Translational profiling of mouse dopaminoceptive neurons reveals region-specific gene expression, exon usage, and striatal PGE2 modulatory effects

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SUPPLEMENTARY METHODS

CONTENT:

Animals

BAC (bacterial artificial chromosome) transgenic mice expressing EGFP fused to the N-terminus of the large subunit ribosomal protein Rpl10a under the control of the *Drd1* or *Drd2* promoter (Drd1-EGFP/Rpl10a or Drd1-EGFP/Rpl10a) were as described[1], and maintained as heterozygotes on a C57Bl/6J background. Males and females were used (**Supplementary Table 1a**). Other transgenic mice were from Jackson laboratories, Drd1-Cre (#Jax 028298), Ai14(RCL-tdT)-D mice (B6;129S6-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J, #Jax 007914), and Tg(Drd1-tdTomato)5Calak or Gensat, (Tg(Drd1-cre) EY262Gsat, Gensat/MMRRC), Drd2-Cre (Tg(Drd2-cre)ER44Gsat/Mmucd). Wild-type male C57Bl/6 mice were purchased from Janvier (France) and used at 10-12 weeks. Mice were maintained on a 12-h light/dark cycle (light on at 7:00 am) and had, before the beginning of specific experiments, free access to water and food. Animal protocols were performed in accordance with the regulations and approved by the geographically relevant committees: the National institutes of Health Guide for the Care and Use of Laboratory Animals, Rockefeller University's Institutional Animal Care and Use Committee (protocol 14753-H) or guidelines of the French Agriculture and Forestry Ministry for handling animals (decree 87-848) under the approval of the "*Direction Départementale de la Protection des Populations de Paris*" (authorization number C-75-828, license B75-05-22, and APAFIS number 15638, license B751317) or animal Ethics Committee of Utrecht University in agreement with the Dutch law (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

mRNA extraction for TRAP-Seq

Cell type-specific ribosome-bound mRNA was purified as described[1,2] with some changes. TRAP transgenic mice were sacrificed by decapitation. The brain was quickly dissected out, placed in cold buffer and then in an ice-cold brain form to cut thick slices from which the prefrontal cortex (PFC) was separated and the nucleus accumbens (NAc) and the dorsal striatum (DS) punched out using ice-cold stainless steel cannulas (**Fig.1b**). Samples containing tissue pieces from 1-3 mice (final proportion of males 0.46-0.50 in each cell population, **Supplementary Table 1a**) were homogenized in 1 mL of lysis buffer (20 mM HEPES KOH, pH 7.4, 5 mM $MgCl_2$, 150 mM KCl, 0.5 mM dithiothreitol, 100 µg.mL^{-1} cycloheximide (Sigma, #C7698-1g), protease (Roche, #11836170001) and RNAse inhibitors (Ambion, #AM2694, Fisher, #PR-N2515) with successively loose and tight glass-glass 2-mL Dounce homogenizers. Homogenates were centrifuged at 2,000 x g, at 4°C, for 10 min. The supernatant was separated from cell debris, and supplemented with NP-40 (EDM Biosciences, 10 µL.mL^{-1}) and 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC, Avanti Polar lipids, 30 mM, final concentrations). After mixing and a 5-min incubation on ice, the lysate was cleared for 10 min at 20,000 x g. A mixture of streptavidin-coated magnetic beads was incubated 35 min at room temperature with biotinylated protein L and then 1 h with EGFP antibody and then added to the supernatant and incubated overnight at 4°C with gentle end-over-end rotation. Beads were collected with a magnetic rack, washed 4 times with high-salt buffer (20 mM HEPES-KOH, pH 7.4, 5 mM $MgCl_2$, 350 mM KCl, 10 µL.mL^{-1} NP-40) and immediately placed in "RTL plus" buffer (Qiagen). mRNA was purified using RNeasy Plus Micro Kit (Qiagen) and in-column DNAse digestion. RNA integrity was evaluated using a

Bioanalyzer with a RNA Pico Chip (Agilent), and the quantity of RNA was measured by fluorimetry using the Quant-IT Ribogreen kit.

Libraries and sequencing

Five ng of RNA were used for reverse-transcription, performed with the Ovation RNA-Seq System V2 (Nugen). cDNA was quantified by fluorimetry, using the Quant-iT Picogreen reagent, and ultra-sonicated using a Covaris S2 sonicator (duty cycle 10 %, intensity 5, 100 cycles per burst, 5 min). Two hundred ng of sonicated cDNA were used for library construction with the Illumina TruSeq RNA sample prep kit, starting at the End-Repair step, and following the manufacturer's instructions. The libraries were quantified with the Bioanalyzer high-sensitivity DNA kit, multiplexed and sequenced on an Illumina HiSeq 2500 instrument. At least 20 million 50-bp paired-end reads were collected for each sample.

Bioinformatics analysis

The quality of the raw data was assessed using FastQC[3] for common issues including low quality of base calling, presence of adaptors among the sequenced reads or any other overrepresented sequences, and abnormal per base nucleotide percentage. The different libraries were then mapped to the *Mus musculus* genome GRCm38 (UCSC mm10) using HISAT2[4]. After RNA-Seq Quality Control, reads were quantified using the RNA-Seq pipeline of SeqMonk[5] and, for each gene product, counts of all reads in all exons were exported with the corresponding gene annotations. Gene products from sex chromosomes were not included in the study. Sequencing data has been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE137153 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE137153). Differential expression was performed with DESeq2 package[6] v1.18 with the option betaPrior set to TRUE. Significance was set at an adjusted p-value of 0.01 and more stringent thresholds were then added to focus on differences most likely to be biologically important (Padj < 10^{-3}, L2FC > 1). Except for differential expression analysis, the raw count files were processed to generate counts per million reads (CPM). BaseMeans are means of normalized counts of all samples within a group, normalized for sequencing depth. Data were then normalized with DESeq2's rlog function. PCA was performed with the prcomp function of the stats R package. GO term enrichment (biological process branch) was performed on lists of significantly differentially expressed genes with WebGestalt, using the list of all expressed genes as background. Only categories with a FDR of 0.01 after Benjamini-Hochberg correction[7] were retained.

To analyze differential exon usage across cell populations, we performed more sample filtering. The technology used for reverse transcription and amplification of TRAP RNA (Nugen Ovation RNAseq v2 kit) causes a slight 3' bias across the body of every gene, which could mask some of the exon usage differences. We computed average read coverages across gene bodies for every sample using the RSeQC package[8] and then calculated the ratio of read coverage across the 3'-most 20% of the gene body over coverage across the 5'-most 20% of the gene body. The largest ratios flag the samples with the largest 3'-bias. We thus filtered out 10% of the samples with the largest 3' to 5' ratios. For the remainder of the samples, we used the DEXseq package[9] and the Ensembl release 70 annotations to calculate read counts

for each exon in each sample. We loaded the count matrix into R and estimated the size factors, dispersions and Exon Fold Changes by using the DEXSeq package with the default parameters.

Network inference

Following the conclusions of the DREAM5 challenge's analysis[10], we used a combination of methods based on different algorithms to infer regulatory networks. We combined the results of CLR[11], a mutual-information-based approach providing undirected edges, and GENIE3[12], a tree-based regression approach providing directed edges. Both methods were the best performers in their category at DREAM5. Both tools were applied using gene expression from all samples, using filtered CPM as described above. Because CLR only provides undirected edges while GENIE3 provides directed ones, the results of CLR were all mirrored with the same score on edges in both directions. Only edges with a positive score in GENIE3's results were used. The edges present in both CLR and GENIE3 results were then ranked according to the product of CLR and GENIE3's scores. This only retains edges that have either an extremely high score with one method, or consistent scores with both methods. Subnetworks were extracted using gene lists as seeds, retaining only the first neighbors with scores above a threshold. Visualization and analysis of the resulting networks was done using Cytoscape[13]. The R script is available as Supplementary material: *Network-Inference.R*

Quantitative reverse transcription PCR (RT-qPCR)

For regional distribution of mRNA (**Supplementary Figures 1** and **2**), mice were sacrificed by cervical dislocation, the PFC was quickly dissected out and microdisks punched out from the NAc and the DS with a stainless steel cannula and placed on ice, as described above. Tissue samples (one mouse per sample) were homogenized in TRIzol with loose and tight glass-glass 2-mL Dounce homogenizers. Total RNA was extracted with TRIzol Reagent (Life Technologies) according to manufacturer's instructions. The RNA was quantified by using the Nanodrop 1000 spectrophotometer and its integrity checked with the Bionalyzer (Agilent RNA 6000 nano kit). Five hundred ng of mRNA from each sample were used for retro-transcription, performed with Reverse Transcriptase II (Life Technologies) following the manufacturer's instructions. Quantitative real time PCR (qPCR) was performed using SYBR Green PCR kit in 96-well plates according to the manufacturer's instructions. Data are normalized to the indicated housekeeping genes and the delta-delta-cycle threshold (ddCT) method was used to obtain a fold change (FC). mRNA levels are presented relative to DS for comparisons DS vs PFC or NAc, and to NAc for NAc vs DS. To do so, the FC obtained for each sample was divided by the average of the FCs of the DS and NAc, respectively. Housekeeping genes for normalization were beta-myosin heavy chain gene (*Myh7*) for DS vs NAc and cyclosporin A binding protein/peptidylprolyl isomerase A gene (*PpiA*) for DS vs PFC.

For Ptger1, 2, and 4 analysis (**Figure 3d-f**) mice were sacrificed by decapitation. The DS and the NAc were quickly dissected out and placed on ice. Cell-type specific ribosome-bound mRNA was purified as described[1,14]. Five ng of RNA were used for reverse-transcription, performed with the Ovation RNA-Seq System V2 (Nugen). qPCR was performed in a LightCycler 1.5 detection system (Roche, Meylan France) using the LightCycler FastStart DNA Master plus SYBR Green I kit (Roche) in 384-well plates according to

the manufacturer's instruction. Results are presented as normalized to the house-keeping gene *Rpl19* and the ddCT method was used to obtain a FC.

Single molecule fluorescent *in situ* hybridization

Analyses of *Ptger1*, *Ptger 2*, *Ptger 4*, *Drd2*, and *Drd1* mRNA expression were performed using single molecule fluorescent *in situ* hybridization (smFISH) as described[15]. Brains from 2 C57/Bl6 male mice were rapidly extracted, snap-frozen on dry ice and stored at -80°C until use. Fourteen-µm coronal sections of the DS (bregma +0.86 mm) were collected directly onto Superfrost Plus slides (Fisherbrand). RNAscope Fluorescent Multiplex labeling kit (ACDBio, #320850) was used to perform the smFISH assay according to the manufacturer's recommendations. Probes used for staining were Mm-Ptger1-C3 (ACDBio, #551308-C3), Mm-Ptger2 (#546481), Mm-Ptger4 (#441461), Mm-Drd1 (#461908), Mm-Drd2-C3 (#406501-C3) and Mm-Drd1-C2 (#461901-C2). After incubation with fluorescent-labeled probes, sections were counterstained with DAPI and mounted with ProLong Diamond Antifade medium (Thermo-Fisher, #P36961). Confocal microscopy and image analyses were carried out at the Montpellier RIO imaging facility. Double- and triple-labeled images were single confocal sections captured using sequential laser scanning confocal microscopy (Leica SP8).

Pharmacological treatments

For acute treatments, misoprostol (Santa Cruz Biotechnology, Santa Cruz, CA, #SC-201264) was dissolved in phosphate-buffered saline (PBS) and injected i.p. (0.1 $mg.kg^{-1}$). PBS was used as vehicle treatment in control mice. Haloperidol (Tocris) was dissolved in saline and injected i.p. (0.5 $mg.kg^{-1}$). For intra-peritoneal misoprostol infusion, 12-week-old WT mice (n = 23-24 per treatment) were deeply anesthetized with pentobarbital (40 $mg.kg^{-1}$) and an osmotic mini-pump was intraperitoneally (i.p.) implanted (model 1004; Alzet, Palo Alto, CA). Vehicle (DMSO in PBS, 1/1) or misoprostol (Santa Cruz Biotechnology, #SC-201264), were infused at a dose of 50 $µg.kg^{-1}.day^{-1}$. For intra-striatal misoprostol infusion, 12-week-old WT mice (n = 9-10 per condition) were deeply anesthetized with pentobarbital (40-60 $mg.kg^{-1}$) and placed in a stereotaxic apparatus for bilateral insertion of a 28-gauge stainless steel cannula (Plastics One, #3280PD-4.0-SPC) into the DS (+0.6 mm anteroposterior to bregma, ±2.0 mm lateral to midline and −3 mm ventral to the bone surface). Cannulas were fixed on the skull with anchor screws and dental cement. Osmotic minipumps (model 2004; Alzet, Palo Alto, CA, USA), previously equilibrated overnight at 37°C in PBS, were implanted subcutaneously in the back of the animal and connected to the cannulas allowing infusion of vehicle (PBS) or misoprostol (0.11 $µl.h^{-1}$), resulting in a misoprostol dose of 0.03 $µg.day^{-1}$ per side.

Immunoblotting

To analyze CNTNAP2/Caspr2 isoforms, DS and NAc were dissected from adult mouse brains and immediately frozen on dry ice. Tissue samples were lysed in RIPA buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 5 $mg.mL^{-1}$ sodium deoxycholate, 10 $µL.mL^{-1}$ NP-40, 1 $mg.mL^{-1}$ SDS) with Complete protease inhibitors (Roche). Equal amounts of protein (BCA protein assay, Thermo-Fisher, #23235) were separated by SDS-

PAGE on NuPAGE 8-12% Bis-Tris gels (Thermo-Fisher) and transferred to nitrocellulose membrane (0.45 mm) in 25 mM Tris-HCl, pH 7.4, 192 mM glycine and 200 $mL.L^{-1}$ ethanol. Membranes were blocked with 50 $g.L^{-1}$ non-fat dry milk in Tris-buffered saline (TBS; 0.25 M Tris, 0.5 M NaCl, pH 7.5) with Tween-20 1 $mL.L^{-1}$ (TBST) for 1 h at room temperature, incubated with rabbit antibody 187 directed against the intracellular domain of Caspr2[16], in the same buffer for 2 h and then 1 h with appropriate IRDye-conjugated secondary antibodies. Blots were quantified with the Odyssey–LI-COR infrared fluorescence detection system (LI-COR). For analysis of striatal phospho-PKA- substrates, mice were sacrificed by cervical dislocation 30 min after i.p. injection of misoprostol (0.1 $mg.kg^{-1}$) or vehicle, and striata dissected as above. Fifteen µg of protein per sample were separated in 4-20% SDS-polyacrylamide gel (BIO-RAD mini-protean-TGX) before electrophoretic transfer onto a nitrocellulose blotting membrane (Amersham, #G9990998). Membranes were blocked 45 min in 30 $g.L^{-1}$ bovine serum albumin and 10 $g.L^{-1}$ non-fat dry milk in 0.1 M PBS. Membranes were then incubated overnight with anti-p(Ser/Thr)-PKA substrates (1:1000; Cell Signaling Technology, Beverly, MA, USA) and then IRDye-conjugated secondary antibodies, visualized with Odyssey–LI-COR. The optical density in the lane after acquisition was assessed using the GELpro32 software.

Histology

Drd1-mice were transcardially perfused with 40 $g.L^{-1}$ paraformaldehyde (PFA) and post-fixed overnight. Coronal sections (80 µm) were prepared on a Vibratome in PBS. Sections were blocked with 5% normal goat serum (NGS) and 25 $g.L^{-1}$ bovine serum albumin (BSA) in PBS containing 0.2% Triton. Afterwards, slices were incubated overnight with at 4°C with a chicken anti-GFP primary antibody (1:500, Aves Labs) mixed in the same blocking solution. Subsequently, slices were rinsed in PBS 3 times for 10 min each at room temperature, after which they were incubated with a corresponding secondary antibody Alexa Fluor 488 (1:500, Aves Labs) for 2 h. Slices were again rinsed 3 times for 10 min in PBS and placed in a PBS-DAPI solution for 10 min. Afterwards, slices were rinsed a final time for 10 min in PBS and then mounted and coverslipped using DAPCO adhesive. Image acquisition was performed with an epifluorescent system (HBO 100, Zeiss) using x 5 and x 10 objectives.

Patch-clamp electrophysiology

For slice electrophysiology experiments, we used the male offspring of Drd1-Cre x Ai14 tdTomato reporter mice, which have red fluorescence in DRD1+ neurons. On a recording day mice were randomly assigned to receive either an i.p. injection with misoprostol (0.1 $mg.kg^{-1}$) or saline. Thirty minutes later animals were anesthetized with isoflurane (Zoetis, UK) between 9.00 a.m. and 11.00 a.m. and then rapidly decapitated. Coronal brain slices of 250 µm were cut on a vibratome (1200 VTs, Leica, Rijswijk, The Netherlands) in ice cold carbogenated (95% O2, 5% CO2) slicing solution, containing (in mM) choline chloride (92), ascorbic acid (10), $CaCl_2$ (0.5), glucose (25), HEPES (20), KCl (2.5), N-acetyl L cysteine (3.1), $NaHCO_3$ (25); NaH_2PO_4 (1.2), N-methyl-D-glucamine (NMDG, 29), $MgCl_2$ (7), sodium pyruvate (3), and thiourea (2). Slices were transferred for 5 min to warmed solution (34°C) of the same composition, before they were incubated at room temperature in carbogenated incubation medium containing (in mM): ascorbic acid (3), $CaCl_2$ (2); glucose (25), HEPES (20), KCl (2.5), NaCl (92), $NaHCO_3$ (20), NaH_2PO_4 (1.2), NMDG (29), $MgCl_2$ (2), sodium pyruvate (3), and thiourea (2). During recordings, slices were immersed in artificial cerebrospinal fluid

(ACSF) containing (in mM): NaCl (124), KCl (2.5), $CaCl_2$ (2.5), glucose (11), HEPES (5), $NaHCO_3$ (26); NaH_2PO_4 (1), $MgCl_2$ (1.3). Slices were continuously superfused at a flow rate of 2 $ml.min^{-1}$ at 32 °C.

In the dorsomedial striatum, spiny projection neurons (SPNs) were identified based on their morphology. D1-SPNs were further identified as fluorescent cells, whereas putative D2-SPNs were identified as non-fluorescent SPNs. Neurons were patch-clamped using borosilicate glass pipettes (2.7–4 MΩ; glass capillaries, GC150-10, Harvard apparatus, UK), under a BX51WI Olympus microscope (Olympus, France). Recordings were made in whole-cell voltage or current clamp in a potassium gluconate-based internal containing (in mM): potassium gluconate (139), HEPES (10), EGTA (0.2), creatine phosphate (10), KCl (5), Na_2ATP (4), Na_3GTP (0.3), and $MgCl_2$ (2). Signals were amplified, low-pass filtered at 2.9 kHz with a 4-pole Bessel filter and digitized at 20 kHz with an EPC9/2 dual patch-clamp amplifier (HEKA Elektronik GmbH). Data were acquired using PatchMaster v2x78.2 software. After break-in, a 10 min waiting time occurred prior to the start of the recording. Series resistance was determined in voltage clamp with a −4 mV step from -65 mV to -69 mV lasting 50 msec, using the resultant capacitive current transients and Ohm's law. To determine current-voltage relationships and biophysical properties, cells were recorded in current clamp. Cells were clamped at 0 pA and from there were subjected to 24 subsequent sweeps. Every sweep started with a 0 pA injection of 400 msec, followed by a current step of 800 msec length of -100 pA (with a +25 pA increasing increment on each subsequent sweep), followed by a return to 0 pA for 1000 msec. Inter-sweep interval was 10 sec.

From the obtained current-voltage relation data, excitability profiles and neuronal biophysical properties were calculated in Igor Pro-8 (Wavemetrics). Excitability profiles were determined as the number of action potentials generated during the 800 msec window of the 24 sweeps where currents were incrementally increased from -100 pA to +475 pA. To assess excitability over a physiologically more relevant range of action potential generation, we statistically assessed occurrence of differences, over the range from average vehicle rheobase [the minimal current required induce an action potential (AP)] to that value plus 200 pA (i.e. from 125 pA to 325 pA for D2-SPNs, and from 225 pA to 425 pA for D1-SPNs). To determine the AP threshold, we differentiated the rheobase sweep, calculated when voltage changes first occurred at a rate surpassing 20 mV/msec, and then related this time point in the original sweep to the matching membrane voltage. This voltage value was set as the AP threshold. The resting membrane potential was determined as membrane voltage when no current was injected (0 pA), which indeed never led to active conductance. Membrane resistance was calculated in voltage-clamp, with a -4 mV step from -65 mV to -69 mV lasting 50 msec, using the steady-state current and Ohm's law.

Fiber photometry

For Drd2 recordings, Drd2-cre mice were anaesthetized with isoflurane and received 10 $mg.kg^{-1}$ intraperitoneal injection (i.p.) of Buprécare® (buprenorphine 0.3 mg) diluted 1/100 in NaCl 9 $g.L^{-1}$ and 10 $mg.kg^{-1}$ of Ketofen® (ketoprofen 100 mg) diluted 1/100 in NaCl 9 $g.L^{-1}$, and placed on a stereotactic frame (Model 940, David Kopf Instruments, California). We unilaterally injected 0.5 µl of virus (pAAV.Syn.Flex.GCaMP6f.WPRE.SV40 (titer $\geq 10^{13}$ genome copy (GC).mL^{-1}, working dilution 1:5 (Addgene viral prep #100833-AAV9 https://www.addgene.org/100833/) into the DS (L = +/-1.5; AP = +0.86; V = -3.25, in mm) or the NAc (L=+/- 1; AP=+1.55, V=-4.5) at a rate of 100 $nl.min^{-1}$. The injection needle was carefully

removed after 5 min waiting at the injection site and 2 min waiting half way to the top. Optical fiber for calcium imaging into the striatum was implanted 100 µm above the viral injection site. A chronically implantable cannula (Doric Lenses, Québec, Canada or RWD Life Science) composed of a bare optical fiber (400 µm core, 0.48 N.A.) and a fiber ferrule was implanted 100 µm above the location of the viral injection site in the DS (L = +/-1.5; AP = +0.86; V = -3.25, in mm) or NAc (L=+/- 1; AP=+1.55, V=-4.5). The fiber was fixed onto the skull using dental cement (Super-Bond C&B, Sun Medical). Real time fluorescence emitted from the calcium sensor GCaMP6f expressed by neurons with the DRD2 receptor was recorded using fiber photometry as described[17]. Fluorescence was collected in the DS using a single optical fiber for both delivery of excitation light streams and collection of emitted fluorescence. The fiber photometry setup used 2 light emitting LEDs: 405 nm LED sinusoidally modulated at 330 Hz and a 465 nm LED sinusoidally modulated at 533 Hz (Doric Lenses) merged in a FMC4 MiniCube (Doric Lenses) that combines the 2 wavelengths excitation light streams and separate them from the emission light. The MiniCube was connected to a fiberoptic rotary joint (Doric Lenses) connected to the cannula. A RZ5P lock-in digital processor controlled by the Synapse software (Tucker-Davis Technologies, TDT, USA), commanded the voltage signal sent to the emitting LEDs via the LED driver (Doric Lenses). The light power before entering the implanted cannula was measured with a power meter (PM100USB, Thorlabs) before the beginning of each recording session. The light intensity to capture fluorescence emitted by 465 nm excitation was between 25-40 µW, for the 405 nm excitation this was between 10-20 µW at the tip of the fiber.

The fluorescence emitted by the GCaMP6f activation in response to light excitation was collected by a femtowatt photoreceiver module (Doric Lenses) through the same fiber patch cord. The signal was then received by the RZ5P processor (TDT). On-line real time demodulation of the fluorescence due to the 405 nm and 465 nm excitations was performed by the Synapse software (TDT). A camera was synchronized with the recording using the Synapse software.

For Drd1 recordings, male Drd1-Cre mice, 9-month old at the time of surgery, were anesthetized with a mixture of ketamine 75 $mg.kg^{-1}$ and dexmedetomidine (Dexdomitor) 1 $mg.kg^{-1}$ and placed on a stereotactic frame (UNO BV, the Netherlands). rAAV5-Syn-FLEX-GCaMP6f-WPRE-SV-40 (500 nL, titer 3.10^{12} $GC.mL^{-1}$) was injected unilaterally into the DS (AP +0.86; ML -1.5; DV -3.25) at a rate of 100 $nL.min^{-1}$. The injection needle was carefully retracted after 8 min. In addition, a chronically implantable fiber optic ferrule (ø 2.5 mm – 440 µm bore size) that contained a bare optical fiber (FT400UMT, Thorlabs, Germany) was implanted 100 µm above the injection site and fixed with a layer of dental cement (Super-Bond C&B, Sun Medical). At the end of surgery, 1 mL of NaCl and 5 $mg.kg^{-1}$ carprofen (Rimadyl) dissolved in NaCl was administered subcutaneously. During the first week of recovery, the animals had access to carprofen dissolved in drinking water.

Fluorescence was collected in the DS using a single optical fiber for both delivery of excitation light streams and collection of emitted fluorescence. The fiber photometry setup used 2 light-emitting LEDs: 405 nm LED sinusoidally modulated at 330 Hz and a 465 nm LED sinusoidally modulated at 533 Hz (Doric Lenses) merged in a FMC6 MiniCube (Doric Lenses) that combined the 2 wavelengths excitation light streams and separated them from the emission light. The MiniCube was connected to a low autofluorescence fiber optic patch cord (400 µm - NA 0.37, Doric Lenses), which in turn was connected to the fiber optic implant of the mouse. Light intensities were measured every time before recording using a fiber optic power meter (Thorlabs, Germany). The light intensity to capture fluorescence emitted by 465

nm excitation was between 25-40 µW, for the 405 nm excitation this was between 10-20 µW at the tip of the fiber. A camera was synchronized with the recording using the Doric Studio software.

Signals were exported to MATLAB R2016b (Mathworks) and analyzed offline. After careful visual examination of all trials, they were clean of artifacts in these time intervals. The timing of events was extracted from the video. For each session, signal analysis was performed on two-time intervals: one extending from -60 to 0 sec (home cage, HC) and the other from 0 to +60 sec (new environment, NE). From a reference window (from -180 to -60 sec), a least-squares linear fit was applied to the 405 nm signal to align it to the 465 nm signal, producing a fitted 405 nm signal. This was then used to calculate the ΔF/F that was used to normalize the 465 nm signal during the test window as follows: ΔF/F = (465 nm $signal_{test}$ - fitted 405 nm $signal_{ref}$)/fitted 405 nm $signal_{ref}$. To compare signal variations between the two conditions (HC versus NE), for each mouse, the value corresponding to the entry point of the animal in the new cage was set at zero.

Behavioral assays

Haloperidol-induced catalepsy: mice were injected with misoprostol (0.1 $mg.kg^{-1}$, i.p.) or vehicle, and 15 min later with haloperidol (0.5 $mg.kg^{-1}$, i.p.). Catalepsy was measured at several time points, 45-180 min after haloperidol injection. Animals were taken out of their home cage and placed in front of a 4-cm-elevated steel bar, with the forelegs upon the bar and hind legs remaining on the ground surface. The time during which animals remained still was measured. A behavioral threshold of 180 sec was set so the animals remaining in the cataleptic position for this duration were put back in their cage until the next time point.

Behavior of mice chronically implanted with osmotic minipumps was explored successively using rotarod and food-cued Y maze, 9-15 days and 20-25 days after implantation, respectively.

Rotarod. Animals were placed on a motorized rod apparatus (30-mm diameter) accelerating linearly from 4 to 40 RPM over 5 min. Training was performed 14 times (4 trials per day x 3 days and 2 trials x 1 day) over consecutive days. The fall latency was recorded. Twenty-four h after the last training session the animals were tested at fixed speed (24 RPM) and the number of falls per min was counted.

Y-maze. Mice were tested for learning and cognitive flexibility in a black Y maze (arm 35-cm length, 25-cm height, 15-cm width). All mice were mildly food deprived (85-90% of original weight) for 3 days prior to starting the experiment. The first day mice were placed in the maze for 15 min for habituation. Then, mice underwent 3 days of training with one arm reinforced with a highly palatable food pellet (TestDiet 5-UTL). Each mouse was placed at a start point and allowed to explore the maze. It was then blocked for 20 sec in the explored arm and then placed again in the starting arm. This process was repeated 10 times per day. At the end of the learning phase all mice showed a > 70% preference for the reinforced arm. The average number of entries in each arm over 5 trials was plotted. Two days of reversal learning followed the training phase during which the reinforced arm was changed and the mice were subjected to 10 trials per day with the reward in the arm opposite to the previously baited one.

Statistical analyses

Compiled data are always reported and represented as mean ± s.e.m., with single data points plotted. Data were statistically analyzed with GraphPad Prism 6. Normal distribution was tested with Shapiro-Wilk test. When n was > 7 and normality test passed, data were analyzed with Student's t test, one-way ANOVA, two-way ANOVA or repeated-measures ANOVA, as applicable and Holm-Sidak's post-hoc tests for two by two comparisons. Otherwise non-parametric Mann-Whitney test or, for paired comparisons, Wilcoxon matched-pairs signed rank test were used. All tests were two-tailed. Significance was considered as $p < 0.05$. Detailed statistical results are reported in **Supplementary Table 19**.

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