Supplemental Material

Maternal high fat diet during lactation reprograms the dopaminergic circuitry in mice.

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Supplemental Methods

Electrophysiology

Experiments were performed on brain slices from 16-week-old male and female C57Bl6, DAT-tdTomato and DRD1-tdTomato mice that expressed tdTomato protein (tdTomato) selectively in mesencephalic DA neurons that express the transporter (DAT) or dopamine receptor type 1 (DRD1), respectively. SN dopaminergic neurons were identified according to their I_H–mediated sag potential upon hyperpolarization, broad action potentials (1-3) and/or by tdTomato fluorescence. MSNs of the Caudate Putamen (CPu) and the Nucleus Accumbens (NAc) expressing DRD1 and VTA dopaminergic neurons were identified by tdTomato fluorescence. Additionally, some neurons were identified post hoc by biocytin-streptavidin labeling in combination with TH/DAT- or tdTomato-immunohistochemistry. Animals were lightly anesthetized with isoflurane (B506; AbbVie Deutschland GmbH and Co KG, Ludwigshafen, Germany) and subsequently decapitated. The brain was rapidly removed and a block of tissue containing the mesencephalon or the striatum was immediately dissected. Coronal slices (250 - 300 µm) containing the SN/VTA or parasaggital slices (330 – 350 µm) containing the CPu and the NAc were cut with a vibration microtome (HM-650 V; Thermo Scientific, Walldorf, Germany) under cold (4°C), carbogenated (95% O_2 and 5% CO_2), glycerol-based modified artificial cerebrospinal fluid (GACSF (4)). GACSF contained (in mM): 250 Glycerol, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 1.2 NaH_2PO_4, 10 HEPES, 21 $NaHCO_3$, 5 glucose adjusted to pH 7.2 (with NaOH). Brain slices were transferred into carbogenated artificial cerebrospinal fluid (ACSF). First, they were kept for 20 min in a 35°C 'recovery bath' and then stored at room temperature (24°C) for at least 30 min prior to recording. ACSF contained (in mM): 125 NaCl, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 1.2 NaH_2PO_4, 21 $NaHCO_3$, 10 HEPES, and 5 Glucose adjusted to pH 7.2 (with NaOH). For the experiments, brain slices were transferred to a recording chamber (~1.5 ml volume) and continuously superfused with carbogenated ACSF at a flow rate of ~2

ml·min^{-1}. Experiments were carried out at ~32°C. Recordings were performed with an EPC10 amplifier (HEKA, Lambrecht, Germany) and a modified ELC03-XS amplifier with improved capacity compensation (NPI Electronic, Tamm, Germany) controlled by the software PatchMaster (version 2.32; HEKA). In parallel, data were sampled at 10 kHz with a CED 1401 using Spike2 (version 7) (both Cambridge Electronic Design, UK) and low-pass filtered at 2 kHz with a four-pole Bessel filter**.** The liquid junction potential between intracellular and extracellular solution was compensated (14.6 mV; calculated with Patcher's Power Tools plug-in for Igor Pro 6 (Wavemetrics, Portland, OR, USA)).

Perforated patch recordings were performed using protocols modified from Horn & Marty (5) and Akaike & Harata (6). Electrodes with tip resistances between 3 and 5 MOhm were fashioned from borosilicate glass (0.86 mm inner diameter; 1.5 mm outer diameter; GB150- 8P; Science Products) with a vertical pipette puller (PP-830; Narishige, London, UK). Patch recordings were performed with ATP and GTP free pipette solution containing (in mM): 140 K-gluconate, 10 KCl, 10 HEPES, 0.1 EGTA, 2 $MgCl_2$ and adjusted to pH 7.3 (with KOH). ATP and GTP were omitted from the intracellular solution to prevent uncontrolled permeabilization of the cell membrane (7). The patch pipette was tip filled with internal solution and back filled with 0.02% tetraethylrhodamine-dextran (D3308, Invitrogen, Eugene, OR, USA) and amphotericin-containing internal solution (~160-200 µg·ml^{-1}; G4888; Sigma-Aldrich, Taufkirchen, Germany) to achieve perforated patch recordings. Amphotericin was dissolved in dimethyl sulfoxide (final concentration: 0.1 - 0.3%; DMSO; D8418, Sigma-Aldrich) (8) and was added to the modified pipette solution shortly before use. The used DMSO concentration had no obvious effect on the investigated neurons. During the recordings, access resistance (R_a) was constantly monitored and experiments were started after R_a and the action potential amplitude were stable (~15 – 20 min). Recordings with $R_a > 50$MOhm were not considered for analysis of intrinsic

electrophysiological parameters. In the analyzed recordings R_a were comparable, did not change significantly over recording time, and were not significantly different between the distinct experimental groups. A change to the whole-cell configuration was indicated by a sudden change in R_a and diffusion of tetraethylrhodamine-dextran into the neuron. Such experiments were rejected. In recordings of SN dopaminergic neurons, GABAergic and glutamatergic synaptic input was blocked by addition of 10^{-4} M PTX (picrotoxin; P1675, Sigma-Aldrich), 5 x 10^{-5} M DL-AP5 (DL-2-amino-5-phosphonopentanoic acid; A5282; Sigma-Aldrich), and 10^{-5} M CNQX (6-cyano-7-nitroquinoxaline-2-3-dione, C127; Sigma-Aldrich) to the ACSF. Resting membrane potential and spontaneous action potential firing frequencies were determined when stable recording conditions were achieved. A cell was considered silent when the spontaneous mean action potential firing was < 0.5 Hz. In order to determine the pacemaking properties of DA neurons, the coefficient of variation (CV) of 100 interspike intervals (ISIs) was calculated. A cell was considered silent when the spontaneous action potential firing was < 0.5 Hz. A cell was considered a pacemaker if the coefficient of variation (CV) of the measured ISIs was < 10% and ISIs were normally distributed. The cell input resistance was calculated from the slope of linear fits to I-V relations of voltage responses to small hyperpolarizing current pulses. To analyze excitability, i.e. evoked action potential firing, cells were held at -70 mV (SN DA neurons) or -80 mV (MSNs) and a series of depolarizing current pulses (0 pA to 200 - 300 pA in 20 pA increments; 1.5 s duration) were injected. For each current pulse the mean action potential frequency was determined.

Data analysis

Data analysis was performed with Spike2 (Cambridge Electronics), GraphPad Prism (version 8.2; GraphPad Software Inc), custom-made analysis scripts written in Python and Igor Pro. To compare the excitability between CC and CH cohorts, data were fit

using a sigmoidal Boltzmann fit. Statistical tests were used as indicated in the text. Statistical significances are indicated as $p < 0.05$ (*). In graphs, data are given as mean ± SEM as indicated. In boxplots, horizontal lines indicate the median of the data, + sign indicates the mean of the data; boxes indicate the 25th and 75th percentile and whiskers indicate minimal and maximal values.

Immunohistochemistry

To label single cells, 1% biocytin (B4261, Sigma) was added to the pipette solution. After the recordings, the brain slices were fixed in Roti-Histofix (P0873, Carl Roth) for ~12 h at 4°C and rinsed in 0.1 M PBS (pH 7.2, 3 times for 10 min each time). Brain slices were incubated in PBS containing 1% Triton X-100 (PBS-T) and 10% normal goat serum (30 min, RT; Serva). Afterwards, the slices were incubated for 2 d at 4 °C in rabbit anti-TH (1:1000, ab112, Abcam, Cambridge, UK) or rat anti-DAT (1:500, ab5990, Abcam, Cambridge, UK) or anti-DsRed (1:1000, 632496, Takara Bio Europe SAS, Paris, France) that was dissolved in PBS-T and 10% normal goat serum. Brain slices were then rinsed in PBS (three times for 15 min each) and incubated with the following secondary antibodies: Alexa 633-conjugated streptavidin (1:400, S21375, Molecular Probes, Karlsruhe, Germany), dylight 488 anti-rabbit IgG (1:200, ab96883, Abcam) or dylight 488 anti-rat IgG (1:200, ab96887, Abcam) or dylight 550 anti-rabbit IgG (1:200, ab96884, Abcam) for 2 h at room temperature in PBS. Brains were rinsed in PBS (5 times for 10 min each time, RT), dehydrated, cleared, and mounted in Permount (SP-15, Thermo Fisher Scientific). Fluorescence images of brain slices were captured with a confocal microscope (SP-8, Leica) equipped with HCX-PL Fluotar × 10 (0.3 NA), HC PL APO ×20 (0.75 NA), and HC PL APO ×63 (1.2 NA) objectives. Fluorescent dyes were excited at 488, 552 and 638nm with solid-state lasers, emission was collected with the Acousto-optical tunable filter (AOTF) of the Leica SP-8 optimized to the emission spectra of the selected fluorescent dyes. Scaling, contrast

enhancement, and *z*-projections were performed with ImageJ v2.0.0. The final figures were prepared with Affinity Designer (Ver. 1.6.1, Serif Ltd, UK).

Fast Scan Cyclic Voltammetry

Mice were anesthetized using isoflurane (3–4% for induction and 1.0–1.5 % for maintenance) and given subcutaneous injections of 5 mg/kg meloxicam (Metacam) and 0.7 ml glucose-saline (0.5 % in 0.9 % saline; Aquapharm). After confirming depth of anesthesia, the head was shaved and secured within a stereotaxic frame. A homeothermic heating blanket was used to ensure maintenance of body temperature at 36–37 °C and coreal dehydration was prevented by the use of ophthalmic ointment (Lacri-Lube, Allergan, UK). The head of the animal was treated with diluted Hibiscrub and Reprochem (diluted 1:20 in water). A local anesthetic bupivacaine (2 mg/kg; AstraZeneca), was then administered under the scalp. After exposure of the skull, holes were drilled for a recording electrode and a stimulating electrode as well as an Ag/AgCl reference electrode and an anchoring screw. After securing the reference electrode in place using dental cement (Kemdent, Swindon, UK), a custom-made carbon fiber electrode was attached to a voltammetric amplifier and lowered toward the dorsal nucleus accumbens (NAc) core (AP: +1.4, ML: 0.75, DV: −3.5 to −4.25 from skull). A new electrode was selected from the batch at random for each surgery and the surgeon was blinded to the dietary group of the animals. This was followed in sequence by implantation of a 2-channel untwisted stimulating electrode (PlasticsOne) into the ipsilateral ventral tegmental area (VTA) (AP: −3.5, ML: 0.35, DV: −4.0 to −4.55 from brain). The anchoring screw as well as reference electrode we placed contralateral to the carbon fiber and stimulating electrodes. Approximately every 3 hours for the duration of the surgery, the mouse was treated with additional boluses of 0.7 ml glucose-saline.

Using FSCV, recordings of in vivo nucleus accumbens (NAc) core dopamine levels were made under anesthesia. The applied potential at the carbon fiber was cycled from −0.4 V (vs Ag/AgCl) to +1.3 V and back at a rate of 400 V/s during each voltammetric scan and subsequently held at −0.4 V between scans. This ramping occurring at a frequency of 60Hz for an initial 20-minute period to condition the electrode followed by a reduced scan rate of 10 Hz for the duration of the experiment and commencement of dopamine detection. The application of electrical stimulation was performed using an isolated current stimulator (DS3, Digitimer) and using Tarheel CV (National Instruments) recordings were collected of generated stimuli. Optimal position of the recording and stimulating electrodes were determined by positioning them in order to determine the maximal changes in dopamine that could be detected after stimulation (50 x 2 ms monophasic pulses, 200 µA current, at 50 Hz).

Once the placement was confirmed, the experiment commenced to determine the effect of 1) electrical stimulation and 2) chemogenetic stimulation of the dopamine neurons on patterns of NAc core dopamine release. For electrical stimulation of dopamine release, 5 different stimulation parameters were utilized (2 recordings with each, 3 min between stimulations): (i) 20 pulses 100 µA, (ii) 30 pulses 100 µA, (iii) 30 pulses 150 µA, (iv) 40 pulses 150 µA, and (v) 50 pulses 200 µA. This protocol was performed 3 times during the course of the study, before treatment with either CNO (0.3 mg/kg body weight) or vehicle (0.6% DMSO in sterile saline), 1 hour after injection, and at the end of the experiment (approx. 145 minutes after initial injection). For the stimulations after the first hour, only parameters (ii), (iv) and (v) were used. To assess spontaneous changes in dopamine due to CNO stimulation of dopamine circuits, we continuously monitored dopamine levels under anesthesia for 10 min prior to injection of either CNO or vehicle, then subsequently for 60 minutes after injection and for a further 60 minutes after the midpoint electrical stimulations.

Voltammetric data analysis was carried out using custom-written scripts generated in Matlab and used previously (9). All data were first low pass filtered at 2kHz. In order to characterize, in each mouse, the rate of spontaneous dopamine transients, we first subtracted the average current recorded between 1.5-0.5 s before the target cyclic voltammogram to account for large changes in capacitance current. Following this, we assessed periods when the cyclic voltammograms recorded for the duration of the experiment correlated with a correlation coefficient of $R \geq 0.86$ with a dopamine 'template' derived from electrical stimulation of the VTA before the onset of the experiment (10, 11). The dopamine transients over time and in total were then compared between groups. In order to extract an estimate dopamine level changes over time across the recording session, a principal component analysis was performed using a standard training set of stimulated dopamine release detected by chronically implanted electrodes, with dopamine being selected as the first principal component among other unrelated electrochemical fluctuations, such as changes in the pH. To perform this analysis, data were divided into non-overlapping bins of 30 seconds in length and, from each, the average current recorded over the initial 1 sec in each bin was subtracted. Given that it is not possible to derive an absolute measurement of dopamine levels with FSCV but rather only a relative amount of dopamine, the extracted dopamine signals in each bin we combined by assuming that the first recorded value in bin N+1 continued relative to the last time point in bin N.

Behavioral Testing

For all behavioral tests animals were allowed to acclimate to the testing room for >30 minutes prior to the onset of the test. For experiments requiring intraperitoneal injection, animals were acclimated to handling and injection for a minimum of 5 days prior to testing.

Open Field Test

At the time of testing, animals were placed in the center of a clear Plexi-Glass chamber (27.31 x 27.31 x 20.32 cm, Med Associates, VT), fitted with infrared beams to remotely monitor movement in the X, Y and Z planes and housed in a sound-attenuating chamber (Med Associates, Vermont, USA). For 30 minutes, the animals were tracked with free movement throughout the chamber. All movements were analyzed using MedActivity software (Med Associates, VT). Animals were then returned to normal housing for a period of recovery followed by sucrose preference testing. In studies assessing changes in locomotion after novel exposure, animals were exposed to the chamber on three subsequent days for 30 minutes each day, with Day 1 being the first ever exposure to the chamber, and total locomotion was determined. In studies using CNO, animals were injected with CNO (0.3 mg/kg BW) and allowed free movement in the chamber for 120 minutes.

In studies using a D1 agonist, SKF38393 (Tocris) , animals were either naïve to the chamber or had repeated exposure to the chamber to reduce novelty-associated increases in locomotion. Animals received either 20mg/kg SKF38393 or 0.9% sterile saline via intraperitoneal injection and were immediately placed into the center of the open field apparatus.

Elevated Zero Maze

For studies using CNO injection animals received the CNO injection and were returned to the homecage for 30 minutes, after which they were introduced to the open arm of the elevated zero maze apparatus consisting of 4 equally sized compartments, 2 open and 2 closed (TSE Systems, Germany). Movements were tracked for 5 minutes using the VideoMot tracking system (TSE, Germany).

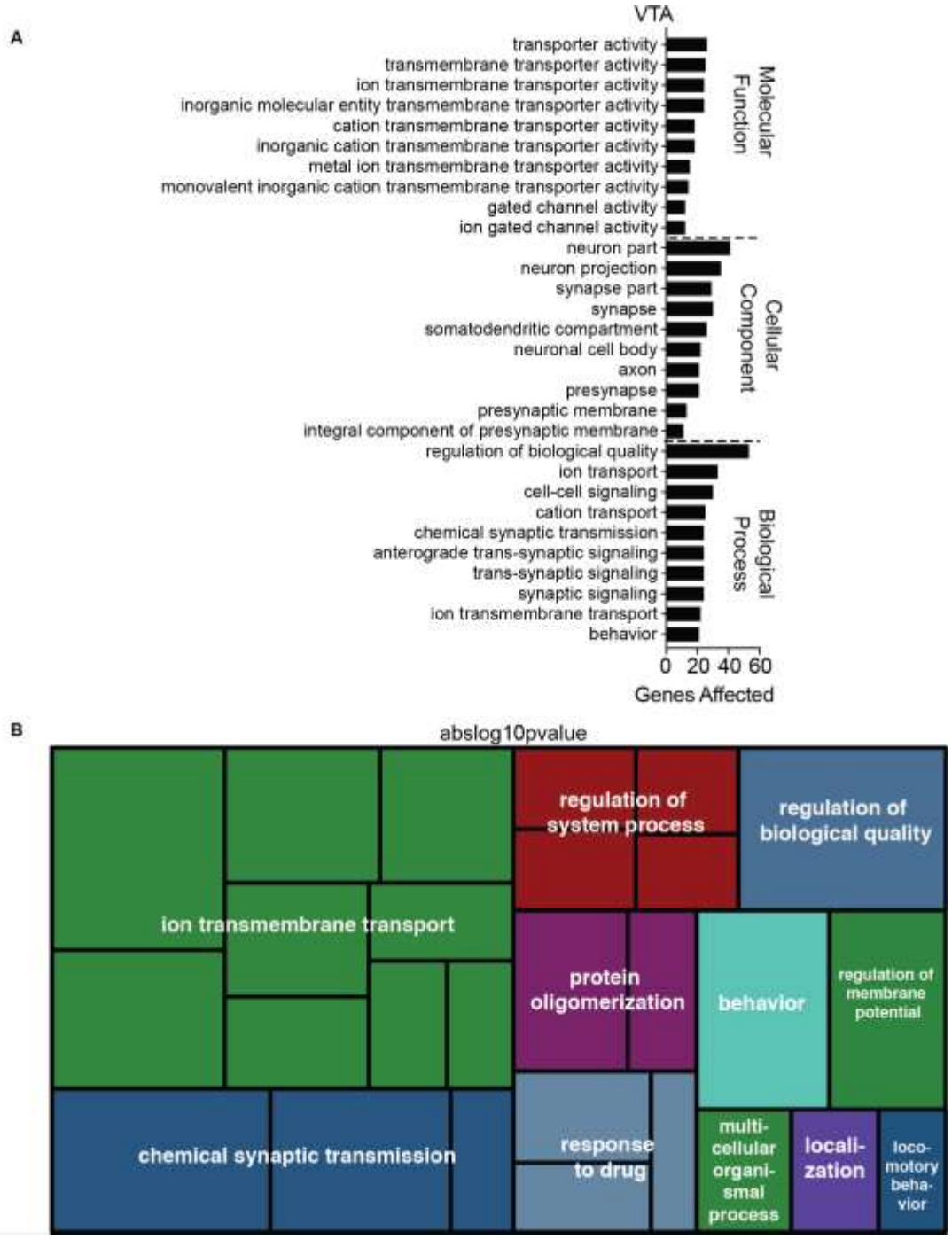
Sucrose Preference

Animals were allowed access to two 15-mL falcons tubes outfitted with sipper tubes in their homecages. In the acclimation phase, only water was available in both tubes, to allow the animals to learn to drink from the tube as well as to determine the animal's preference for a sipper. On the testing days, the non-preferred sipper tube was filled with a 4% sucrose solution (Sigma, Cat No. S7903), and the animal was given free access to both the water and sucrose solution. Total intake was monitored by daily weighing of the solutions to determine amount consumed. Animals were determined to be 'high' or 'low' preferers based on a cutoff of 65% preference, which reflects an established cutoff for anhedonic behaviors (reviewed in (12))

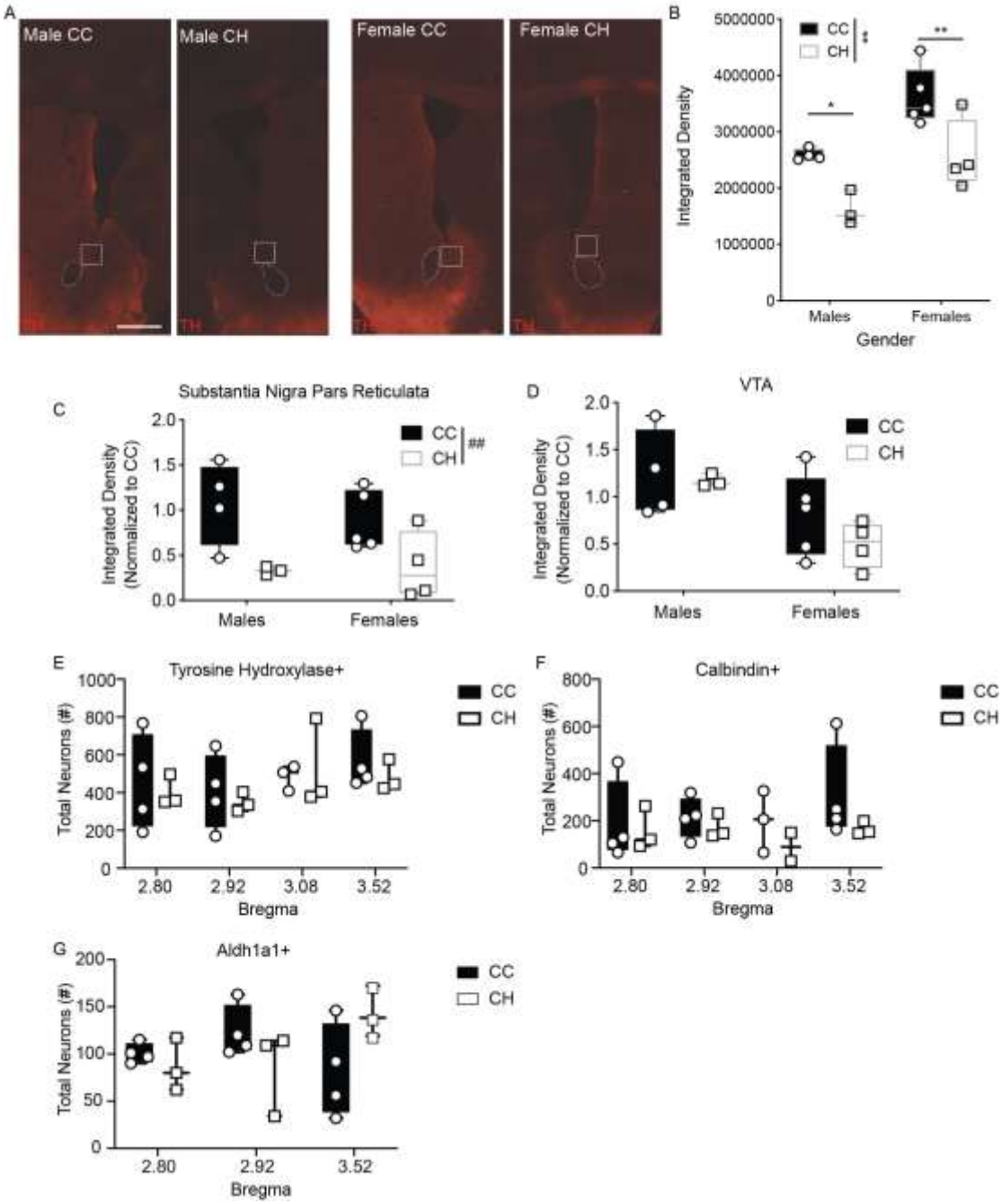
Whole brain imaging

Animals at 4 months of age were deeply anesthetized using Avertin solution and transcardially perfused first with ice cold sterile 1X PBS followed by 4% PFA:Borax solution (pH 9.5). Brain tissue was dissected and post-fixed in 4% PFA:Borax solution for 24 hours. Tissue was then stored in 1X PBS plus sodium azide (0.002%) until further processing. Clearing was performed using a publicly available protocol (13). Briefly, samples were incubated in increasing concentrations of tert-butanol (35%, 50%, 70%, 80% and 96%) for approximately 12 hours at each concentration at 35°C with constant rotation and protected from light. The brains were then incubated 60 minutes in dichloromethane at RT with rotation followed by an overnight incubation in a 4:1 solution of 1:2 benzyl alcohol:benzyl benzoate to diphenyl ether. Samples were then imaged using a Light Sheet Fluorescence Microscope (LaVision BioTec, Germany) and collected images were processed using Arivis software (Germany).

Supplemental Figures

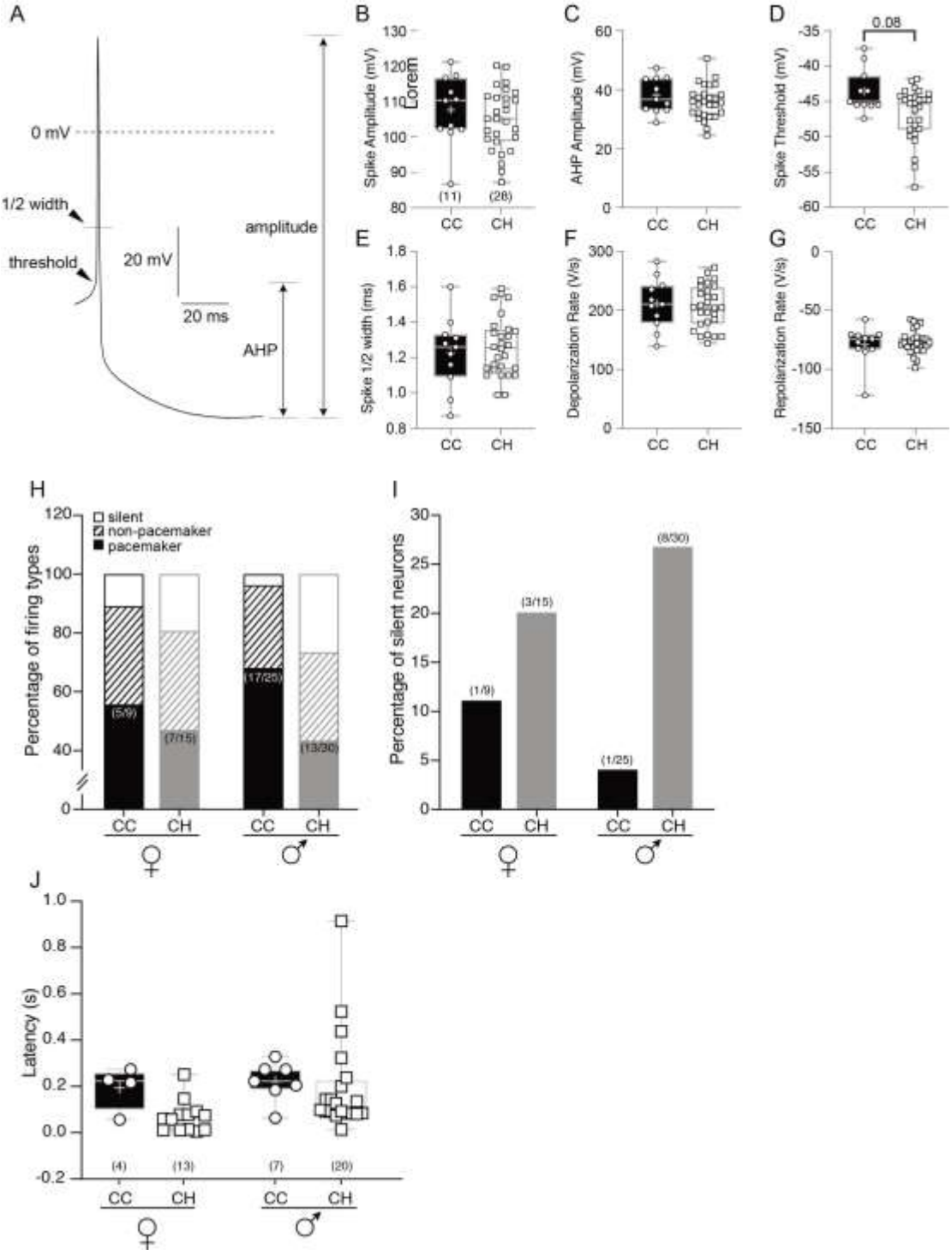


Supplemental Figure 1. Gene Ontology analysis of VTA RNASequencing. A) Gene Ontology analysis of top hits in Molecular Function, Cellular Component and Biological Function in VTA samples (n=5-6 samples per gender and diet group). L) Treemap clustering GO terms based on common themes, square size indicants the absolute log of the p value (larger=more significan



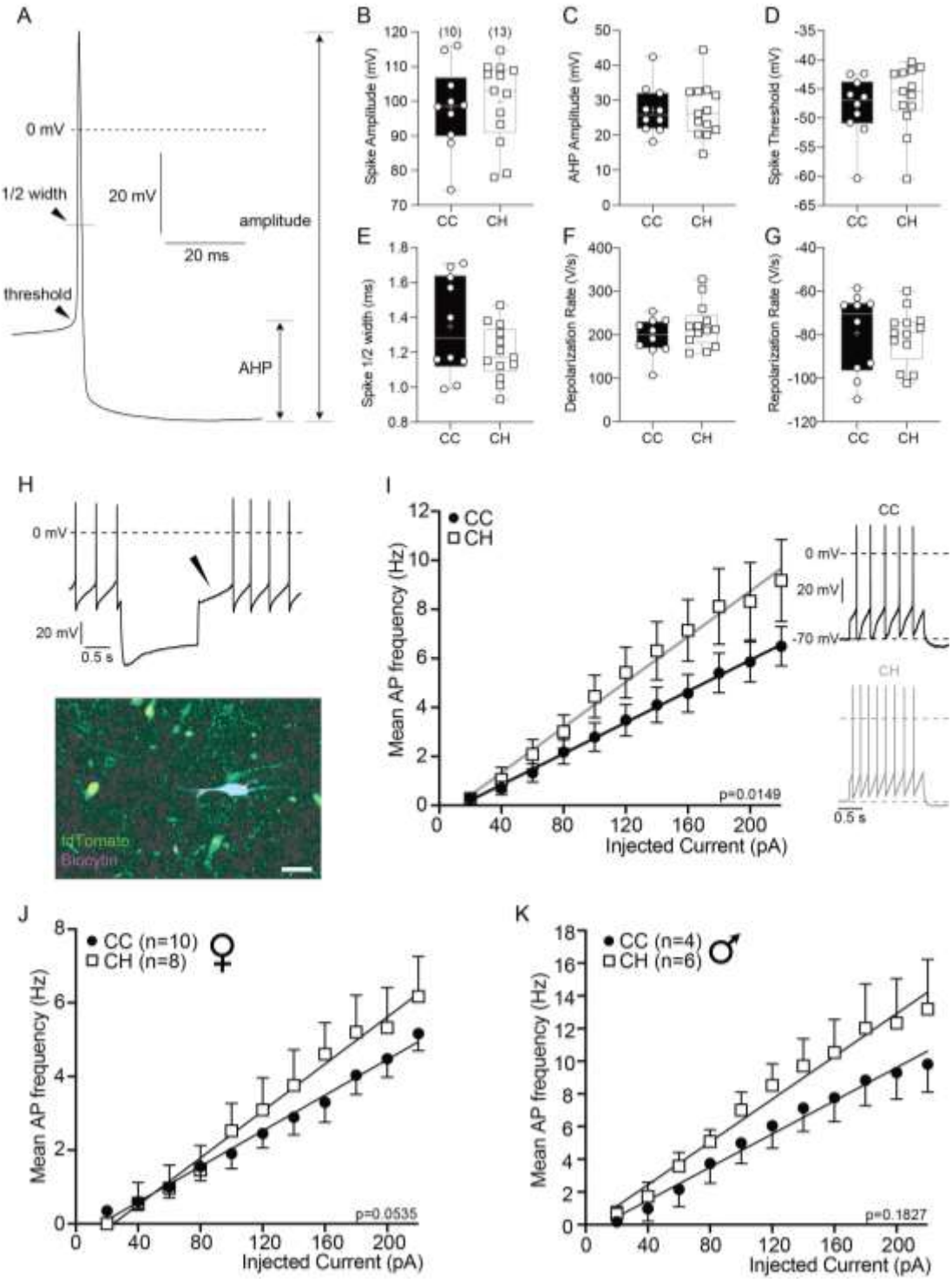
Supplemental Figure 2. Changes in TH immunoreactivity but no differences in DA neuronal number using various DA markers. A) TH staining in male and female CC and CH animals in the dorsal and ventral striatum. White box indicates region of NuAcc Core quantified in B. B) Quantification of signal intensity in males and females in the NuAcc Core. C) Gender specific staining of results shown in Figure 2E. D) TH labeling in a subsection of the VTA in males and females. Quantification of immunohistochemical labeling of E) TH-, F) Calbindin- and G) Aldh1a1-positive

neurons in rostral to caudal sections of the SN and VTA of CC (n=4) versus CH (n=3) animals. **=p<0.01, *=p<0.05 data analyzed via Two Way ANOVA with Bonferonni's post hoc analysis. ##=p<0.01 between diet groups.



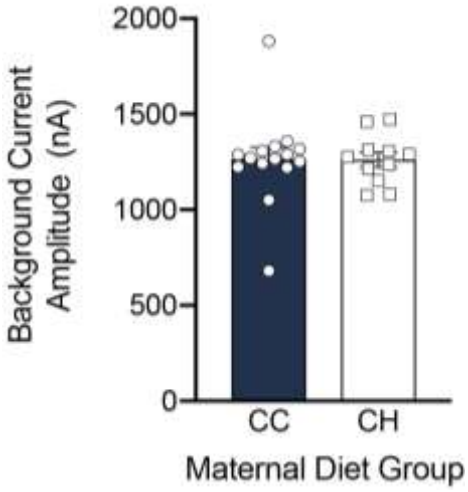
Supplemental Figure 3. Action potential parameters in the Substantia Nigra pars compacta. A) Representative action potential trace in the substantia nigra pars compacta depicting regions and parameters measured. In CC versus CH males and females measurement of B) spike amplitude, C) AHP amplitude, D) Spike threshold, E) Spike ½ width, F) Depolarization Rate, and G) Repolarization rate. For n-values and

statistics, please see Supplemental Table 1. H) Data from Figure 3C analyzed by gender, for description of coloration please see Figure 3 legend. I) Data from figure 3D analyzed by gender. J) Data from Figure 3F analyzed by gender.

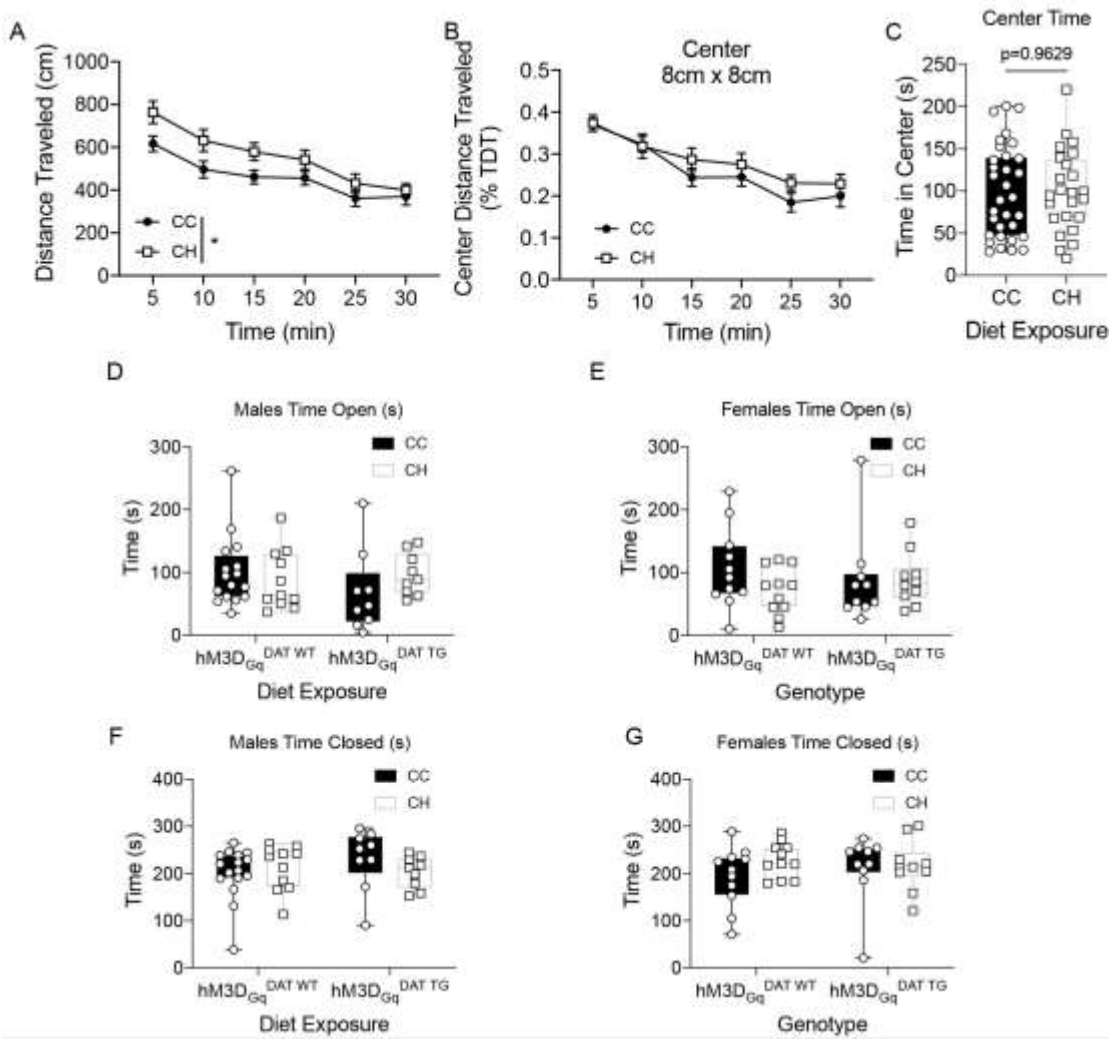


Supplemental Figure 4. Action potential parameters in the lateral Ventral Tegmental Area (lVTA). A) Representative action potential trace in the lateral VTA depicting regions and parameters measured. In CC versus CH males and females measurement of B) spike amplitude, C) AHP amplitude, D) Spike threshold, E) Spike ½ width, F) Depolarization Rate, and G) Repolarization rate. For n-values and

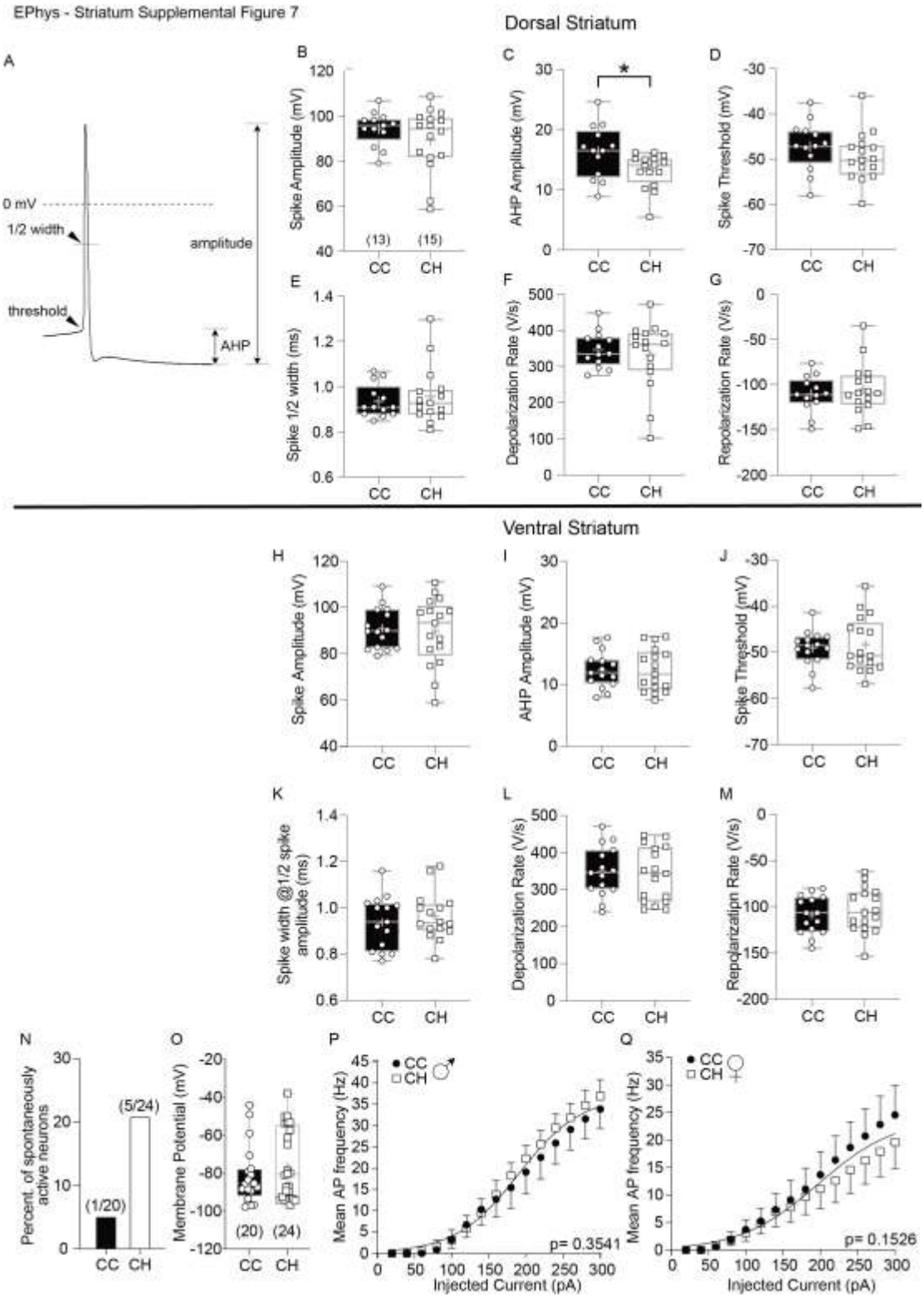
statistics, please see Supplemental Table 2. H) Representative trace of dopamine neuron located in the lVTA (top). Post hoc analysis of recorded neuron (bottom) shows colocalization of biocytin (recorded neuron; magenta) and the dopamine transporter (green). I) Quantification of mean AP frequency with increasing current injection in CC versus CH animals. Comparison of two different traces in CC (top) and CH (bottom) animals. J) As in 1) for females only. K) as in I) for males only. Analysis of Current Injection at was performed using a sigmoidal Boltzmann fit for both data sets, to determine curve fit.



Supplemental Figure 5. Background current in FSCV not different between diet groups. Analysis of background current amplitude in CC versus CH animals subjected to FSCV.

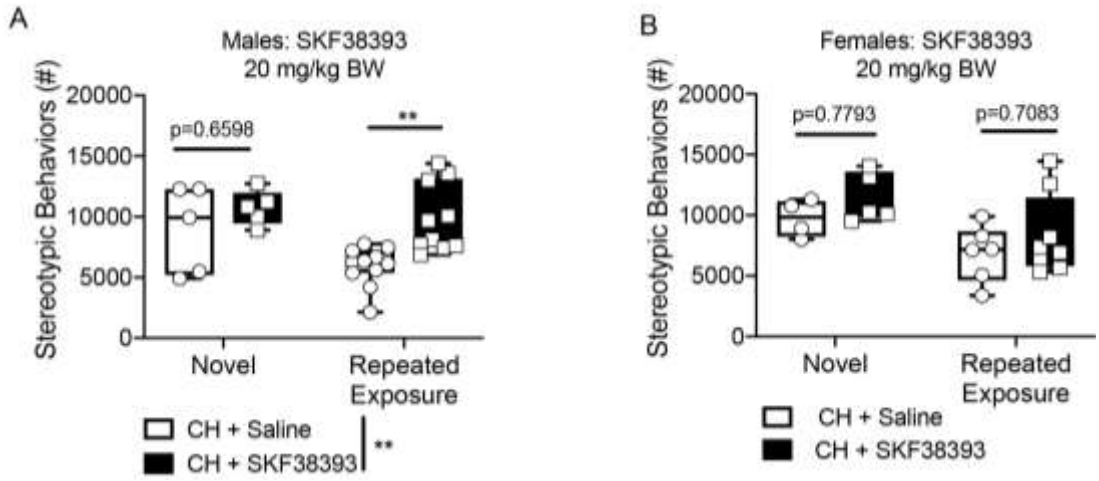


Supplemental Figure 6. Lack of anxiety-like phenotype in CH animals. A) Analysis of distance traveled in the open field test over time in CC versus CH males. B) Center distance as a proportion of total distance traveled over time in CC versus CH males. C) Time spent in the center of the open field test in the initial 5 minutes in CC versus CH males. Time spent in the open arm of the elevated zero maze in D) male and E) female $hM3D_{Gq}^{WT}$ and $hM3D_{Gq}^{TG}$ CC and CH animals. Time spent in the closed arms of the elevated zero maze in F) male and G) female $hM3D_{Gq}^{WT}$ and $hM3D_{Gq}^{TG}$ CC and CH animals. Statistics were performed using a Student *t*-test (C) Two Way ANOVA with Bonferroni's post hoc analysis (D-G) or Two Way ANOVA with repeated measures (A-B). *=$p<0.05$.



Supplemental Figure 7. Action potential parameters in the Dorsal and Ventral Striatum. A) Representative action potential trace in the dorsal striatum depicting regions and parameters measured. In CC versus CH males and females measurement of B) spike amplitude, C) AHP amplitude, D) Spike threshold, E) Spike ½ width, F)

Depolarization Rate, and G) Repolarization rate. For n-values and statistics, please see Supplemental Table 3. In CC versus CH males and females within the ventral striatum, measurement of H) spike amplitude, I) AHP amplitude, J) Spike threshold, K) Spike ½ width, L) Depolarization Rate, and M) Repolarization rate. For n-values and statistics, please see Supplemental Table 4. N) Percentage of spontaneously firing D1 MSNs in the ventral striatum. O) Membrane potential in CC (n=20) versus CH (n=22) D1 MSNs in the ventral striatum. Quantification of mean AP frequency with increasing current injection in CC versus CH P) males and Q) females. Statistics performed using a two-tailed Mann-Whitney test (C). Analysis of Current Injection at was performed using a sigmoidal Boltzmann fit for both data sets, to determine curve fit. *=$p<0.05$.



Supplemental Figure 8. D1R activation in novel environment mimics D1R pharmacological activation. Stereotypic behaviors recorded in A) male and B) female CH animals with either novel (n=4-5 per gender and treatment) or repeated exposure (n=6-11 per gender and treatment) to an open field chamber receiving either saline or the D1R agonist SKF38393 (20mg/kg i.p.). Statistics were performed using Two Way ANOVA with Bonferroni's post hoc analysis. **=p<0.01.

Supplemental Table 1. Electrophysiological parameters SNc DA neurons

	CC				CH				
	n	mean	SD	SEM	n	mean	SD	SEM	p-value
AP Frequency (Hz)	32	2.79	1.168	0.206	34	3.25	1.38	0.237	0.14†
% of silent neurons	5.9 (2/34)				24.4 (11/45)				**0.0338****
Female % of silent neurons	11.1 (1/9)				20 (3/15)				>0.999**
Male % of silent neurons	4 (1/25)				26.7 (8/30)				**0.0307****
% of pacemaker neurons	64.7 (22/34)				44.4 (20/45)				0.1106**
Female % of pacemaker neurons	55.6 (5/9)				46.7 (7/15)				>0.999**
Male % of pacemaker neurons	68 (17/25)				43.3 (13/30)				0.1029*
1st spike latency (s)	11	0.21	0.085	0.026	33	0.15	0.181	0.032	**0.02†**
Female 1st spike latency (s)	4	0.19	0.093	0.047	13	0.07	0.07	0.019	0.07†
Male 1st spike latency (s)	7	0.22	0.085	0.032	20	0.2	0.212	0.047	0.2†
Action Potential Parameters									
Amplitude (mV)	11	107.7	9.79	2.95	28	105.0	8.69	1.64	0.4*
AHP (mV)	11	38.2	5.98	1.80	28	35.8	5.68	1.07	0.26*
Threshold (mV)	11	-43.6	3.08	0.93	28	-46.7	3.75	0.71	0.08†
½ width (ms)	11	1.23	0.203	0.061	28	1.26	0.168	0.032	0.57*
Depolarization Rate (V/s)	11	211.6	43.09	12.99	28	206.5	36.45	6.89	0.71*
Repolarization Rate (V/s)	11	-78.8	16.25	4.9	28	-77	9.68	1.83	0.71†
***: two-tailed unpaired t-test, †: two-tailed Mann-Whitney test, **: Fisher's exact test**									

Supplemental Table 2. Electrophysiological parameters lVTA DA neurons

Current Injections

	CC				CH		
Injected current (pA)	n	mean	SD	SEM	n	mean	SD	SEM
20	14	0.30	0.54	0.14	14	0.28	0.77	0.21
40	14	0.71	0.98	0.26	14	1.06	1.85	0.49
60	14	1.33	1.39	0.37	14	2.09	2.26	0.60
80	14	2.18	1.86	0.50	14	3.01	2.57	0.69
100	14	2.79	2.17	0.58	14	4.45	3.24	0.86
120	14	3.48	2.38	0.64	14	5.42	3.88	1.04
140	14	4.11	2.72	0.73	14	6.31	4.45	1.19
160	14	4.58	2.90	0.77	14	7.14	4.68	1.25
180	14	5.41	3.03	0.81	14	8.12	5.79	1.55
200	14	5.86	3.05	0.82	14	8.33	5.92	1.58
220	14	6.49	2.99	0.80	14	9.18	6.27	1.68

Female	CC				CH		
Injected current (pA)	n	mean	SD	SEM	n	mean	SD	SEM
20	10	0.353	0.605	0.1913	8	0	0	0
40	10	0.598	0.7432	0.235	8	0.56	1.584	0.56
60	10	0.999	0.9374	0.2964	8	0.9675	1.758	0.6214
80	10	1.554	1.231	0.3894	8	1.456	1.889	0.6678
100	10	1.907	1.304	0.4125	8	2.525	2.107	0.745
120	10	2.452	1.237	0.391	8	3.093	2.463	0.8709
140	10	2.892	1.496	0.4731	8	3.759	2.73	0.965
160	10	3.299	1.71	0.5406	8	4.606	2.42	0.8556
180	10	4.035	1.661	0.5252	8	5.201	2.837	1.003
200	10	4.483	1.596	0.5046	8	5.325	3.074	1.087
220	10	5.166	1.48	0.4682	8	6.171	3.078	1.088

Male	CC				CH		
Injected current (pA)	n	mean	SD	SEM	n	mean	SD	SEM
20	4	0.1675	0.335	0.1675	6	0.6617	1.118	0.4564
40	4	0.975	1.536	0.7681	6	1.727	2.106	0.8599
60	4	2.155	2.115	1.058	6	3.58	2.055	0.8388
80	4	3.74	2.413	1.206	6	5.083	1.767	0.7212
100	4	4.99	2.51	1.255	6	7.008	2.688	1.097
120	4	6.053	2.751	1.376	6	8.525	3.221	1.315
140	4	7.138	2.874	1.437	6	9.708	4.084	1.667
160	4	7.765	2.93	1.465	6	10.53	4.959	2.025
180	4	8.835	3.095	1.548	6	12.02	6.635	2.709
200	4	9.3	3.259	1.629	6	12.34	6.644	2.712
220	4	9.815	3.416	1.708	6	13.19	7.434	3.035

Firing Properties and Action potential parameters

	CC				CH				
	n	mean	SD	SEM	n	mean	SD	SEM	p-value
AP Frequency (Hz)	11	3.49	2.844	0.858	13	3.68	2.068	0.574	0.46†
% of silent neurons	21.4 (3/14)				23.5 (4/17)				>0.999**
Female % of silent neurons	42.9 (3/7)				30 (3/10)				0.6437**
Male % of silent neurons	0 (0/4)				28.6 (2/7)				0.4909**
% of pacemaker neurons	45.5 (5/11)				30.8 (4/13)				0.6752**
Female % of pacemaker neurons	57.1 (4/7)				25 (2/8)				0.3147**
Male % of pacemaker neurons	25 (1/4)				40 (2/5)				>0.999**
Action Potential Parameters									
Amplitude (mV)	10	98.12	12.34	3.90	13	99.85	11.97	3.32	0.74*
AHP (mV)	10	27.21	7.03	2.22	13	26.92	7.70	2.14	0.93*
Threshold (mV)	10	-48.13	5.40	1.71	13	-46.44	5.66	1.57	0.32†
½ width (ms)	10	1.35	0.28	0.09	13	1.2	0.16	0.04	0.12*
Depolarization Rate (V/s)	10	195.7	43.3	13.69	13	220.6	51.39	14.25	0.23*
Repolarization Rate (V/s)	10	-79.46	18.50	5.85	13	-80.89	12.59	3.49	0.53†
***: two-tailed unpaired t-test, †: two-tailed Mann-Whitney test, **: Fisher's exact test**									

Supplemental Table 3. Electrophysiological parameters CPu MSN

	CC				CH				
	n	mean	SD	SEM	n	mean	SD	SEM	p-value
Resting Membrane Potential (mV)	20	-82.2	13.2	2.95	22	-68.9	18.91	4.03	**0.02†**
Male Resting Membrane Potential (mV)	12	-77.76	14.64	4.23	10	-71.35	12.03	3.80	0.28*
Female Resting Membrane Potential (mV)	8	-88.84	7.19	2.54	12	-66.82	23.55	6.78	0.08†
Input Resistance at rest (MOhm)	14	101.2	34.18	9.14	12	126.3	55.48	16.02	0.17*
Input Resistance (hyperpolarized) (MOhm)	13	76.1	32.9	9.13	19	79	35.35	8.11	0.81*
Action Potential Parameters									
Amplitude (mV)	13	94.4	7.54	2.09	16	89.5	14.09	3.52	0.28*
AHP (mV)	13	16.3	4.43	1.23	16	13.2	2.89	0.72	**0.03†**
Threshold (mV)	13	-47.1	5.52	1.53	16	-49.4	5.41	1.35	0.26*
½ width (ms)	13	0.94	0.073	0.02	16	0.97	0.127	0.032	0.67†
Depolarization Rate (V/s)	13	346.9	49.81	13.82	16	331.7	94.84	23.71	0.88†
Repolarization Rate (V/s)	13	-110.5	20.34	5.64	16	-105.1	28.81	7.20	0.58*
***: two-tailed unpaired t-test, †: two-tailed Mann-Whitney test**									

Electrophysiological parameters CPu MSN
Current Injections

Male	CC				CH			
Injected current (pA)	n	mean	SD	SEM	n	mean	SD	SEM
20	11	0	0	0	10	0	0	0
40	11	0	0	0	10	0	0	0
60	11	0.73	2.403	0.725	10	1.05	2.571	0.775
80	11	1.36	4.499	1.356	10	2.87	4.479	1.35
100	11	2.39	6.536	1.971	10	5.25	6.947	2.095
120	11	3.2	8.215	2.477	10	7.99	9.984	3.01
140	11	4.54	9.831	2.964	10	10.42	11.84	3.569
160	11	5.63	11.18	3.37	10	12.78	12.97	3.912
180	11	7.11	12.28	3.704	10	15.07	14.51	4.375
200	11	9	14.88	4.487	10	18.8	15.48	4.896
220	11	10.96	15.81	4.767	10	20.71	16.37	5.177
240	11	14.55	15.08	4.546	10	22.36	17	5.377
260	11	17.7	14.97	4.515	10	23.76	17.43	5.513
280	11	20.7	15.43	4.652	10	24.75	17.82	5.635
300	11	23.76	15.96	4.812	10	25.93	18.38	5.811

Boltzmann sigmoidal fit parameters				
	Best fit values	Std. Error	Best fit values	Std. Error
Top	38.24	27.52	35.48	8.111

V50	270.1	89.97	208.8	28.51
Slope	60.3	26.13	45.56	15.62
p-value	**0.0009**			

Female	**CC**				**CH**			
Injected current (pA)	n	mean	SD	SEM	n	mean	SD	SEM
20	8	0	0	0	11	0	0	0
40	8	0.08	0.237	0.084	11	0	0	0
60	8	1.68	3.039	1.074	11	1.05	2.571	0.775
80	8	3.77	5.622	1.988	11	2.87	4.479	1.35
100	8	6.03	7.41	2.62	11	5.25	6.947	2.095
120	8	8.37	9.951	3.518	11	7.99	9.984	3.01
140	8	10.79	11.37	4.021	11	10.42	11.84	3.569
160	8	12.69	12.57	4.445	11	12.78	12.97	3.912
180	8	15.13	12.99	4.594	11	15.07	14.51	4.375
200	8	17.26	13.7	4.845	11	18.8	15.48	4.896
220	8	19.92	14.13	4.996	11	20.71	16.37	5.177
240	8	22.26	13.86	4.899	11	22.36	17	5.377
260	8	24.22	14.14	4.998	11	23.76	17.43	5.513
280	8	26.39	14.23	5.032	11	24.75	17.82	5.635
300	8	28.6	12.87	4.549	11	25.93	18.38	5.811

Boltzmann sigmoidal fit parameters				
	Best fit values	Std. Error	Best fit values	Std. Error
Top	30.54	5.919	26.18	3.482
V50	181.7	29.04	162.1	18.6
Slope	53.47	17.23	41.59	13.54
p-value	0.9323			

Supplemental Table 4. Electrophysiological parameters NAc MSN

	CC				CH				
	n	mean	SD	SEM	n	mean	SD	SEM	p-value
Resting Membrane Potential (mV)	20	-81.6	15.2	3.4	24	-75.7	18.6	3.8	0.46†
Male Resting Membrane Potential (mV)	7	-83.56	8.38	3.17	7	-82.69	12.12	4.58	0.71†
Female Resting Membrane Potential (mV)	13	-80.56	18.08	5.01	17	-72.75	20.25	4.91	0.46†
Input Resistance at rest (MOhm)	10	87.3	32.5	10.3	9	95.8	41.4	13.8	0.63*
Input Resistance (hyperpolarized) (MOhm)	18	60.9	18.9	4.5	19	61.0	20.1	4.6	0.99†
Action Potential Parameters									
Amplitude (mV)	15	91.0	8.87	2.29	17	89.82	14.62	3.55	0.79*
AHP (mV)	15	12.37	2.95	0.76	17	12.42	3.48	0.84	0.99*
Threshold (mV)	15	-49.1	3.88	1.0	17	-48.33	5.9	1.42	0.68*
½ width (ms)	15	0.94	0.12	0.03	17	0.96	0.10	0.03	0.72†
Depolarization Rate (V/s)	15	349.2	67.91	17.54	17	340.0	73.48	17.82	0.72†
Repolarization Rate (V/s)	15	-108.7	20.65	5.33	17	-103.7	24.34	5.90	0.54*
***: two-tailed unpaired t-test, †: two-tailed Mann-Whitney test**									

Electrophysiological parameters NAc MSN
Current Injections

Male	CC				CH			
Injected current (pA)	n	mean	SD	SEM	n	mean	SD	SEM
20	7	0.00	0.00	0.00	7	0.00	0.00	0.00
40	7	0.00	0.00	0.00	7	0.00	0.00	0.00
60	7	0.00	0.00	0.00	7	0.56	1.49	0.56
80	7	0.81	2.14	0.81	7	1.58	4.18	1.58
100	7	3.24	5.33	2.02	7	3.09	6.71	2.54
120	7	6.73	7.86	2.97	7	5.87	8.03	3.03
140	7	10.31	9.79	3.70	7	9.57	8.58	3.24
160	7	12.71	11.08	4.19	7	13.79	8.67	3.28
180	7	15.45	12.89	4.87	7	18.28	7.95	3.00
200	7	19.09	13.60	5.14	7	22.22	8.34	3.15
220	7	22.54	13.67	5.17	7	25.59	8.68	3.28
240	7	25.87	13.12	4.96	7	29.45	8.57	3.24
260	7	29.07	12.73	4.81	7	31.84	8.85	3.35
280	7	31.55	12.16	4.60	7	34.71	9.60	3.63
300	7	33.88	11.93	4.51	7	36.89	10.17	3.85

Boltzmann sigmoidal fit parameters				
	Best fit values	Std. Error	Best fit values	Std. Error
Top	36.06	5.317	37.80	3.102

V50	193.3	19.28	186.2	10.34
Slope	44.95	11.75	39.74	7.008
p-value	0.3541			

Female		**CC**				**CH**		
Injected current (pA)	n	mean	SD	SEM	n	mean	SD	SEM
20	11	0.00	0.00	0.00	14	0.00	0.00	0.00
40	11	0.00	0.00	0.00	14	0.00	0.00	0.00
60	11	0.58	1.70	0.51	14	0.88	2.48	0.66
80	11	1.91	4.42	1.33	14	1.83	3.82	1.02
100	11	3.71	5.97	1.80	14	3.12	5.64	1.51
120	11	5.26	7.85	2.37	14	4.84	7.18	1.92
140	11	7.31	9.72	2.93	14	6.99	10.29	2.75
160	11	9.11	11.72	3.53	14	7.79	10.38	2.77
180	11	11.06	12.73	3.84	14	9.65	12.07	3.23
200	11	13.67	14.02	4.23	14	11.12	13.47	3.60
220	11	16.34	14.61	4.41	14	12.61	14.93	3.99
240	11	18.64	15.50	4.68	14	14.55	15.99	4.27
260	11	20.71	16.42	4.95	14	16.29	16.33	4.37
280	11	22.81	17.14	5.17	14	17.89	17.02	4.55
300	11	24.55	17.99	5.42	14	19.58	17.88	4.78

Boltzmann sigmoidal fit parameters				
	Best fit values	Std. Error	Best fit values	Std. Error
Top	27.27	6.352	21.60	5.871
V50	198.4	32.02	194.9	39.53
Slope	50.31	17.70	54.15	21.44
p-value	0.1526			

Supplemental Table 5. Litter Statistics

		CC			CH		
Wild-Type	n	mean	SEM	n	mean	SEM	p-value
Litter Size	19	5.579	0.3447	15	5.933	0.3581	0.4852
M/F ratio (%)		51.70	4.617		46.28	4.634	0.4198
Transgenic Lines							
Litter Size	29	5.759	0.2610	32	6.125	0.2570	0.3220
M/F ratio (%)		51.89	4.379		50.66	3.964	0.8357

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