**Dopamine D1 receptor activation leads to object recognition memory in a coral reef fish**

**Authors:** Trevor J. Hamilton1,2\*, Martin Tresguerres3,⌘, David I. Kline3,4,⌘

**Electronic Supplementary Material**

*Experimental Testing*

Damselfish were first placed in the ‘dosing tank’ a 20 cm X 13 cm X 14 cm plastic aquarium, and then in the ‘experimental tank,’ a 32 cm × 22 cm × 18 cm plastic aquarium, for trials 1 and 2. In between trials 1 and 2, fish were placed in the ‘holding tank,’ which was identical to the dosing tank. The walls and the floors of the outside of the tanks were white. Fish were exposed to the objects in the experimental tank and held in the holding tank in between trials.

Prior to experimentation, the experimental and holding tanks were filled with seawater to a height of 4 cm in the dosing tank and 7 cm in the experimental tank, which completely submerged the objects. First, the fish were individually placed in the dosing tank for a total of 10 min in order to allow acclimatization to the rectangular tank environment and expose it to control, control + DMSO or SKF + DMSO solutions. Secondly, the fish were transferred to the middle section of the experimental tank for trial 1, where two identical objects were presented for 10 min. The objects were LEGO® figures (Figure 1A), which were chosen because they are non-toxic and can be easily obtained throughout the world. The two objects were approximately 4 cm wide, 5 cm long and 3.5 cm tall. Fish were then placed into the holding tank for a retention interval (RI) of 10 min. In trial 2, one familiar object was switched for one novel object (this was systematically counterbalanced with two fish per treatment in each configuration, Figure 1B). Location preference was quantified by dividing the arena into two lateral zones and a middle third zone. EthoVision XT motion tracking software (version 10, Noldus, VA) quantified the time the fish spent in each lateral zone and the total distance moved (cm).

*Statistical Analysis*

Normality of the data was assessed using D’Agostino & Pearson omnibus normality test. Familiar object preference indices FOP1, and FOP2 ([15,17]) were calculated to determine whether the fish explored one object more than the other. FOP1 was calculated by subtracting the time fish spent in the novel object zone from the time fish spent in the familiar object zone in trial 2. A positive value of FOP1 indicates a preference for the familiar object and a negative value indicates preference for the familiar object. FOP2 was calculated by dividing FOP1 by the total time the fish spent in both zones combined. One-sample t-tests were used to determine whether FOP1 and FOP2 were significantly different from zero (to determine whether there was an object preference) and unpaired two sample t-tests were used to determine whether the control and SKF groups were different (to determine whether there was a different level of discrimination between treatment conditions) [1]. All tests were two-tailed. A Kruskal-Wallis test was used to determine whether there were significant differences between distance traveled for both groups in trials 1 and 2. Data was quantified for first 5 minutes of T1 and T2, the last 5 minutes of T1 and T2, and combined for the total 10 minutes to assess any time epoch related locomotion differences. The α for all tests was set at .05. Control fish (n=4) and control + DMSO fish (n=4) showed no significant difference in preference (SKF; t(7)=0.9714, P= 0.3689) so these groups were combined. Data was analyzed using GraphPad Prism software (v6, San Diego, CA).

References:

Akkerman, S., Prickaerts, J., Steinbusch, H. W. M., & Blokland, A. 2012. Object recognition testing: Statistical considerations. *Behav. Brain Res*. **232,** 317-322.

(doi:10.1016/j.bbr.2012.03.024 )