Materials and Methods

Animals: A total of 29 male Long Evans rats with an average age of 18.4 (SEM=1.2) weeks were used in this study. Rats were maintained on a 12/12 light/dark cycle. The local ethical committee on animal experimentation (canton of Fribourg), approved all experimental procedures.

Operant chamber: Behavioral sessions were conducted in an operant chamber (32 × 32 × 55 cm) equipped with one lever, a house light and a reward light at the feeding trough. A camera and a speaker were mounted 60 cm above the arena. The operant chamber (lever press read out, house light, food light and the reward dispenser) were controlled by a PC with custom MATLAB scripts.

Lever press training: Following recovery from the viral injection surgery, animals were lightly food restricted to be within 90-95% of their free feeding weight. Animals were then trained to press a lever to receive a food reward, (45mg, chocolate flavored sucrose pellets, TestDiet Richmond IN). Following acquisition of lever pressing the number of presses required to receive a reward were increased until animals were consistently responding on fixed ration (FR) 14 schedule, i.e. the animal had to press the lever 14 times in order to receive a single reward. Following acquisition of the FR schedule animals were transitioned to a variable interval (VI) schedule until stable lever pressing was achieved at a VI interval 30 +/-5s, and they had received a minimum of 600 rewards. Each behavioral session consisted of 56 trials with a trial duration of 60±5s and inter-trial intervals of 9±1s with the house light off. Additional lever press re-training sessions were conducted following the recovery period of optic fiber/electrode implantation prior to the auditory discrimination task.

Viral injections: Anesthesia was induced with ketamine (100 mg/kg) and xylazine (20 mg/kg), and maintained using 1-2% isoflurane in pure O_2 inhalation. Animals were placed into a

stereotaxic device, and a midline incision was made on the scalp. Two small burr holes were made over the left and right basal forebrain targets AP: −0.8; ML: ± 2.8, and a custom-made 26 gauge infuser was lowered to the target site (DV: -8mm). One of four viral constructs (University of North Carolina Vector Core; n=12 rats with AAV5-hSynhChR2(H134R)-mCherry; n=13 AAV5-hSyn-eArch3.0-EYFP, n=2 AAV5-hSyn-mCherry, n=2 AAV5-hSyn-EYFP) were injected bilaterally (volume: 1 µL), using a Hamilton syringe driven by a dual syringe pump (Kd Scientiffic, Holliston, MA) at 0.1 µL/min. The viral infuser was left in place for 10 minutes following each injection before it was slowly removed.

Implantation of electrodes and optic fibers: After viral infusion and initial lever press training in the operant chamber, animals underwent a second surgery to insert optrodes, (240 µm diameter; Doric lenses, or custom made optrodes; 225 µm diameter) bilaterally into the VP (AP −0.8; ML ± 2.8; DV −8). Additionally two tungsten microelectrodes with tip resistances of ~400 kΩ (FHC Inc Bowdoin ME) were implanted bilaterally in the ACC (AP 3; ML ± 0.8; DV -3). Electrodes and optrodes were secured to the skull surface with five stainless steel screws and dental cement. An additional screw over the cerebellum served as a reference. Electrodes and EEG screws were wired to a customized 10 pin connector/PCB board attached to a ziff clip (TDT Apalucha, FL) head stage. The skull was covered with dental acrylic, and sutures were placed at both the anterior and posterior terminations of the incision. Postoperatively the animal received a single dose of Buprenorphine (.05 mg/Kg) and Vetramil ointment was applied to the margins of the incision. Animals received postoperative analgesia in the form of Paracetamol dissolved in their drinking water 2mg/ml for 3 to 4 days following the surgery. Experiments were conducted >5 weeks post viral injection and at least one week following optic fiber/electrode implantation.

Electrophysiology: LFP data was acquired through a unity gain head stage (TDT Alachua, FL) and was sampled at 2.4 kHz and band pass filtered between .5 and 300 Hz using an RZ5 amplifier (TDT) and stored on a PC for offline analysis. To minimize the risk of cable disturbances during freely moving animal recordings, cables were connected to a commutator (TDT, ACO 32) or a rotary joint (Doric lenses, HRJ-OE_FC_12_HARW).

Optical stimulation: A 200-µm dual branching patch cord was connected to the external portion of the chronically implanted optical fibers (Doric Lenses, M3-thread). Patch cords were attached to a 473-nm blue laser or to a 594-nm yellow laser (Changchun New Industries Optoelectronics, China). Laser intensity was set daily at the output of the patch cord to be ~40 mW/mm^2 using an optical power meter (PM 100D, Thorlabs Newton NJ). In ChR2 animals, stimulation was delivered using 40 Hz square pulses with a duty cyle of 50%, for 8 seconds followed by a one second inter-stimulus interval. The stimulation protocol was controlled by a pulse generator (Rigol, Beaverton OR) triggered by a TTL from the RZ5 recording device. In Arch animals, stimulation was delivered using continuous light for 8 seconds followed by one second inter-stimulus interval controlled directly by the RZ5.

Home cage optogenetics: A plastic box (30 x 50 x 40 cm) with nesting material and a drinking bottle attached, served as a home cage like environment for recording sessions to validate VP optogenetics in the absence of operant behavior.

Operant chamber VI30 sessions: To examine the impact of VP optogenetics on lever press behavior we conducted sessions in the operant chamber with VI30 reward schedule. A session consisted of 28 trials per stimulation condition (ChR2/Arch on and off) in pseudo-randomized order, with the caveat that no single condition was allowed to repeat more than three times.

Auditory stimuli: We imported four instrumental music recordings (Chopin Fantasy Op.49 in F-minor; Bach Cello Suite Nr.1 Prelude; Beethoven March in D, Smetana Moldau) as MP3 files

in MATLAB (2018a). Music was up-sampled by a factor of three, shifting auditory stimulus frequencies to higher registers, in accordance with the frequency sensitivity of the rat. Suitable music segments of 8s were selected for discrimination studies. Fourier amplitude was equalized among the four 8s music segments, ensuring that all music segments possessed equal spectral content. Music stimuli were ramped (100ms) to avoid sudden sounds at the beginning and end of the stimulus.

Auditory discrimination task: For auditory discrimination training, four unique musical segments of 8 second duration were used, with 2 segments serving as S+ and 2 segments serving as S- conditions ($S+_A$, $S-_A$, $S+_B$, $S-_B$). In stimulus set A Beethoven and Chopin served either as S+ or S- and in stimulus set B Smetana or Bach served as S+ or S-. Optogenetic stimulation was paired with one S+ and one S- segment, while the other segments served as control conditions. Each session consisted of 56 trials with trial durations randomized at 60±5 s and matched such that the animal spent an equal amount of time in each condition. Each musical segment was presented 14 times, with no condition allowed to repeat more than three times in a row. Responses during S+ trials were rewarded on a VI 30 schedule, with the maximum VI time being less than the minimum trial time, meaning that the animal had the possibility of getting a reward in any S+ trial. The 8s musical stimuli were looped during the duration of the trial, with an inter-stimulus interval of 1s. Optogenetic stimulation was presented throughout the duration of the appropriate trials. Discrimination performance was measured independently for the stimulated vs non stimulated S+ S- pairs, as percent correct, S^+ lever presses/(S^+ + S^- lever presses). In order to make certain that differences in performance between the control and opto conditions were not due to simple differences in discriminability between the stimulus sets, performance without optogenetic intervention was assessed for the different stimuli. Specifically we measured performance for both

stimulus pairs by comparing the number of sessions to reach a discrimination performance better than 70%, with animals learning each pairing equally well, (Stimulus Set A; n=9, mean=14.7±3 sessions, Stimulus Set B; n=9 , mean=13.6±1.8 sessions, t-test, p=0.76). To compare learning performance between control and opto conditions within groups of animals, learning curve segments of seven subsequent sessions were selected per animal when the performance of one of the two stimulus sets went over 50% correct.

Generalization task: To study rat's ability to generalize to other, similar musical pairings, we presented novel segments of the same musical pieces. Generalization sessions were conducted without optogenetics, but were otherwise identical to the learning conditions.

LFP power analyses: To calculate VP power spectrograms (see Fig.3A, E), we extracted power over time by using multi-taper-method implemented in the Fieldtrip toolbox (http://fieldtriptoolbox.org; Donders Institute for Brain, Cognition and Behaviour, Radboud University, Netherlands) (*42*) with a Hanning time window of 500ms (step size: 10ms) and frequency steps of 1Hz. Extracted power data over frequency and time was averaged over trials and plotted for the frequency and time range of interest. For the spectrogram shown in Fig.7E, the VP LFP was down-sampled to 500Hz and fast Fourier transform with $\log_{10}$-scale was computed for 2s data epochs. Finally, power data was plotted over time with a frequency resolution of 1Hz. For power spectrum analyses of VP and ACC data (Fig.3B, C, F), fast Fourier transform was performed with $\log_{10}$-scale of 8s epochs and averaged over 64 trials of each stimulation condition (on/off) per Arch (n=6) and ChR2 (n=8) rat. For statistical comparison between stimulation conditions, in Arch rats (Fig.3C) power values were averaged in the gamma range of 40 to 60Hz. In ChR2 rats (Fig.3F), the 40Hz power peak was detected per animal of the averaged ChR2 on trials and divided by the 40Hz power value of the ChR2 off condition. To analyze the gamma power during grooming, segments of the LFP during

grooming were concatenated and divided into 1 second epochs and FFT analysis computed. For lever pressing the LFP was selected at ±1s to the lever press and FFT analysis was again performed on one second epochs. For *other behaviors* LFP epochs were taken from periods that contained no grooming or lever pressing and aging analysis was performed on one second epochs of the LFP. All LFP data was down sampled to 500 Hz and FFT transformations were $\log_{10}$-scaled, with a frequency resolution of 1Hz). Only behavioral segments without any optogenetic stimulation were included. Gamma power peaks were detected between 40 and 60Hz per behavior condition and animal for subsequent comparison analyses (Fig.7G).

Behavioral Monitoring: Based on video recordings of operant chamber learning sessions, time segments with different behaviors were manually scored by using the ELAN behavioral tracking software (https://tla.mpi.nl/tools/tla-tools/elan/; Max Planck Institute for Psycholinguistics). Behavioral scorings were then aligned with electrophysiological data based on recording starting times.

Gamma burst detection: VP gamma bursts were detected by detrending and bandpass filtering (45–60 Hz) the LFP signal. The amplitude threshold was calculated individually for each animal and session as 2 SDs from the mean. Burst beginning and end times were then defined as the points at which the amplitude fell below 1.5 SDs before and after the detected burst. Only gamma bursts with a length of 100–500 ms were included in subsequent analyses.

Granger Causality Analyses: To study directional influences in the gamma band in the VP and ACC, Granger causality spectral analyses were performed during VP gamma burst epochs, band pass filtered between 45-60 Hz. Unfiltered LFP signals were segmented into epochs of 160 ms centered on the detected bursts. Based on those epochs, we performed bivariate (n=12 animals) Granger causality analyses by using the MVGC multivariate Granger causality

toolbox (*44*). Here, the LFP epochs were transformed to auto-covariance data by setting the maximal model order of 20 according to the Akaike Information Criterion.

Histology: Rats were deeply anesthetized with 100 mg/Kg pentobarbital, then perfused transcardially with 400mL 0.1M phosphate buffer Saline (PBS) (pH 7.4) followed by 400 mL cold 4% paraformaldehyde in 0.1M PBS. Brains, then were placed in the same fixative overnight at 4°C. Whole brains where then rinsed 3 times for 20 minutes each, in cold 0.1M PBS and cryoprotected by immersion in sucrose gradient (15 and 30% w/v sucrose) until they sank. Brains were then blocked and fast frozen in dry-ice chilled isopentane and stored at least overnight at -20°C before cryosectioning. Brains were cut into 40um-thick coronal sections using a sliding microtome (MICROM HM 440E, Microm International GmbH, Walldorf, Germany). Every third section was kept in a storage solution (30% ethylene glycol and 30% glycerol in 0.1M Phosphate Buffer) at -20°C. To determine electrode track positions, sections were mounted on Super Frost ® Plus Adhesion slides (Fisher Scientific AG, Reinach CH), stained for nuclei and Nissl bodies, using a 0.5% Cresyl violet (acetate) and coverslipped using EuKitt ® Quick-hardening mounting medium (Sigma-Aldrich Chemie GmbH, Switzerland). To visualize the virus-mediated expression of ChR2-mCherry and Arch-eYFP by fluorescence, sections where mounted, air-dried, and cover slipped using an aqueous Vectashield ® antifade mounting media with Dapi (Vector Laboratories, Inc., Burlingare, CA, USA, H-1200). Nissl Stain and virus expression were imaged using a NanoZoomer 2.0-HT slide scanner (Hamamatsu Photonics), with a 2x20 0.75 NA air objective and at a resolution of 0.23µm/pixel. Visualization and analysis was done with the NDP.view 2 freeware (Hamamatsu Photonics).

Immunohistochemistry: We performed immunohistochemistry for the specific neuronal markers ChAT and GAD67 on adjacent sections from the same brain specimens extending anterior to posterior through the ventral pallidum (coordinates from Bregma -0.48/-0.60 to

Bregma -1.08) spaced every sixth 40-μm thick section for each marker at regular intervals. Free-floating sections were prepared by rinsing in 0.1 M phosphate buffered saline (PBS) for 20 min, then sections were treated with Heat Induced Epitope Retrieval (HIER) in a Tris-EDTA based solution at pH 9.0 to unmask the antigens and epitopes and thus enhance staining intensity of antibodies. Then sections were permeabilized for 90 min with 0.1 M PBS + 0.2% Triton X-100, followed by incubation in blocking solution for 2 h. at RT containing 0.1M PBS-0.05% Triton X-10, 0.3 M Glycine, and 10% Normal Donkey Serum (NDS) (Abcam, AB 7475). Thereafter, sections were incubated with mouse anti-GAD67 (1:1000; Millipore, MAB5406) and goat anti-ChAT (1:200; Millipore, AB144P) in 0.05% Tween 20, 5% NDS and 0.02% NaN_3 in 0.1M PBS for 48 h at 4°C. Sections were washed in 0.1M PBS + 0.05% Tween 20 + 1% NDS four times, 20 min each, and then incubated in Alexa Fluor-488-tagged donkey anti-mouse IgG (1:400; Jackson Immuno Research, Europe Ltd, UK; Cat N.: 715-545-151) or Alexa Fluor-488-tagged donkey anti-goat IgG (1:200; Sigma-Aldrich Co., St Louis, MO, USA, Cat N.: SAB4600032), or Alexa Fluor-594- tagged donkey anti-mouse IgG (1:800; Jackson Immuno Research, Europe Ltd, UK; Cat N.: 715-585-150) or Alexa Fluor-594-tagged donkey anti-goat IgG (1:200, Jackson Immuno Research, Europe Ltd, UK; Cat N.: 705-585-147 for 16 h at 4°C. Sections were then counterstained with DAPI at 0.5 μg/mL for 5 min. and mounted on Super Frost Plus microscopic slides, and cover-slipped with 90% glycerol + 0.5% N-propyl gallate in 20mM Tris pH 8.0. Images were collected using a 1.30 NA 63X glycerol-immersion objective on a Leica TCS SP5 Laser scanning confocal microscope (Leica Microsystems AG, Switzerland)) and analyzed using LAS X software (version 3.3.0, Leica Microsystems). Image processing was performed with the ImageJ/Fiji software (NIH, Bethesda, MD, USA).

Quantification using stereological methodology: To estimate the number ChR2-mCherry and Arch-eYFP positive cells that co-labeled with ChAT positive neurons and GAD67 positive

neurons in the Ventral Pallidum (VP), we employed the unbiased stereological technique using the optical fractionator workflow in MBF Stereo Investigator (Version 2019.1.3, MicroBrightField, Williston, VT, USA), in combination with a Zeiss Axioplan Fluorescence microscope (Zeiss, Jena, Germany), connected to an Orca-05G monochrome digital Camera (Hamamatsu, Japan) and coupled with a motorized x-y precision stage system BioPoint 2 (Ludl Electronic Products, LTD, NY, USA). Cells were counted with a 63x NA 1.40 immersion oil objective with a mean section thickness of 28.6 µm. The VP was sample in every sixth consecutive 40µm section, yielding 6 to 8 for analysis (GAD67 (n=3-4) or ChAT (n=3-4) respectively. The VP region of interest was first identified under 5x magnification. A 70× 70 µm counting frame was used and a sampling grid area (xy) that covered the region of interest. At each sampling location, the microscope was focused down through the dissector sample to count any cell within that particular counting frame. Only clear cell bodies were counted. Guard zones of 0.5µm at the top and bottom of each slice were used with an optical dissector height of 25 µm. The VP region of interest extended from -0.48/-0.60mm anterior to -1.08 mm posterior to bregma. Two ChR2- and two Arch- animals were analyzed. Counts represent the mean from 3-4 four sections per animal.