

Supplementary Information for

D1 dopamine receptor antagonists as a new therapeutic strategy to treat autistic-like behaviours in lysosomal storage disorders

Maria De Risi^{1,2*}, Lorenzo Cusimano¹, Xabier Bujanda Cundin¹, Mariateresa Pizzo¹, Ylenia Gigante¹, Mariagrazia Monaco¹, Chiara Di Eugenio², Elvira De Leonibus^{1,2*}

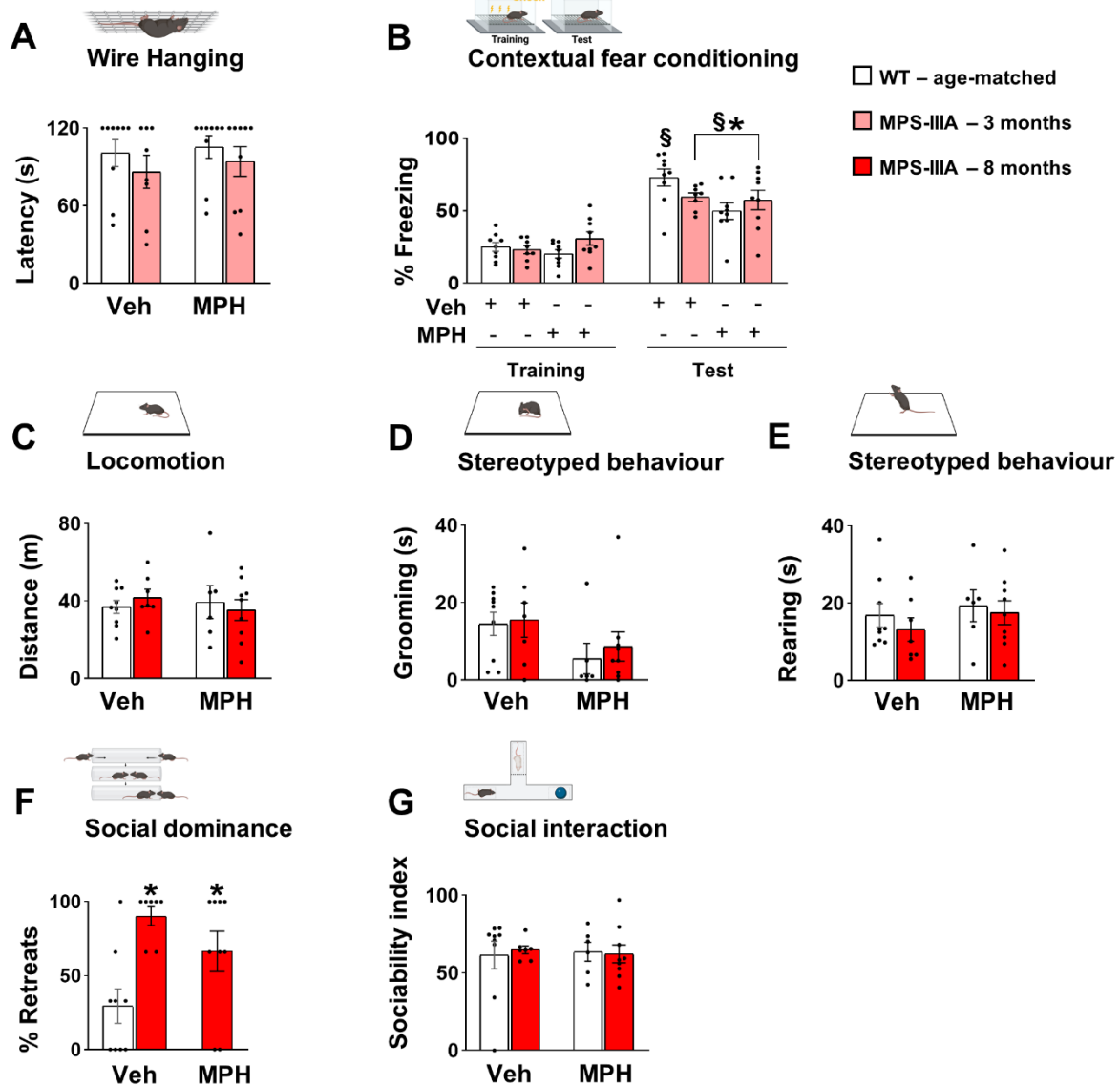
¹ Telethon Institute of Genetics and Medicine, Via Campi Flegrei 34, 80078 Pozzuoli, Naples, Italy

² Institute of Biochemistry and Cell Biology, CNR, Via Ramarini 32, 00015 Monterotondo Scalo, Rome, Italy

This PDF file includes:

- Supplementary Figures 1 to 3
- Supplementary Table 1 to 5

Chronic Methylphenidate treatment

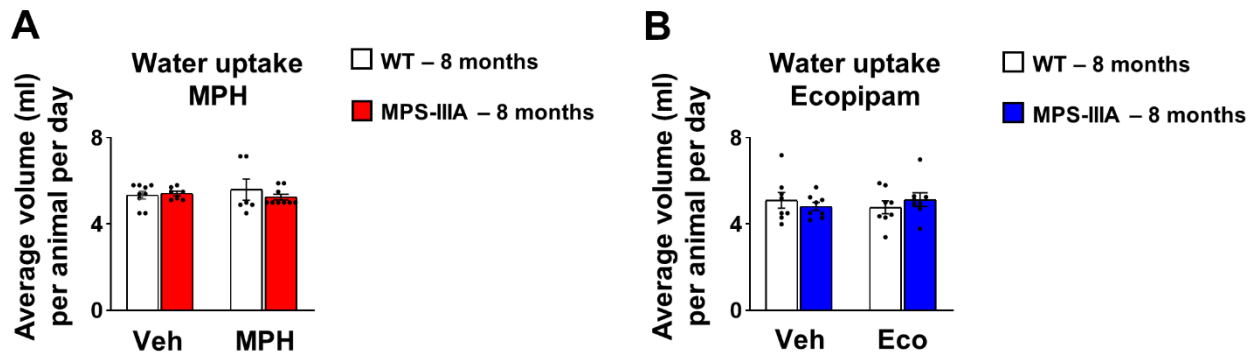


Supplementary Figure 1

(A-B) 3-month-old vehicle-treated (oral administration) MPS-IIIa (light red bars) show no defect in the hanging wire test compared to age-matched WT. In the contextual fear conditioning test, even if there is a decrease in the percentage of freezing compared to the vehicle-treated WT in the test phase, there is a significant increase compared to the training phase (Repeated measures ANOVA Genotype x Treatment $F_{1,31} = 6.22$; $p = 0.01$; % Freezing $F_{1,31} = 118.7$; $p < 0.001$; % Freezing x Treatment $F_{1,31} = 4.7$; $p = 0.03$). MPH has no significant effects on both hanging wire and fear conditioning test. WT veh N = 9; WT MPH N = 9; MPS veh N = 8; MPS MPH N = 9.

(C-G) At 8 months of age, no significant difference was observed between vehicle-treated WT and MPS-IIIa mice (dark red bars) due to the regression of autistic-like behaviours at this age, except for

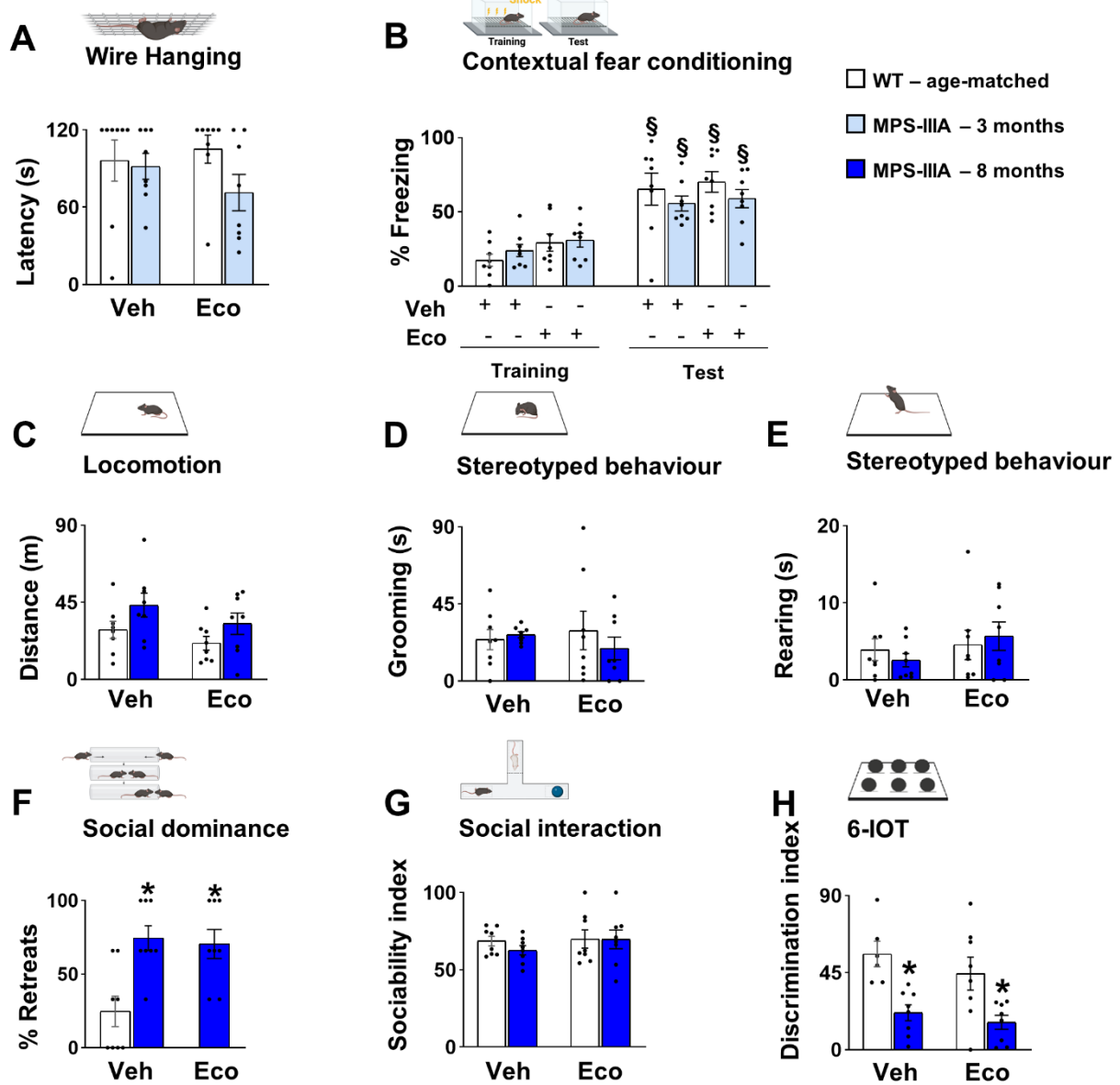
the social dominance tube test (χ^2 vehicle = 0.02 ; χ^2 MPH = 0.005). No significant effects of MPH are observed (WT veh N = 9; WT MPH N = 7; MPS veh N = 7; MPS MPH N = 9). Histograms represent mean $\pm$ S.E.M. * p < 0.05, between genotype, within treatment; § p < 0.05 test phase vs training phase, Bonferroni *post-hoc*.



Supplementary Figure 2

(A-B) Water uptake expressed as average volume (ml) consumed per animal per day for both MPH and ecopipam. Statistical analysis show no significant effects for the genotype and treatment (Kruskal-Wallis test for MPH: $p=0.6$; Kruskal-Wallis test for ecopipam: $p=0.8$). Histograms represent mean $\pm$ S.E.M.

Chronic Ecopipam treatment



Supplementary Figure 3

(A-B) 3-month-old vehicle-treated MPS-IIIa (blue bars) show no defect in the hanging wire test and in the fear conditioning test compared to age-matched WT. No significant effects of ecopipam are observed.

(C-G) At 8 months of age no significant difference was observed between vehicle-treated WT and MPS-IIIa mice (blue bars) due to the regression of autistic-like behaviours at this age, except for the social dominance tube test (χ^2 vehicle < 0.05; χ^2 eco < 0.05). Additionally, no significant effects of ecopipam were observed.

(H) At 8 months of age, vehicle-treated MPS-IIIA mice show an impairment in 6-IOT, which was not rescued by ecopipam. (Two-way ANOVA: Genotype $F_{1,26}=20.9$; $p=0.0001$). $N = 8$ for each group, except for 6-IOT WT veh $N = 6$. Histograms represent mean $\pm$ S.E.M. * $p < 0.05$, between genotype, within treatment; § $p < 0.05$ test phase vs training phase, Bonferroni *post-hoc*.

Experimental group	Total exploration (s) - Habituation phase
WT vehicle	150.8 ± 21.0
WT risp 0.1	85.2 ± 19.0
WT risp 0.2	105.0 ± 29.5
MPS vehicle	115.4 ± 13.6
MPS risp 0.1	142.3 ± 30.3
MPS risp 0.2	113.6 ± 29.5

Supplementary Table 1. No significant differences were found in the total exploration time (seconds) during the habituation phase of the social interaction test after vehicle or acute risperidone treatment (Two-way ANOVA: Genotype $F_{1,42} = 0.24$; $p = 0.6$; Treatment $F_{2,42} = 0.5$; $p = 0.6$; Genotype x Treatment $F_{2,42} = 1.6$; $p = 0.2$). Data are expressed as mean ± SEM.

Experimental group	Total exploration (s) - Habituation phase
WT vehicle – 3 months	198.4 ± 41.2
WT MPH – 3 months	215.8 ± 14.0
MPS vehicle – 3 months	187.5 ± 14.8
MPS MPH – 3 months	180.1 ± 14.9
WT vehicle – 8 months	126.1 ± 35.8
WT MPH – 8 months	202.3 ± 17.4
MPS vehicle – 8 months	191.8 ± 23.6
MPS MPH – 8 months	168.8 ± 26.4

Supplementary Table 2. No significant differences were found in the total exploration time (seconds) during the habituation phase of the social interaction test after vehicle or MPH chronic treatment (3 months Two-way ANOVA: Genotype $F_{1,31} = 0.99$; $p = 0.32$; Treatment $F_{1,31} = 0.83$; $p = 0.8$; Genotype x Treatment $F_{1,31} = 0.28$; $p = 0.2$; 8 months Two-way ANOVA: Genotype $F_{1,27} = 0.29$; $p = 0.5$; Treatment $F_{1,27} = 0.8$; $p = 0.38$; Genotype x Treatment $F_{1,27} = 2.7$; $p = 0.11$). Data are expressed as mean ± SEM.

Experimental group	Total exploration (s) - Habituation phase
WT vehicle	175.9 ± 39.3
WT eco 0.015	168.3 ± 43.2
WT eco 0.03	124.9 ± 26.0
MPS vehicle	159.0 ± 15.3
MPS eco 0.015	163.6 ± 50.8
MPS eco 0.03	99.2 ± 27.6

Supplementary Table 3. No significant differences were found in the total exploration time (seconds) during the habituation phase of the social interaction test after vehicle or acute ecopipam treatment (Two-way ANOVA: Genotype $F_{1,48} = 0.29$; $p = 0.59$; Treatment $F_{2,48} = 1.5$; $p = 0.22$; Genotype x Treatment $F_{2,48} = 0.04$; $p = 0.95$). Data are expressed as mean ± SEM.

Experimental group	Total exploration (s) - Habituation phase
WT vehicle – 3 months	198.6 ± 36.4
WT eco – 3 months	162.9 ± 13.5
MPS vehicle – 3 months	163.1 ± 16.6
MPS eco – 3 months	198.3 ± 12.1
WT vehicle – 8 months	170.5 ± 27.1
WT eco – 8 months	166.5 ± 23.9
MPS vehicle – 8 months	176.6 ± 19.7
MPS eco – 8 months	220.5 ± 29.8

Supplementary Table 4. No significant differences were found in the total exploration time (seconds) during the habituation phase of the social interaction test after vehicle or ecopipam chronic treatment (3 months Two-way ANOVA: Genotype $F_{1,28} = 7.4$; $p = 0.99$; Treatment $F_{1,28} = 1.22$; $p = 0.99$; Genotype x Treatment $F_{1,28} = 2.5$; $p = 0.11$; 8 months Two-way ANOVA: Genotype $F_{1,28} = 1.3$; $p = 0.24$; Treatment $F_{1,28} = 0.61$; $p = 0.43$; Genotype x Treatment $F_{1,28} = 0.88$; $p = 0.35$). Data are expressed as mean ± SEM.

Experimental group	Total exploration (s) - Study phase
WT vehicle – 3 months	53.775 ± 7.048
WT eco – 3 months	83.038 ± 16.867
MPS vehicle – 3 months	92.338 ± 11.497
MPS eco – 3 months	85.938 ± 7.834
WT vehicle – 8 months	67.300 ± 8.911
WT eco – 8 months	93.775 ± 8.102
MPS vehicle – 8 months	98.375 ± 11.757*
MPS eco – 8 months	107.925 ± 12.18*

Supplementary Table 5. No significant differences were found at 3 months of age in the total exploration time (seconds) during the study phase of the 6-IOT after vehicle or ecopipam chronic treatment (Two-way ANOVA: Genotype $F_{1,28} = 1.7$; $p = 0.19$; Treatment $F_{1,28} = 0.04$; $p = 0.83$; Genotype x Treatment $F_{1,28} = 0.82$; $p = 0.37$). At 8 months, a significant effect for genotype was found (Two-way ANOVA: Genotype $F_{1,26} = 4.43$; $p = 0.04$; Treatment $F_{1,26} = 1.8$; $p = 0.18$; Genotype x Treatment $F_{1,26} = 1.4$; $p = 0.23$). Data are expressed as mean ± SEM. * $p < 0.05$.