

**Sex-dependent effects of stress on immobility behavior and VTA dopamine
neuron activity: modulation by ketamine**

Supplementary Material

Supplementary Methods

Electrophysiological Recordings

VTA Sampling

The VTA was sampled in 6-9 sequential electrode tracks separated by 0.2mm and arranged in a predetermined grid pattern to assess the distribution of dopamine neuron activity across the medial-lateral and antero-posterior extent of the A10 region (see schematic below). This procedure was developed to sample dopamine neurons with a variety of different projection targets (Ikemoto, 2007) and has been published by our group in multiple prior studies (Belujon *et al*, 2016; Chang and Grace, 2014; Valenti *et al*, 2012). Animals were required to have a minimum of six tracks within 0.4mm of target coordinates to be included in the study.

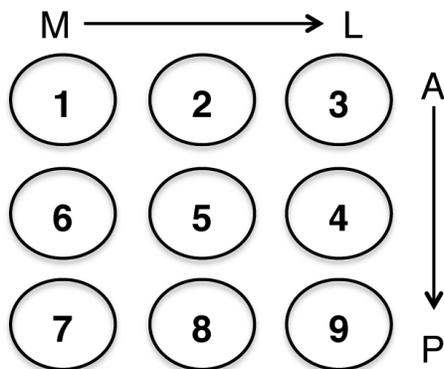


Figure S1 Schematic representing the pattern of the cells/track sampling of the VTA. The population activity of DA neurons is determined by counting the number of spontaneously firing DA neurons encountered while making 6-9 vertical tracks separated by 200um across the anterior-posterior (A-P) and medial-lateral (M-L) extent of the VTA. All spontaneously active DA

neurons encountered per electrode track were counted. The number of cells/track is determined by dividing the total number of active DA neurons recorded by the total number of tracks performed.

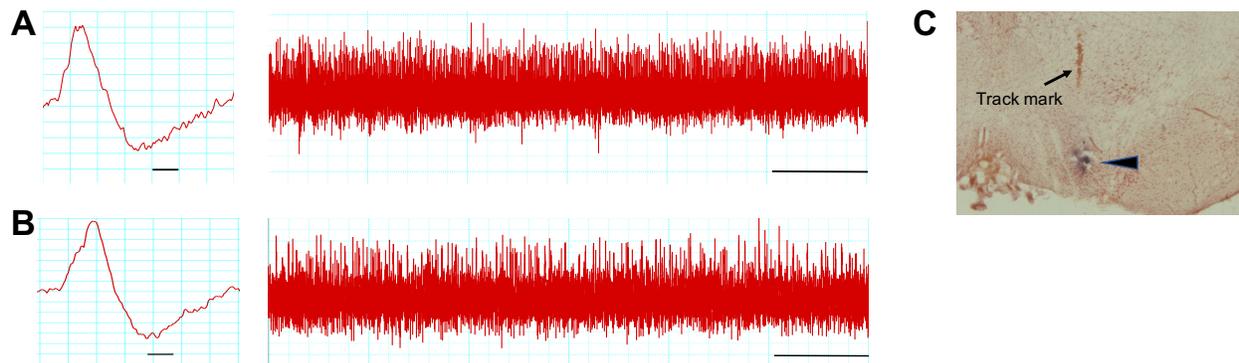


Figure S2 VTA DA neuron activity in male and female rats. A) Representative waveform of DA neuron (scale bar : 0.5ms) and 1 minute segment of spontaneous activity (scale bar : 10s) in male rats. B) Representative waveform of DA neuron (scale bar : 0.5ms) and 1 minute segment of spontaneous activity (scale bar : 10s) in female rats. C) A representative Pontamine sky blue deposit in which the final location of the last track was marked by electrophoretic injection of dye for histological verification (arrow indicates track mark, arrowhead indicates final location of electrode).

Supplementary References

Belujon P, Jakobowski NL, Dollish HK, Grace AA (2016). Withdrawal from Acute Amphetamine Induces an Amygdala-Driven Attenuation of Dopamine Neuron Activity: Reversal by Ketamine. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **41**(2): 619-627.

Chang CH, Grace AA (2014). Amygdala-ventral pallidum pathway decreases dopamine activity after chronic mild stress in rats. *Biological psychiatry* **76**(3): 223-230.

Ikemoto S (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain research reviews* **56**(1): 27-78.

Valenti O, Gill KM, Grace AA (2012). Different stressors produce excitation or inhibition of mesolimbic dopamine neuron activity: response alteration by stress pre-exposure. *The European journal of neuroscience* **35**(8): 1312-1321.