Brain reward function after chronic and binge methamphetamine regimens in mice expressing the HIV-1 TAT protein.

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SUPPLEMENTARY METHODS

Intracranial self-stimulation

The intracranial self-stimulation (ICSS) procedure was conducted as previously described (1-3). At 4 months of age, mice were anesthetized with 1-3% isoflurane/oxygen mixture and a stainless steel bipolar electrode (0.20 mm diameter; 6 mm length; Plastics One, Roanoke, VA, USA) was implanted into the medial forebrain bundle at the level of the lateral hypothalamus using the following coordinates: anterior/posterior, +1.6 mm; medial/lateral, -1.0 mm; dorsal/ventral, -5.3 mm; from flat skull (4). Four stainless steel screws (3.2 mm long, Plastics One, Roanoke, VA, USA) were fixed to the skull to keep the electrode in place, together with the application of a resin ionomer (Den-Mat, Santa Maria, CA, USA) and dental acrylic (Ortho-Jet, Lang Dental, Wheeling, IL, USA). The mice were allowed seven post-surgery recovery days prior to commencing training.

ICSS training and testing were conducted in eight Plexiglas operant chambers (30.5 cm × 24 cm × 27 cm; Med Associates, St. Albans, VT, USA). Each operant chamber was enclosed within a light- and sound-attenuated chamber (40 cm × 60 cm × 63.5 cm). Intracranial electrical stimulation was delivered by constant-current stimulators (Stimtek model 1200c). The mice were connected to the stimulation circuit through flexible bipolar leads (Plastics One, Roanoke, VA, USA) attached to gold-contact swivel commutators (model SL2C, Plastics One, Roanoke, VA, USA) mounted above the operant chamber. Initially, the mice were trained to turn a wheel manipulandum (5.5 cm diameter, 4 cm width) on a fixed-ratio 1 schedule of reinforcement. After successful acquisition of this schedule mice were tested in the discrete-trial current-threshold procedure. Each trial began with the delivery of a noncontingent electrical stimulus followed by an 8 s response window within which the subject could make a positive response to receive a second contingent stimulus identical in all parameters to the initial noncontingent stimulus. The intertrial interval that followed either a positive response or the end of the response window (in the case of no

response) had an average duration of 10 s (ranging from 7.5 to 12.5 s). Responses that occurred during the intertrial interval, time-out responses, resulted in a further 12.5 s delay of the onset of the next trial. During training on the discrete-trial procedure, the duration of the intertrial interval and delay periods induced by time-out responses were gradually increased until both reached a duration of 10 s (ranging from 1 to 10 s during training). Subsequently, the animals were tested in the current-threshold procedure.

A test session consisted of four alternating series of descending and ascending current intensities, starting with a descending series. Blocks of three trials were presented to the subject at a given stimulation intensity, and the intensity changed by 4-5 μA steps between blocks of trials. The initial stimulus intensity was set approximately 30–40 μA above the baseline current-threshold for each animal. Each test session lasted 30–40 min and provided several dependent variables: threshold, response latency and timeout responses. The threshold value of each series was defined as the midpoint in microamperes between the current intensity level at which the animal made two or more positive responses out of the three stimulus presentations and the level at which the animal made less than two positive responses. The animal's estimated current-threshold for each test session was the mean of the four series' thresholds. The response latency was defined as the average time in seconds that elapsed between the delivery of the electrical stimulus and the turning of the wheel manipulandum for all of the trials that led to a positive response. Timeout responses were defined as the total number of responses that occurred during the intertrial interval for a test session. Extra responses were additional turns of the wheel manipulandum after a successful reponse.

Real Time-PCR

All Primers were purchased from Qiagen (Valencia, CA).

Receptor	Species	Detected transcript	Cat number
DRD1	Mus musculus	NM_010076	PPM04267A
DRD2	Mus musculus	NM_010077	PPM04288A
DRD4	Mus musculus	NM_007878	PPM04830C
DRD5	Mus musculus	NM_013503	PPM04828A
Adora1	Mus musculus	NM_001008533	PPM04290B
Adora2A	Mus musculus	NM_009630	PPM03472F
Adora2B	Mus musculus	NM_007413	PPM04282B

SUPPLEMENTARY RESULTS

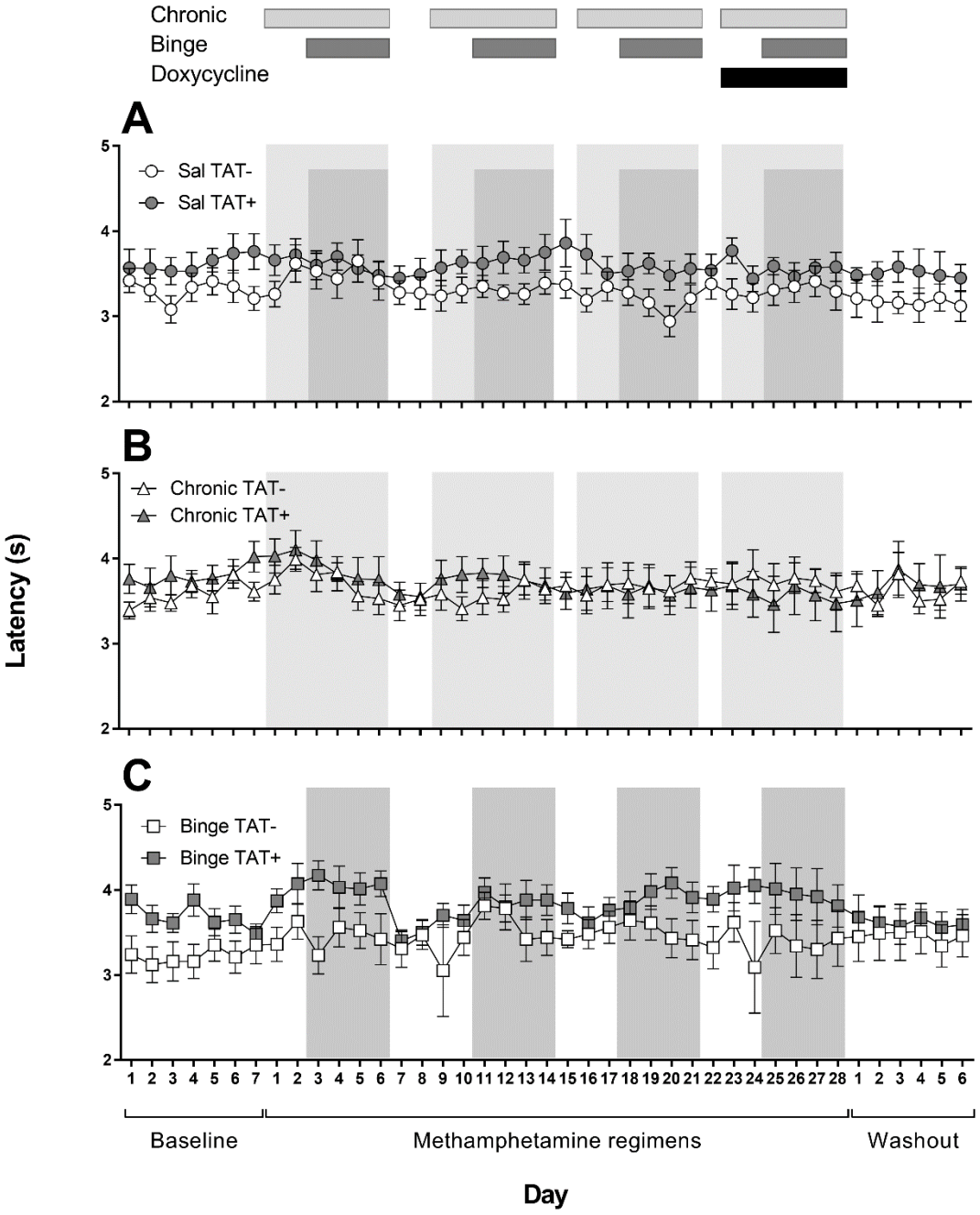

Figure S1. Latency to respond in the intracranial self-stimulation procedure in TAT- and TAT+ mice administered saline (**A**), or chronic (**B**) and binge methamphetamine regimens (**C**). Time points assessed 12 h after a day of methamphetamine administration are represented by the shaded regions with lighter gray for the chronic regimen and darker gray for the binge regimen. The days of doxycycline administration are shown by the black bar (top). Data are expressed as mean ± SEM.

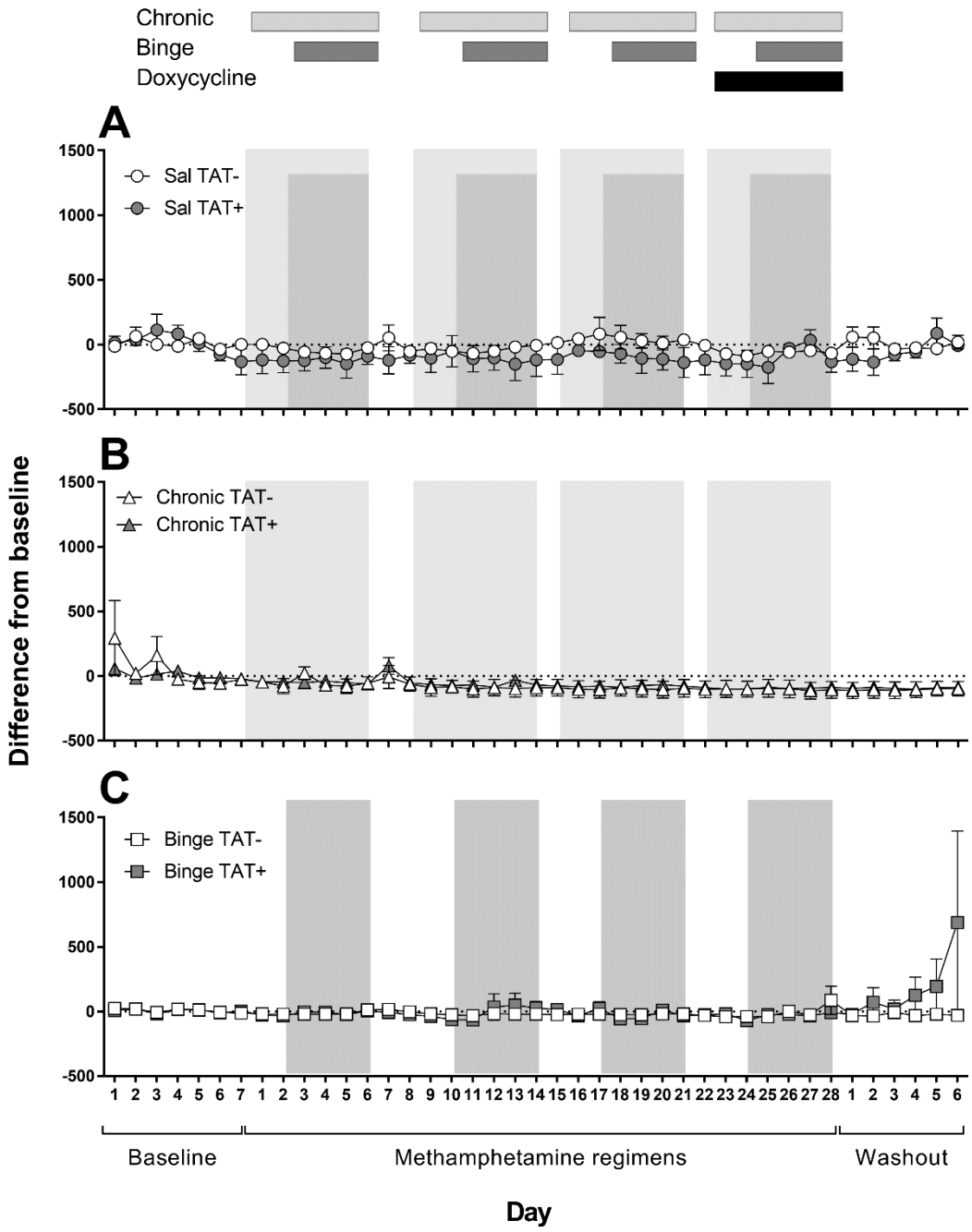

Figure S2. Timeout responses in the intracranial self-stimulation procedure in TAT- and TAT+ mice administered saline (**A**), or chronic (**B**) and binge methamphetamine regimens (**C**). Timeout responses are presented as the difference from the baseline average over the 5 days prior to beginning the methamphetamine regimens. Time points assessed 12 h after a day of methamphetamine administration are represented by the shaded regions with lighter gray for the chronic regimen and darker gray for the binge regimen. The days of doxycycline administration are shown by the black bar (top). Data are expressed as mean ± SEM.

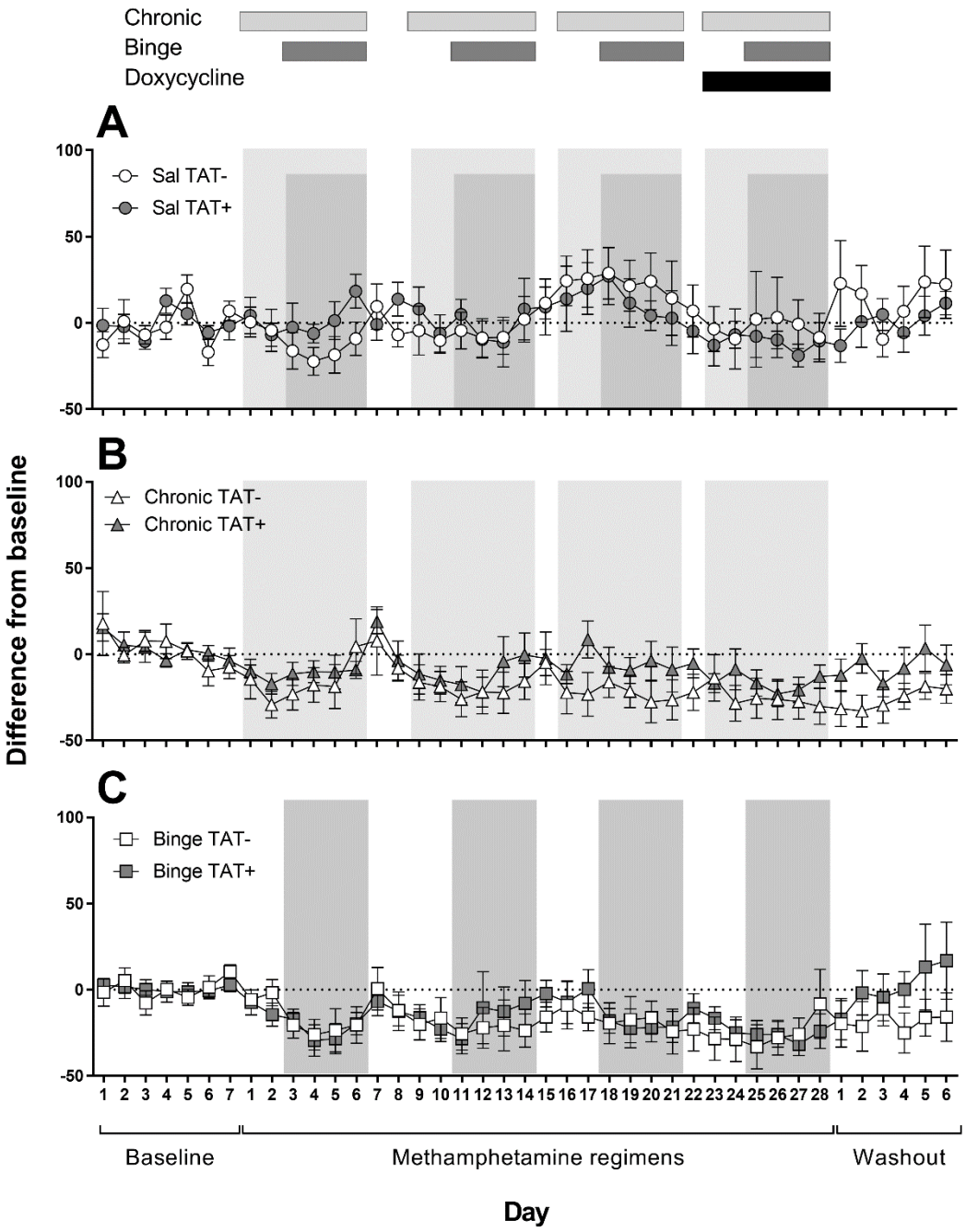

Figure S3. Extra responses in the intracranial self-stimulation procedure in TAT- and TAT+ mice administered saline (**A**), or chronic (**B**) and binge methamphetamine regimens (**C**). Extra responses are presented as the difference from the baseline average over the 5 days prior to beginning the methamphetamine regimens. Time points assessed 12 h after a day of methamphetamine administration are represented by the shaded regions with lighter gray for the chronic regimen and darker gray for the binge regimen. The days of doxycycline administration are shown by the black bar (top). Data are expressed as mean ± SEM.

SUPPLEMENTARY REFERENCES

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