

Neuron, Volume 73

Supplemental Information

GABA Neurons of the VTA

Drive Conditioned Place Aversion

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This manuscript has four (4) supplemental figures associated with the main figures.

Figure S1. *In vitro* functional validation of the selective expression of ChR2-eYFP in GABA VTA cells. *Is associated with main Fig. 1*

Figure S2. *In vivo* electrophysiological characterization of VTA neurons in anaesthetized mice. *Is associated with main Fig. 2*

Figure S3. The footshock induced-inhibition of DA neurons is abolished with bicuculline, a GABA_A receptor antagonist. *Is associated with main Fig. 3*

Figure S4. Operant conditioned place aversion with GADcre mice infected with AAV5-flox-ChR2-eYFP and THcre mice infected with AAV5-flox-eNpHR3.0-eYFP or AAV5-flox-eYFP. *Is associated with main Fig. 4*

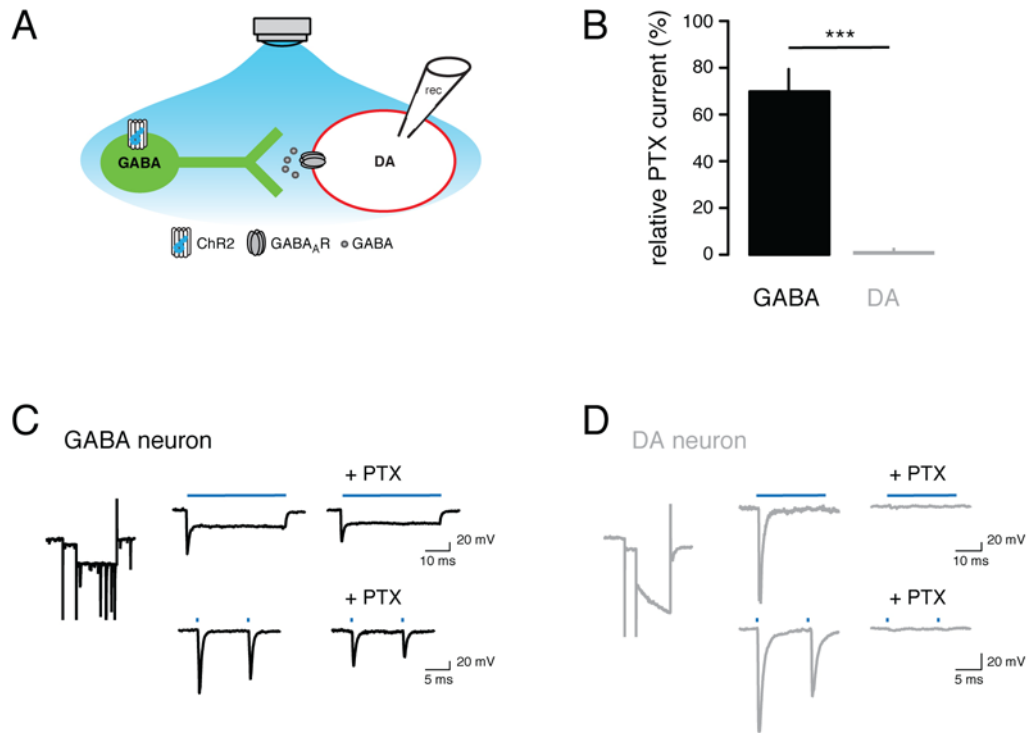


Figure S1. *In vitro* functional validation of the selective expression of ChR2-eYFP in GABA VTA cells.

(A) Schematic showing ChR2-eYFP protein selectively expressed in VTA GABA neurons of GADcre mice. Excitation of GABA neuron is induced by blue light, resulting in a fast GABA_A receptor-mediated IPSC recorded from VTA DA neurons. (B) Bargraph showing the relative amplitude of remaining photocurrents and blue light-evoked synaptic IPSCs in GABA and DA cells, respectively after bath application of PTX (100 μ M, n=3-4, GABA: 71 ± 9 % vs DA 2 ± 1 % $t_{(4;5)} = 8.8$ *** $p < 0.001$). (C) Example traces of blue light-evoked photocurrent and IPSCs recorded from a GABA neuron (infected green cell, absence of I_h current). (D) Example traces of blue light-evoked synaptic IPSCs recorded from a DA neuron (non green cell, presence of I_h current). Only synaptic currents are abolished with picrotoxin (100 μ M). Blue bars indicate blue light stimulation (one pulse for 400ms or two pulses for 4ms 50ms apart).

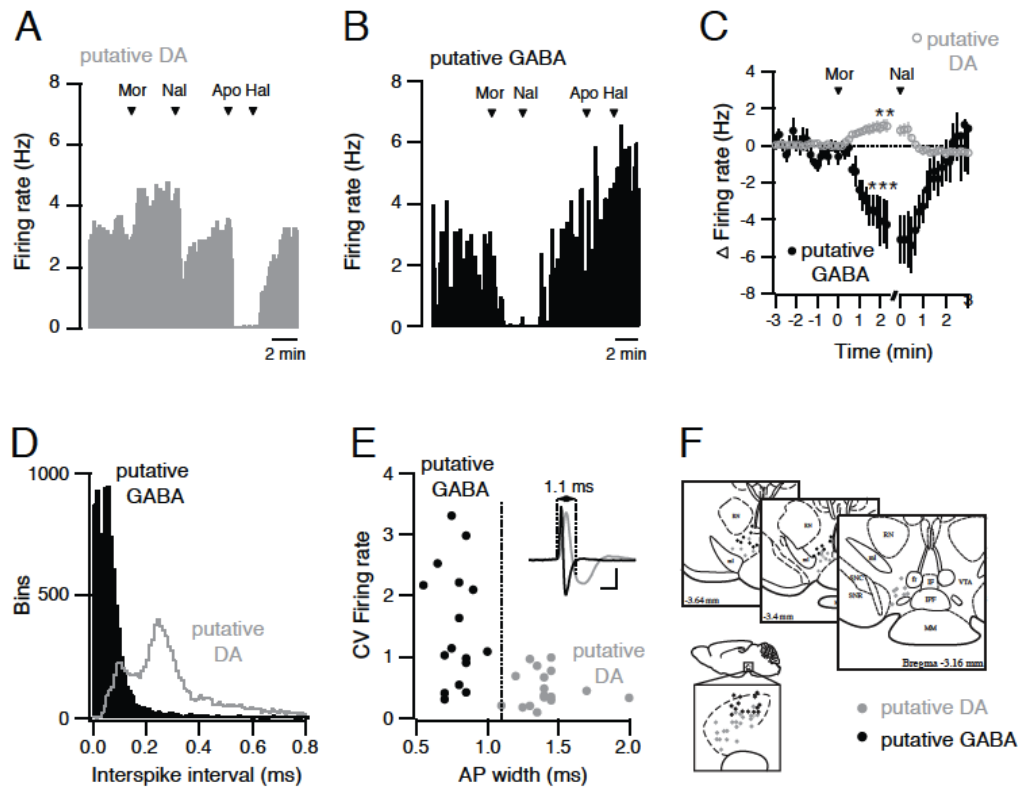


Figure S2. *In vivo* electrophysiological characterization of VTA neurons in anaesthetized mice.

(A) Representative firing frequency plot of a putative DA neuron and (B) of a putative GABA neuron of the VTA during consecutive i.v. injections of morphine (2 mg/kg), naloxone (1 mg/kg), apomorphine (0.05 mg/kg) and haloperidol (0.2 mg/kg). (C) Normalized firing rate of putative DA and GABA cells in response to consecutive i.v. injections of morphine and naloxone. ** $p < 0.01$, *** $p < 0.001$, $n = 12-17$. (D) Distribution of interspike intervals of recorded putative DA and GABA neurons. (E) Plot showing CV firing rate as a function of action potential width for both types of cells. The vertical bar and the inset represent the limit (1.1ms) for which a cell is considered a DA or a GABA cell. (F) Mapping of recorded sites within the VTA. Diagrams adapted from Paxinos and Franklin, 2004.

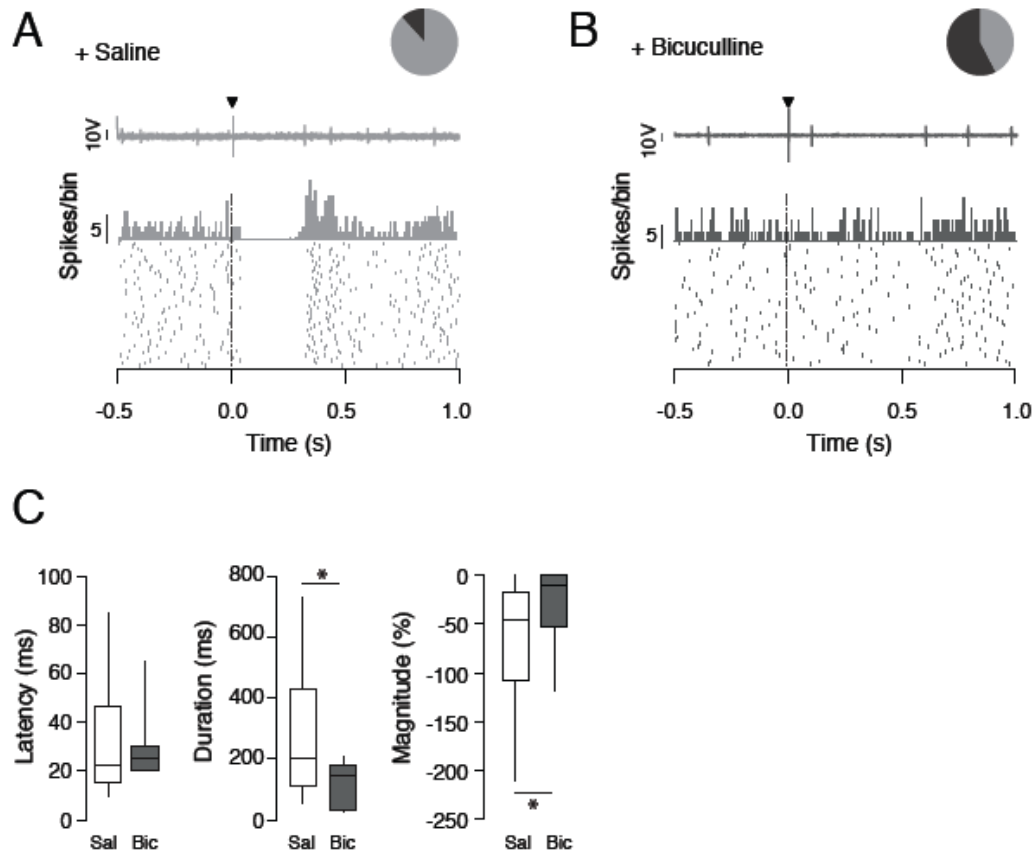


Figure S3. The footshock induced-inhibition of DA neurons is abolished with bicuculline, a GABA_A receptor antagonist.

(A) Single unit recording, PSTH and raster plot of a representative putative DA neuron inhibited by an electrical stimulation while saline was added in the recording pipette. The inset shows that 88% of the recorded cells were inhibited whereas the other 12 % did not respond. (B) Same as in A. for a representative putative DA neuron not inhibited by an electrical stimulation while bicuculline was added in the recording pipette. The inset is a pie chart showing that 58% of the cells were non-responsive to the electrical stimulation in presence of bicuculline in the recording pipette. (C) Boxplot representation of the response latency, duration and magnitude for saline and bicuculline in WT mice, n=12-26. Even in the 14 cells that still showed inhibition with bicuculline, the duration of inhibition was significantly reduced (latency: saline 32 ± 21 ms vs bicuculline 21 ± 13 ms, $t_{(33)} = 0.3$ p=0.76, duration: saline 265 ± 189 ms vs bicuculline 130 ± 70 ms p=0.05 $t_{(1.9; 33)}$, magnitude saline $-57 \pm 11\%$ vs bicuculline $-28 \pm 7\%$ *p= 0.003 $t_{(47)} = 2.17$. Data are expressed as median (line), interquartile (box) and 75th and 25th percentiles.

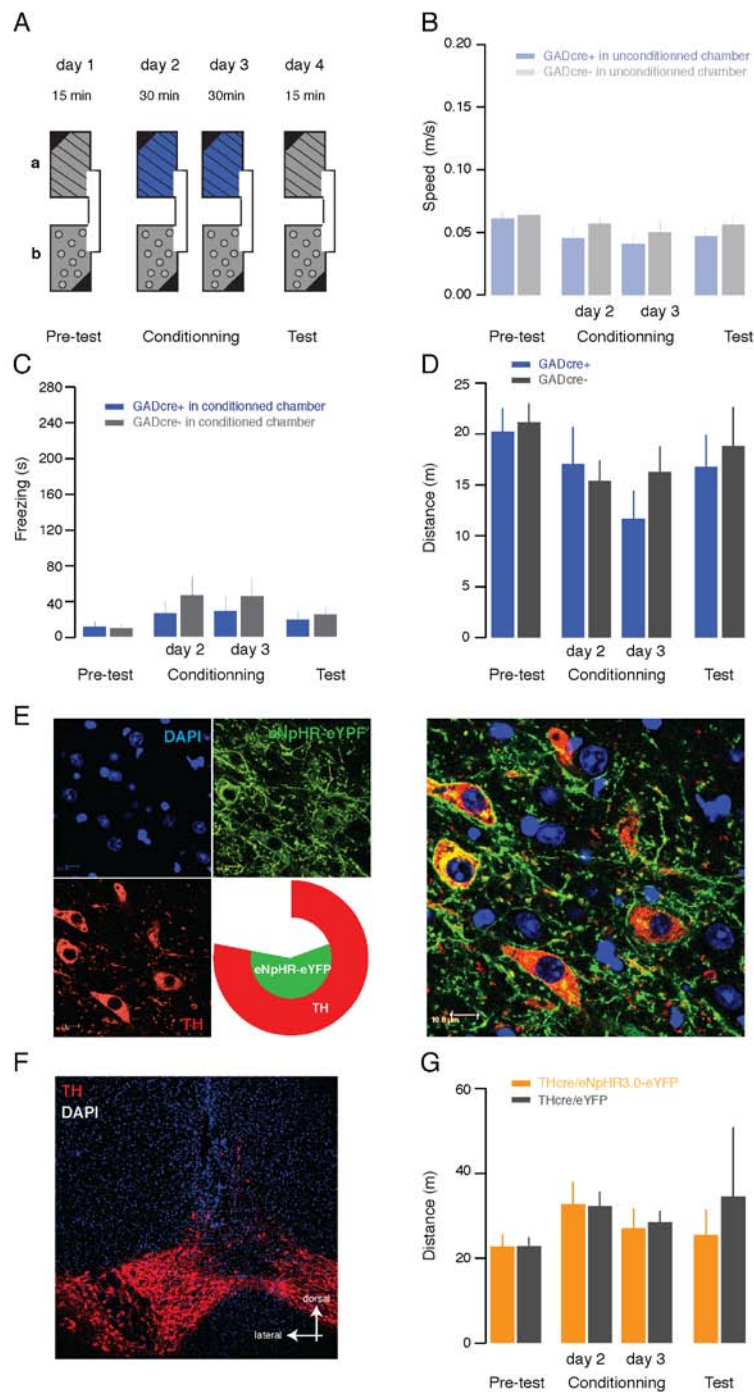


Figure S4. Operant conditioned place aversion with GADcre mice infected with AAV5-flox-ChR2-eYFP and THcre mice infected with AAV5-flox-eNpHR3.0-eYFP or AAV5-flox-eYFP.

(A) Conditioning paradigm for blue light-induced CPA. AAV5-flox-ChR2-eYFP VTA infected GADcre+ and GADcre- mice were conditioned with GABA cells excitation through blue light stimulation in the chamber a. In the paradigm, the laser was turned on as soon as the mouse entered the conditioned chamber a and switched off when the mouse left the chamber. The laser was not used during pre-test and test days. Mice could freely explore the entire apparatus in all sessions. (B)

Bargraph showing the average speed for each group in the unconditioned chamber. Bars are means \pm SEM (n=9-14) $p > 0.05$. (C) Bargraph showing the time spent in freezing status for each group in the conditioned chamber. Average speed and freezing parameters were comparable for both group in the mentioned chamber $p > 0.05$. (D) Bargraph showing the locomotor activity on each session for GADcre- and GADcre+ mice. (E) Immunohistochemical staining for tyrosine hydroxylase (TH, red) and nucleoli (DAPI, blue) in VTA slices of THcre+ mice infected with AAV5-flox-eNpHR3.0-eYFP (green) in the VTA. The pie chart is a quantitative representation of the staining. The inner segment corresponds to the fraction of eNpHR3.0-YFP-positive cells and the outer segment shows quantification of the TH-positive cells (n=4 mice). Overlap between inner and outer segments represents colocalization. (F) Coronal slice of a VTA expressing NpHR3.0-eYFP in DA cells showing the restriction of the expression of NpHR3.0-eYFP to the VTA and the path of the guide cannula down to the VTA. (G) Bargraph showing the locomotor activity on each session for THcre/eNpHR3.0-YFP and THcre/eYFP mice. Amber light itself does not perturb locomotor activity. Bars are means \pm SEM (n=10), $p > 0.05$.