**Data Supplement:**

# Male DAT Val559 Mice Exhibit Compulsive Behavior Under Devalued Reward Conditions Accompanied by Cellular and Pharmacological Changes

Adele Stewart PhD[1,2,#], Gwynne L. Davis PhD[1,#], Lorena B. Areal PhD[1], Maximilian J. Rabil[1], Vuong Tran[1], Felix P. Mayer PhD[1], and Randy D. Blakely PhD[1,2,*]

[1]Department of Biomedical Science and [2]Brain Institute, Florida Atlantic University, Jupiter, FL.

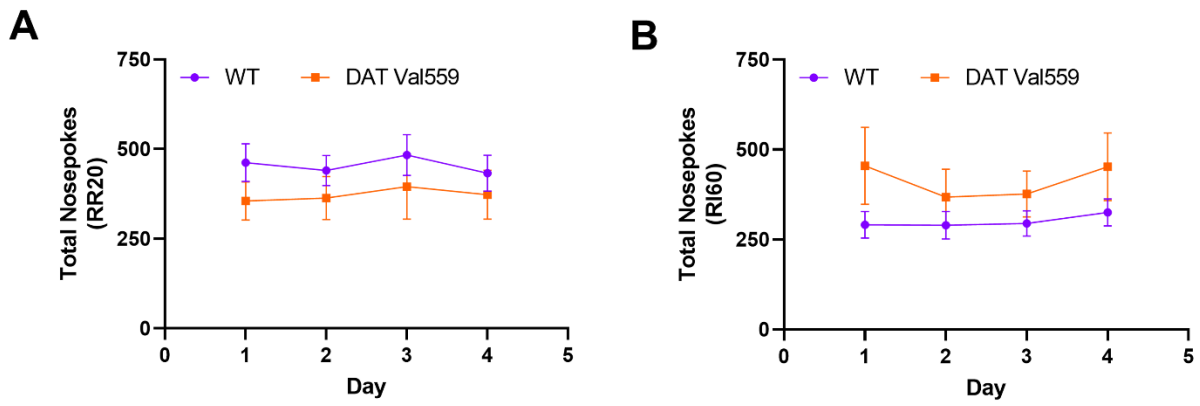


**Figure S1. Instrumental responding is stable across the final days of training in the Goal vs Habit nose poke paradigm.** WT (n=12) and DAT Val559 (n=10) males were subjected to a within subject lever pressing paradigm that utilizes contextual cues coupled to random ratio (RR) or random interval (RI) reinforcement schedules to bias animals toward goal-directed or habitual actions, respectively. Total nose pokes during the RR20/RI60 phase of the paradigm are depicted for the (A) RR and (B) RI context. There was no significant impact of genotype or day on total nose pokes for either context (See Table S2). Data were analyzed by two-way RM-ANOVA. Data are presented as mean ± SEM.

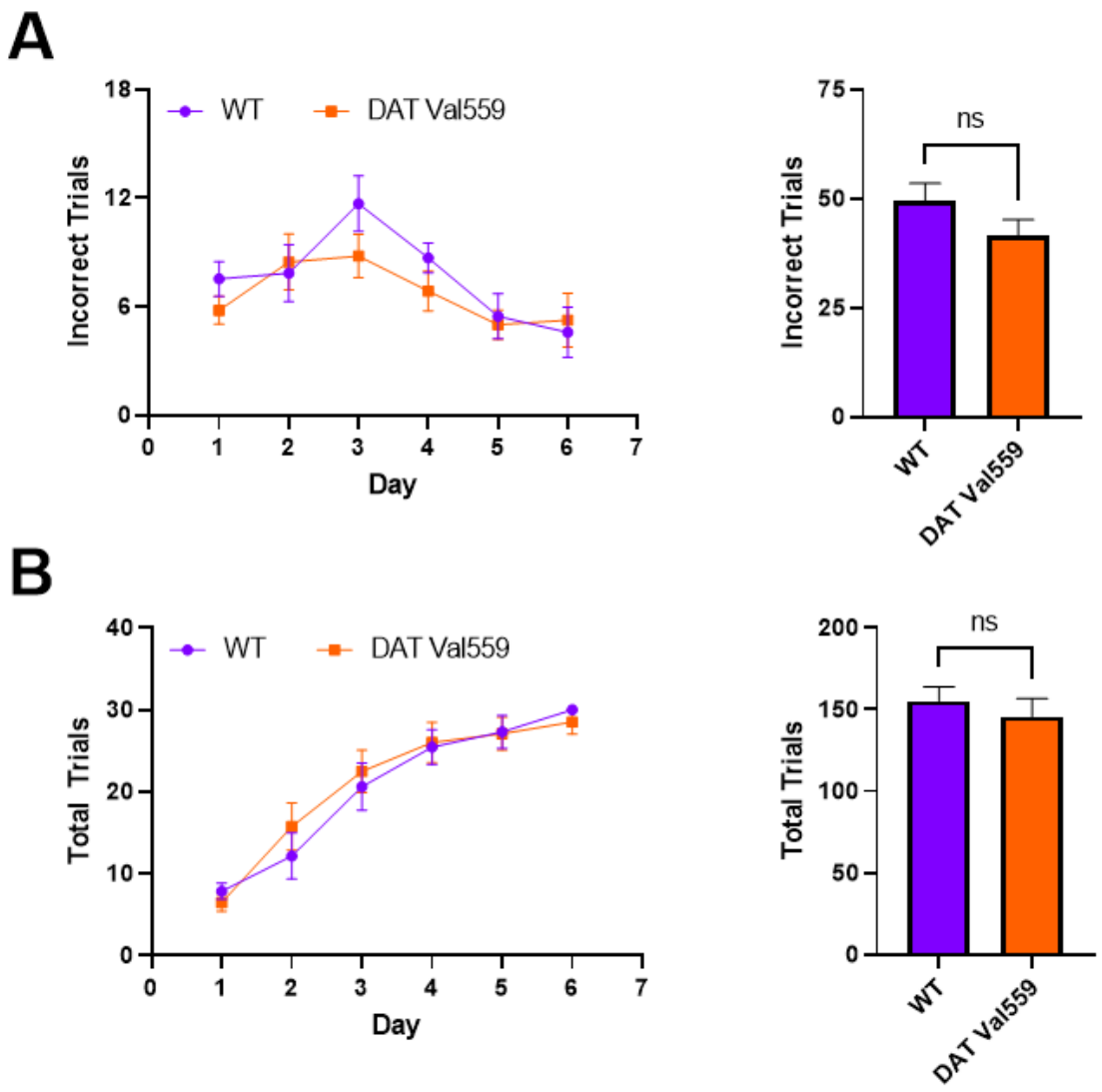


**Figure S2. Supplemental reversal learning data.** WT (n=14) and DAT Val559 (n=15) mice underwent pairwise discrimination training followed by a reversal phase. (A) Incorrect trials by day (left) and summed (right) during the reversal learning phase. (B) Trials completed by day (left) and summed (right) during the reversal learning phase. Data were analyzed by two-tailed student's t-test or two-way RM-ANOVA with Sidak's multiple comparison's test. ns = not significant. Data are presented as mean ± SEM.

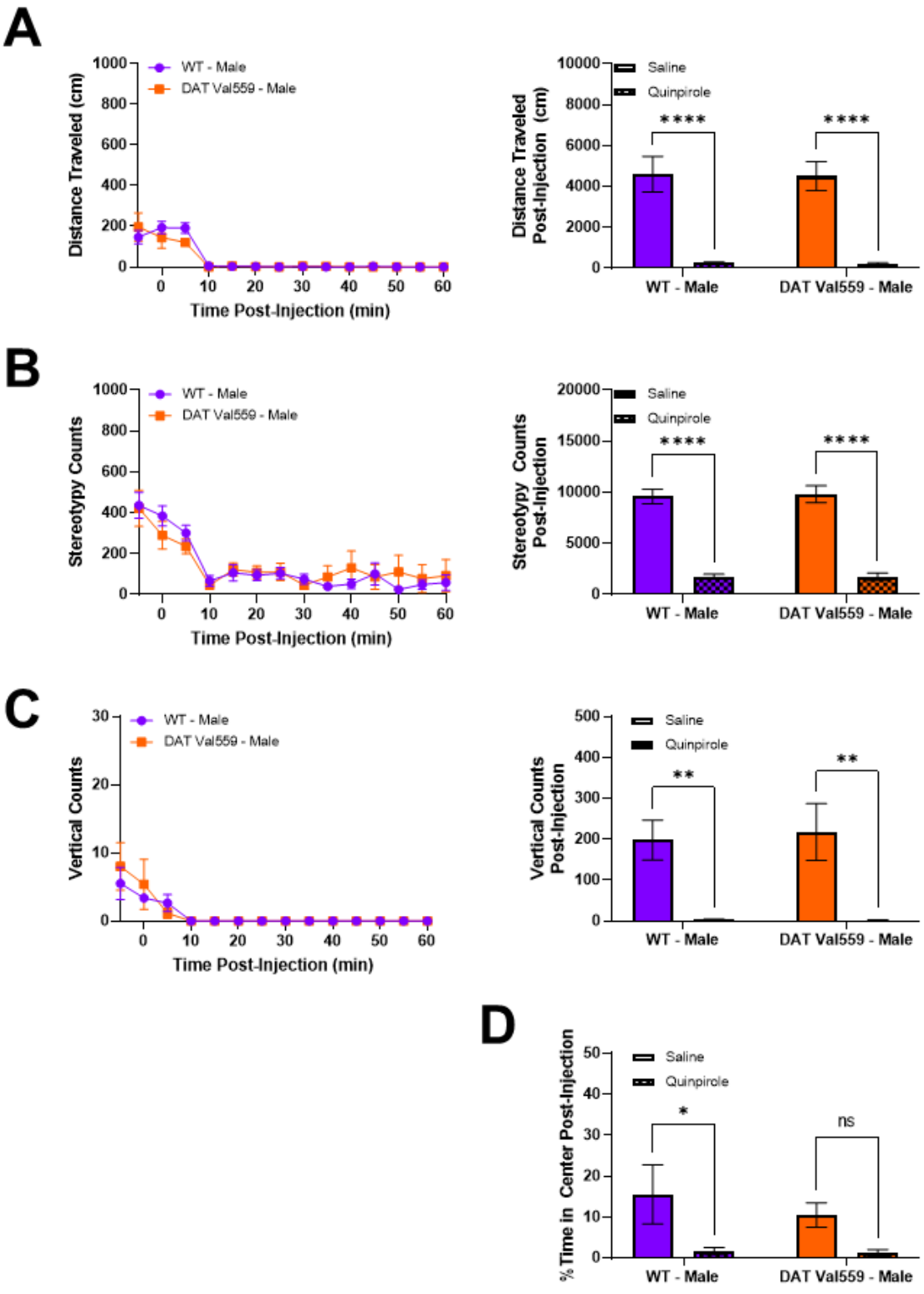
A
Distance Traveled (cm)
WT - Male
DAT Val559 - Male
Time Post-Injection (min)
Distance Traveled Post-Injection (cm)
Saline
Quinpirole
****
****
WT - Male
DAT Val559 - Male
B
Stereotypy Counts
Time Post-Injection (min)
Stereotypy Counts Post-Injection
C
Vertical Counts
Time Post-Injection (min)
Vertical Counts Post-Injection
**
**
D
% Time in Center Post-Injection
*
ns

**Figure S3. Quinpirole-dependent locomotor suppression is comparable in WT and DAT Val559 mice.** WT (n=8) and DAT Val559 (n=8) mice were given a single injection of the D2/D3 agonist quinpirole (1 mg/kg, i.p.) and locomotor activity recorded for 60 minutes post-injection. Datasets are presented in 5-minute time bins across the recording period and as summary data adding up all activity post-injection. (A) Horizontal distance traveled. (B) Stereotypic motor movements. (C) Vertical locomotor activity. (D) % Time spent in the center of the chamber. Data were analyzed by two-way ANOVA with Sidak's multiple comparison's test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. ns = not significant. Data are presented as mean ± SEM.

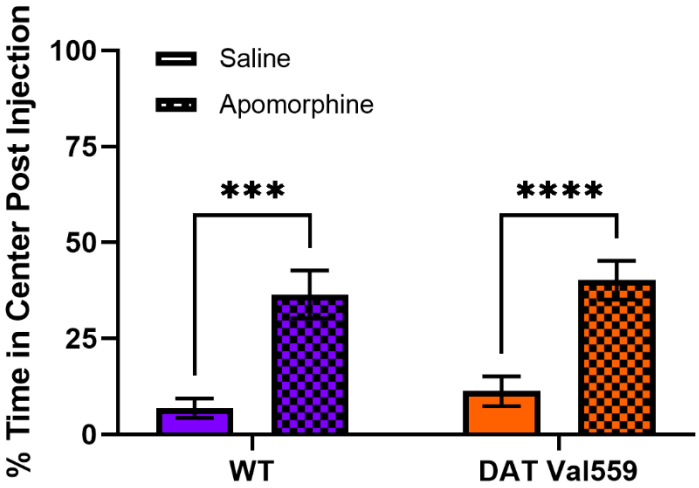


**Figure S4. WT and DAT Val559 mice increase center occupancy in response to apomorphine.** WT (n=13) and DAT Val559 (n=15) mice were given a single injection of the DA agonist apomorphine (5 mg/kg, s.c.) and locomotor activity recorded for 60 minutes post-injection. % Time spent in the center of the chamber after drug administration is depicted. Data were analyzed by two-way ANOVA with Sidak's multiple comparison's test. ***P < 0.001, ****P < 0.0001. Data are presented as mean ± SEM.

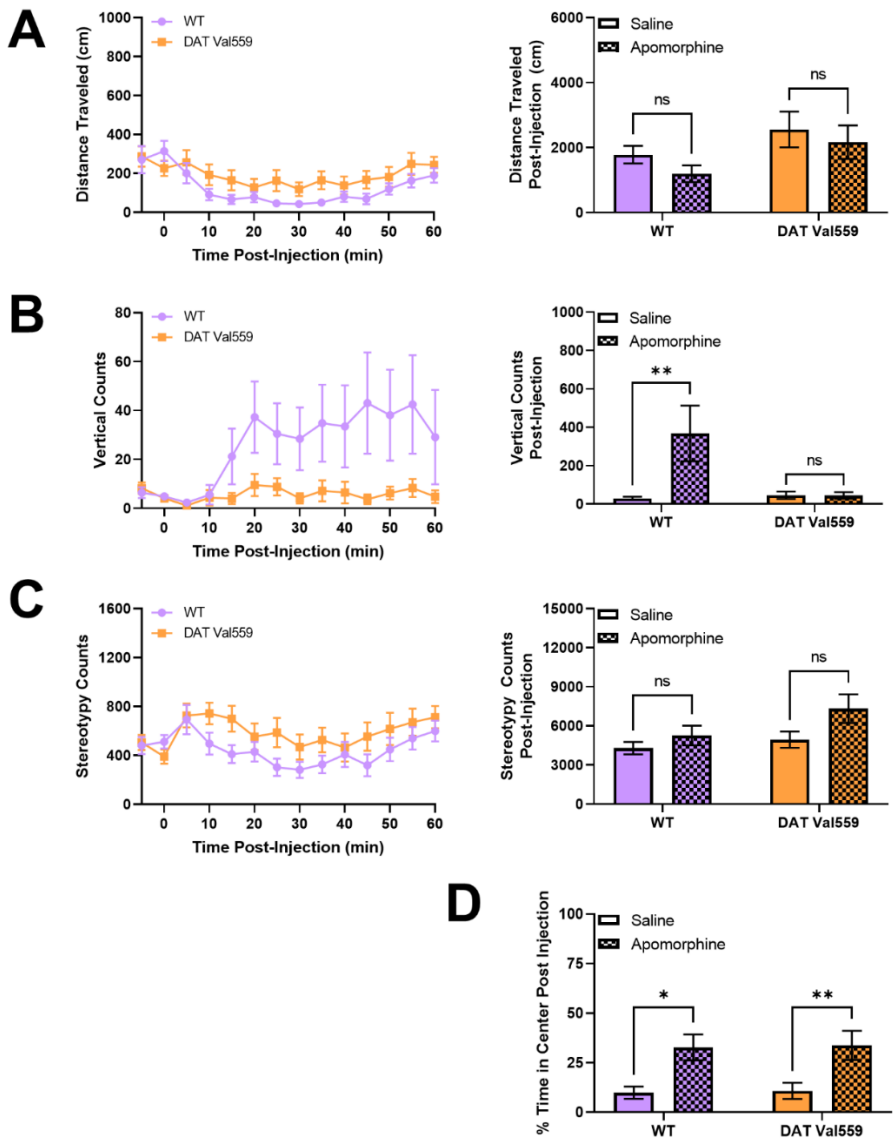


**Figure S5. DAT Val559 females display a loss of apomorphine-dependent rearing.** WT (n=14) and DAT Val559 (n=14) female mice were given a single injection of the DA agonist apomorphine (5 mg/kg, s.c.) and locomotor activity recorded for 60 minutes post-injection. Datasets are presented in 5-minute time bins across the recording period and as summary data adding up all activity post-injection. (A) Horizontal distance traveled. (B) Vertical locomotor activity. (C) Stereotypic motor movements. (D) Center occupancy. Data were analyzed by two-way ANOVA with Sidak's multiple comparison's test. *P < 0.05, **P < 0.01. ns = not significant. Data are presented as mean ± SEM.

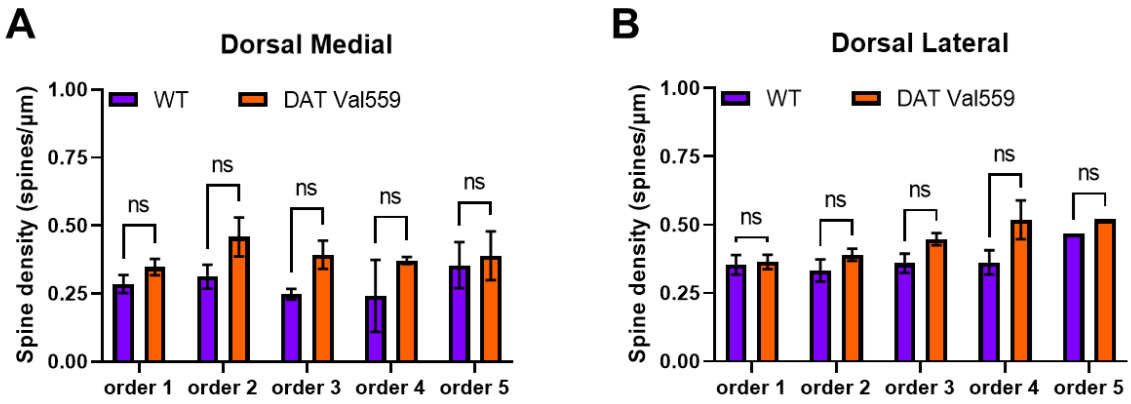


**Figure S6. Supplemental dendritic spine data.** Spine density in sections from WT (n=4) and DAT Val559 (n=4) mice was assessed utilizing Golgi staining coupled to brightfield microscopy. Spine densities were stratified based on degree of separation from the cell soma (order 1-5) in the (A) DMS and (B) DLS. Data were analyzed by two-way ANOVA with Sidak's multiple comparison's test. ns = not significant. Data are presented as mean ± SEM.

**Table S1:** Complete statistical analysis for data analyzed by Student's t-test. Cohen's *d* is provided as an indicator of effect size. For reference, *d* = 0.2 would be considered a 'small' effect size, 0.5 represents a 'medium' effect size and 0.8 a 'large' effect size

| Figure | t, df | P value | Significant? | Effect Size, Cohen's *d* |
|---|---|---|---|---|
| 2A | t=1.339, df=40 | 0.1881 | | 0.412 |
| 2B | t=2.233, df=41 | 0.0311 | * | 0.676 |
| 2C | t=2.141, df=41 | 0.0383 | * | 0.653 |
| 2D | t=0.3684, df=41 | 0.7145 | | 0.112 |
| 3C | t=2.045, df=27 | 0.0507 | | 0.755 |
| 3D | t=2.419, df=27 | 0.0226 | * | 0.894 |
| 3E | t=1.904, df=25 | 0.0685 | | 0.729 |
| 3F | t=0.7967, df=27 | 0.4326 | | 0.295 |
| 5B | t=2.577, df=6 | 0.0419 | * | 1.821 |
| 5C | t=1.852, df=6 | 0.1135 | | 1.310 |
| S1A | t=1.473, df=26 | 0.1528 | | 0.558 |
| S1B | t=0.6684, df=26 | 0.5098 | | 0.256 |

**Table S2**: Complete statistical analysis for data analyzed by two-way ANOVA. Eta squared ($\eta^2$) is provided as an indicator of effect size. For reference, $\eta^2 = 0.01$ indicates a small effect; $\eta^2 = 0.06$ indicates a medium effect; $\eta^2 = 0.14$ indicates a large effect.

| Figure | Source of Variation | F (DFn, DFd) | P value | Significant? | Effect Size, $\eta^2$ |
|---|---|---|---|---|---|
| 1C | Interaction | F (3, 80) = 0.07582 | P=0.9729 | | 0.0028 |
| | Genotype | F (1, 80) = 0.002305 | P=0.9618 | | 0.0000 |
| | Context | F (3, 80) = 32.32 | P<0.0001 | **** | 0.5480 |
| 1D | Interaction | F (1, 37) = 0.1362 | P=0.7142 | | 0.0037 |
| | Context | F (1, 37) = 1.271 | P=0.2668 | | 0.0332 |
| | Genotype | F (1, 37) = 14.82 | P=0.0005 | *** | 0.2860 |
| 1E | Interaction | F (1, 40) = 1.549 | P=0.2205 | | 0.0373 |
| | Food type | F (1, 40) = 245.2 | P<0.0001 | **** | 0.8598 |
| | Genotype | F (1, 40) = 2.661 | P=0.1107 | | 0.0624 |
| 4A | Interaction | F (1, 52) = 0.1150 | P=0.7359 | | 0.0022 |
| | Genotype | F (1, 52) = 1.656 | P=0.2038 | | 0.0309 |
| | Drug | F (1, 52) = 0.1098 | P=0.7417 | | 0.0021 |
| 4B | Interaction | F (1, 52) = 0.1796 | P=0.6734 | | 0.0034 |
| | Genotype | F (1, 52) = 3.799 | P=0.0567 | | 0.0681 |
| | Drug | F (1, 52) = 1.371 | P=0.2469 | | 0.0257 |
| 4C | Interaction | F (1, 51) = 3.455 | P=0.0688 | | 0.0634 |
| | Genotype | F (1, 51) = 7.226 | P=0.0097 | ** | 0.1241 |
| | Drug | F (1, 51) = 16.10 | P=0.0002 | *** | 0.2400 |
| 5B | Interaction | F (2, 18) = 1.008 | P=0.3847 | | 0.1007 |
| | Spine Type | F (2, 18) = 55.46 | P<0.0001 | **** | 0.8604 |
| | Genotype | F (1, 18) = 6.901e-017 | P>0.9999 | | 0.0000 |
| 5C | Interaction | F (2, 18) = 1.041 | P=0.3733 | | 0.1037 |
| | Spine Type | F (2, 18) = 62.42 | P<0.0001 | **** | 0.8740 |
| | Genotype | F (1, 18) = 0.01895 | P=0.8920 | | 0.0011 |
| S1A | Day x Genotype | F (3, 60) = 0.1960 | P=0.8987 | | 0.0097 |
| | Day | F (2.515, 50.31) = 0.6441 | P=0.5638 | | 0.0312 |
| | Genotype | F (1, 20) = 1.223 | P=0.2819 | | 0.1897 |
| S1B | Day x Genotype | F (3, 60) = 0.6364 | P=0.5945 | | 0.0308 |
| | Day | F (3, 60) = 1.316 | P=0.2775 | | 0.0617 |
| | Genotype | F (1, 20) = 2.097 | P=0.1631 | | 0.2477 |
| S3A | Interaction | F (1, 28) = 0.001124 | P=0.9735 | | 0.0000 |
| | Genotype | F (1, 28) = 0.01867 | P=0.8923 | | 0.0007 |
| | Drug | F (1, 28) = 58.76 | P<0.0001 | **** | 0.6773 |
| S3B | Interaction | F (1, 28) = 0.03167 | P=0.8600 | | 0.0011 |
| | Genotype | F (1, 28) = 0.03833 | P=0.8462 | | 0.0014 |

| | | | | | |
|---|---|---|---|---|---|
| | Drug | F (1, 28) = 182.4 | P<0.0001 | **** | 0.8669 |
| S3C | Interaction | F (1, 28) = 0.06037 | P=0.8077 | | 0.0022 |
| | Genotype | F (1, 28) = 0.04678 | P=0.8303 | | 0.0017 |
| | Drug | F (1, 28) = 23.33 | P<0.0001 | **** | 0.4545 |
| S3D | Interaction | F (1, 27) = 0.3251 | P=0.5733 | | 0.0119 |
| | Genotype | F (1, 27) = 0.4466 | P=0.5096 | | 0.0163 |
| | Drug | F (1, 27) = 8.076 | P=0.0084 | ** | 0.2303 |
| S4 | Interaction | F (1, 52) = 0.006125 | P=0.9379 | | 0.0001 |
| | Genotype | F (1, 52) = 0.7662 | P=0.3854 | | 0.0145 |
| | Drug | F (1, 52) = 39.89 | P<0.0001 | **** | 0.4341 |
| S5A | Interaction | F (1, 52) = 0.05409 | P=0.8170 | | 0.0010 |
| | Genotype | F (1, 52) = 4.308 | P=0.0429 | * | 0.0765 |
| | Drug | F (1, 52) = 1.354 | P=0.2499 | | 0.0254 |
| S5B | Interaction | F (1, 52) = 0.8340 | P=0.3653 | | 0.0158 |
| | Genotype | F (1, 52) = 3.071 | P=0.0856 | | 0.0558 |
| | Drug | F (1, 52) = 4.654 | P=0.0356 | * | 0.0821 |
| S5C | Interaction | F (1, 51) = 5.100 | P=0.0282 | * | 0.0909 |
| | Genotype | F (1, 51) = 4.162 | P=0.0465 | * | 0.0754 |
| | Drug | F (1, 51) = 5.019 | P=0.0295 | * | 0.0896 |
| S5D | Interaction | F (1, 52) = 0.0002238 | P=0.9881 | | 0.0000 |
| | Genotype | F (1, 52) = 0.03106 | P=0.8608 | | 0.0006 |
| | Drug | F (1, 52) = 17.19 | P=0.0001 | *** | 0.2484 |
| S6A | Interaction | F (4, 20) = 0.3888 | P=0.8141 | | 0.0721 |
| | Spine Type | F (4, 20) = 0.8154 | P=0.5303 | | 0.1402 |
| | Genotype | F (1, 20) = 7.600 | P=0.0122 | * | 0.2754 |
| S6B | Interaction | F (4, 23) = 0.9695 | P=0.4432 | | 0.1442 |
| | Spine Type | F (4, 23) = 2.519 | P=0.0690 | | 0.3045 |
| | Genotype | F (1, 23) = 6.004 | P=0.0223 | * | 0.2069 |