Online Methods

Animals: 2-3 week (*in vitro* electrophysiology) and 20-32 week (*in vivo* electrophysiology/behavior) old WT C57BL/6 mice, Pitx3-GFP knock-in mice[1], GAD67-GFP Δneo mice[2], α1(H101R) knock-in mice[3] and α3(H126R) knock-in mice[4]. All procedures were approved by the local ethics committee as well as the cantonal authorities of Geneva.

In vitro **electrophysiology:** Horizontal slices (250 μm thick) of the midbrain were prepared as described previously[5]. Slices were kept in artificial cerebrospinal fluid (ACSF) containing in mM 119 NaCl, 2.5 KCl, 1.3 $MgCl_2$, 2.5 $CaCl_2$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 11 glucose and bubbled with 95% O_2 and 5% CO_2. The whole-cell voltage-clamp recording technique was used (31–33 °C, 2 ml/ min, submerged slices) to measure synaptic responses of DA neurons, mIPSCs and holding currents of DA or GABA neurons of the VTA. The holding potential was -60 mV and the access resistance was monitored by a hyperpolarizing step to -90 mV with each sweep every 10 s. Experiments were terminated if the access resistance varied more than 20%. Synaptic currents were evoked by stimuli (0.1 ms) at 0.05 Hz through bipolar stainless steel electrodes positioned rostral to the VTA. When EPSCs were recorded, the internal solution was composed of (mM): CsCl 130, NaCl 4, $MgCl_2$ 2, EGTA 1.1, HEPES 5, Na_2ATP 2, Na_2-creatine-phosphate 5, Na_3GTP 0.6 and spermine 0.1, while for mIPSCs the internal solution used contained K-Gluconate 30, KCl 100, $MgCl_2$ 4, EGTA 1.1, HEPES 5, Na_2ATP 3.4, creatine-phosphate 10, Na_3GTP 0.1. Currents were amplified (Multiclamp 700A, Molecular Devices, Sunnyvale, CA) filtered at 1 kHz and digitized at 5 kHz (National Instruments Board PCI-MIO-16E4, Igor, Wave Metrics, Lake Oswego, OR). As the liquid junction potential was -3 mV, traces were not corrected. Recordings of EPSCs were carried out in the presence of picrotoxin (100 μM) and d-APV (50 μM). The rectification index (RI) was calculated by dividing the amplitude of the AMPAR-EPSC measured at -65 mV by the amplitude at +35 mV. sIPSCs were recorded with continuous bath-application of kynuric acid (2 mM), and TTX (500 nM) was added to measure mIPSCs. When sIPSCs were recorded (Fig. 4), the bath-applied ACSF contained a Ca^{2+}/Mg^{2+} ratio of 3-6. The goal was to increase the number of interneuronal spikes while interfering with the GABAergic output per spike as little as possible. The multiplicity factor was calculated following the protocol of Hsia et al[6]. At the end of each experiment, picrotoxin (100 μM) was bath-applied to verify that the recorded current was mediated by $GABA_A$Rs.

In vivo **electrophysiology:** Mice were initially anaesthetized with 4% chloral hydrate (480 mg/kg, i.p.), supplemented each hour with a lower dose (120 mg/kg i.p.) to maintain optimal anaesthesia

throughout the experiment. Animals were positioned in a stereotaxic frame (MyNeurolab, St. Louis, MO) and body temperature was maintained at 36-37 °C using a feedback-controlled heating pad (Harvard Apparatus, Holliston, MA). An incision was made in the midline to expose the skull. A burr hole was unilaterally drilled above the VTA (AP: -3.0 to 3.4, ML: -1.1 to 1.4, DV -4 to 4.5 mm from the bregma (Paxinos and Franklin, 2004) and the dura was carefully retracted. Electrodes were broken back to give a final tip diameter of 1-2 µm and filled with 2% Chicago Sky Blue dye in 0.5 M Na-acetate. All electrodes had impedance of 15-25 MΩ. They were angled by 10° from the vertical, slowly lowered through the burr hole with a micro drive (Luigs Neumann, Ratingen, FRG) and positioned in the VTA. All electrode descents within a single animal were a minimum of 100 µm apart. A reference electrode was placed in the subcutaneous tissue. Electrical signals were AC-coupled, amplified (Neurodata, USA), and monitored in real time using an audiomonitor (homemade). Signals were filtered on-line (Humbug, Quest scientific) and digitized at 20 kHz (for waveform analysis) or 5 kHz (Igor, WaveMetrics, Lake Oswego, OR). The bandpass filter was set between 0.3 and 5 kHz. Extracellular identification of VTA neurons was based on their location as well as on their established electrophysiological properties (DA neurons: biphasic action potential of more than 1.1 ms duration, firing frequency of 0.5 to 7 Hz and spike height accommodation during bursts)[7,8]. In addition we discriminated between the two populations using an aversive electrical footshock and response to morphine. The drugs were injected through the tail vein via a cannula. Following completion of recordings, Chicago Sky Blue dye was deposited by iontophoresis (-15 µA, 15 min) to mark the position of the final recording site. At the end of the experiment, the brain was kept at -20°C in a solution of methyl butane. 50 µm thick coronal sections were cut on a cryostat, stained with luxol fast blue/cresyl violet and the recording site was verified by light microscopy.

Stereotaxic injection: WT and α1(H101) mice were anaesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). The animal was then placed in a stereotaxic frame (MyNeurolab, St. Louis, MO). The VTA coordinates were ML ± 0.8, AP -2.4, DV - 4.4 mm from Bregma and verified with ink injections. 5 µl of a 8 mg/ml MDZ solution or 5 uL ACSF were injected bilaterally over 10 minutes. The animal was sutured and recovered for 24 h until *in vitro* recordings were made.

Immunohistochemistry: GAD67-GFP Δneo mice were anaesthetized with Nembutal (50 mg/kg) and perfused transcardially with 4% paraformaldehyde in phosphate buffer. The brain was extracted and post-fixed for 3 h, cryoprotected in 30% sucrose in PBS, frozen, and cut at 40 µm with a sliding microtome. Triple immunofluorescence with guinea pig antibody against the α1 subunit or the α3

subunit, a mouse antibody against TH, and a rabbit antibody against eGFP was performed as previously described[9] in perfusion-fixed transverse sections from the brain of GAD67-GFP Δneo mice. Images were taken with a laser scanning confocal microscope using a 20x (N.A. 0.8) or a 63x (N.A. 1.4) objective, using sequential acquisition of separate channels to avoid cross-talk. The fraction of neurons single and double-labeled for these markers was assessed pair-wise (e.g., α1/TH or α3/GAD67-GFP) in 4 equally spaced sections through the VTA per mouse (n=4) and expressed as % of the total number of cells counted.

Oral self-administration: Mice were habituated to handling for one week and housed with free access to two 450-ml plastic bottles in their home cage. Two days before the test, 4% sucrose was added to both bottles. During the test mice had access to bottles containing either MDZ (0.005 mg/ml) in sucrose or sucrose alone. For the sucrose preference experiment in α1(H101R) mice, sucrose was compared against water. In cases where mice spontaneously preferred one bottle to another during the pretest phase, MDZ was always added to the least-preferred bottle during the test phase. To determine MDZ preference, the relative consumption of MDZ solution to the control solution was calculated.

Drugs: MDZ, DZ, FZ and Flu were provided by Roche. ZOL and L- (L-838 417) were supllied by Tocris and Morphine-HCl by the pharmacy of the University hospital of Geneva. Drugs were dissolved in saline for i.p. and i.v. injections, in ACSF for intra-VTA injections and in DMSO for bath-applications. The final DMSO concentration was 0.1%.

Statistics: Grouped data are expressed as means ± SEM or box-plots (median, interquartile, and 90th and 10th percentiles). For statistical comparisons the one-way Anova, Bonferroni matched or the paired Student's t-tests were used. The levels of significance are indicated as follows: * $P<0.05$, ** $P<0.01$, *** $P<0.001$. The Kolmogorov Smirnov test was used to compare cumulative probability plots.

References

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