Supplementary Material (Supplemental Methods + Supplemental Figures 1-4)

Supplemental Methods

Electrophysiology

The electrophysiological experiments were performed on 5 Gabrg2^{DATCreER} and 4 WT control mice that were anesthetized with urethane. Neuronal activity was extracellularly recorded, and DA-like cells were identified based on electrophysiological criteria (Grace *et al*, 1983; Ungless *et al*, 2012) and localization in VTA and SNc (Fig. S1). The responses of DA-like neurons to bicuculline and muscimol were tested by local, iontophoretic application of the drugs. All technical details are described below.

Surgical procedure: During surgical procedure animals' body temperature was monitored using an automatic heating pad (temperature controller TCP-02, WMT) and maintained at 37°C. Animals were mounted in a stereotaxic frame (SF-1450AP, ASI Instruments Inc.; Warren, MI, USA) on standard ear (EB-918, 18° tip) and incisor bars (RA-200). Recording electrodes were positioned in the VTA/SNc using the following stereotaxic coordinates: 3.1 - 3.2 mm caudal from bregma, 0.7 - 0.9 mm lateral from the middle of the sagittal sinus, 4.5 - 5 mm ventral from the skull surface at bregma.

Recording and iontophoretic drug application: Extracellular recordings were performed using 4-barrel microiontophoretic pipettes that were prepared from borosilicate glass capillaries (o.d.: 1.5 mm, i.d.: 0.86 mm, with internal filament; Sutter Instruments, USA) and pulled using a vertical puller (model PE-21, Narishinge International Instruments, Japan). The recording barrel was filled with 2% Chicago Sky Blue in 2 M NaCl (impedance 7-10 MΩ at 1000 kHz). The surrounding barrels were filled with muscimol (20 mM in 0.9% NaCl, pH 4.5, Tocris, Bristol, UK), bicuculline (20mM in 0.9% NaCl, pH 4, Tocris, Bristol, United Kingdom) and 0.9% NaCl (current balancing barrel). A positive current in the range of 5-10 nA was used for the bicuculline and muscimol ejections and negative retention current (5nA) was used between the drug applications. All drug applications and automatic current balancing were performed using a 2-channel iontophoresis amplifier (MVCS-02C, NPI electronic GmbH, Germany). Extracellular signal was amplified (10 000x) and filtered (300-5000 Hz) using an A-M Systems microelectrode amplifier (model 1800; Carlsborg, WA, USA). Experimental control and signal digitalisation was performed using a Micro 1401 mkII interface equipped with Spike2 software (Cambridge Electronic Design Inc.; Cambridge, UK). After the end of the recordings, a negative current of 15 μA (isolated pulse stimulator; model 2100; A-M Systems; Carlsborg, WA, USA) was passed through the recording pipette for 15 minutes to deposit Chicago Sky Blue at the recording site.

Identification of dopamine neurons: Neurons were classified as dopamine-like when they met the following, previously established criteria (Grace and Bunney, 1984): broad (>1.1 ms; length measured

from AP initiation to the minimum of the negative phase), triphasic action potential and a firing rate below 10 Hz. Additionally, a portion of the recorded neurons were tested for their reaction to systemic (i.p.) application of D2 receptor agonist (Fig. S2; quinpirole, 0.5 mg/kg, diluted in saline, Tocris, Bristol, UK) followed by application of D2 receptor antagonist (Fig. S2; eticlopride, 5 mg/kg, diluted in saline, Tocris, Bristol, UK). The final classification of the neurons that were DA-like was performed after a histological confirmation of the recording sites within the VTA or SNc (Fig. S1).

Histology: At the end of the experiments, the animals were sacrificed by decapitation, and their brains were extracted, fixed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.2) for at least 48 hours and then sliced (50 µm thick sections) in the coronal plane using a microtome (Leica VT1000S, Heidelberg, Germany). Sections containing dye deposits were photographed to verify the placement of the recording with reference to a stereotaxic atlas of the mouse brain (Fig. S1).

Data analysis: The spontaneous basal activity was recorded in each putative DA-like neuron for at least 100 s before the drug was applied. For further analysis, two 40 s-long periods were used, one of which referred to basal activity and one that was performed during drug application (muscimol or bicuculline). The calculation of action potential durations and spontaneous firing rates, the analysis of interspike intervals, and the detection and analysis of bursts of action potentials were performed off-line using custom-made Spike2 scripts (Cambridge Electronic Design Inc.; Cambridge, UK). Bursts were detected based on the criteria of Grace and Bunney (Grace *et al*, 1984). An analysis of burst parameters was performed only in the cells that generated at least one burst within the subjected periods (baseline and drug application). The pattern of activity in the recorded DA-like neurons was classified as either non-bursting or bursting based on the percentage of action potentials that were generated in bursts (<10%: non-bursting; >=10%: bursting).

Go/no-go test

Animals were trained in mouse operant chambers (ENV-307W-CT, Med Associates Inc., USA) enclosed in cubicles equipped with a fan. Each chamber was equipped with a dual cup liquid receptacle, a nose-poke port containing a cue-light, located on the left or right side of a liquid receptacle, counterbalanced between animals and a stimulus light located above the nose-poke port. House light and a 65 dB, 2.9 kHz tone generator were located on the wall opposite to the liquid receptacle. A 0.01% saccharin (Sigma-Aldrich) dissolved in deionized water was delivered into individual cup by an infusion pump (PHM-100, Med Associates Inc., USA) containing a 10 ml syringe connected to the liquid receptacle via silicone tube.

A cohort of 20 male wild-type mice (mean age 20.75 ± 0.92 weeks; mean weight 30.19 ± 0.43 g) and 13 male Gabrg2^{DATCreER} mice (mean age 19.92 ± 0.76 weeks; mean weight 31.66 ± 0.69 g) was restricted to 1.5 ml of water per day, a week before behavioral testing. This water restriction schedule was maintained for the duration of the experiment. Mice were trained 5-7 days per week and their body weight was checked on each day. Additional water access was provided in cases when mice reached below 80% of their initial body weight. One Gabrg2^{DATCreER} mouse did not drink water when it was provided and was excluded from the experiment.

Mice were first familiarized with the operant chamber and liquid reward for 30 minutes. A 20 µl of water sweetened with 0.01% saccharin was delivered into receptacle in 60 s intervals. On subsequent days mice were trained under continuous reinforcement schedule (CRF) and were rewarded with 10 µl of saccharin solution by poking their noses into the active port (with cue-light on). Animals were trained until they reached the criterion of 60 rewarded responses in 45 minutes. All animals met the criterion and advanced to the next phase.

Animals underwent two training phases during which they learned to respond to a 'go' signal (cue-light in the nose-poke port), presented for 30 s during the first phase and for 10 s during the second phase. Beginning of each trial was signaled by a stimulus light located above the nose-poke port. At the same time, pre-cue period ranging from 9 to 24 s (with 3 s increments) was initiated, after which the 'go' signal was presented. Responses during the final 3 s of the pre-cue period caused the trial to reset. Correct 'go' response (a 'hit') resulted in delivery of 10 µl of saccharin solution. Then, a 10 s inter-trial interval (ITI) followed. Trials in which animals failed to respond to a 'go' signal (a 'miss') were punished by 5 s time-out period during which the house light was lit, and after which the 10 s ITI followed (with the house light off). The completion criterion for both phases were 60 completed trials and fewer than 10 misses over two consecutive sessions. 3 wild-type mice did not pass the criterion in the first training phase, and additional 4 wild-type mice did not complete the second phase.

In the go/no-go discrimination task, animals had to discriminate between 'go' (cue-light in the nose-poke port presented for 5 s) and 'no-go' signals (cue-light and a continuous 65 dB 2.9 kHz tone presented for 5 s). Refraining from responding during 'no-go' signal presentation (a 'correct rejection') was rewarded with delivery of 10 µl of saccharin solution. Conversely, a nose-poke during the 'no-go' signal (a 'false alarm') resulted in immediate trial termination and transition into 5 s time-out period, followed by 10 s ITI. Responses made during presentation of 'go' signal had same consequences as described above. Animals were tested for ten consecutive sessions (each comprising 60 trials), during which 'go' and 'no-go' signals were presented randomly. A single session contained 30 'go' and 30 'no-go' trials.

Supplementary figures

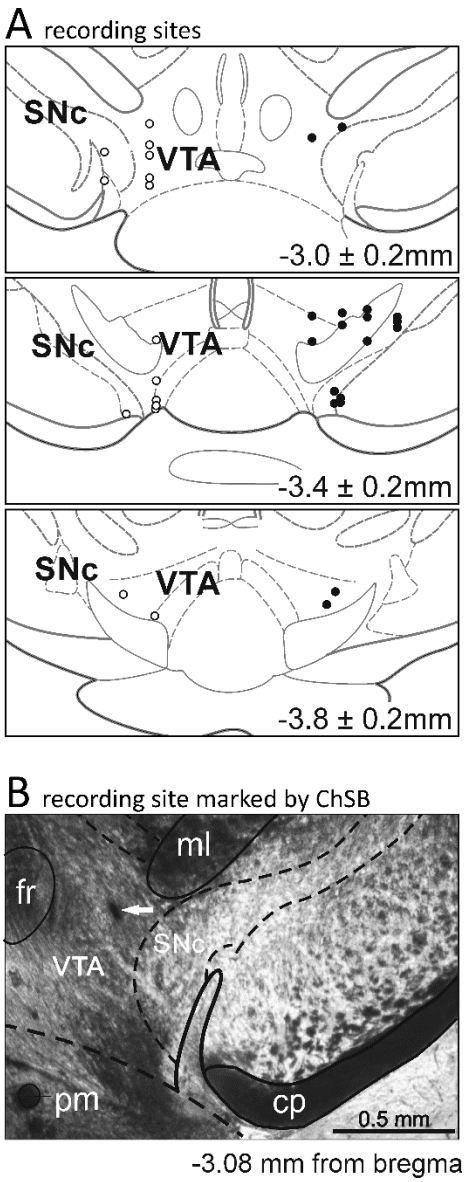

Figure S1. Histological verification of recording sites of DA-like neurons in VTA and SNc. Panel A - localization of recorded DA-like cells in WT animals (left side; ○) and Gabrg2^{DATCreER} animals (right side; ●) depicted on coronal sections based on mouse brain stereotaxic atlas. Anterior-posterior distance from bregma point is indicated in the bottom-right corner of each section. For clarity, recording sites from control and mutant animals were depicted unilaterally (left - WT; right - mutants). B - example of coronal section from the mouse brain with recording site marked by Chicago Sky Blue deposition (pointed by white arrow) within VTA. VTA – ventral tegmental area; SNc – substantia nigra pars compacta; ml – medial lemniscus; cp – cerebral peduncle; fr – fasciculus retroflexus; pm – principal mammillary tract.

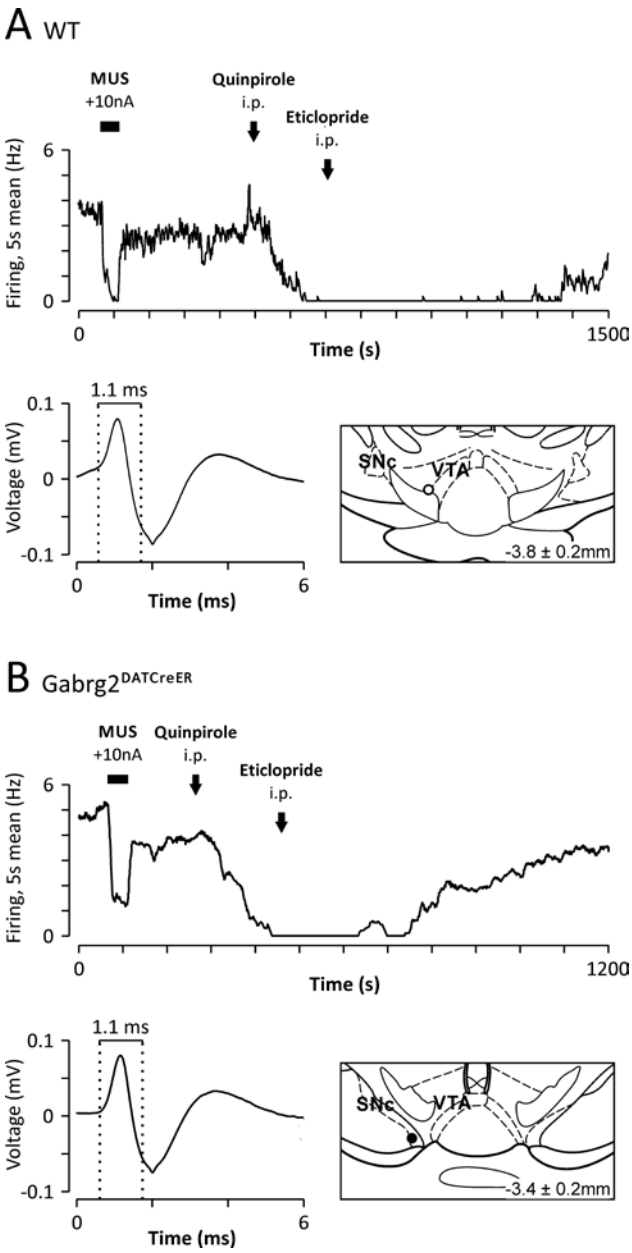

Figure S2. DA-like neurons were additionally identified basing on their reaction to i.p. application of D2 receptor agonist (quinpirole) and antagonist (eticlopride). A- effect of i.p. injections of quinpirole and eticlopride on spontaneous activity of DA-like neuron in VTA of control animal. B -effect of i.p. injections of quinpirole and eticlopride on spontaneous activity of a DA-like neuron in SNc of a Gabrg2^{DATCreER} animal. In both panels (A and B): top graphs depict firing rate of single DA-like neuron in baseline conditions, during muscimol (MUS) Iontophoretic application and after i.p. injection of quinpirole followed by eticlopride; bottom-left panels show averaged extracellular action potential (AP) waveform of tested DA-like neuron (the dashed lines mark AP length criterion for identification of DA-like neurons); bottom-right panels depict location of recorded neurons (O - WT; ● - Gabrg2^{DATCreER}) on coronal section from mouse brain atlas. Intraperitoneal (i.p.) injections of drugs are marked with arrows and iontophoretic application of muscimol is indicated by the black bars.

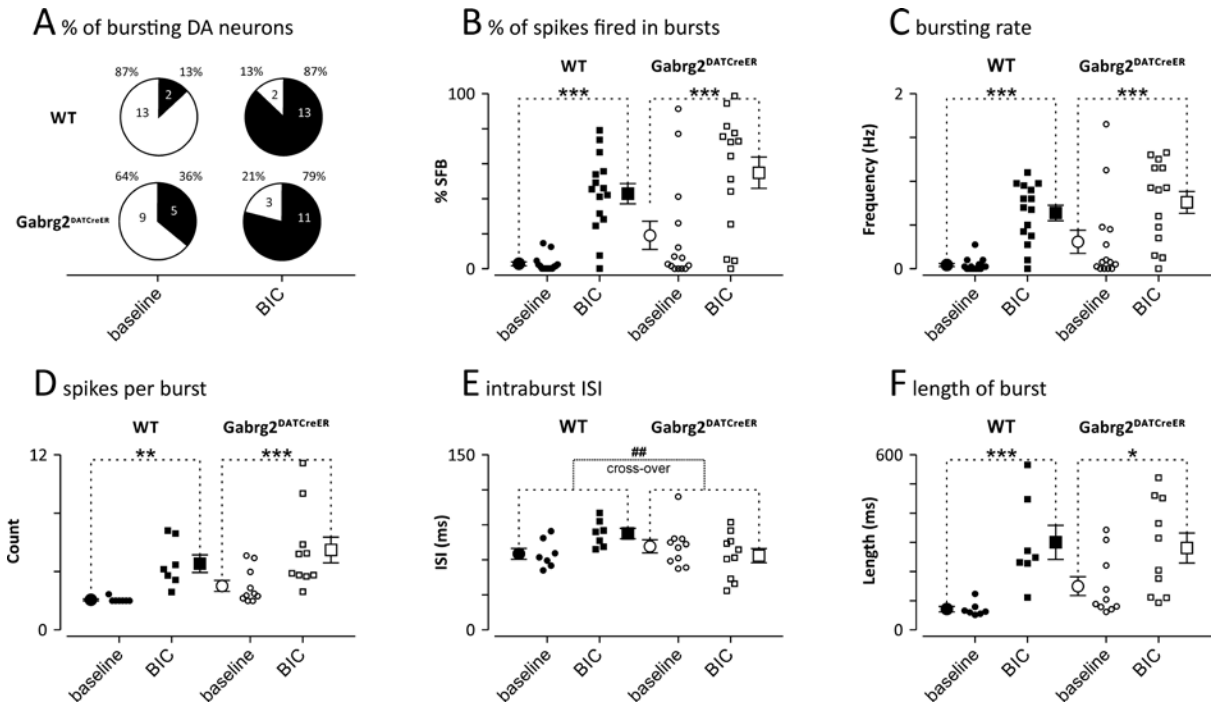

Figure S3. Bicuculline induced bursting of DA-like neurons of Gabrg2^{DATCreER}and WT mice don't differ. Pie charts in panel A show that bicuculline (BIC) induced similar increase in the percentage of bursting DA-like neurons in the VTA and SNc of Gabrg2^{DATCreER} and WT mice. Similarly all parameters of bursting activity (B, C, D and F), except intraburts ISI (panel E), were similarly increased by the application of bicuculline to DA-like neurons in Gabrg2^{DATCreER}and WT mice. Note that although neither genotype nor treatment had not significantly influenced intraburst ISI, there is significant cross-over interaction (i.e. bicuculline application induces opposite effects in Gabrg2^{DATCreER} and WT mice). WT: ● - baseline, ■ - bicuculline; Gabrg2^{DATCreER}: O - baseline, □ - bicuculline; Vertical lines with whiskers indicate the mean value and SEM. '*'indicates Bonferroni corrected t-test that resulted in $p < 0.05$. ** or $^{\#\#}$ indicates $p < 0.01$, *** indicates $p < 0.001$.

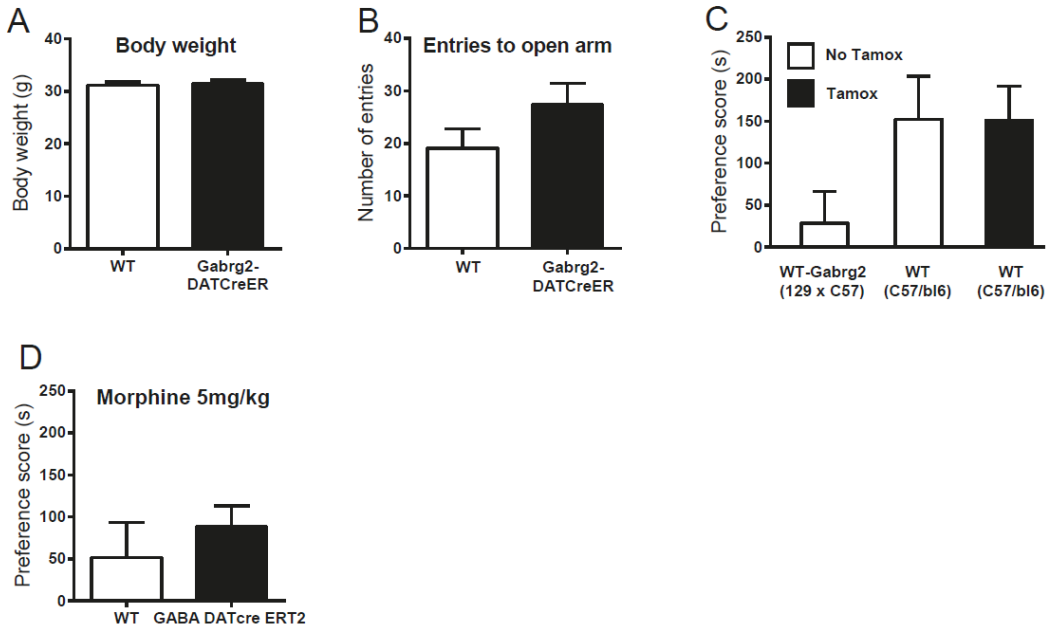

Figure S4. Control experiments for the behavioral analysis. A, Body weight of two cohorts of mice at the start of the behavioral experiments. B, Entries to the open arm in the elevated plus-maze experiment. C, Ethanol-induced CPP in mice with different background. Some groups were pre-treated with tamoxifen and some not. D, CPP induced by a low dose (5 mg/kg) of morphine.