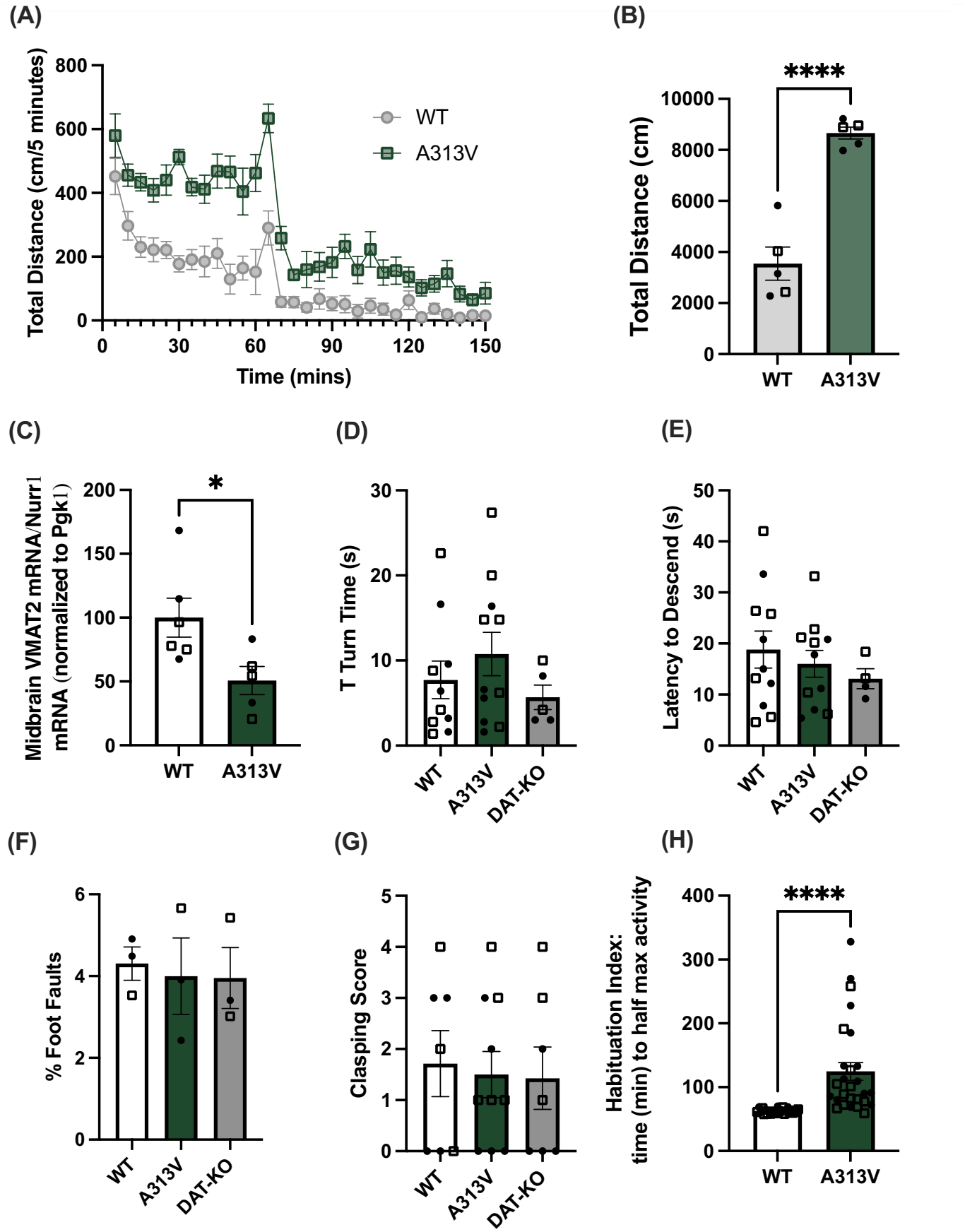
Expanded View Figures

Figure EV1. A313V mice do not display dystonic behaviors and have decreased VMAT2 mRNA and habituation.

(**A**, **B**) Wild-type (WT) ($n = 5$) and A313V knock-in (A313V) ($n = 5$) mice were aged to 15 months old. Total distance was recorded in 5 min bins for 150 min in an open field chamber. An i.p. injection of saline was given at 60 min. A313V mice showed significantly greater summed levels of total distance traveled (WT: 3548 ± 648.59 beam breaks vs A313V: 9650 ± 233.9- beam breaks, $t = 7.413$, df = 8, $P < 0.0001$). (**C**) RT-qPCR performed on midbrain samples showed that WT mice have approximately twofold greater *VMAT2* (WT: 100 ± 15.33 % of WT average vs A313V: 50.70 ± 10.98 % of WT average, $t = 2.615$, df = 8.612, $P = 0.0291$) mRNA compared to A313V mice. There was no observation of dystonia in A313V mice compared to WT mice as assessed during the pole reversal test in either (**D**) T turn latency (WT: 7.72 ± 2.21 s vs A313V: 10.76 ± 2.54 s vs DAT-KO: 5.68 ± 1.44 s, $F_{2,23}$=0.9805, $P = 0.3902$) or (**E**) latency to descend (WT: 18.82 ± 3.64 s vs A313V: 16.02 ± 2.63 s vs DAT-KO: 13.1 ± 1.95 s, $F_{2,23}$=0.5415, $P = 0.5892$). Similarly, there was no difference in (**F**) Percentage of foot faults between WT, A313V, and DAT-KO mice as assessed through the foot fault test (WT: 4.31±0.41 % of foot faults vs A313V: 3.99±0.95 % of foot faults vs DAT-KO: 3.95±0.75 % of foot faults, $F_{2,6} = 0.070$, $P = 0.933$ A tertiary measure of dystonia, the tail suspension test and severity of clasping was recorded. There was no difference in (**G**) Clasping severity between WT, A313V, and DAT-KO mice (WT: 1.71 ± 0.64 clasping severity score vs A313V: 1.5 ± 0.45 clasping severity score vs DAT-KO: 1.43 ± 0.61 clasping severity score, $F_{2,21} = 0.06414$, $P = 0.9381$). (**H**) Habituation of locomotor exploration was quantified using a habituation index (time in minutes to reach half of the maximal locomotor activity divided by total distance traveled using linear regression). Habituation rate in A313V mice was roughly half that of WT controls (WT: 62.93 ± 0.72 min vs A313V: 124.71± 13.75 min, $t = 4.402$, df = 51, $P < 0.0001$). Closed circular points represent values from female mice, and open square points represent values from male mice. Results are presented as mean ± SEM. Student's unpaired, two-tailed *t* tests and one-way ANOVAs to assess the effect of genotype with Tukey's multiple comparisons were conducted. *$P \leq 0.05$, ****$P < 0.0001$. Source data are available online for this figure.


(A)
Total Distance (cm/5 minutes)
800
600
400
200
0
0
30
60
90
120
150
Time (mins)
WT
A313V
(B)
****
Total Distance (cm)
10000
8000
6000
4000
2000
0
WT
A313V
(C)
Midbrain VMAT2 mRNA/Nurr1 mRNA (normalized to Pgk1)
*
200
150
100
50
0
WT
A313V
(D)
T Turn Time (s)
30
20
10
0
WT
A313V
DAT-KO
(E)
Latency to Descend (s)
50
40
30
20
10
0
WT
A313V
DAT-KO
(F)
% Foot Faults
6
4
2
0
WT
A313V
DAT-KO
(G)
Clasping Score
5
4
3
2
1
0
WT
A313V
DAT-KO
(H)
Habituation Index: time (min) to half max activity
****
400
300
200
100
0
WT
A313V

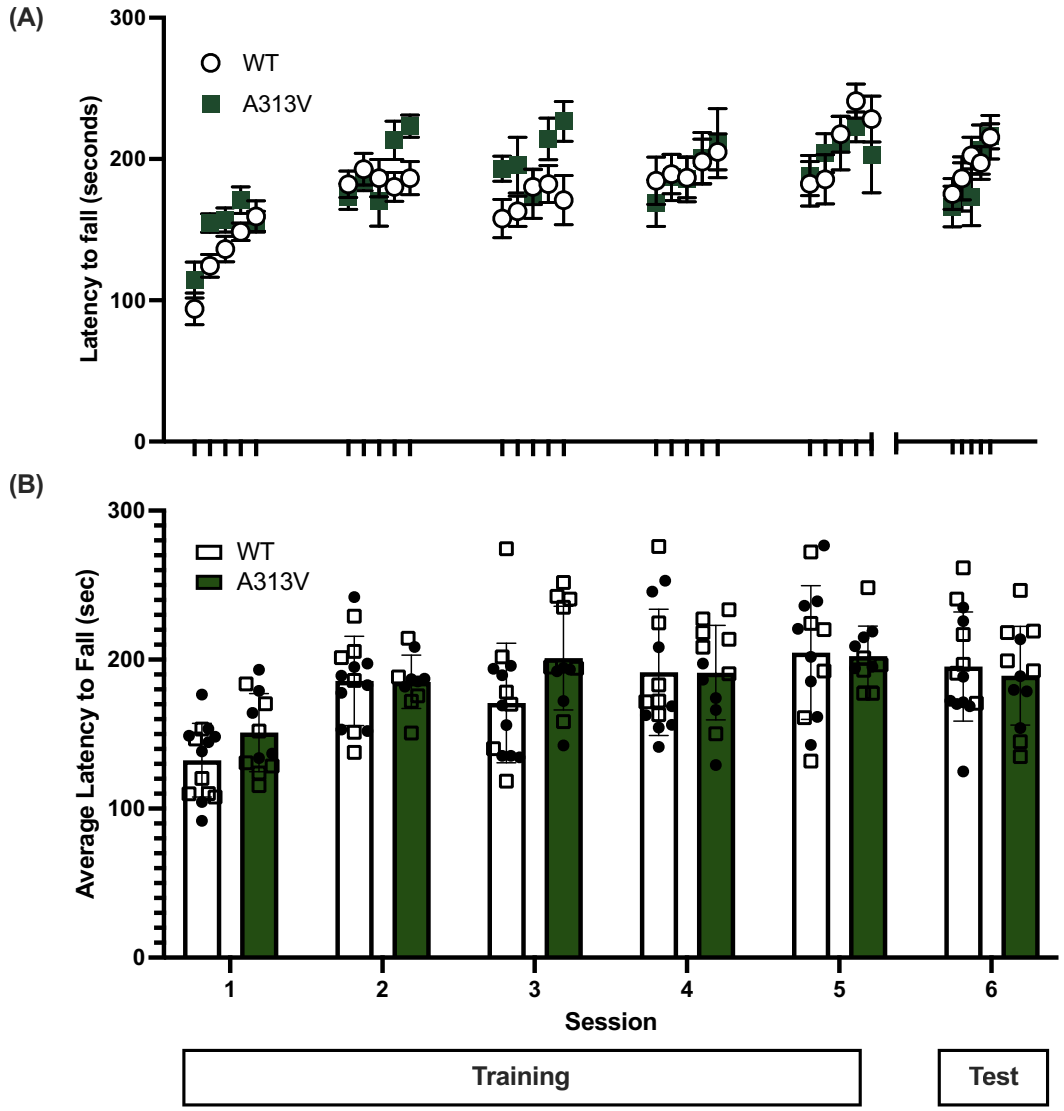


Figure EV2. Motor learning and coordination in A313V and WT mice.

(**A**) A nine-day rotarod experiment was conducted in wild-type (WT) ($n = 14$) and A313V dopamine transporter (DAT) knock-in (A313V) ($n = 12$) mice. Latency to fall was recorded for 5 sessions per day (training). After day 5, animals were rested for 3 days, and performance was tested again on the 9th day (test). (**B**) A mixed model analysis conducted on the average latency to fall on each day revealed no significant effect of genotype ($F_{1,13} = 0.6625$, $P = 0.4303$), or a genotype by trial interaction ($F_{5,50} = 1.727$, $P = 0.1455$). There was a significant effect of trial ($F_{5.65} = 14.79$, $P < 0.0001$). Results are presented as mean ± SEM. Source data are available online for this figure.

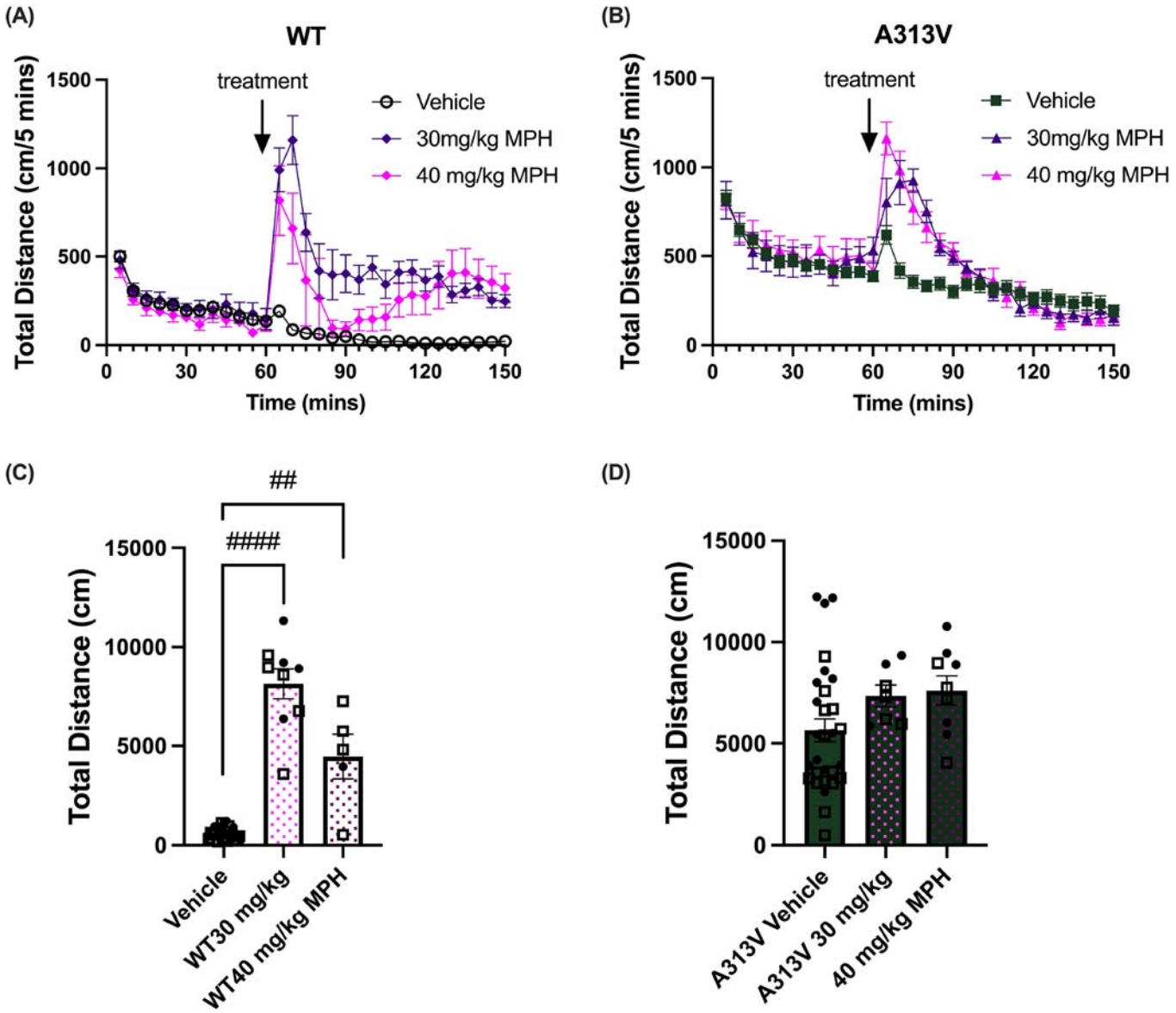


Figure EV3. MPH does not reduce locomotor activity in A313V mice.

Total distance was recorded in 5-min bins for 150 min in wild-type (WT) and dopamine transporter (DAT) A313V knock-in (A313V) mice. The test began with a 60-min baseline followed by intraperitoneal (i.p.) administration of vehicle, 30 mg/kg or 40 mg/kg methylphenidate (MPH) at the 60-min timepoint. Drug intervention is marked with an arrow. (**A**) Total distance traveled over time in WT and A313V mice treated with 30 or 40 mg/kg MPH (**A**, **B**) and cumulatively (**C**, **D**). MPH administration was not able to decrease hyperactivity in the A313V mice compared to vehicle-treated animals, as revealed by a two-way ANOVA with Tukey's multiple comparisons performed on the sum of total distance traveled post-treatment. There was a significant main effect of treatment ($F_{2,79} = 30.25$, $P<0.0001$), genotype ($F_{1,79} = 17.31$, $P<0.0001$), and a significant genotype by treatment interaction ($F_{2,79} = 10.59$, $P < 0.0001$). In WT mice, total distance traveled upon treatment of both 30 mg/kg and 40 mg/kg was increased compared to vehicle (vehicle: 652.62 ± 63.32 cm vs 30 mg/kg MPH: 8156.67 ± 750.17 cm, $P < 0.0001$; vehicle: 652.62 ± 63.32 cm vs 40 mg/kg MPH: 4468.40 ± 1127.82, $P = 0.0020$, respectively). In A313V mice, there was no significant differences between vehicle and 30 mg/kg MPH (vehicle: 5658.48 ± 576.75 cm vs 30 mg/kg MPH: 7357.29 ± 535.62 cm, $P = 0.1693$), and vehicle and 40 mg/kg MPH (vehicle: 5658.48 ± 576.75 cm vs 40 mg/kg MPH: 7623.44 ± 715.31 cm, $P = 0.0581$). In bar graphs, closed circular points represent values from female mice, and open square points represent values from male mice. Results are presented as mean ± SEM. A two-way ANOVA examining treatment, genotype, and interactions, with Šidák's multiple comparisons was conducted. For post hoc effects: $^{\#\#}P \leq 0.01$, $^{\#\#\#\#}P<0.0001$. Source data are available online for this figure.

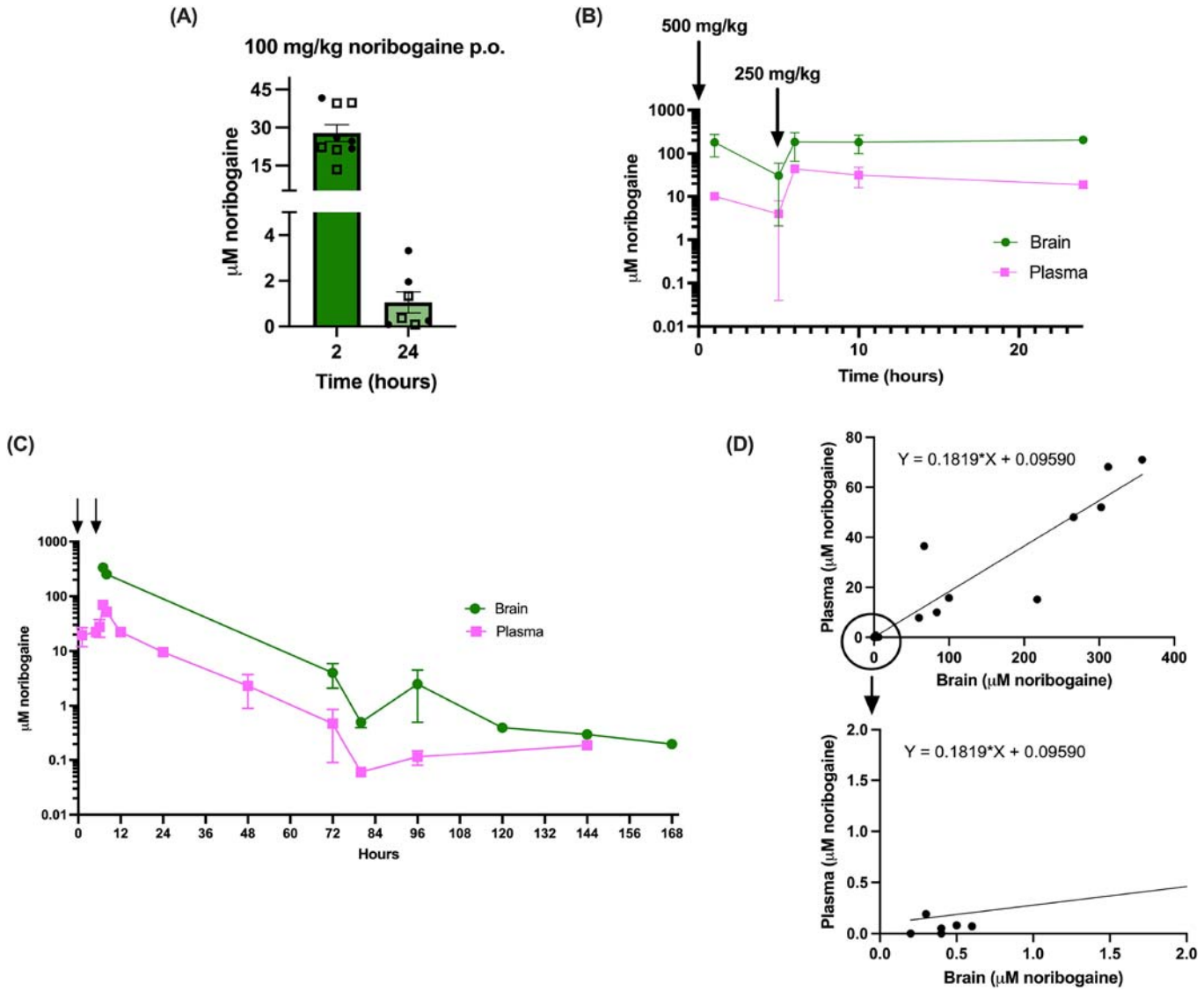


Figure EV4. Noribogaine PK analysis shows that 500 mg/kg + 250 mg/kg noribogaine is sufficient to achieve 100 μM brain noribogaine for at least 24 h.

(**A**) Mean noribogaine concentrations were measured by LC/MS/MS in cerebellar brain tissues at 2 and 24 h after oral administration of 100 mg/kg. This dose resulted in 27.84 μM ± 3.34 and 1.06 ± 0.46 μM of noribogaine in the brain after 2 and 24 h, respectively. (**B**) The dosing regimen was modified, and another cohort of mice received 500 mg/kg of noribogaine via oral gavage followed by a second 250 mg/kg dose (oral gavage) 5 h later. Brain noribogaine levels were above 100 μM (179.56 ± 95.94 μM) at 1 h and following the second injection and remained above this for at least 24 h. (**C**) 500 mg/kg noribogaine via oral gavage followed by 250 mg/kg 5 h later was carried out (arrows represent noribogaine administration) and plasma was collected at different time points (1, 5, 6, 12, 24, 48, 72, 80, 96, 120, and 144 h) after the initial 500 mg/kg noribogaine dose. Brains were collected at the 72, 80, 96, 120, 144, and 168 h timepoints. After the 500+250 mg/kg oral gavage, brain noribogaine concentrations fell below 100 μM at approximately 24 h and below 50 μM at ~36 h after first oral gavage. (**D**) Regression analysis of plasma vs. brain μM noribogaine gave an R-squared value of 0.8731. In bar graphs, closed circular points represent values from female mice, and open square points represent values from male mice. Results are presented as mean ± SEM. Source data are available online for this figure.

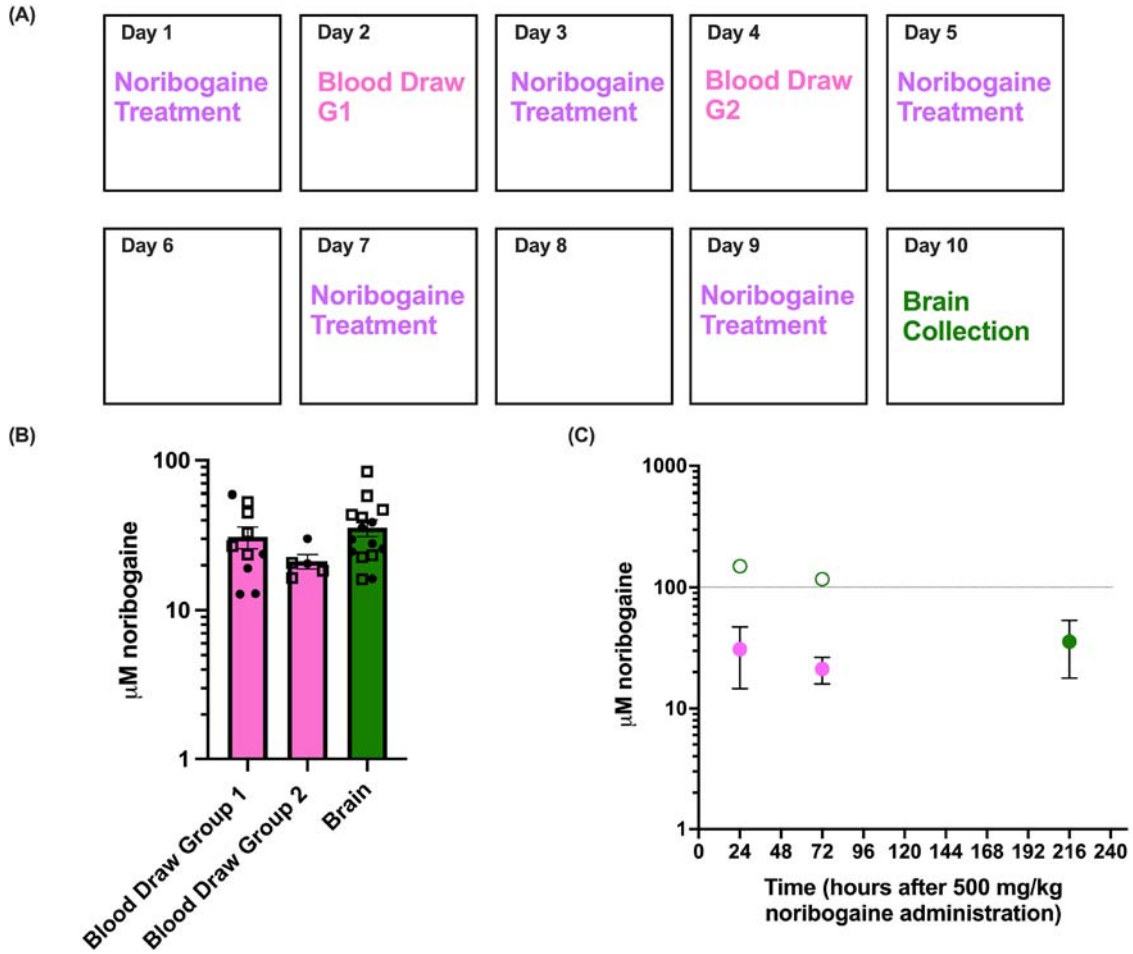


Figure EV5. Noribogaine levels in the brain fall below 100 μM at approximately 96 h.

(**A**) 500 + 250 mg/kg noribogaine was administered every 48 h over the course of 9 days, with brains collected on day 10, 24 h after the 4th noribogaine dose. Blood was also collected from two groups of animals on day 2 (group 1) and 4 (group 2), 24 h after the 500 mg/kg noribogaine administration on days 1 and 3, respectively. (**B**) The mean noribogaine plasma concentration on day 2 was 30.93 μM ± 5.17 and on day 4 was 21.23 ± 2.36 μM. The mean noribogaine brain concentration on day 10 was 35.68 μM ± 4.60. (**C**) The brain concentrations of noribogaine (open circles) were estimated based on the previously established relationship between plasma and brain noribogaine concentrations. This indicated that on day 2, brain noribogaine concentrations were approximately 169.94 μM and 116.64 μM on day 4. In bar graphs, closed circular points represent values from female mice, and open square points represent values from male mice. Results are presented as mean ± SEM. Source data are available online for this figure.