

Figure S1. The tVTA neurons project to the SNc. **(a)** Experimental protocol for BDA injection into the tVTA ($n = 7$). **(b)** Example of a BDA injection site into the tVTA (black) with TH co-labeling (brown). **(c)** Example of the anterograde labeling observed in the SNc following BDA injection into the tVTA with TH co-labeling. **(d)** Example of a BDA injection site into the tVTA. **(e, f)** Example of the anterograde labeling observed in the SNc following BDA injection into the tVTA. **(e,** larger view; **f,** details of boxed area). **(g)** Examples of tVTA terminals making appositions (black arrows) on dopamine neurons in the SNc. BDA, biotinylated dextran amine; CLi, caudal linear nucleus of the raphe; CTb, cholera toxin β -subunit; IP, interpeduncular nucleus; ml, medial lemniscus; MT, medial terminal nucleus of the accessory optic tract; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; TH, tyrosine hydroxylase; VTA, ventral tegmental area. Scale bars = 500 μm in **(b-e)**, 10 μm in **(f, g)**.

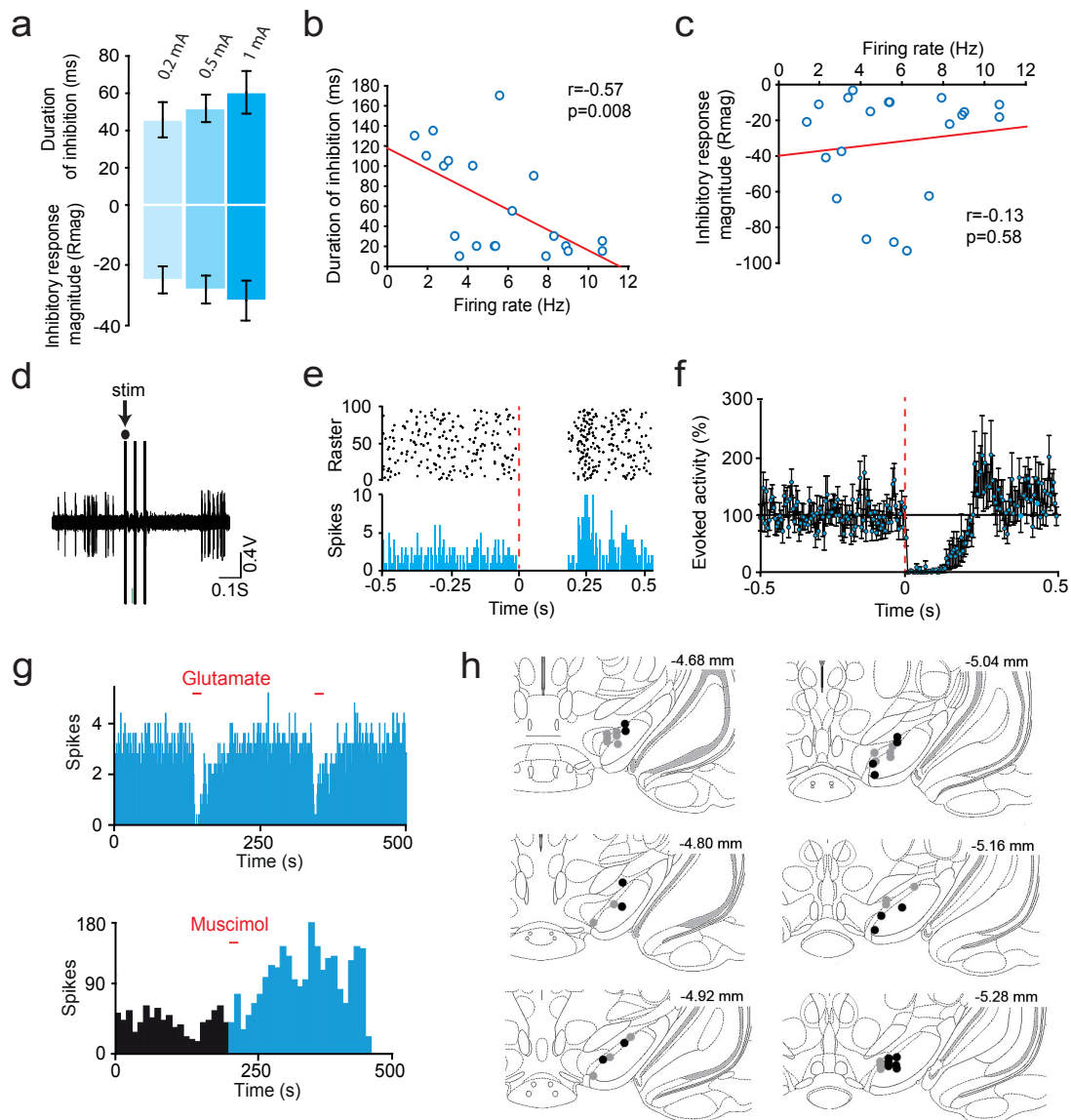


Figure S2. The tVTA exerts an inhibitory influence over SNc dopamine neurons. **(a)** The inhibition duration and inhibitory response magnitude of SNc dopamine neurons for tVTA stimulation at 0.2 ($n = 13$), 0.5 ($n = 24$) and 1 mA ($n = 19$). At 1 mA stimulation, 95% of neurons were inhibited (18 out of 19 neurons) with an onset of inhibition of 3.2 ± 1.3 ms, an inhibition duration of 60.5 ± 11.4 ms and an inhibition magnitude of -32.2 ± 6.6 . **(b)** The duration of inhibition of SNc dopamine neurons is inversely correlated to their spontaneous activity (1 mA, $n = 19$; linear regression, $r = -0.57$, $p < 0.01$). **(c)** The magnitude of the inhibitory response of SNc dopamine neurons is not correlated to their spontaneous activity (1 mA, $n = 19$; linear regression, $r = 0.13$, $p = 0.58$). **(d)** Example of a spike trace of a SNc dopamine neuron with 3 pulses of tVTA stimulation at 1 mA. **(e)** Consequence of 3 pulses of tVTA stimulation on a single SNc dopamine neuron **(f)** and on the mean of 9 neurons **(f, $n = 9$, $F_{98,784} = 5.6$, $p < 0.001$)**. **(g)** Examples of unitary activities from single SNc dopamine neurons after tVTA chemical manipulation. For glutamate, the chemical stimulation was repeated twice for the presented neuron. **(h)** Estimated locations of the recorded neurons in the SNc corresponding to Figure 4b (grey circles, control group; black circles, tVTA lesion group). Graphs represent mean \pm SEM.