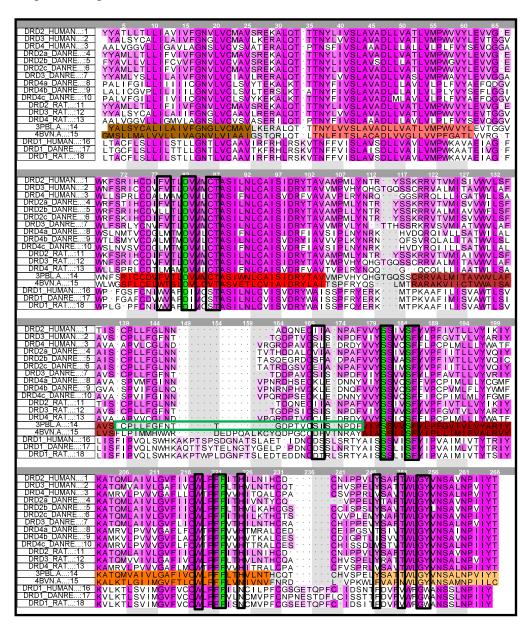
# Behavioral Analysis of Dopaminergic Activation in Zebrafish and Rats Reveals Similar Phenotypes

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# **Supporting information**

Sequence alignment



**Figur 1s**. A refined multiple alignment including a selection of human, zebrafish and rat dopamine receptor sequences. Dark purple indicate conserved amino acids, while light purple indicate less conserved but still similar amino acids. The sequences used as template in the receptor modelling, the turkey adrenergic  $\beta_1$  (4BVN) and the human dopamine  $D_3$  receptor structure (3PBL) are also included. The transmembrane regions in the crystal structures are highlight with seven boxes (TM1–7). The N-and C-terminus have been excised as well as the third intracellular loop, between TM5 and TM6. The second extracellular loop (EC2) which is important for ligand binding and used as template in the modeling of the  $D_2$ -like dopamine receptors is highlighted with a green box. The residues in the black boxes are those within 4Å from apomorphine in the zebrafish dopamine  $D_2$  receptor model (drd2a\_danre) and the green highlighted are the agonist key amino acids.

**Table 1s**. Sequence similarity based on the complete alignment above

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1:DRD2_HUMAN		72.1	49.2	89.5	79.2	88.3	76.2	49.6	50.8	49.2	97.9	71.4	48.3	71.7	39.7	37.7	37.2	37.6
2:DRD3_HUMAN	73.3		51.3	72.6	70.0	71.7	82.0	47.1	50.4	47.9	73.3	95.9	50.4	99.2	37.7	35.8	36.8	34.5
3:DRD4_HUMAN	50.0	51.3		50.2	52.9	49.6	53.6	66.7	69.2	68.3	50.0	50.2	90.8	51.3	35.6	34.2	34.5	33.7
4:DRD2a_DANR	89.8	71.7	49.6		79.2	92.1	75.7	49.2	48.8	48.3	89.8	71.4	48.3	70.8	40.9	37.7	37.6	38.0
5:DRD2b_DANR	80.5	70.0	52.9	80.2		81.3	72.0	49.6	53.3	50.4	81.4	69.3	51.7	69.6	36.4	37.0	36.4	36.4
6:DRD2c_DANR	89.8	71.7	49.6	93.2	81.3		76.2	50.0	50.4	49.6	89.8	71.4	48.7	70.8	39.7	38.9	37.6	38.4
7:DRD3_DANRE	77.1	81.7	53.3	76.4	71.7	75.8		50.0	51.7	49.2	78.0	80.5	50.8	80.8	40.9	39.7	39.9	38.4
8:DRD4a_DANR	50.4	47.1	66.7	49.8	49.6	50.0	50.2		72.1	87.9	50.4	47.7	67.6	47.1	32.0	34.6	33.7	34.9
9:DRD4b_DANR	51.7	50.4	69.2	49.4	53.3	50.4	51.9	72.1		72.9	52.1	50.2	68.5	50.0	32.0	36.2	36.4	36.0
10:DRD4c_DANR	50.0	47.9	68.3	48.9	50.4	49.6	49.4	87.9	72.9		49.2	47.7	70.2	47.9	31.6	37.4	36.4	36.8
11:DRD2_RAT P	97.9	72.1	49.2	89.5	80.0	88.3	77.0	49.6	51.3	48.3		71.4	48.7	71.7	39.7	37.7	36.8	37.6
12:DRD3_RAT P	72.9	96.3	50.4	72.6	69.6	71.7	81.2	47.9	50.4	47.9	72.9		49.2	95.4	37.7	36.6	36.8	35.3
13:DRD4_RAT P	48.7	50.0	90.0	48.5	51.3	48.3	50.6	67.1	67.9	69.6	49.2	48.5		50.0	35.6	34.2	34.9	34.1
14:3PBL.A	72.9	99.2	51.3	71.7	69.6	70.8	81.2	47.1	50.0	47.9	72.9	95.0	50.4		37.7	35.4	36.8	34.1
15:4BVN.A	41.5	38.8	36.7	42.6	37.5	40.8	42.3	32.9	32.9	32.5	41.5	38.6	37.0	38.8		40.5	41.5	39.9
16:DRD1_HUMAN	41.1	38.3	36.7	40.9	39.6	41.7	42.7	37.1	38.8	40.0	41.1	39.0	37.0	37.9	42.1		82.6	92.2
17:DRD1_DANRE	40.7	39.6	37.1	40.9	39.2	40.4	43.1	36.3	39.2	39.2	40.3	39.4	37.8	39.6	43.3	82.9		81.0
18:DRD1_RAT P	41.1	37.1	36.3	41.4	39.2	41.3	41.4	37.5	38.8	39.6	41.1	37.8	37.0	36.7	41.7	92.6	81.0	

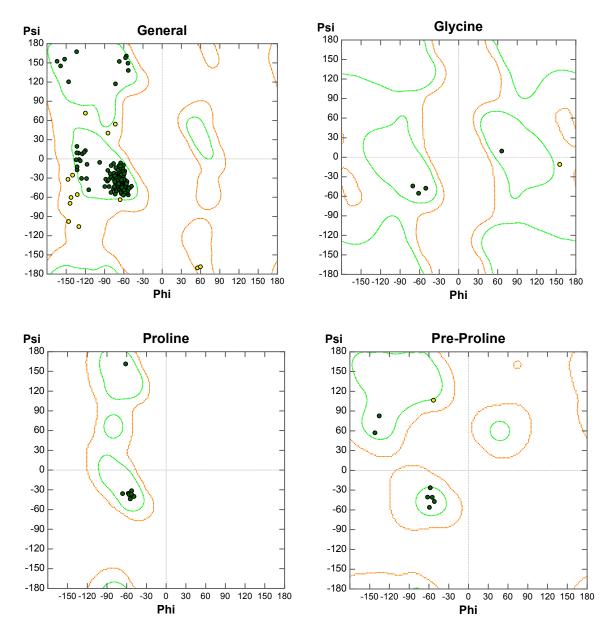
Table 2s. Sequence similarity transmembrane (TM) region

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1:DRD2_HUMAN		79.5	53.5	93.6	81.4	93.6	82.0	55.8	58.1	54.7	97.7	77.9	54.4	78.9	48.3	49.7	49.1	49.7
2:DRD3_HUMAN	79.1		55.2	77.9	76.7	77.9	84.3	54.7	55.8	52.3	79.1	96.5	53.2	99.4	44.8	46.8	46.8	45.6
3:DRD4_HUMAN	53.5	55.6		53.5	58.7	53.5	55.8	75.0	76.2	76.7	53.5	54.1	93.6	55.6	40.1	42.1	42.7	41.5
4:DRD2a_DANR	93.6	78.4	53.5		82.6	97.1	82.0	57.0	56.4	55.2	93.6	77.3	54.4	77.8	49.4	50.9	50.3	50.9
5:DRD2b_DANR	81.4	77.2	58.7	82.6		83.7	77.9	55.8	59.9	55.2	82.6	75.6	57.9	76.6	44.2	46.8	47.4	46.2
6:DRD2c_DANR	93.6	78.4	53.5	97.1	83.7		82.0	56.4	57.6	55.2	93.6	77.3	54.4	77.8	48.3	50.9	50.3	50.9
7:DRD3_DANRE	82.0	84.8	55.8	82.0	77.9	82.0		57.0	57.0	55.2	83.1	82.6	55.0	84.2	49.4	51.5	51.5	50.3
8:DRD4a_DANR	55.8	55.0	75.0	57.0	55.8	56.4	57.0		79.1	90.7	55.8	55.2	77.2	55.0	39.0	43.3	42.7	43.3
9:DRD4b_DANR	58.1	56.1	76.2	56.4	59.9	57.6	57.0	79.1		79.1	58.7	56.4	75.4	55.6	40.1	45.6	46.2	45.0
10:DRD4c_DANR	54.7	52.6	76.7	55.2	55.2	55.2	55.2	90.7	79.1		53.5	52.3	78.4	52.6	38.4	45.0	43.9	44.4
11:DRD2_RAT P	97.7	79.5	53.5	93.6	82.6	93.6	83.1	55.8	58.7	53.5		77.9	55.0	78.9	48.3	49.1	48.5	49.1
12:DRD3_RAT P	77.9	97.1	54.1	77.3	75.6	77.3	82.6	55.2	56.4	52.3	77.9		52.0	96.5	44.8	47.4	47.4	46.2
13:DRD4_RAT P	54.1	53.2	93.0	54.1	57.6	54.1	54.7	76.7	75.0	77.9	54.7	51.7		53.2	39.5	42.7	43.3	42.7
14:3PBL.A	78.5	99.4	55.2	77.3	76.2	77.3	83.7	54.7	55.2	52.3	78.5	95.9	53.2		44.8	46.2	46.2	45.0
15:4BVN.A	48.3	45.0	40.1	49.4	44.2	48.3	49.4	39.0	40.1	38.4	48.3	44.8	39.8	45.0		50.3	49.7	49.7
16:DRD1_HUMAN	49.4	46.8	41.9	50.6	46.5	50.6	51.2	43.0	45.3	44.8	48.8	47.1	42.7	46.2	50.0		90.6	97.1
17:DRD1_DANRE	48.8	46.8	42.4	50.0	47.1	50.0	51.2	42.4	45.9	43.6	48.3	47.1	43.3	46.2	49.4	90.6		91.2
18:DRD1_RAT P	49.4	45.6	41.3	50.6	45.9	50.6	50.0	43.0	44.8	44.2	48.8	45.9	42.7	45.0	49.4	97.1	91.2	

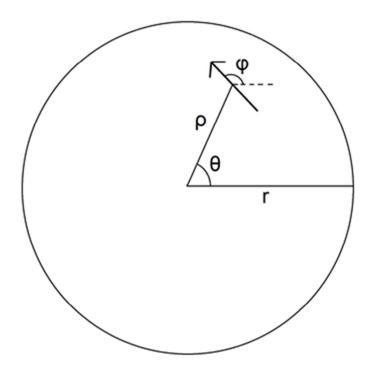
**Table 3s**. Sequence similarity within 4 Å from apomorphine defining the binding pocket (20 amino acids)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1:DRD2_HUMAN		95.0	75.0	95.0	80.0	95.0	95.0	75.0	75.0	75.0	100.0	95.0	75.0	95.0	60.0	55.0	55.0	55.0
2:DRD3_HUMAN	95.0		75.0	95.0	85.0	95.0	100.0	75.0	75.0	75.0	95.0	100.0	75.0	100.0	60.0	55.0	55.0	55.0
3:DRD4_HUMAN	75.0	75.0		75.0	80.0	75.0	75.0	90.0	90.0	90.0	75.0	75.0	95.0	75.0	60.0	50.0	50.0	50.0
4:DRD2a_DANR	95.0	95.0	75.0		80.0	95.0	95.0	75.0	75.0	75.0	95.0	95.0	75.0	95.0	60.0	55.0	55.0	55.0
5:DRD2b_DANR	80.0	85.0	80.0	80.0		80.0	85.0	80.0	80.0	80.0	80.0	85.0	80.0	85.0	65.0	50.0	50.0	50.0
6:DRD2c_DANR	95.0	95.0	75.0	95.0	80.0		95.0	75.0	75.0	75.0	95.0	95.0	75.0	95.0	60.0	55.0	55.0	55.0
7:DRD3_DANRE	95.0	100.0	75.0	95.0	85.0	95.0		75.0	75.0	75.0	95.0	100.0	75.0	100.0	60.0	55.0	55.0	55.0
8:DRD4a_DANR	75.0	75.0	90.0	75.0	80.0	75.0	75.0		95.0	95.0	75.0	75.0	90.0	75.0	60.0	50.0	50.0	50.0
9:DRD4b_DANR	75.0	75.0	90.0	75.0	80.0	75.0	75.0	95.0		95.0	75.0	75.0	90.0	75.0	60.0	50.0	50.0	50.0
10:DRD4c_DANR	75.0	75.0	90.0	75.0	80.0	75.0	75.0	95.0	95.0		75.0	75.0	90.0	75.0	60.0	50.0	50.0	50.0
11:DRD2_RAT P	100.0	95.0	75.0	95.0	80.0	95.0	95.0	75.0	75.0	75.0		95.0	75.0	95.0	60.0	55.0	55.0	55.0
12:DRD3_RAT P	95.0	100.0	75.0	95.0	85.0	95.0	100.0	75.0	75.0	75.0	95.0		75.0	100.0	60.0	55.0	55.0	55.0
13:DRD4_RAT P	75.0	75.0	95.0	75.0	80.0	75.0	75.0	90.0	90.0	90.0	75.0	75.0		75.0	60.0	50.0	50.0	50.0
14:3PBL.A	95.0	100.0	75.0	95.0	85.0	95.0	100.0	75.0	75.0	75.0	95.0	100.0	75.0		60.0	55.0	55.0	55.0
15:4BVN.A	60.0	60.0	60.0	60.0	65.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0		70.0	70.0	70.0
16:DRD1_HUMAN	55.0	55.0	50.0	55.0	50.0	55.0	55.0	50.0	50.0	50.0	55.0	55.0	50.0	55.0	70.0		100.0	95.0
17:DRD1_DANRE	55.0	55.0	50.0	55.0	50.0	55.0	55.0	50.0	50.0	50.0	55.0	55.0	50.0	55.0	70.0	100.0		95.0
18:DRD1_RAT P	55.0	55.0	50.0	55.0	50.0	55.0	55.0	50.0	50.0	50.0	55.0	55.0	50.0	55.0	70.0	95.0	95.0	

Three dimensional reconstruction models of zebrafish DRD2(a-c) and DRD4(a-c)



**Figure 2s**. Ramachandran plots for glycines, prolines, pre-prolines and for general residues of the selected zebrafish dopamine  $D_2$  (drd2a) homology model. The contours indicate allowed (orange) and core (green) regions of  $\varphi$  and  $\Psi$  angles, and the filled green rings indicate amino acids within the core regions. The yellow rings indicate allowed regions. No outliers are present.



**Figure 3s**. Definition of radial distance, Rho ( $\rho$ ) and angles Theta ( $\theta$ ) and Phi ( $\varphi$ ).

## **Definitions**

## **Bout**

Zebrafish larvae swim by performing discrete bouts of activity that are well-defined and precisely quantifiable. A swim bout is here defined as swim events with more movement than a certain threshold (0.02 pixels/ frame). The distance the zebrafish larvae swim is calculated by three time resolutions (48, 24, and 12 frames) and all three must result in a movement above the threshold for the event to be treated as a bout.

## **Turn**

A turn is defined as the total angular change in phi  $(\phi)$  in a bout.

# **Definitions of behavioral parameters**

## Distance travelled

The distance per minute averaged over the time window. Calculates the total distance the zebrafish has swum divided by the length of the recording (in minutes).

# **Bouts per min**

The total number of bouts executed per minute averaged over the time window.

#### **Bouts**

The number of bouts performed within a time window. The zebrafish will be counted as swimming when in a swim bout and at a standstill when not moving.

# Standstills\_T1-T2 (5-10 s, 10-15 s and 15-30 s)

The number of periods the zebrafish has been at a standstill for the time interval T1–T2. The zebrafish is treated as standing still when not performing a bout.

#### **Bout distance**

The distance the zebrafish has swum per swim bout averaged over all swim bouts in the time window

#### **Bout Duration**

The time that the zebrafish has swum per swim bout averaged over all swim bouts in each time window

#### **Bout mean speed**

The average zebrafish swim speed during a bout averaged over all swim bouts in the time window.

## **Bout max speed**

The zebrafish's maximum speed during a bout averaged over all swim bouts in the time window.

#### Bout mean acceleration

The average acceleration for the zebrafish's swim bout averaged over all swim bouts in the time window.

## Bout max acceleration,

Th maximum acceleration for fish swim for a bout averaged over all swim bouts in the time window.

# Center/wall preference (Center/Periphery 0-R1, R1-R2, R2-R3 or R3-R4)

How much time the zebrafish spend in the center compared to the periphery of the well. There are four radial segments of the same area, which is defined as annuli with inner radius R and outer radius R, n+1 (except the center area is a circle of radius R1). Since the areas are to be equal, then the radii R1-R4 are 0.5r, 0.71r, 0.87r, and r, where r is the well radius. The value is the fraction of time the zebrafish has been in each area (all four parameters thus always sum to one).

## Distance from center (MeanRhoPos)

The distance from the zebrafish to the center of the well per bout averaged over all bouts in a time window.

## **Change in distance from center (RhoPosChange)**

How much the distance has changed in absolute value between the zebrafish and center of the well per bout and averaged over all bouts in a time window.

## Small and Large angle turn (Turn1 or 2)

The number of turns below (Turn1) or above (Turn2)  $60^{\circ}$ . The number of turns is then calculated and normalized so that Turn1 + Turn2 = 1.

## Average rotational speed

The zebrafish rotational speed using Phi ( $\varphi$ ). Calculated similar to MeanSPD.

**Phi angle change** The total change in the absolute value of Phi  $(\phi)$  during the time window, It is calculated per swim bout and averaged over all the bouts in the time window.

## Theta change

The total change in the absolute value of Theta  $(\theta)$  during the time window. It is calculated per swim bout and averaged over all the bouts in the time window.

## Theta mean speed

The zebrafish's average speed in  $\theta$ -direction (thus only the velocity component in  $\theta$ -direction counts). Calculated otherwise as MeanSPD.

## Theta max speed

The same as ThetaMeanSPD but using the maximum velocity instead.

## RightRevPref

The time the larvae swims in right turns divided by the sum of the time it swims in right and left turns (a number between 0 and 1). The zebrafish is assumed to swim in right turns when the angle between the body's directional vector and vector r (Figure 1) from the center to the zebrafish is.  $\pi/6 < \theta$ - $\Phi$  (mod  $2\pi$ )  $< \pi$ 

# RightOrLeft

The relationship between the time the zebrafish swims to the right or left and total swim time. If the angles have an absolute value that is less than  $\pi$  / 6, the zebrafish is considered to be facing the wall and not performing a left or right turn.

To calculate the octant parameters, the areas below the wells are divided into nine zones with the same area. Eight of them are annular sectors with a sector angle of  $\pi$  / 4 and the ninth is the center sector (a circle with a radius that is one third of the well), thus the same area as each wedge.

#### OctPos

The percentage of time that the zebrafish remained in their "home base" throughout the time window.

#### OctSwim

The percentage of time the zebrafish has been swimming in their "home base" compared to the total swimming time.

#### **OctVis**

The percentage of time the zebrafish swim into their "home base" (visit it) compared to the total number of visits in all areas. The zebrafish is assumed to be visiting an area when it moves from one area to another.

For the parameters below the sum of the ratio for each sector and the ratio for the center area, is equal to 1.

# Oct(1-8)Pos

The amount of time that the zebrafish spent in each annular sector reported as a ratio between each annular sector (1-8) and the total time.

#### **CenterPos**

The amount of time that the zebrafish spent in the center area reported as a ratio between the center and the total time.

# Oct(1-8)Swim

The amount of time that the zebrafish swim in each annular sector reported as a ratio between each annular sector (1–8) and the total time.

#### CenterSwim

The amount of time that the zebrafish swim in the center area and the total time reported as a ratio between the center and the total time.

# Oct(1-8)Vis

The ratio between the number of visits that the zebrafish make in each annular sector and the total number of visits in all sectors.

#### **CenterVis**

The ratio between the number of visits that the zebrafish make in the center area and the total number of visits in all sectors.

Definition of classified turns

**Scoot**: Total angle change  $< 15^{\circ}$ . Also bouts with no turn is classified as scoot.

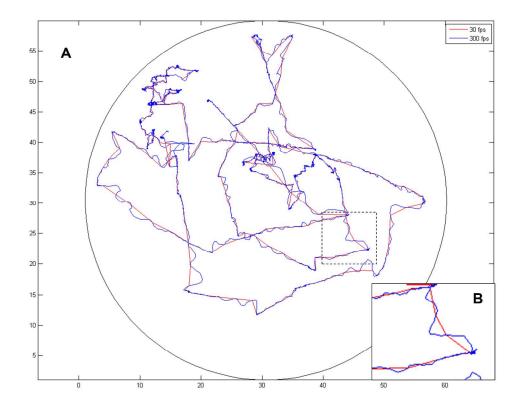
**JBend**: Total angle change in the interval  $10^{0}$  to  $80^{0}$ . Most of the movement is done sideways. Fraction between total angle change and distance travelled have to be  $>40^{0}$ /mm

**CBend**: Total angle change  $> 80^{\circ}$ . The first angle change in the turn must be in the interval  $80^{\circ}$  to  $130^{\circ}$ .

**OBend**: Total angle change  $> 80^{\circ}$ . The first angle change in the turn must be  $> 130^{\circ}$ .

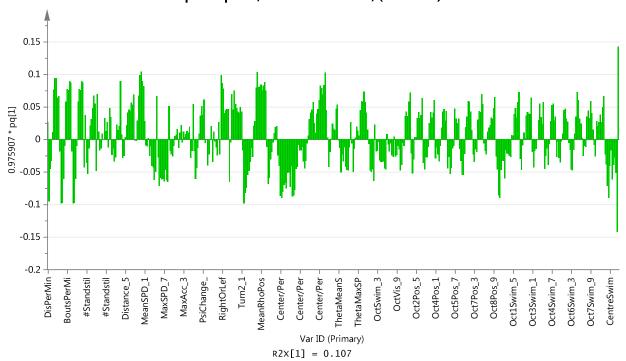
**Startle**: Maximum angle change  $> 45^{\circ}$  /frame and maximum speed > 0.065 m/s.

Routine turn: Turn that has not been classified as scoot, J-bend, C-bend or O-bend.

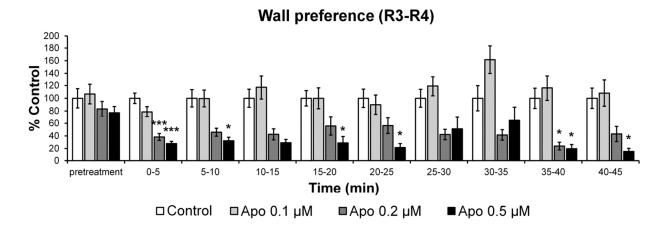


**Figure 4s**. (A) Trajectory pattern for the same video file analyzed using all frames (300 fps) or only 10% of frames (30 fps). Expansion (B) highlights a section of the trajectory pattern that demonstrates that an increased frame rate improves tracking of fast behavioral features.

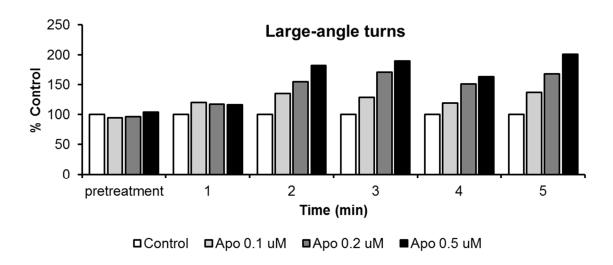
## Apomorphine, 0.5 uM and 50 uM, (OPLS-DA)



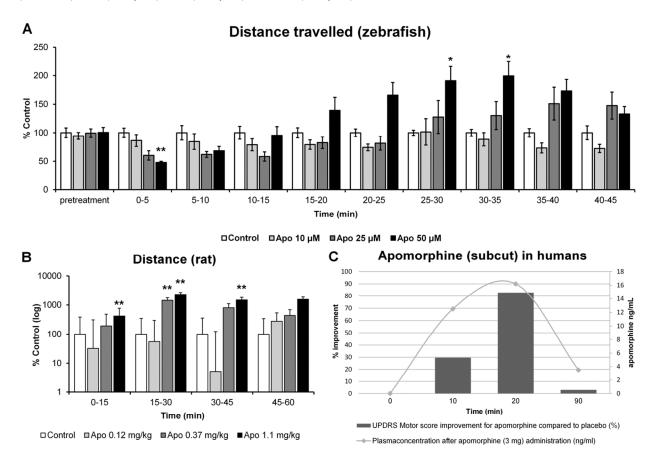
**Figure 5s.** OPLS-DA loading plots for all parameters separating groups treated with 50 and 0.5  $\mu$ M in multivariate analysis.



**Figure 6s.** Detailed analysis of wall preference (R3-R4) for the low-dose phenotype, n=36 (Control), 35 (0.5  $\mu$ M), 35 (0.2  $\mu$ M) and 34 (0.1  $\mu$ M). Significance reported when comparing treatment with control groups: \* p < 0.05, \*\* p < 0.001, \*\*\* p < 0.0001. Significance reported when comparing 0.5  $\mu$ M with 0.1  $\mu$ M: † p < 0.05, †† p < 0.001, ††† p < 0.0001. Bars represent mean over 5 min  $\pm$  SEM.



**Figure 7s.** Turn 2 after low dose apomorphine using 1 min time frames for the first 5 min, n = 36 (Control), 35 (0.5  $\mu$ M), 35 (0.2  $\mu$ M) and 34 (0.1  $\mu$ M).



**Figure 8s**. Treatment with apomorphine increases locomotor activity in zebrafish (A), rats (B), and humans (C) in an analog way.