Repeated Ketamine Exposure Induces an Enduring Resilient Phenotype in Adolescent and Adult Rats

Supplemental Information

Supplemental Methods and Materials

Forced Swim Test

The forced swim test (FST) is a 2 day procedure in which rats are forced to swim under inescapable conditions (1). On day 1, rats are forced to swim for 15 min. Initially, they engage in escape-like behaviors but eventually adopt a posture of immobility in which they make only the movements necessary to maintain their head above water. When retested 24 h later, rats become immobile very quickly; however, antidepressant treatment between the forced swim exposures can significantly increase their escape-like behaviors, an effect that has been correlated with antidepressant activity in humans (2). At the start of the experiment, rats were placed in plastic cylinders (75 x 30 cm) filled to 54 cm depth with 25°C water, as described previously (3). Afterward, rats were removed from the water, dried with towels, and placed in a warmed enclosure for 30 min. All cylinders were emptied and rinsed between rats. On day 2 (24 h later), rats were retested for 5 min under identical conditions. Here, the latency to become immobile, total immobility, and swimming, climbing, and immobility counts were measured. Behavioral counts were taken at 5 s intervals during the 5 min retest. Latency to immobility was defined as the time at which the rat first initiated a stationary posture that did not reflect attempts to escape from the water (4). To qualify as immobility, this posture had to be clearly visible and maintained for ≥ 2.0 s (5, 6).

Chronic Unpredictable Stress

Adolescent rats (postnatal day 31 at start) were housed in isolation and exposed to a single unpredictable stressor for 15 consecutive days. The stressors included overnight cage flooding, food and water deprivation, restraint (2 h), overnight light exposure, 45° tilted cages (24 h), cage shaking, lights on overnight, strobe light overnight, cold exposure (4°C for 2 h), and cold swim (see Table S2). As a control, a group of adolescent rats were double housed and did not receive any stressors during this time. On day 15, rats received an overnight sucrose preference test to assess stress-induced anhedonia (see Figure 2). On day 16, stressed rats received either saline or ketamine (20 mg/kg), 60 minutes before being placed in the FST day 2. Control rats did not receive any treatment prior to FST exposure. Seventy-two hours later, each group was sacrificed and trunk blood was taken to assess serum corticosterone. Half of the non-stressed controls were subjected to an acute stressor (5 minute FST) immediately before being sacrificed.

Corticosterone Enzyme Immunoassay

Trunk blood from each animal was individually collected in EDTA lined tubes and kept on ice until use. Whole blood samples were centrifuged at 1500 x g for 30 min at 4°C. Serum supernatant was decanted for analysis with the corticosterone enzyme immunoassay per manufacturer's instructions (Assay Designs). Briefly, serum was diluted to 10% using the provided buffer and added to the wells of an immuno-lined 96-well plate and allowed to incubate for 2 h with provided antibodies. The plate was washed with a provided wash buffer, developed, and optical density was read using a 96-well plate reader (Biotek). Serum

corticosterone was calculated by comparing these values to optical density values obtained from corticosterone standards.

Sucrose Preference

The sucrose preference test consisted of a two bottle choice procedure in which rats were given the choice between consuming water and a sucrose solution. This paradigm has been used extensively to assess the effects of stress-induced anhedonia (7). Rats were habituated to drink water from two bottles for 5 days. At the start of the experiment, they were exposed to ascending concentrations of sucrose (0, 0.25, 0.5, and 1%) for two days per sucrose concentration (8). Water and sucrose consumption was measured at 0800 and 1700 each testing day. The position of the sucrose bottle (left or right) was counterbalanced between groups and changed daily. Preference for sucrose over water [sucrose/(sucrose + water)] was used as a measure for rats' sensitivity to reward.

Elevated Plus Maze

Saline and ketamine pretreated rats were tested on the elevated plus maze, a behavioral task assessing anxiety-like behavior (9). The maze consisted of two perpendicular, intersecting runways (12 cm wide x 100 cm long) made from gray plastic. One runway had tall walls (40 cm high), termed "closed arms," while the other had no walls, termed "open arms." The arms were connected together by a central area, and the maze was elevated 1 m from the floor. Testing was conducted between 0900 and 1300 under controlled light conditions (~90 lux) as described previously (10, 3). At the start of the test, rats were positioned in the central

area, facing one of the open arms and allowed to explore freely for 5 min, and the percentage time that the rat's center point (as determined by Noldus Ethovision XT) spent in the open versus closed arms was measured [100 X (total time spent in open arms/total time spent in closed arms)].

Basal Locomotor Activity

Locomotor activity was measured in a Plexiglas open-field apparatus (40.64 cm × 40.64 cm × 40.64 cm) surrounded by photobeams (Coulbourn Instruments; Allentown, PA). A computer was used to record all horizontal beam breaks and determine distance travelled (TruScan, v. 2.01; Coulbourn Instruments).

Place Preference Conditioning

Place conditioning for ketamine was performed in a three-compartment apparatus (FSU Psychology Engineering) as described previously (11-13). Compartments differed in floor texture, wall coloring, and lighting. On the preconditioning day (day 0), rats were allowed to explore the entire apparatus for 30 min to obtain baseline preference to any of the three compartments (length by width by height: side compartments, 35 x 27 x 25 cm; middle compartment, 10 x 27 x 25 cm). Rats showing a preference (before ketamine exposure) to either side compartment received drug pairing in the non-preferred side. Conditioning trials occurred over 4 consecutive days. During conditioning, rats received saline (1 ml/kg, IP) at 1000 hours and were confined to one of the side compartments of the apparatus for 30 min. After 3 h, (1300 hours) rats received ketamine (0, 5, 10, or 20 mg/kg, IP) and were confined to the

opposite side compartment (drug-paired compartment) for 30 min. On the test day (day 5), between 1100 and 1200 hours, rats received saline and were allowed to explore the entire apparatus for 30 min and time spent in the drug-paired compartment was assessed (drug side minus saline side).

Table S1. Chronic Unpredictable Stress Schedule

Day	PD	Stressor	Duration
1	31	Cage Flooding	Overnight
2	32	Food Deprivation	24 h
3	33	Restraint	2 h
4	34	Light Exposure	Overnight
5	35	Water Deprivation	24 h
6	36	Cage Tilt	24 h
7	37	Cold Exposure	2 h
8	38	Cage Flooding	Overnight
9	39	Cage Shaking	1 h
10	40	Cold Swim	15 min
11	41	Strobe Light	Overnight
12	42	Cage Shaking	2 h
13	43	Cold Exposure	2 h
14	44	Restraint	2 h
15	45	Forced Swim	15 min
16	46	Forced Swim Test	5 min

PD, postnatal day.

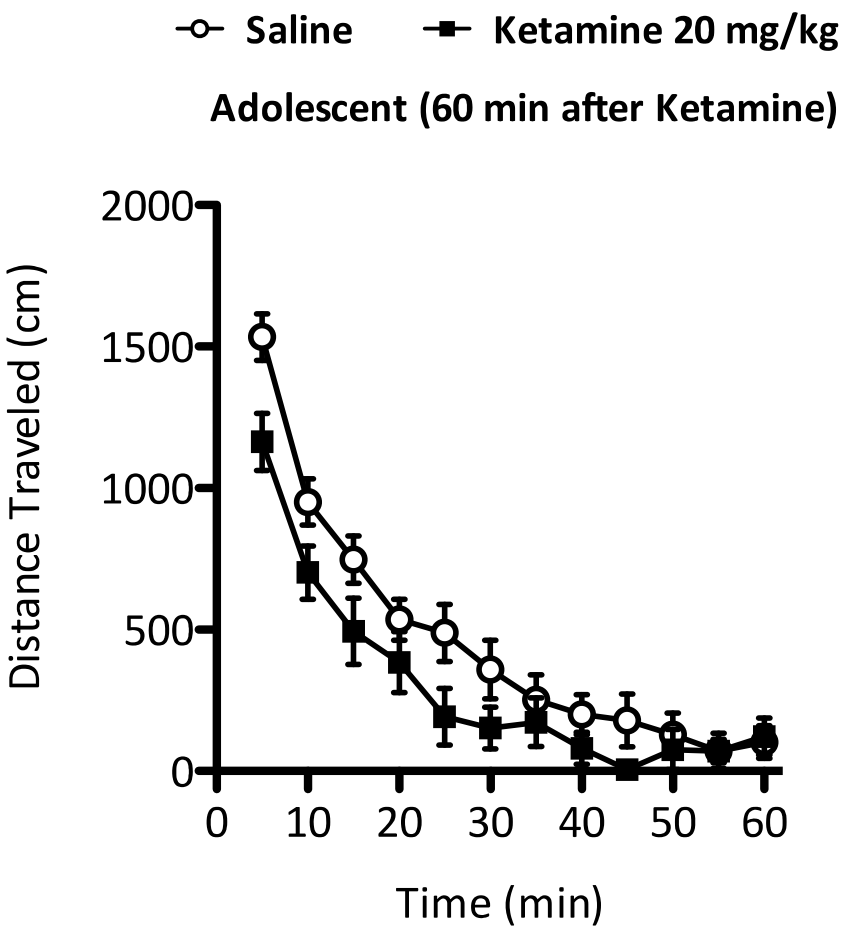

Figure S1. The effects of a single injection of ketamine (20 mg/kg) on locomotor activity 60 minutes after exposure in adolescent rats. Adolescent rats treated with ketamine show no difference in locomotor activity 60 min after exposure when compared to controls (*n* = 9-10/condition). Data are represented as mean distance traveled (mean ± SEM, in cm).

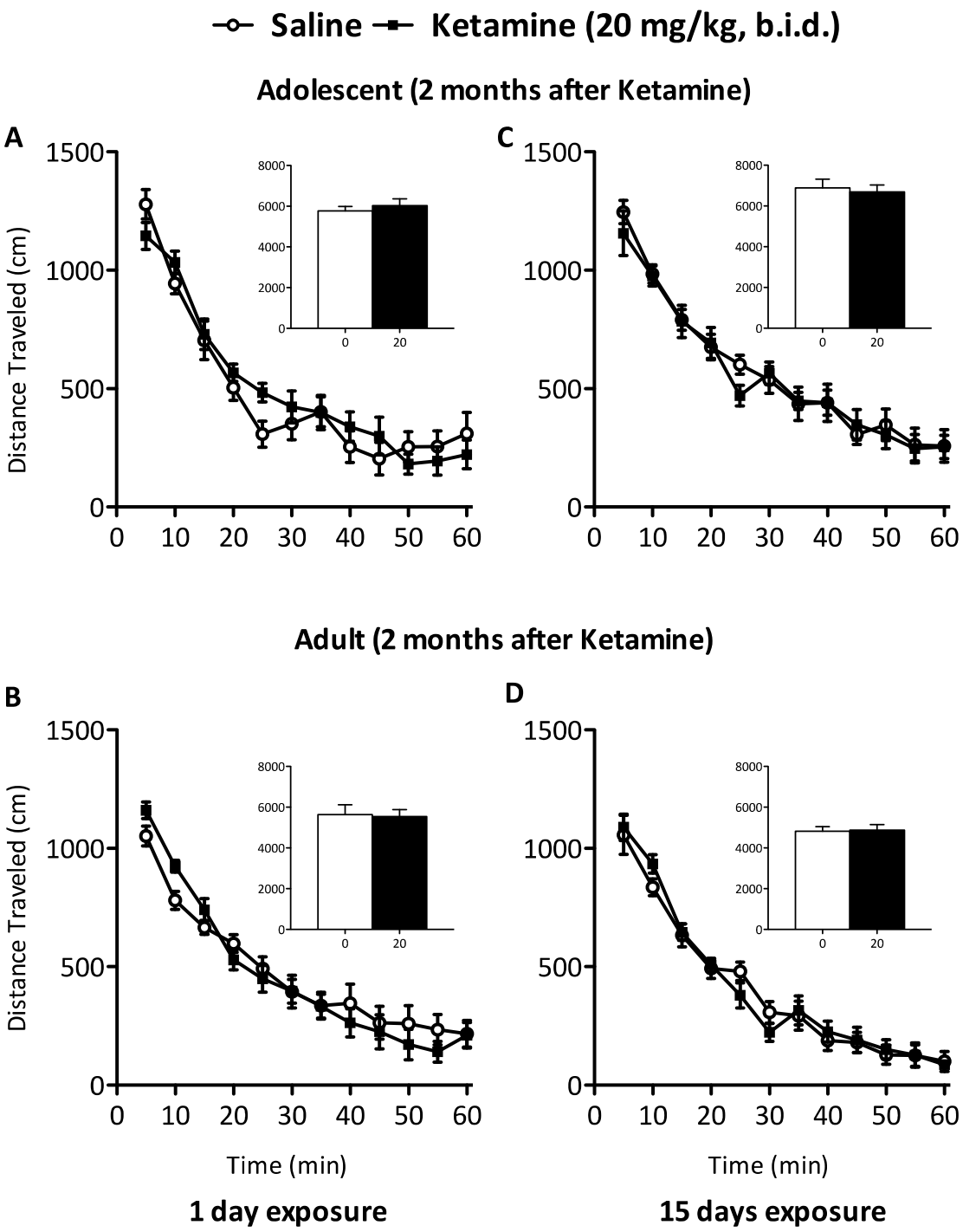

Figure S2. Long-term effects of ketamine (20 mg/kg) exposure on locomotor activity in adolescent and adult rats. **(A-B)** Exposure to 1 or 15 days of ketamine during adolescence did not influence locomotor activity 2 months after exposure (*n* = 9-10/condition). **(C-D)** Similarly, 1 or 15 days of ketamine exposure during adulthood did not influence locomotor activity 2 months after drug exposure (*n* = 10/condition). Data are represented as mean distance traveled (mean ± SEM, in cm).

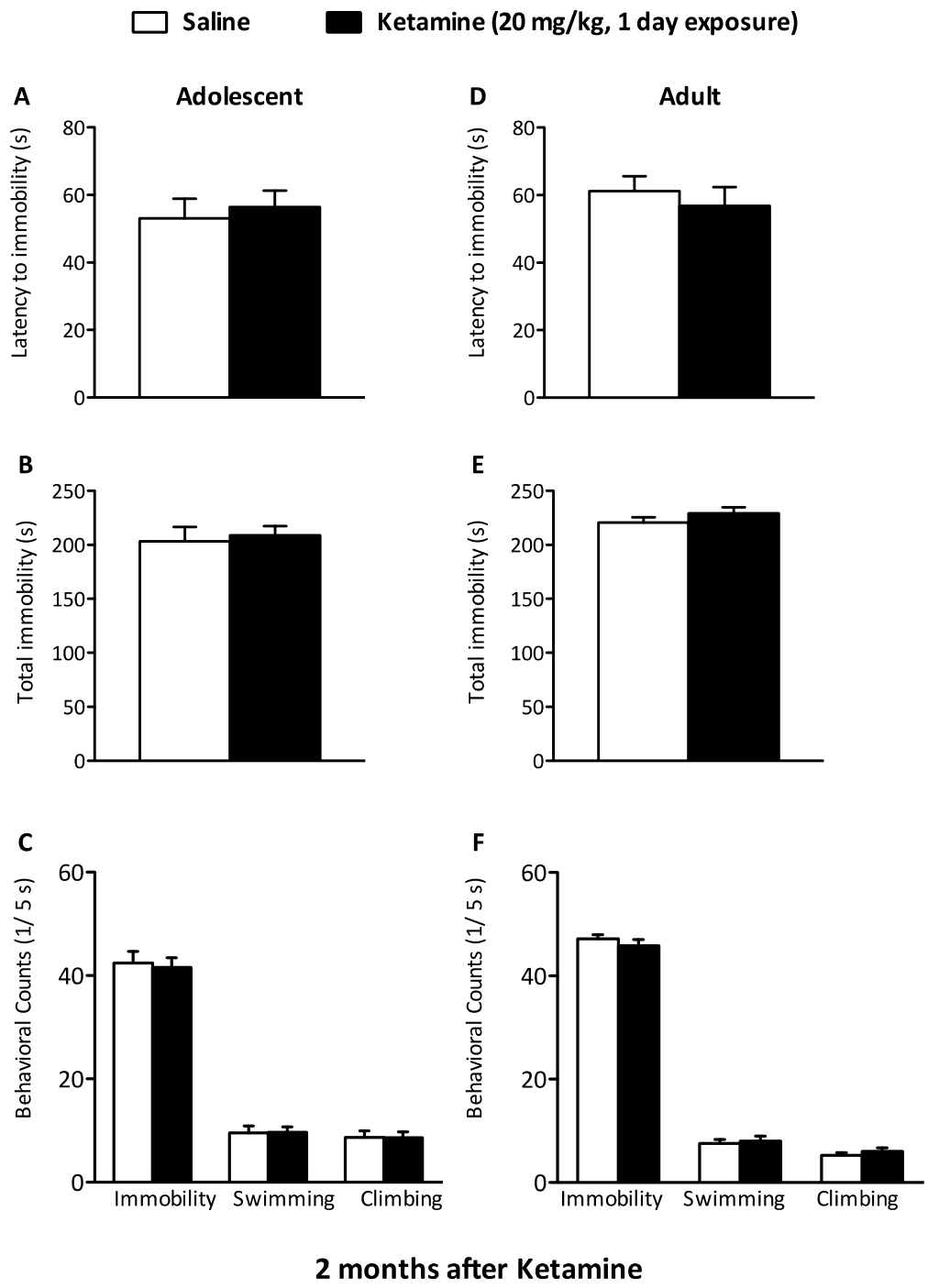

Figure S3. Lasting effects of 1-day exposure to ketamine (20 mg/kg, twice daily) on behavioral despair using the forced swim test (FST) paradigm, 2 months after drug exposure. **(A-C)** Ketamine treatment during adolescence did not yield changes on any of the escape-like behavioral measures assessed in the FST when compared to saline-treated controls (*n* = 10/group). **(D-F)** Similar effects were seen in adult rats (*n* = 10/group). Data are presented as latencies to become immobile and total immobility (in seconds) and as cumulative 5-second intervals of swimming, climbing, and immobile counts (mean ± SEM).

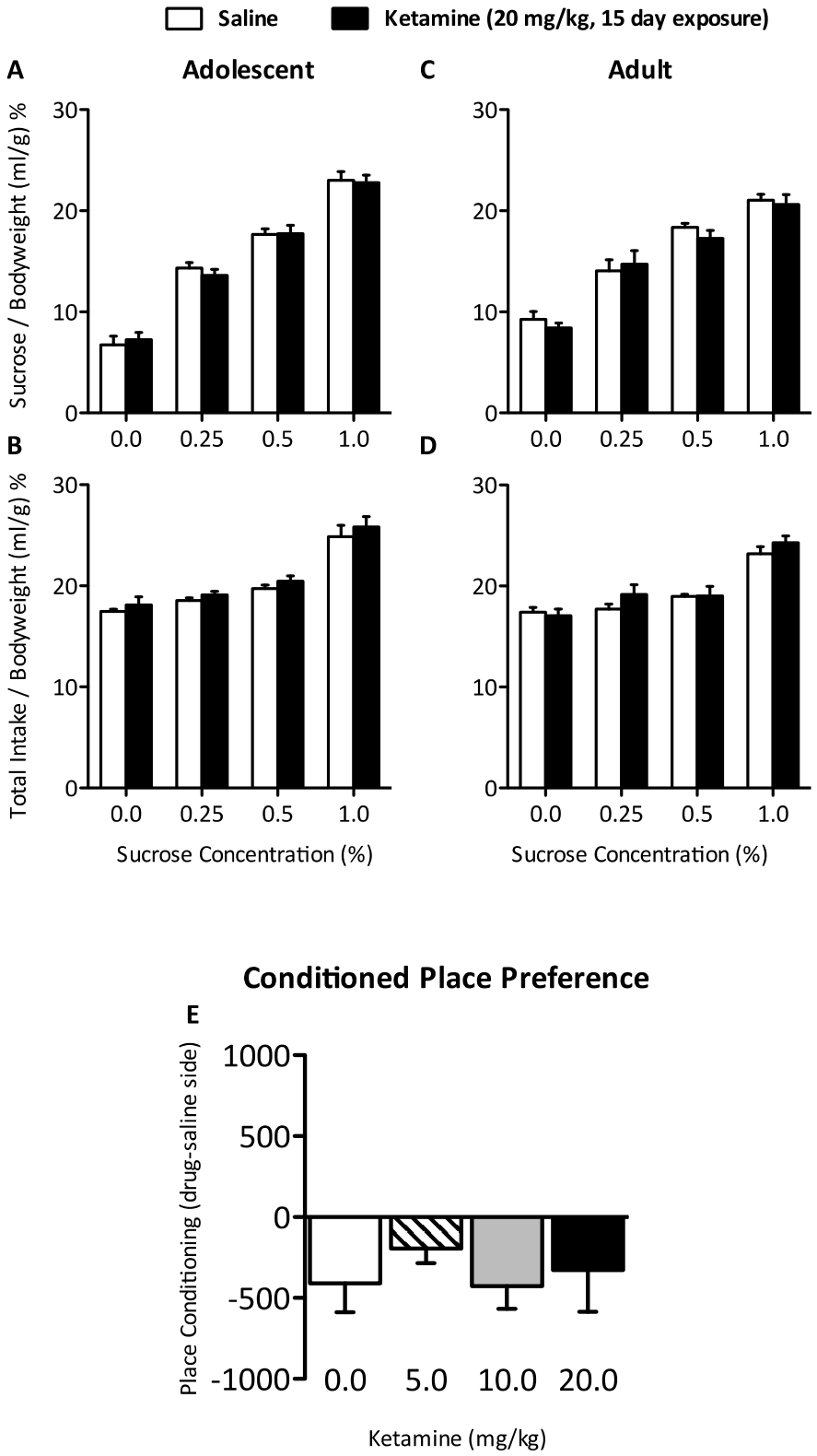

Figure S4. Effects of ketamine (20 mg/kg) exposure on reward-related behaviors. **(A-D)** Fifteen consecutive days of ketamine exposure during adolescent or adulthood did not influence sucrose preference or total liquid intake 2 months after drug exposure (*n* = 10-12/condition).

Data are presented as percent of total sucrose consumed by body weight (**A** and **C**) or percent of total mL consumed by body weight (**B** and **D**) in saline- and ketamine-treated rats (mean ± SEM). **(E)** Ketamine failed to induce place preference conditioning in adolescent rats (PD35) at any of the doses tested (*n* = 6-10/condition). Data are presented as difference scores for time spent in the drug-paired minus the saline-paired side (mean ± SEM in minutes).

Supplemental References

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