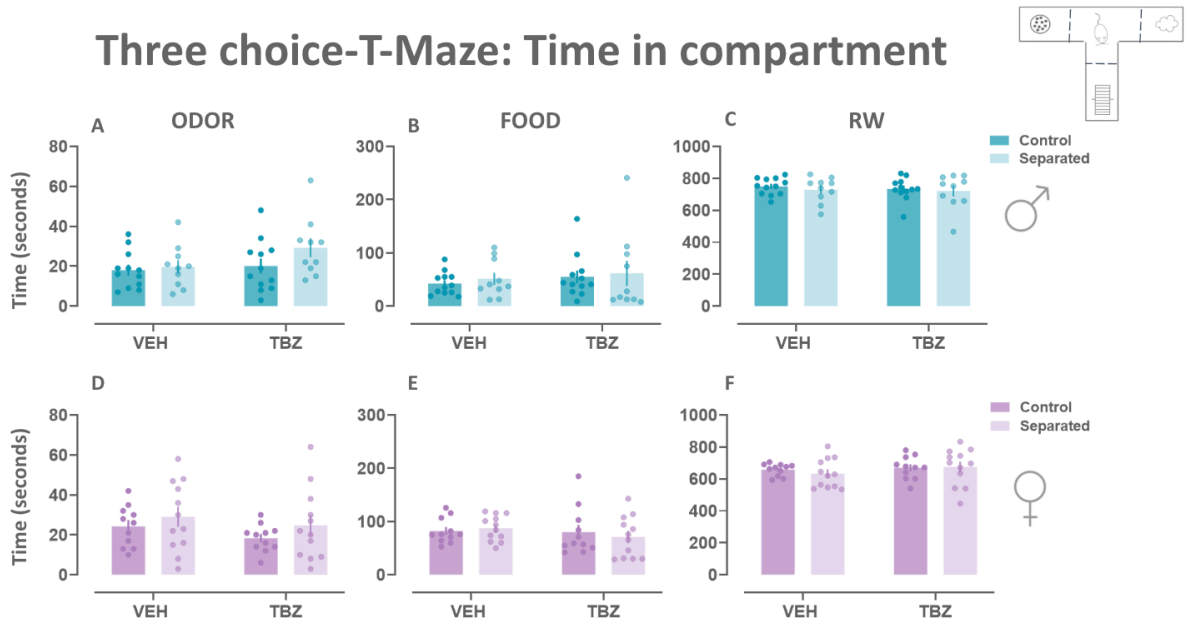
Supplemental material

Sup. Exp. 1. Impact of MS and TBZ on time spent in compartments in male and female mice.

The two-way ANOVAs on time spent in each one of the T-maze compartments showed that neither treatment nor separation changed time spent in proximity of any of the stimuli, neither on males nor on females, indicating no avoidance of the stimuli. Food compartment: treatment ($F(1,21)=0.99$, $p=0.33$, $\eta p^2=0.047$), separation ($F(1,21)=0.24$, $p=0.63$, $\eta p^2=0.012$), and interaction ($F(1,21)=0.01$, $p=0.92$, $\eta p^2=0.001$). RW compartment: treatment ($F(1,20)=0.48$, $p=0.50$, $\eta p^2=0.023$), separation ($F(1,20)=0.33$, $p=0.57$, $\eta p^2=0.016$), and interaction ($F(1,20)=0.051$, $p=0.82$, $\eta p^2=0.003$). Odor compartment: treatment ($F(1,20)=2.31$, $p=0.14$, $\eta p^2=0.103$), separation ($F(1,20)=2.43$, $p=0.14$, $\eta p^2=0.108$), and interaction ($F(1,20)=0.95$, $p=0.34$, $\eta p^2=0.045$).

The same pattern was observed for females. Food compartment: treatment ($F(1,21)=1.52$, $p=0.23$, $\eta p^2=0.067$), separation ($F(1,21)=0.03$, $p=0.88$, $\eta p^2=0.001$), and interaction ($F(1,21)=0.92$, $p=0.35$, $\eta p^2=0.042$). RW compartment, the two-way ANOVAs revealed no effects of main factors treatment ($F(1,21)=2.32$, $p=0.14$, $\eta p^2=0.099$), or separation ($F(1,21)=0.09$, $p=0.77$, $\eta p^2=0.004$), and no significant interaction ($F(1,21)=0.75$, $p=0.40$, $\eta p^2=0.035$). Odor compartmente: treatment ($F(1,21)=3.30$, $p=0.08$, $\eta p^2=0.136$), separation ($F(1,21)=1.14$, $p=0.30$, $\eta p^2=0.052$), and interaction ($F(1,21)=0.07$, $p=0.79$, $\eta p^2=0.003$).



Sup. Fig. 1: Impact of MS and TBZ on time spent in each compartment: odor, food, and RW in the T-maze task assessed in males (A,B,C), and in females (D,E,F). Data are expressed as mean ± S.E.M. of accumulated seconds during 15 min.

In conclusion, there are no differences between control and separated animals. Moreover, TBZ did not affect the time mice spent in each compartment. DA depletion and mild MS did

not produce increase in preference or avoidance to the compartment where the reinforcers are present in spite of changes in time interacting with the stimulus itself.

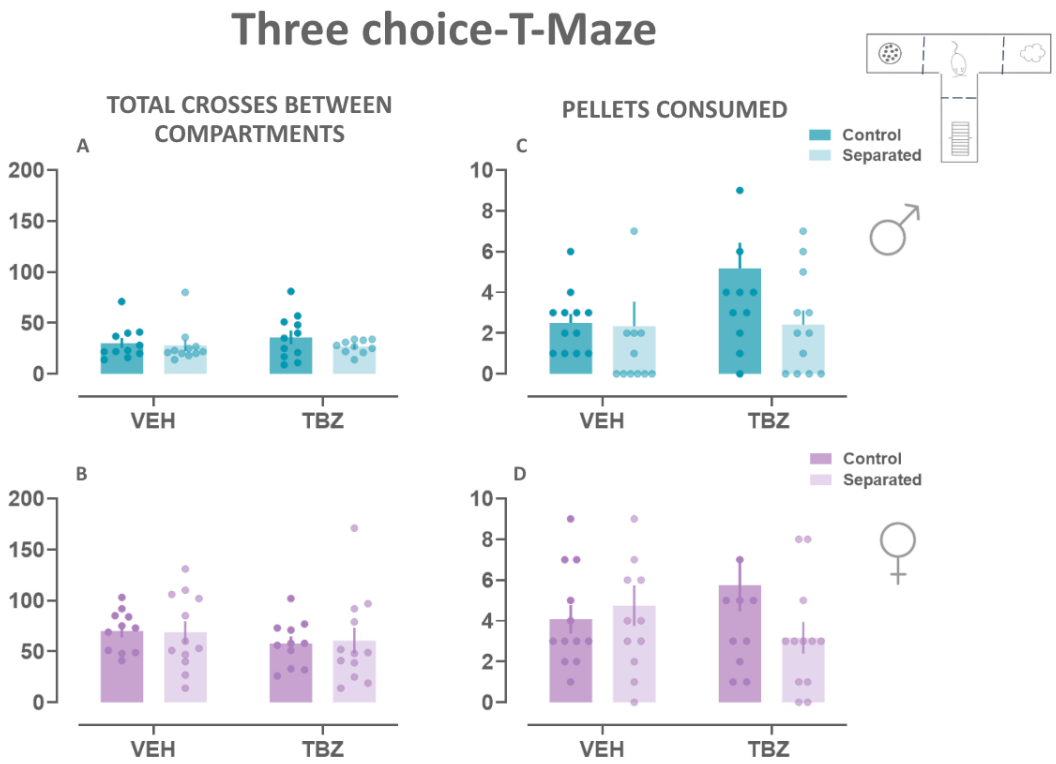
Sup. Exp 2. Impact of MS and TBZ on total number of pellets consumed and locomotion in male and female mice in the 3-choice-T-maze.

The total number of crosses between compartments in the T-maze was analyzed as a measure of locomotor exploration.

The two-way ANOVAs, showed no significant effect of treatment ($F(1,19)=0.27$, $p=0.61$, $\eta p^2=0.014$), or separation ($F(1,20)=0.81$, $p=0.43$, $\eta p^2=0.041$), and no interaction ($F(1,19)=0.81$, $p=0.38$, $\eta p^2=0.041$) in males. In females there was no effect of treatment ($F(1,21)=3.77$, $p=0.07$, $\eta p^2=0.152$), separation ($F(1,21)=0.00$, $p=0.96$, $\eta p^2=0.000$) and no significant interaction ($F(1,21)=0.13$, $p=0.72$, $\eta p^2=0.006$) either. See Sup. Fig. 2 (A, B).

The total number of pellets consumed in the T-maze was analyzed to study the impact of MS and TBZ on food consumption.

The two-way ANOVA for males showed no effect of treatment ($F(1,22)=3.12$, $p=0.09$, $\eta p^2=0.124$), separation ($F(1,22)=1.66$, $p=0.21$, $\eta p^2=0.070$), and no significant interaction ($F(1,22)=2.76$, $p=0.11$, $\eta p^2=0.111$). In females, there was no significant effect of treatment ($F(1,22)=0.00$, $p=0.96$, $\eta p^2=0.000$), separation ($F(1,22)=0.76$, $p=0.39$, $\eta p^2=0.033$), and no interaction ($F(1,22)=4.011$, $p=0.06$, $\eta p^2=0.154$). See Sup. Fig. 2 (C, D).



Sup. Fig. 2: Impact of MS and TBZ on total number of crosses in males (A) and in females (B), and on the total amount of pellets consumed for males (C) and females (D) in the T-maze.

Data are expressed as mean ± S.E.M. of number of crosses and number of pellets during 15 minutes.

Thus, TBZ and MS did not affect exploration and pellet consumption on the T-maze in either sex, indicating that DA depletion changed time running in the RW with no effect on exploratory locomotion in this paradigm and with no effect on food consumption. In general, it seems that females explore the T-maze more than males; they spend more time in the food compartment, and they eat more.

Sup. Exp 3. Impact of MS and TBZ on locomotor exploration in the DL, EPM and SI paradigms in male and female mice.

The total number of crosses between compartments or between arms was analyzed as a marker of exploratory locomotion under anxiogenic conditions.

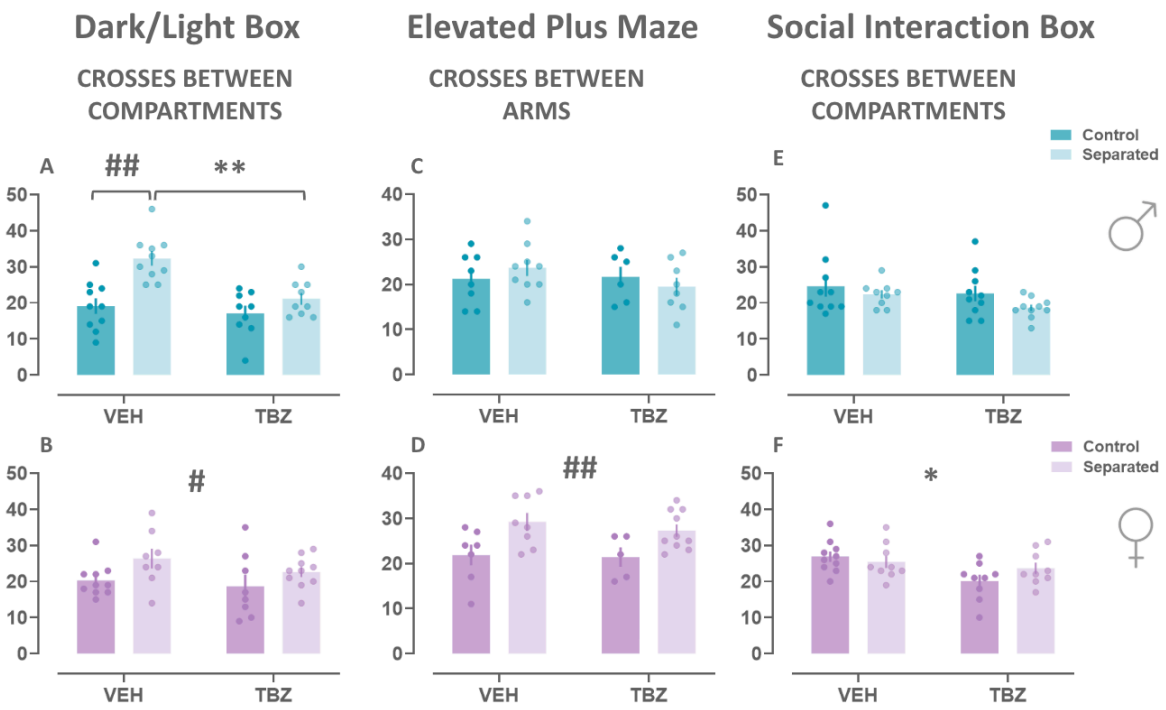
DL. For the total crosses between compartments, there were significant effects of treatment ($F(1,35)=10.94$, $p=0.002$, $\eta p^2=0.243$), and separation ($F(1,35)=18.63$, $p=0.0001$, $\eta p^2=0.354$), and also a significant interaction ($F(1,35)=5.33$, $p=0.027$, $\eta p^2=0.136$). Sidak's multiple comparisons revealed that separated males crossed more between compartments than non-separated males under the vehicle condition ($p<0.01$), but separated animals that received TBZ had a lower number of crosses ($p<0.01$) than separated controls.

In females, for total crosses between compartments there was a significant effect of separation ($F(1,32)=5.34$, $p=0.028$, $\eta p^2=0.143$), but not of treatment ($F(1,32)=1.57$, $p=0.219$, $\eta p^2=0.047$), and no interaction ($F(1,32)=0.23$, $p=0.63$, $\eta p^2=0.007$).

EPM. For total crosses between arms in males, there was no effect of separation ($F(1,27)=0.00$, $p=0.951$, $\eta p^2=0.000$), no effect of treatment ($F(1,27)=0.87$, $p=0.358$, $\eta p^2=0.031$), and no significant interaction ($F(1,27)=1.31$, $p=0.263$, $\eta p^2=0.046$). As for the total number of crosses between arms in females, there was a main effect of separation ($F(1,26)11.77$, $p=0.002$, $\eta p^2=0.312$), but no effect of treatment ($F(1,26)=0.39$, $p=0.54$, $\eta p^2=0.015$), and no significant interaction ($F(1,26)=0.15$, $p=0.70$, $\eta p^2=0.006$).

These results suggest that in general, separated mice were more active than controls, they performed more crosses in both paradigms, although male mice were more sensitive to DA depletion, reducing the exploratory locomotion in the DL paradigm but not in the EPM.

SI. In terms of locomotor exploration in the SI box, the two-way ANOVA showed no main effect of treatment ($F(1,35)=2.13$, $p=0.15$, $\eta p^2=0.057$), no main effect of separation ($F(1,35)=2.55$, $p=0.12$, $\eta p^2=0.068$), and no significant interaction ($F(1,35)=0.19$, $p=0.66$, $\eta p^2=0.006$). Thus, neither separation nor treatment affect the locomotor exploration in that paradigm in males. However, in females, the two-way ANOVA for locomotor exploration of the SI box revealed a main effect of treatment ($F(1,33)=7.28$, $p=0.01$, $\eta p^2=0.181$), although no significant effect of separation ($F(1,33)=0.44$, $p=0.51$, $\eta p^2=0.013$) or interaction ($F(1,33)=2.49$, $p=0.12$, $\eta p^2=0.070$).



Sup. Fig. 3: Impact of MS and TBZ on the total number of crosses between compartments in the DL in males (A) and in females (B), in the EPM in males (C) and in females (D), and in the SI box in males (E) and in females (F). Data are expressed as mean ± S.E.M. of number of crosses during 5 minutes in DL and EPM and 10 minutes in the SI. *p<0.05, **p<0.01 significant differences due to treatment. #p<0.05, ##p<0.01 significant differences based on separation..