Restoring Mood Balance in Depression: Ketamine Reverses Deficit in Dopamine-Dependent Synaptic Plasticity

Supplemental Information

Supplemental Methods & Materials

Learned Helplessness Paradigm

Inescapable shocks: the average duration of each shock was 15 s (range 7–22 s) with an average inter-trial interval of 60 s (range 35–85 s). The inescapable session consisted of 120 inescapable, uncontrollable electric foot-shocks at 1.0 mA in the shocking chambers.

Active avoidance: on the first day of testing (day 2), animals were allowed to explore the shuttle box for 5 min. Each trial began with a 5-sec warning tone and light followed by a 0.8 mA shock. Helpless behavior was evaluated over 25 trials of unexpected and escapable foot shock, which required two crossings of the shuttlebox to terminate (FR2). If the escape response did not occur within 15 seconds, the trial was automatically terminated. If an animal crossed during the 15s shocks, the mean escape latency was measured.

Electrophysiological Recordings

Recordings were performed 24 h after the last active avoidance task and as previously described (1-5). Rats were injected with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic frame (Kopf, Tujunga, CA). Anesthesia was maintained by subsequent i.p. injections of chloral hydrate as needed to maintain suppression of the hindlimb compression withdrawal reflex. The internal temperature of the rats was held constant at 37°C using a temperature-controlled heating pad (Fine Science Tools). *In vivo* extracellular recordings were performed using WPI glass electrode with an impedance of 12-16 MΩ for nucleus accumbens (NAc) recordings and 6-8 MΩ for ventral tegmental area (VTA) recordings and filled with a 2% Chicago Sky Blue solution in 2M NaCl.

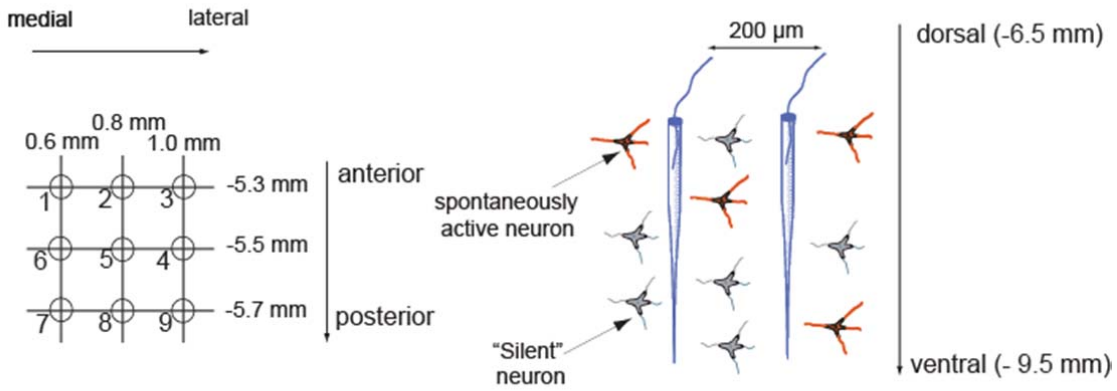
VTA recordings. The population activity of dopaminergic neurons was determined by counting the number of spontaneously firing dopamine (DA) neurons encountered while making 5 to 9 vertical tracks separated by 200 µm. The number of

cells per track is determined by dividing the total number of active DA neurons recorded by the total number of tracks performed (Figure S1). Spontaneously active DA neurons were identified with open filter setting (low pass, 50 Hz; high pass 16 kHz) and distinguished from other VTA neurons based on their unique long-duration waveform, slow irregular firing rate, and other well-established electrophysiological criteria previously described (6-8).

NAc recordings. Single-pulse, and high-frequency stimulation were applied via a concentric bipolar stimulating electrode (NEX-100X; Rhodes Medical Instruments). While searching for a responsive NAc neuron, single pulse stimuli were applied to the fimbria every 2 sec using a dual output stimulator (S8800; Grass Technologies; intensity 1 mA; pulse-width 0.25 msec). Once a neuron responded to the stimulation monosynaptically (latency: 6-11 ms; (9, 10)), a similar stimulation procedure was used (pulse duration 0.25 msec; every 2 sec) and the current administered to the fimbria was adjusted to evoke an action potential approximately 50% of the time. Spike probabilities were measured by dividing the number of spikes observed by the number of stimuli in 5 min intervals. After recording stable baseline activity, one of 3 experimental paradigms was administered for NAc recordings: high-frequency stimulation (HFS) (50 Hz; 2 s at suprathreshold) of the fimbria pathway, injection of ketamine/saline i.p. followed by HFS to the fimbria, infusion of the D1 antagonist SCH23390 (0.5 µg/0.5 µl) in the NAc followed by HFS to the fimbria. No more than one neuron per animal was recorded. Changes in spike probabilities were used as an index of changes in the influence that hippocampal inputs exert over NAc neuronal activity.

Electrophysiological signals were amplified (x1000) and filtered (low pass: 400 Hz (NAc) or 50 Hz (VTA), high pass: 16 kHz) using a Fintronics amplifier (model WDR-420). Recordings were displayed on an oscilloscope (B&K precision) and transferred via a Powerlab interface (AD Instruments) to a computer equipped with LabChart v.7 software. Neuronal activity with a signal-to-noise ratio >3 was recorded and used for analyses.

A



B

Track Number (coordinates)	Rat1	Rat2	Rat3	Rat4	Rat5
	Number of spontaneously active DA neurons				
1 (-5.3mm AP/ 0.6 mm lat)		0	1	0	1
2 (-5.3 mm AP/0.8 mm lat)	2	2	1	2	
3 (-5.3 mm AP/1.0 mm lat)	0	0	0	1	
4 (-5.5 mm AP/1.0 mm lat)	3	0	1	2	0
5 (-5.5 mm AP/0.8 mm lat)	1		1	2	1
6 (-5.5 mm AP/0.6 mm lat)	0		1	2	0
7 (-5.7 mm AP/0.6 mm lat)	0	0	0	0	1
8 (-5.7 mm AP/0.8 mm lat)	1	1	0	0	
9 (-5.7 mm AP/1.0 mmlet)	1	1	1	1	
Total number of DA neuronsrecordedper rat	8	4	6	10	3
Total number of valid tracksper rat	8	7	9	9	5
Number of cells per valid track per rat	1	0.571429	0.666667	1.111111	0.6

Figure S1. Example of the analysis of the population activity of the ventral tegmental area (VTA) in home cage control rats. (**A**) Schematic representing the pattern of the cell/track sampling of the VTA. The population activity of dopamine (DA) neurons is determined by counting the number of spontaneously firing DA neurons encountered while making 5 to 9 vertical tracks separated by 200 µm. (**B**) Example of the number of DA neurons recorded for each electrode track in home cage control rats. The number of cells per track is determined by dividing the total number of active DA neurons recorded by the total number of tracks performed. Gray boxes represent non-valid tracks.

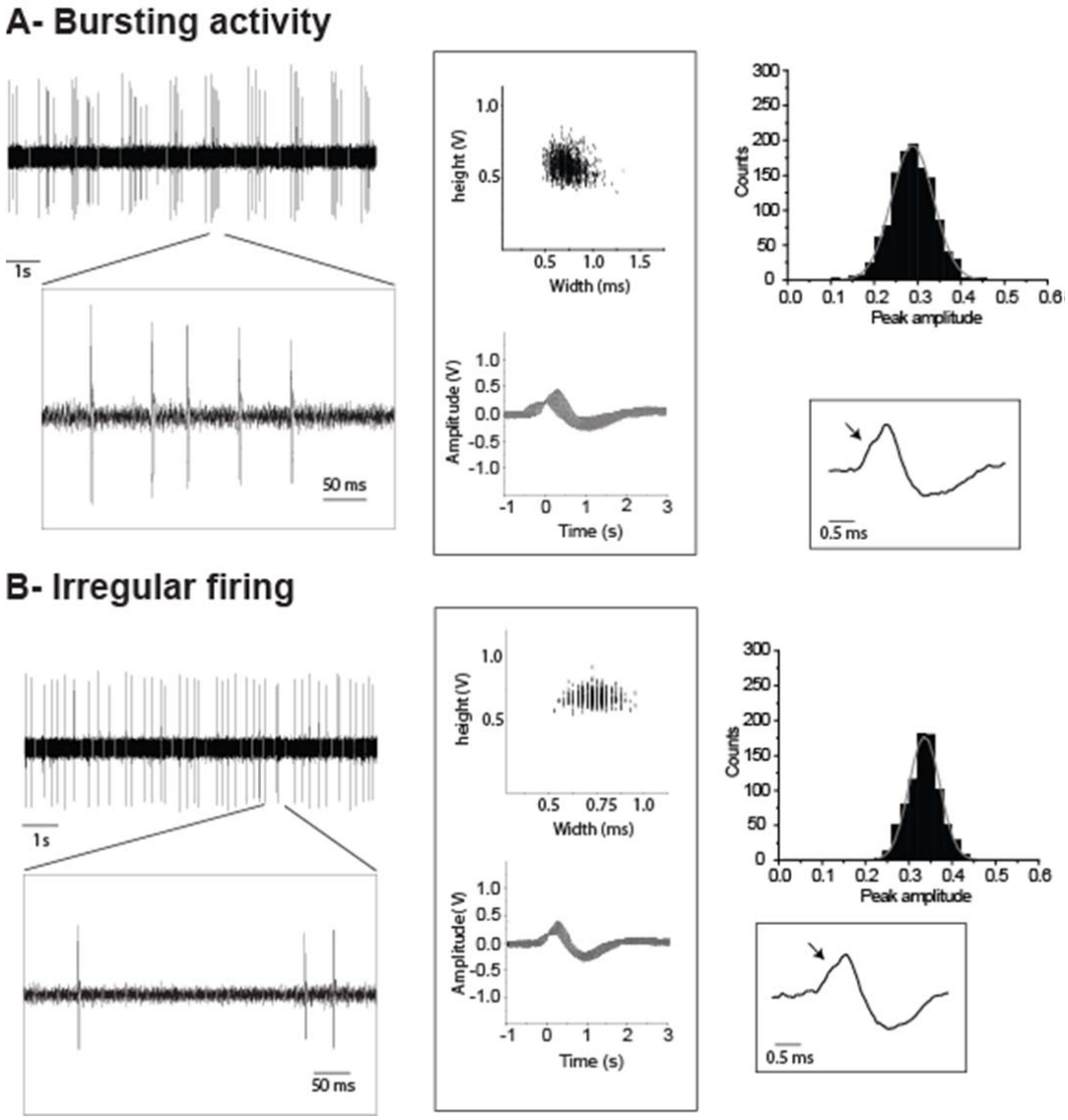


Figure S2. Electrophysiological identification of dopaminergic neurons. Two types of activity are represented (**A**) bursting pattern and (**B**) irregular firing pattern. (**Left**) Representative example of a 10-sec recording of spontaneous activity of a single dopamine neuron displaying bursting (top) or irregular (bottom) activity. Each insert represent a 400-msec close-up. (**Middle**) Discriminator view from the software Lab chart allowing the selection of one unit. (**Right**) Each selected unit exhibited a narrow amplitude distribution with a single population which could be fitted by one Gaussian curve around 0.3 mV (0.28 for bursting neuron and 0.33 mV for irregular neuron). Insert represents the waveform of an extracellular action potential for each neuron presented as an example. As previously described (6), neurons display a biphasic (positive-negative) action potential, typically with a “notch” in the rising phase corresponding to the calcium–dependent initial segment spike (open arrow) and a prominent negative component, and with a total duration >2.2 ms overall. The duration from the spike initiation to the maximal negative phase of the action potential was ≥1.1 ms.

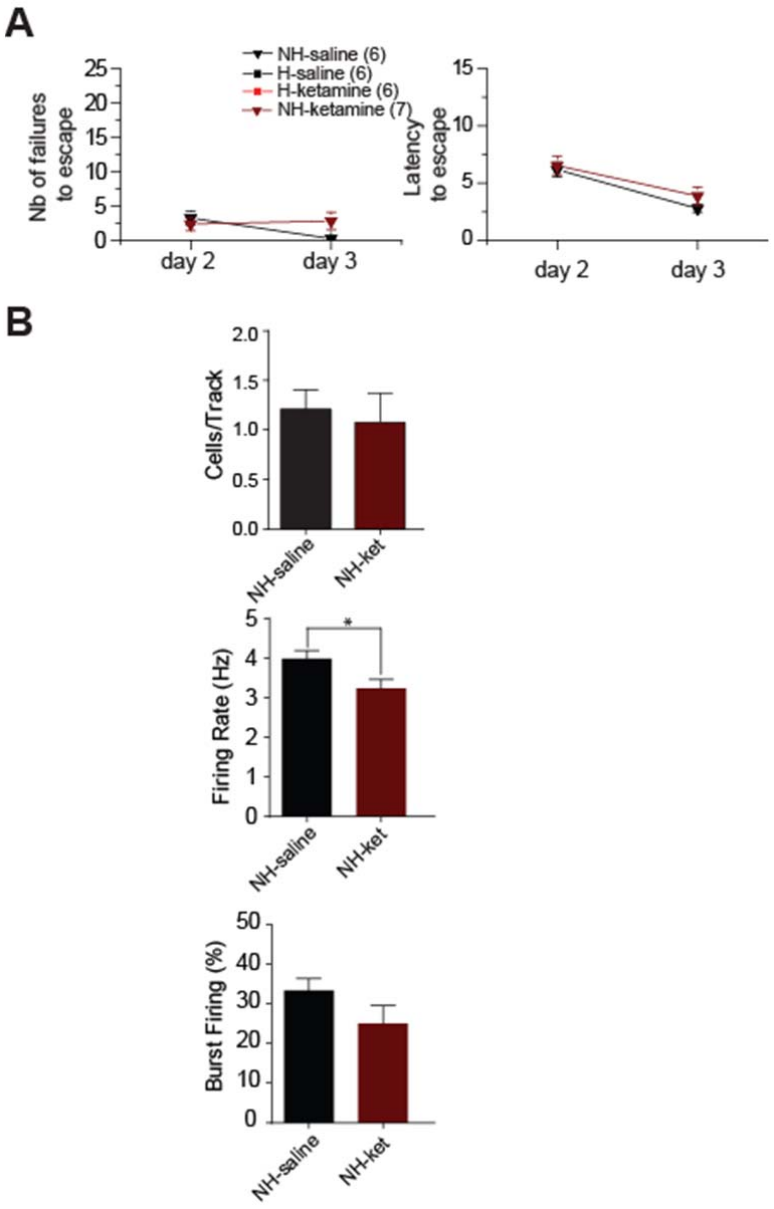


Figure S3. Effect of acute injection of ketamine in non-helpless (NH) rat. (**A**) In non-helpless rats, ketamine (n = 7 rats) had no effect on escape behavior, in comparison to saline injection (failures, one-way analysis of variance (ANOVA) on Ranks, H = 4.13, p = 0.072; latency, one-way ANOVA, $F_{(1,11)}$ = 1.378, p = 0.265). (**B**) Ketamine had no effect on the dopamine neuron population activity (top) or the burst firing (bottom) in the ventral tegmental area in non-helpless rats (n = 5 rats, 48 neurons; population activity, one-way ANOVA, $F_{(1,9)}$ = 0.184, p = 0.678; bursts: one-way ANOVA on Ranks, H = 0.389, p = 0.53). Ketamine induces a decrease in the firing rate (middle, one-way ANOVA, $F_{(1,111)}$ = 5.449, p < 0.05). Even though there was a significant difference between saline and ketamine injection, the firing rate in non-helpless rats after ketamine injection was not different in comparison to the firing rate observed in home cage control animals (one-way ANOVA, $F_{(1,79)}$ = 2.067, p = 0.154).

Supplemental References

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