attentional processing in all trials), we equipped the chambers with an infrared photobeam running between the sidewalls in front of the reward port (**Figure S1A**). Approaching of the reward port broke the beam and while the pushing of the reward port defined trial initiation after both correct and error trials, reward port pushing did not start of the delay. Instead, the delay was not started until the animal had left the reward port and turned to face the cue presentation holes, an event defined by release of the beam break (**Movie S1-S4**).

All sessions were videotaped and reviewed for elimination of trials where the animals did not behave stereotypically, i.e. trials where the animal unexpectedly did not attend to the cue presentation holes. These trials were not used in any type of analysis.

Microdrive construction and implantation

For chronic *in vivo* electrophysiology we employed the flexDrive (Voigts et al., 2013), a small size (~2 cm height, ~1.5 cm diameter) and low weight (~2 g) system which allows for independent day-to-day positioning of tetrodes for large-scale and long-term recordings of single neurons in the brain. Tetrodes consisted of four twisted fine

wires (polyimide insulated ni-chrome wire, 12 μm, Sandvik-Kanthal) that were gold-plated to reduce the impedance to 0.2-0.4 MΩ at 1 kHz. Four movable tetrodes were loaded into medical-grade polyimide carrier tubes (0.005 inch OD, Phelps Dodge) in the microdrive.

The animals were anaesthetized with isoflurane (2%) in O_2 and the body temperature maintained at 37° with a temperature controller system. The animals were fixed in a stereotaxic frame and a hole was drilled through the skull (1.76 mm anterior to Bregma, and 0.25 mm lateral to midline). The microdrive was positioned above the craniotomy and gradually lowered to the PL area (1.25 mm ventral to brain surface). Four miniature anchoring screws were used to attach the microdrive to the skull (two on the anterior and two on the posterior part of the scull). Two Teflon-coated stainless steel wires (0.005 inch bare, A-M systems) from the electrode interface board (EIB) of the microdrive were connected to the screws for grounding. The microdrive was secured onto the skull using dental adhesive cement (Super Bond C&B, Sun Medical). The animals were injected with analgesic (Buprenorphine 0.1 mg/kg s.c.) at the end of surgery and thereafter single-housed.

Chronic electrophysiological recordings during 3-CSRTT

3 fully trained PV-Cre mice were implanted with a flexDrive (Voigts et al., 2013) (1 animal in left hemisphere, and 2 animals in right hemisphere, for details see *Microdrive construction and implantation*). The animals were allowed to recover and acclimate to the microdrive for 7 days and thereafter re-trained in the 3-CSRTT for 7-14 days to assure continued performance at the target criteria.

The neural activity was recorded in a total of 54 3-CSRTT sessions using a Digital Lynx 4SX acquisition system and the Cheetah data acquisition software

(Neuralynx). 3,857 trials were recorded (64-100 trials / session). The tetrodes were individually lowered 20-40 μm after every recording session. Unit signals were amplified with the gain of 10,000, filtered with bandwidth 600-6,000 Hz, digitized at 32 kHz, and stored on a PC. Local field potentials (LFPs) were acquired from one electrode of each tetrode at a sampling rate of 32 kHz. The signal was band-pass filtered between 0.1 and 500 Hz. The last recording day the final position of each tetrode was marked with electrolytic lesion and the animals were thereafter perfused. The tetrode tracks were reconstructed using histological analysis.

Data analysis. All data analysis was conducted using custom software written in MATLAB (Math Works).

Unit sorting

Single units were manually sorted and identified by various spike waveform features (energy, peak, area, spike width, principal components, and fast Fourier transform) using MClust offline sorter (A.D. Redish). Only well-isolated units (Schmitzer-Torbert et al., 2005) with isolation distance > 15, L-ratio < 0.2, and the spikes < 0.01% at ISI < 2 ms were included in the data analysis.

Unit classification

The units were first classified into wide-spiking (WS) putative pyramidal neurons and narrow-spiking (NS) putative interneurons based on the distribution of (1) the peak-to-valley ratio (the ratio between the amplitude of the initial peak (*a*) and the following trough (*b*)) and (2) the half-valley width (*c*) of each spike waveform. For objective classification of units, a Gaussian mixture model (GMM) was fit to the units

(Stark et al., 2013). Units with low classification confidence ($P > 0.05$) were not classified.

To identify putative FS-PV interneurons, the NS population was further classified based on firing rate (data from all trials). NS neurons with a peak-to-valley ratio < 1.1 and a mean firing rate > 10 Hz were classified as FS-PV neurons. NS neurons with peak-to-valley ratio > 1.1 and mean firing rate ≤ 10 Hz were classified as NS1 neurons and the remaining NS neurons as NS2 or NS3 based on distinct clustering.

To evaluate the similarity between optically tagged FS-PV neurons and FS-PV neurons recorded during 3-CSRTT we compared **a,** spike shapes and **b,** cluster distance between the opto-tagged FS-PV neurons and the four classes of NS neurons (FS-PV, NS1-3). Normalized cross-correlation (*r*) values between spike waveforms were calculated to quantify waveform similarity (Jackson and Fetz, 2007). The value of 1 indicates identical spike waveforms. For Mahalanobis distances a lower value indicates higher similarity.

Optogenetic identification of FS-PV interneurons (opto-tagging) during active behavior

For *in vivo* optical tagging of FS-PV neurons 4 PV-Cre mice were injected with AAV DIO ChR2-mCherry (Cardin et al., 2009, 2010) (for details see *Viral injections*) and implanted with the flexDrive (Voigts et al., 2013) (2 animals in left hemisphere, and 2 animals in right hemisphere). The microdrives were equipped with a 200 μm multimode optical fiber (numeric aperture (NA) 0.22, Thorlabs) and 4 movable tetrode wires were positioned in carrier tubes in a circular pattern around the optical fiber. Electrophysiological recordings from the PL/IL were initiated 2-4 w after viral

injection and the tetrodes were lowered individually 20-40 μm after every recording session (for details see *Microdrive construction and implantation* and *Chronic electrophysiological recordings during 3-CSRTT*). Light (5-10 mW at the tip of the fiber, 3-5 ms light pulses, 10-90 Hz) was delivered from a DPSS blue laser (Cobolt MLD^{TM} 473 nm, Cobolt) controlled by custom software written in LabVIEW.

We recorded a total of 252 single units from PL and IL during active behavior (a total of 46 sessions from 4 animals). To identify directly light-activated FS-PV interneurons we employed an automated, unsupervised optical-tagging test (Stimulus-Associated spike Latency Test, SALT) (Kvitsiani et al., 2013). Using SALT it is possible to statistically determinate whether the timing of spikes is significantly changed in relation to the onset of optogenetic activation for identification of directly activated neurons displaying short latency spikes with low jitter. To ensure that the spike sorting was not compromised by light-application, the waveforms of light evoked and spontaneous spike were compared using Pearson's correlation coefficient. Using these measures (SALT P-value < 0.01, Pearson's correlation coefficient $r > 0.9$) 12 units were identified as FS-PV interneurons (half-valley width 248 ± 21 μs, mean firing rate 22 ± 8 Hz) and used for comparison to FS-PV neurons recorded during 3-CSRTT.

Temporal dynamics of neuronal activity during attention

Peri-event time histograms (PETHs) of the firing rate of each FS-PV and WS unit were calculated by a 500 ms sliding window in 100 ms steps with the relevant task events (trial start and cue onset) at time 0 across all trials. To examine population activity PETHs for each unit were normalized in z-score and averaged across different trials (correct, incorrect, and omission). To estimate the probability of spike

occurrence over time, we computed a mean spike density function (SDF) using a Gaussian kernel of 100 ms.

WS clustering

For WS cell sub-classification, we first calculated PCA (principal component analysis) scores using the singular value decomposition of the z-scores of firing rate during the delay of correct trials. Unsupervised hierarchical clustering (Ward's method) was thereafter performed using the first three PCs of the z-scores. Based on the clustering, WS cells were separated into 2 sub-populations, one with increased and one with decreased firing during attention.

Attentional modulation index (AMI)

To quantify how the FS-PV and WS neurons were modulated by attention we computed the AMI (Treue and Maunsell, 1999) by comparing for each neuron the responses 1 s (-1 to 0 s) before cue onset for correct and error trials (FRcorr-FRerror)/(FRcorr+FRerror). AMI ranges from -1 to 1. Positive values represent enhanced spiking in correct trials, negative values enhanced spiking in error trials. The value is zero if there is no modulation.

Cross-correlation analysis

To identify inhibitory putative monosynaptic connections and short-latency interactions between FS-PV and WS cells, we calculated cross-correlations of spike trains for pairs of simultaneously recorded neurons across correct and error trials using a bin size of 1 ms. Only units recorded from different tetrodes were included in the analysis to avoid artificial troughs at time 0. The cross-correlation values were

normalized by dividing by the number of spikes of the reference cell. We used a jittering method to estimate the expected cross-correlogram with 95% confidence interval (Fujisawa et al., 2008). For each cell pair the spikes of one spike train were jittered 1,000 times randomly in the [-5, 5] ms interval. Significant troughs within 10 ms of the center bin were considered to be due to inhibition only when two or more bins were below the 95% confidence interval. For averages of FS-PV--WS pairs, cross-correlograms were standardized by subtracting the mean and dividing by the standard deviation of the jittered cross-correlograms.

Relationship between FS-PV activity and the reaction time (RT)

For investigation of the correlation between the FS-PV activity during attention and the reaction time we compared the firing rate of all FS-PV neurons 1 s (-1 to 0 s, 1 s epoch) before cue onset to the response latency in all correct trials (response latency = time from cue onset to nose poke response, i.e. the reaction time). To remove a potential confounding effect of time, residual values were obtained by subtracting the mean firing rate and reaction time from each value on a trial-by-trial basis (Janssen and Shadlen, 2005). We then calculated the correlation coefficient (r) using these residual values for each cell. Fast and slow RTs: RTs faster than the median reaction time were defined as fast RTs and RT slower than the median as slow RTs (Hayden et al., 2009).

Spectral analysis

Local field potential (LFP) signals were processed in Matlab. The signals were down-sampled to 1 kHz and LFP power spectrum was computed using the Chronux toolbox (*mtspectrumc*). LFP power spectrograms (20-100 Hz) were constructed using the

continuous wavelet transform (complex Morlet wavelet, *cmor1.5-1* in the Matlab wavelet toolbox) and normalized by the peak power. To investigate the relative power of the LFP during attentional processing (-2 to 0 s before cue onset) and cue period (0 to 1 s after cue onset) the power within the band of interest (5-10, 12-24, 30-40 and 60-80 Hz) was divided by the total power of frequencies in the 1-100 Hz range and averaged across trials.

Phase locking analysis

To investigate the relationship between single-unit activity and local field potentials during attentional processing we performed spike-LFP phase locking analysis based on the behavioral outcome (correct, incorrect, omission) the last 2 s of the delay (-2 to 0 s before cue onset). Only neurons with ≥ 50 spikes during the period analysed were used for phase locking estimation. First, the LFP signal (recorded from the same tetrode as single units) was band-pass filtered (30-40 Hz) using the *eegfilt* function from the EEGLAB toolbox. The phase vector of the filtered LFP was then estimated using the Hilbert transform and the mean spike-gamma phase angle for each unit thereafter calculated. We tested the significance of spike-LFP phase locking using circular statistics (CirStat toolbox for Matlab). Rayleigh test was used to assess the circular distribution of the mean phase angle of neurons and to test the non-uniformity of each neuron's spike phase distribution to gamma. The neurons were considered significantly phase-locked if $P < 0.05$. Phase-locking of populations of FS-PV and WS neurons was evaluated by calculation of the concentration parameter (κ) and the length of the mean resultant vector (MRL, range 0-1) of the spike-gamma phase angle distribution of a population. A MRL value of 1 indicates exact phase synchrony, whereas a value of 0 indicates no phase synchrony. A larger value of kappa (κ)

indicates smaller circular variance of the mean spike-gamma phase angles of the neurons in the population. The mean phase angle (μ) was computed as the circular direction of the MRL.

Viral injections

The animals were anaesthetized with isoflurane (2%) in O_2 and the body temperature maintained at 37° with a temperature controller system. The animals were fixed in a stereotaxic frame and a small craniotomy was made 1.76 mm anterior to Bregma, and 0.25 mm lateral to midline, unilaterally for opto-tagging and bilaterally for characterization of SwiChR *in vivo* and optogenetic manipulation during behavior. The virus was delivered by a glass capillary attached to a motorized stereotaxic injector (Stoelting) at 0.1 μl min^{-1} and the center of the injection was targeted to PL (AP 1.76 mm, LM ± 0.25 mm, DV -1.3). The pipette was held in place for 5 min after injection before being slowly retracted from the brain. The incision was closed with tissue glue (Vetbond, 3M) and the animals were injected with analgesic (buprenorphine 0.1 mg/kg s.c.) at the end of surgery.

For opto-tagging and optogenetic activation of FS-PV neurons the adeno-associated viral vector AAV DIO ChR2-mCherry (Cardin et al., 2009) (pAAV-Ef1A-DIO-hChR2(H132R)-mCherry-WPRE-pA, for vector outline see www.optogenetics.org) was used (0.6 μl for opto-tagging, 0.4 μl / hemisphere for optogenetic activation during behavior, 1x10e11-1x10e12 viral particles / ml).

For inhibition of FS-PV neurons the adeno-associated viral vector AAV DIO SwiChR-EYFP (Berndt et al., 2014) (pAAV-EF1A-DIO $SwiChR_{CA}$-TS-EYFP-WPRE, for vector outline see www.optogenetics.org) was used (0.4 μl / hemisphere, 4x10e12 viral particles / ml).

Characterization of the inhibitory action of SwiChR *in vivo*

To characterize the inhibitory action of SwiChR (Berndt et al., 2014) *in vivo,* animals (4 PV-Cre mice) were injected bilaterally with the adeno-associated viral vector AAV DIO SwiChR-EYFP (for details see *Viral injections*). 4-5 weeks later the animals were anaesthetized with isoflurane (2%) in O_2. The body temperature was maintained at 37° with a temperature controller system. The animals were fixed in a stereotaxic frame and a small craniotomy was made 1.76 mm anterior to Bregma, and 0.25 mm lateral to midline. For the acute recordings 32-channel silicon probes (4 shanks with 2 tetrodes, NeuroNexus) and a Digital Lynx 4SX acquisition system with Cheetah data acquisition software (Neuralynx) were used. The silicon probe was targeted to PL/IL and light (5 mW at the fiber tip) was delivered via an optical fiber (200 µm) placed at the surface of the target brain area. The optical fiber was connected to a patch cable (Doric Lenses) coupled to a blue DPSS laser (Cobolt MLD^{TM} 473 nm, Cobolt) and a red DPSS laser (Cobolt MLD^{TM} 638 nm, Cobolt) controlled by custom software written in LabVIEW. 1 s blue light was delivered and 1 s red light was used to terminate SwiChR activation 3, 4 or 5 s later.

Optogenetics during behavior

Inhibition of FS-PV neurons during attentional processing in the 3-CSRTT

The animals (5 PV-Cre mice) were habituated to the experimenter and the operant chambers for 7 days and thereafter injected bilaterally with the adeno-associated viral vector AAV DIO SwiChR-EYFP (for details see *Viral injections*). After 7 days of recovery the animals started training in the 3-CSRTT. After fulfilling the criteria of level 5 the animals were implanted with optical fibers for optogenetic application. In brief, the animals were anaesthetized with isoflurane (2%) in O_2 and the body

temperature maintained at 37° with a temperature controller system. The animals were fixed in a stereotaxic frame and a small craniotomy was made bilaterally 1.76 mm anterior to Bregma, and 0.25 mm lateral to midline. Optical fibers (200 µm, 0.22 NA) with ceramic ferrules (1.25 mm ID, Precision Fiber Products (PFP)) were bilaterally implanted (10° angle) in mPFC. Two anchoring screws were attached in the skull (one in the left frontal and the other in the right parietal bone) to achieve better fixation of the dental cement. UV cured dental cement (Tetric EvoFlow, Ivoclar Vivadent) with adhesion primer (Optibond FL, Kerr) was used to secure the optic ferrules on the skull. After implantation, the optical fibers were covered with custom-made protectors to keep the fiber cores clean and good in condition. The incision was closed with tissue glue (Vetbond, 3M). The animals were injected with analgesic (buprenorphine 0.1 mg/kg s.c.) at the end of surgery and thereafter single-housed. After 7 days of recovery the animals continued training in the 3-CSRTT and after fulfilling the criteria of level 6 optogenetic application was initiated.

Light was delivered *via* a fiber optic rotary joint/patch cable system (Doric Lenses) coupled to a blue DPSS laser (Cobolt MLD^{TM} 473 nm, Cobolt) and a red DPSS laser (Cobolt MLD^{TM} 638 nm, Cobolt) controlled by custom software written in LabVIEW. The fiber optic cable was connected to the implants using ceramic ferrule sleeves and the connection between the patch cable and the implanted optic fiber was completely covered with heat shrink tubing to prevent visualization of the light from becoming a cue to the animals. 0.5, 1.0 or 2.0 s blue light (different sessions; 5 or 7 mW at the fiber tip) was delivered at trial start pseudorandomly in 50% of the trials (4,362 trials in total from 5 animals). Thus in every session 50% of the trials were no light trials for comparison to the trials with light in that specific session. SwiChR activation was terminated with 1 s red light directly after ending of

the pseudorandom delay at all occasions.

Activation of FS-PV neurons during attentional processing in the 3-CSRTT

The animals (5 PV-Cre mice) were habituated to the experimenter and the operant chambers for 7 days and thereafter injected bilaterally with the adeno-associated viral vector AAV DIO ChR2-mCherry (Cardin et al., 2009) (for details see *Viral injections*). After 7 days of recovery the animals started training in the 3-CSRTT. After fulfilling the criteria of level 5 the animals were implanted with optical fibers for optogenetic application. In brief, the animals were anaesthetized with isoflurane (2%) in O_2 and the body temperature maintained at 37° with a temperature controller system. Optical fibers (200 μm, 0.22 NA) with ceramic ferrules (1.25 mm ID, Precision Fiber Products (PFP)) were bilaterally implanted (10° angle) in mPFC. Two anchoring screws were attached in the skull (one in the left frontal and the other in the right parietal bone) to achieve better fixation of the dental cement. UV cured dental cement (Tetric EvoFlow, Ivoclar Vivadent) with adhesion primer (Optibond FL, Kerr) was used to secure the optic ferrules on the skull. After implantation, the optical fibers were covered with custom-made protectors to keep the fiber cores clean and good in condition. The incision was closed with tissue glue (Vetbond, 3M). The animals were injected with analgesic (buprenorphine 0.1 mg/kg s.c.) at the end of surgery and thereafter single-housed. After 7 days of recovery the animals continued training in the 3-CSRTT and after fulfilling the criteria of level 6 optogenetic application was initiated.

Light was delivered *via* a fiber optic rotary joint/patch cable system (Doric Lenses) coupled to a blue DPSS laser (Cobolt MLD^{TM} 473 nm, Cobolt) controlled by custom software written in LabVIEW. The fiber optic cable was connected to the

implants using ceramic ferrule sleeves and the connection between the patch cable and the implanted optic fiber was completely covered with heat shrink tubing to prevent visualization of the light from becoming a cue to the animals. 5 ms blue light (5 or 7 mW at the fiber tip in different sessions) was delivered at different frequencies (1, 5, 10, 20, 30, 40, 60 Hz, one frequency per session) throughout the delay or during the last 2 s of the delay pseudorandomly in 50% of the trials (10,302 trials in total from 5 animals). Thus in every session 50% of the trials were no light trials for comparison to the trials with light in that specific session.

Anatomical analysis

The mice were deeply anesthetized with pentobarbital and transcardially perfused using 100 mM PBS followed by 4% paraformaldehyde in PBS. Brains were carefully removed and postfixed in 4% paraformaldehyde in PBS for 18h at 4°C. After thorough washing in PBS the entire PFC was sectioned (50 μm thickness) using a vibratome (Leica VT1000, Leica Microsystems).

Targeting of electrodes and optical fibers to PL/IL was confirmed using microscopy (Leica DM6000B fluorescent microscope with a Hamamatsu Orca-FLASH 4.0 C11440 digital camera at 16-bit depth resolution) and anatomical reconstructions (Allen brain atlas, Allen Institute).

For specificity and efficacy of opsin labeling every second 50 μm brain slice were collected for staining. The sections were incubated in blocking solution (0.3% Triton X100 and 10% Normal Donkey Serum in 1x TBS) for 2H followed by incubation with primary antibody (1:1000 rabbit anti Parvalbumin, PV25, Swant) for 18h in room temperature. After washing the sections were incubated with a species-

specific fluorophore conjugated secondary antibody (1:1000 goat anti-rabbit Cy5, Jackson) for 2H, washed, mounted on glass slides and coverslipped.

The specificity and efficacy of opsin labeling was score by hand around identified sites of fiber optic placements (3 sections / animal, n = 5 SwiChR injected PV-Cre mice and 5 ChR2 injected PV-Cre mice) using a Leica DM6000B fluorescent microscope with a Hamamatsu Orca-FLASH 4.0 C11440 digital camera at 16-bit depth resolution. Images were acquired (20x magnification) and individual cells were examined for the presence of PV and/or opsin labeling.

Statistical analysis. Statistical significance was tested with t-test, ANOVA, or Wilcoxon rank sum test. All values are expressed as mean ± s.e.m. unless noted otherwise.

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