Modeling anxiety in zebrafish

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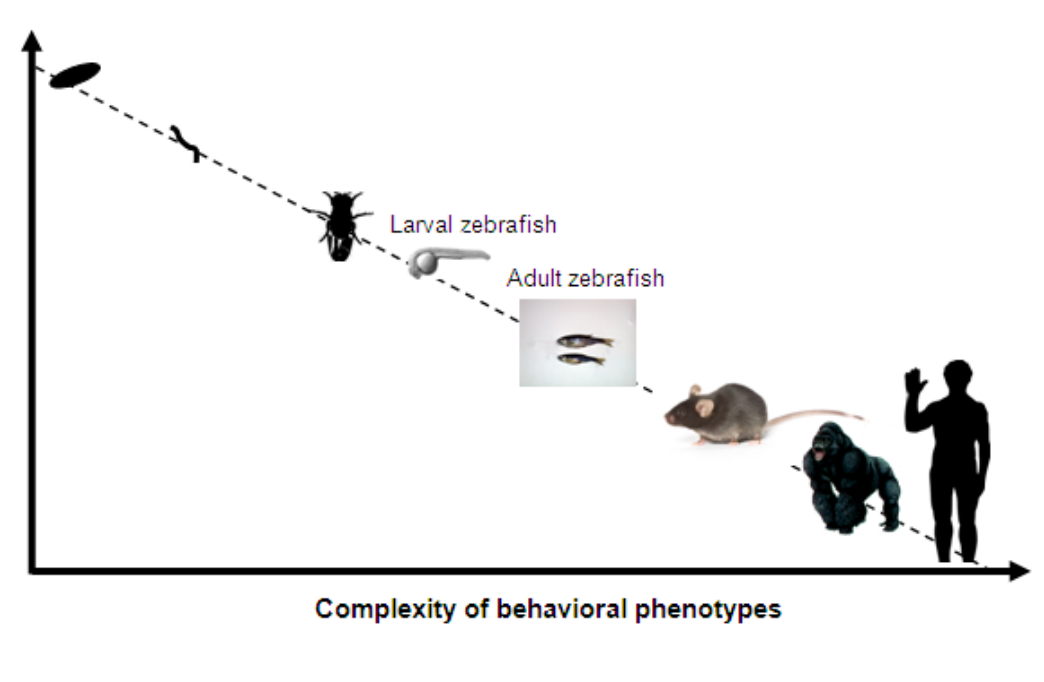
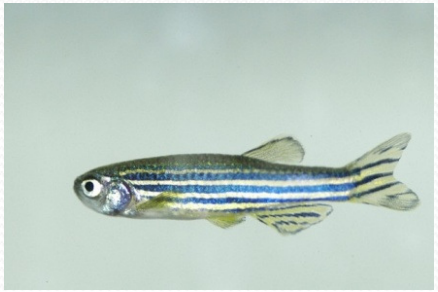
- Constant innovative development of new paradigms together with **widening the spectrum of** behavioral domains and **model organisms** remain the three key priorities in translational biological psychiatry research

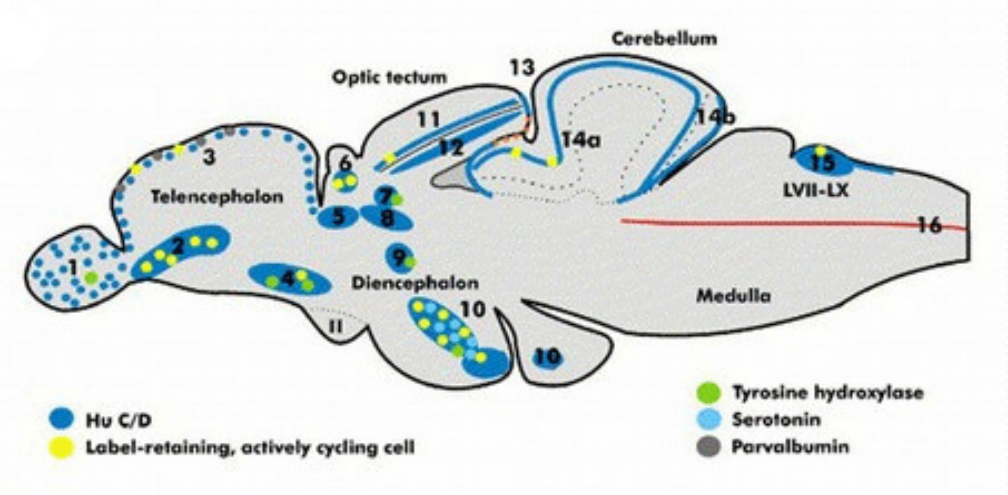


Advantages of zebrafish

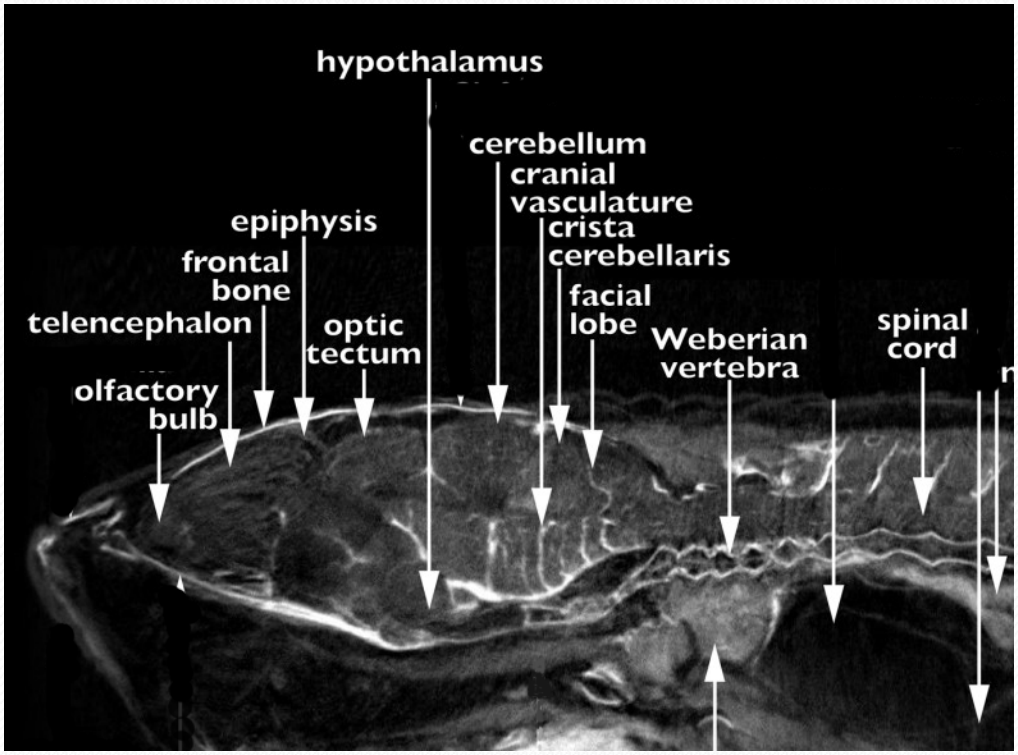


- Vertebrate species
- Physiological similarity
- Reproduce quickly and abundantly
- Rapid development + live longer (than mice)
- Fully sequenced genome
- Ease of genetic manipulation
- History of genetic and developmental research
- Low-cost model
- 3D Behavior

Zebrafish Brain Anatomy



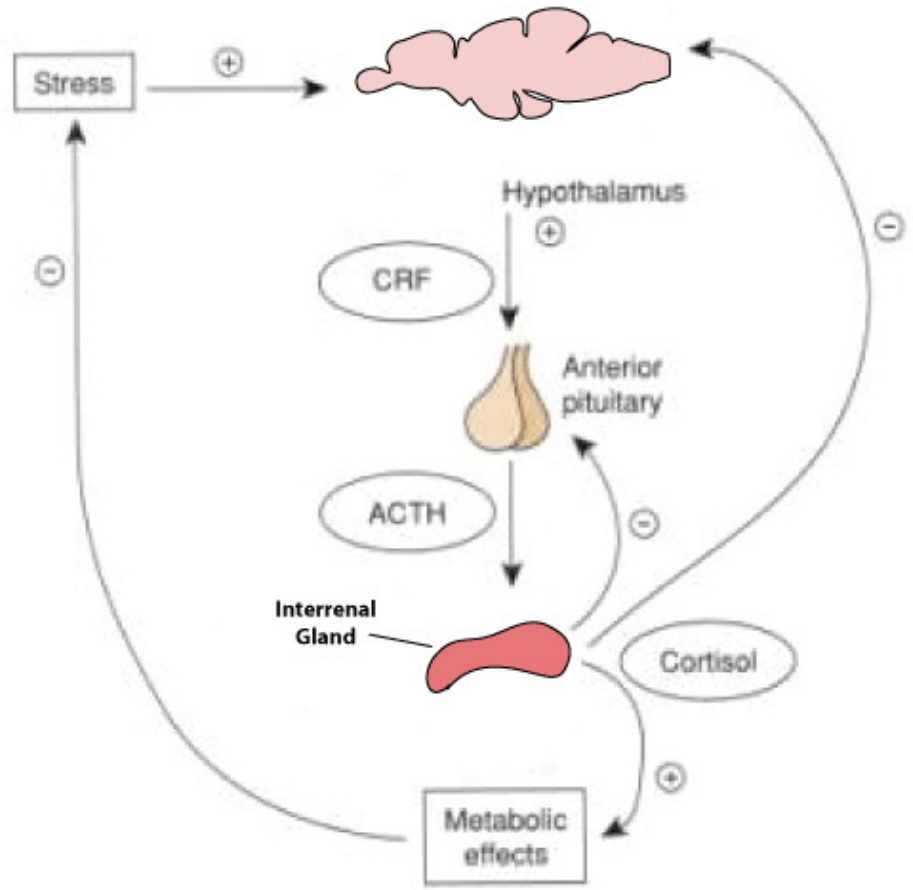
Micheal Brand
(http://www.sfb655.de/Members/mbrand/index_html-en)



www.fishnet.org.au

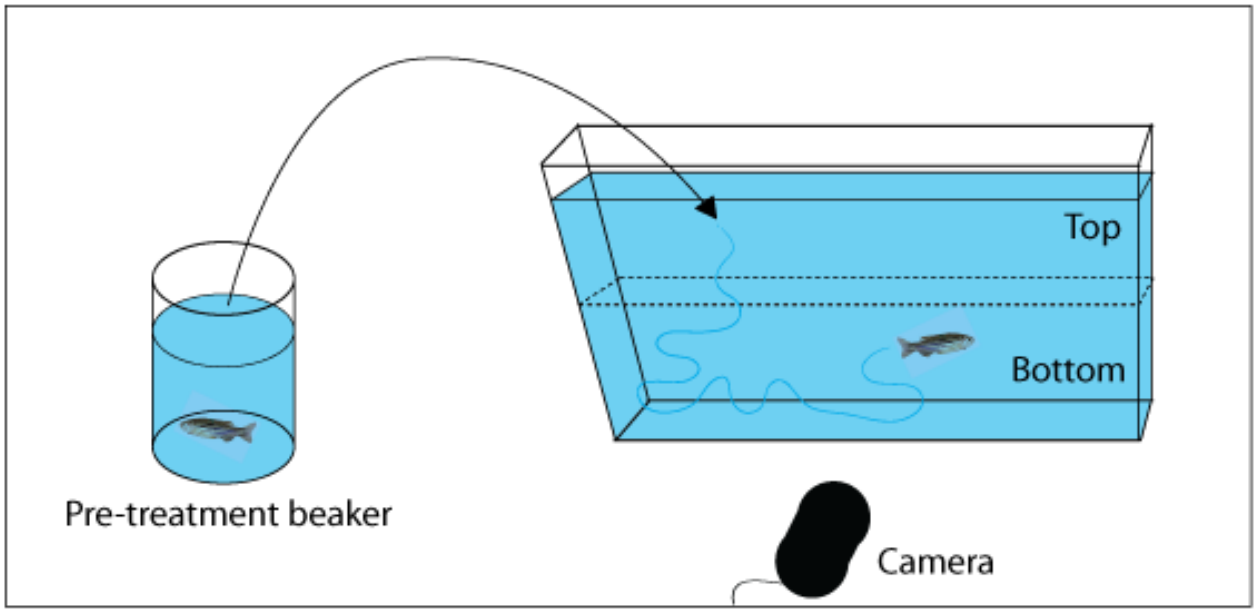
Endocrine responses

- **Humans = Cortisol**
- **Zebrafish = Cortisol**
- Mice = Corticosterone



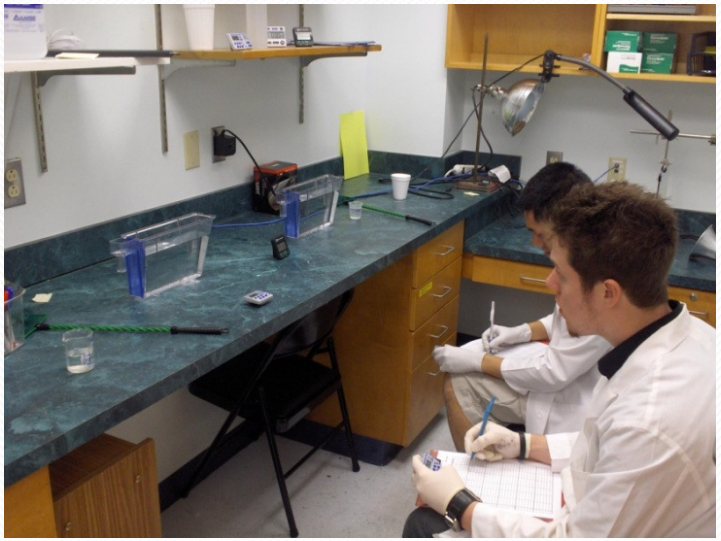
Novel tank test

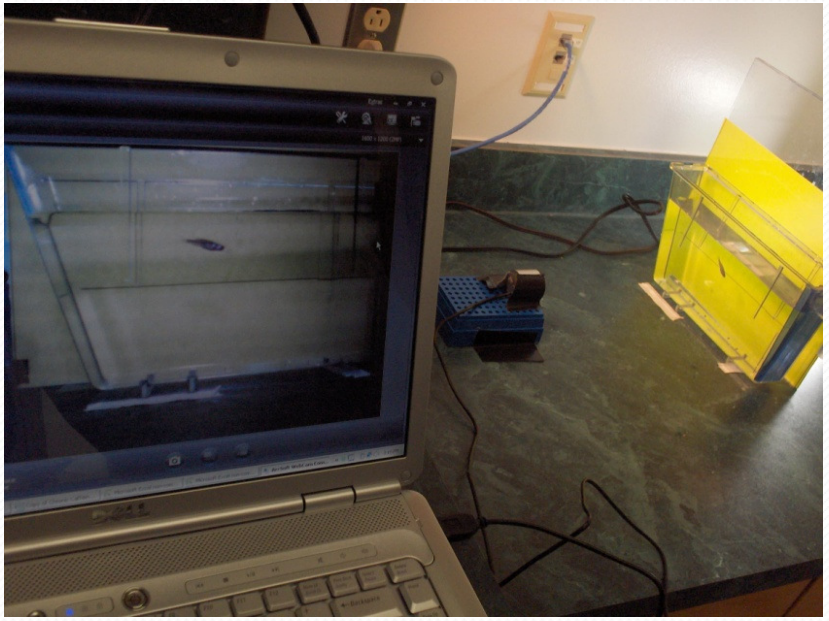
- Manual Observation
  - Latency, transitions and duration in upper half
  - Freezing freq. and duration
  - Erratic movement freq.



Behavioral Endpoints

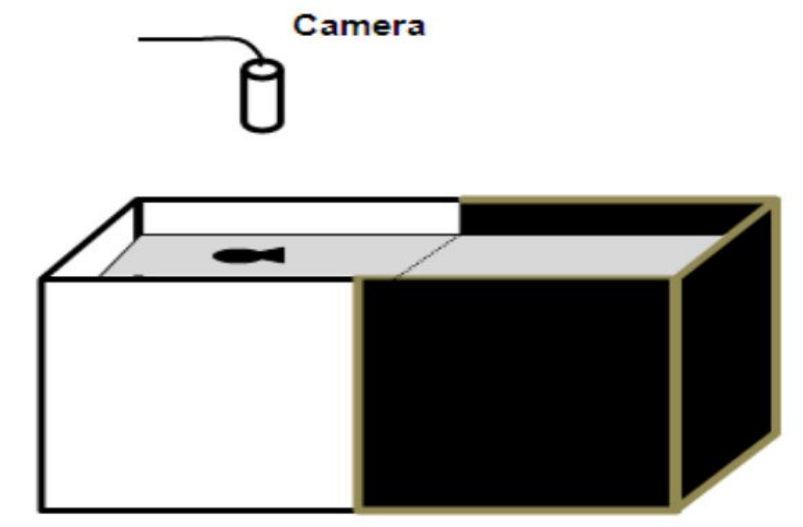
- Manual
  - Time to Top
  - # Top Entries
  - Time in Top
  - Erratic Movements
  - Freezing Bouts
  - Time Frozen
- Video-Tracking
  - # Entries (Top/Bottom)
  - Duration (Top/Bottom)
  - Distance Traveled (mm) (Top/Bottom)
  - Average Velocity (mm/s) (Top/Bottom)



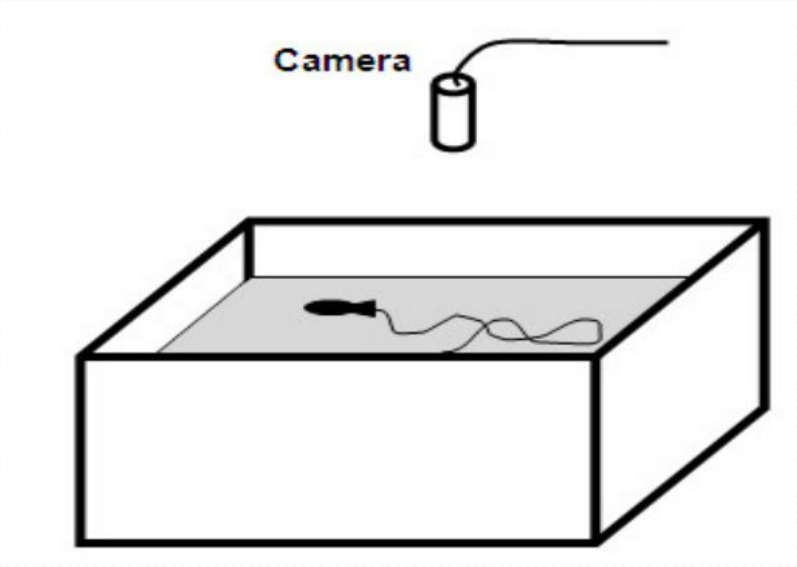


Light Dark Box test

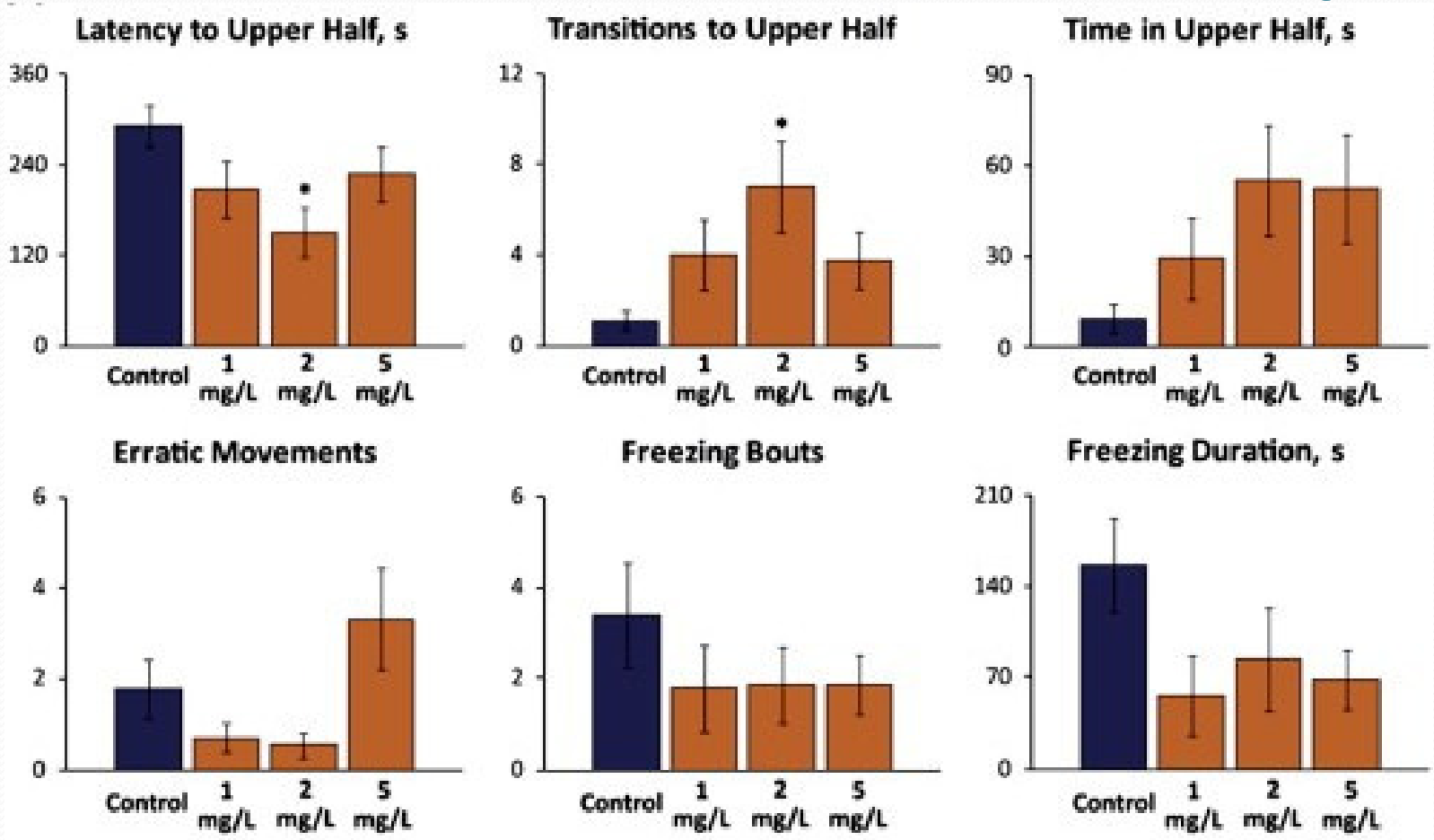
1. Latency to enter the white
2. Time spent in the white half
3. Number of entries to the white
4. White:total time spent ratios
5. Distance traveled in white



Open Field test



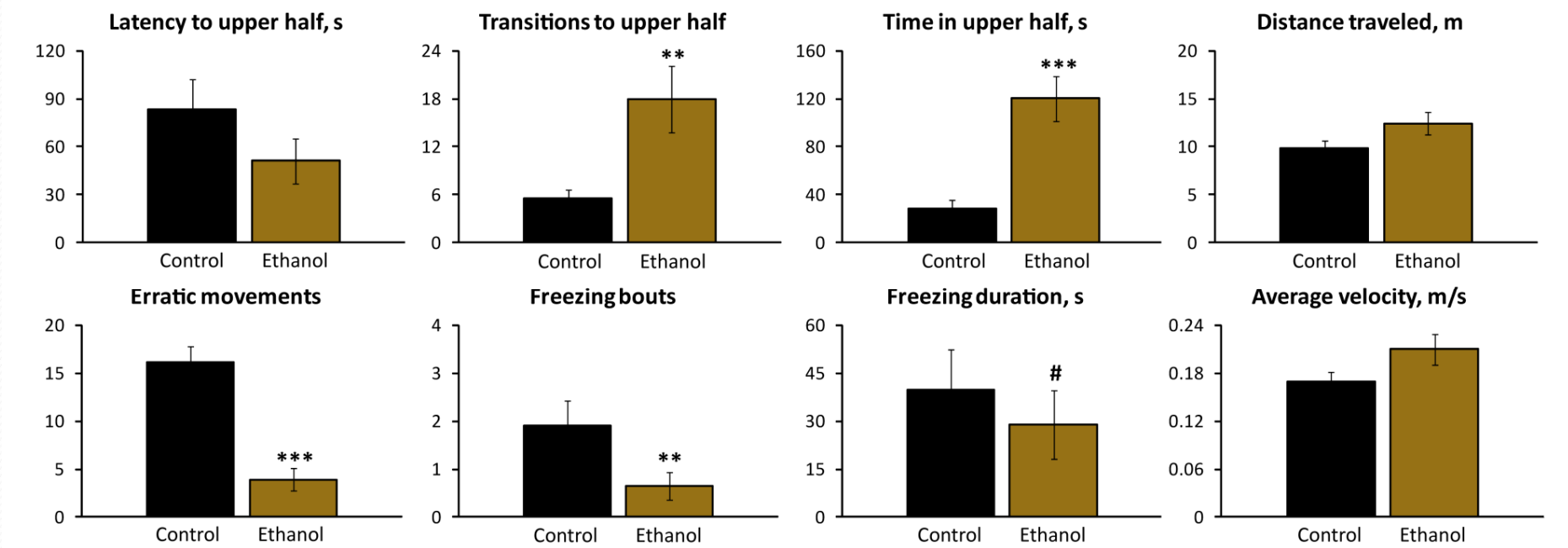
Behavioral effects of Morphine



Morphine (1–5 mg/L) significantly affects the latency to enter the top (F(3, 51) =2.9, P b 0.005) and the number of top transitions (F(3, 51) =2.8, P b 0.005). Data are presented as mean ±SEM (n =13–16 per group), *P b 0.05, ***P b 0.005 vs. control; post-hoc Tukey test for significant ANOVA data.

Pharmacological modulation of anxiety-like phenotypes in adult zebrafish behavioral models

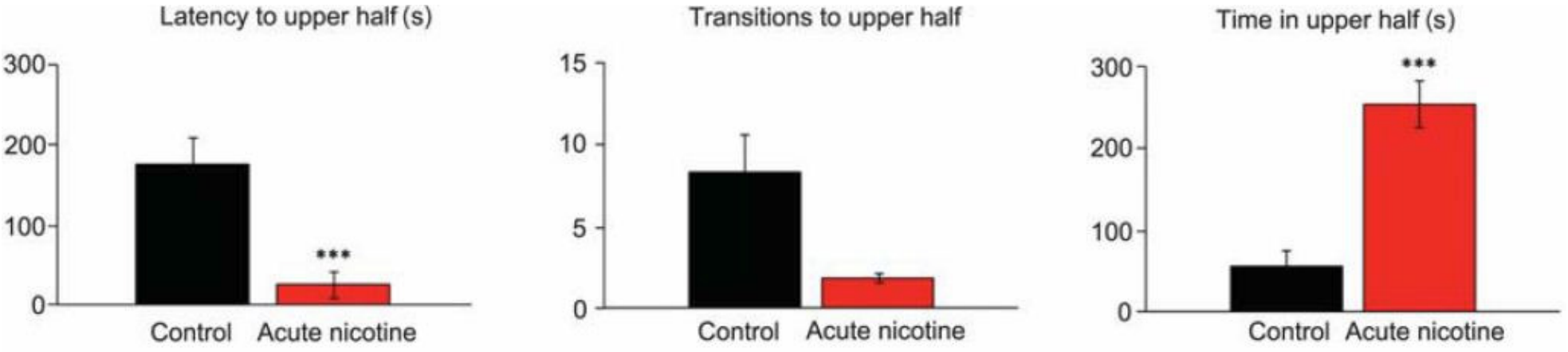
Behavioral effects of Ethanol



Behavioral effects of anxiolytic and anxiogenic manipulations in adult zebrafish tested in the 6-min novel tank test. Anxiolytic treatments included chronic ethanol (0.3% (vol/vol) for 1 week) analyzed manually and by using Noldus EthoVision XT7 video-tracking system.

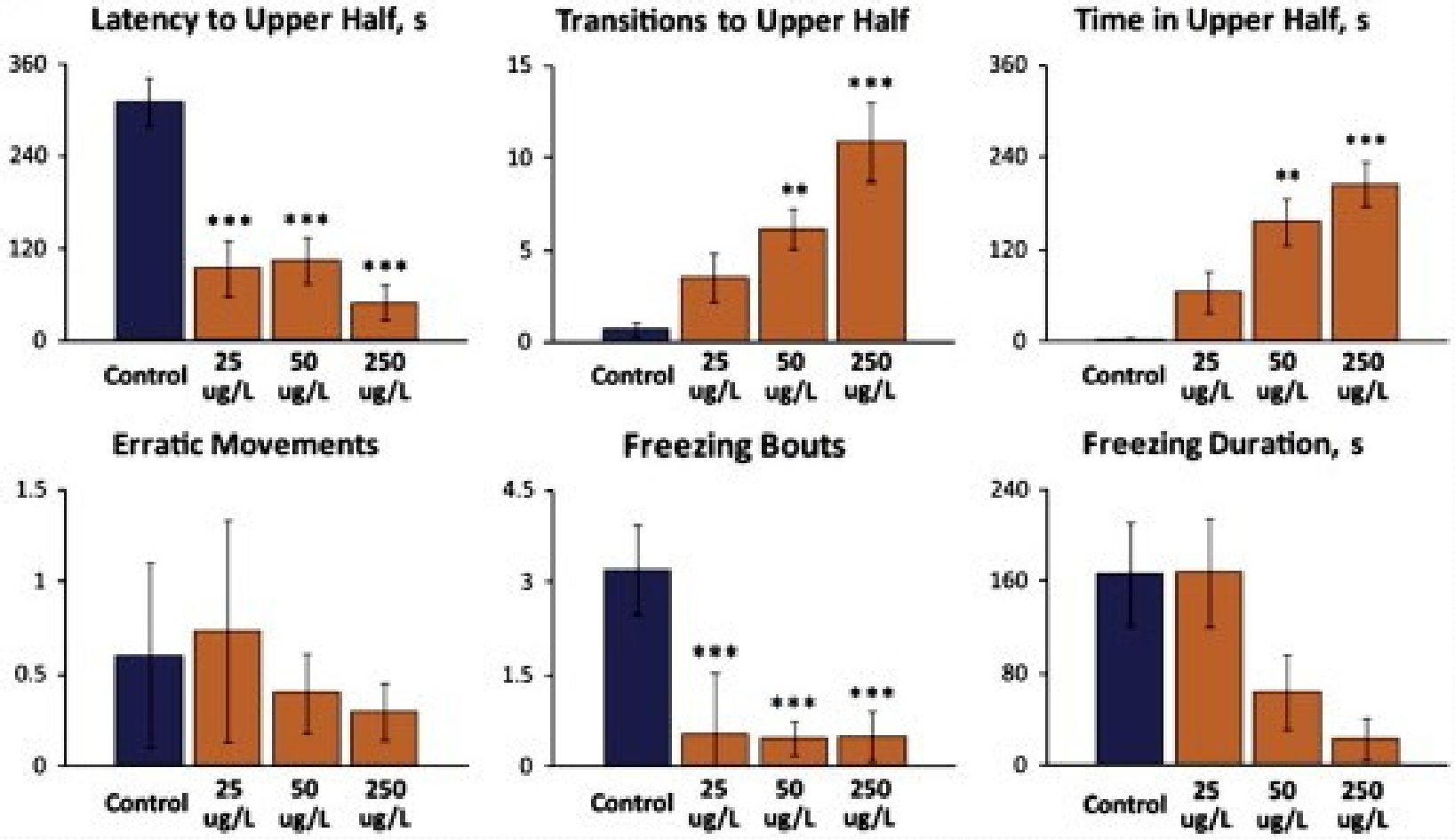
Cachat et al. 2010 Nature Protocols VOL.5 NO.11, 1786-1799

Behavioral effects of nicotine



Acute nicotine administration (10 mg/l for 5 min; n = 15 per each group). ** $p < 0.01$, *** $p < 0.001$, *U-test.*

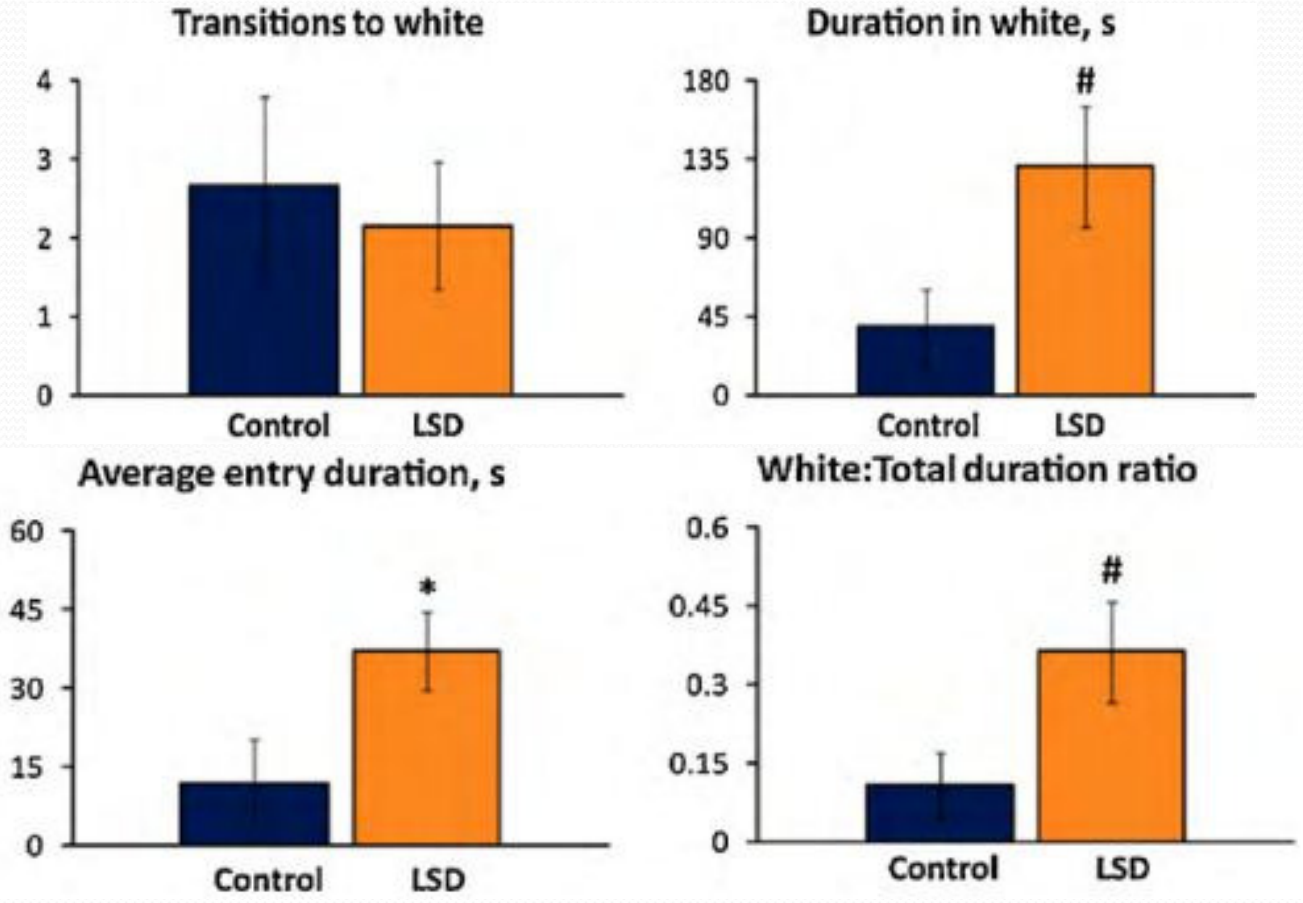
Behavioral effects of LSD



LSD (25–250 μg/L) significantly affected the latency to enter the top, number of top transitions, time spent in top, and freezing bouts in adult wild type (short-fin) zebrafish. Data are presented as mean±SEM (n =10–16 per group), **P b 0.01, ***P b 0.005 vs. control; post-hoc Tukey test for significant ANOVA data

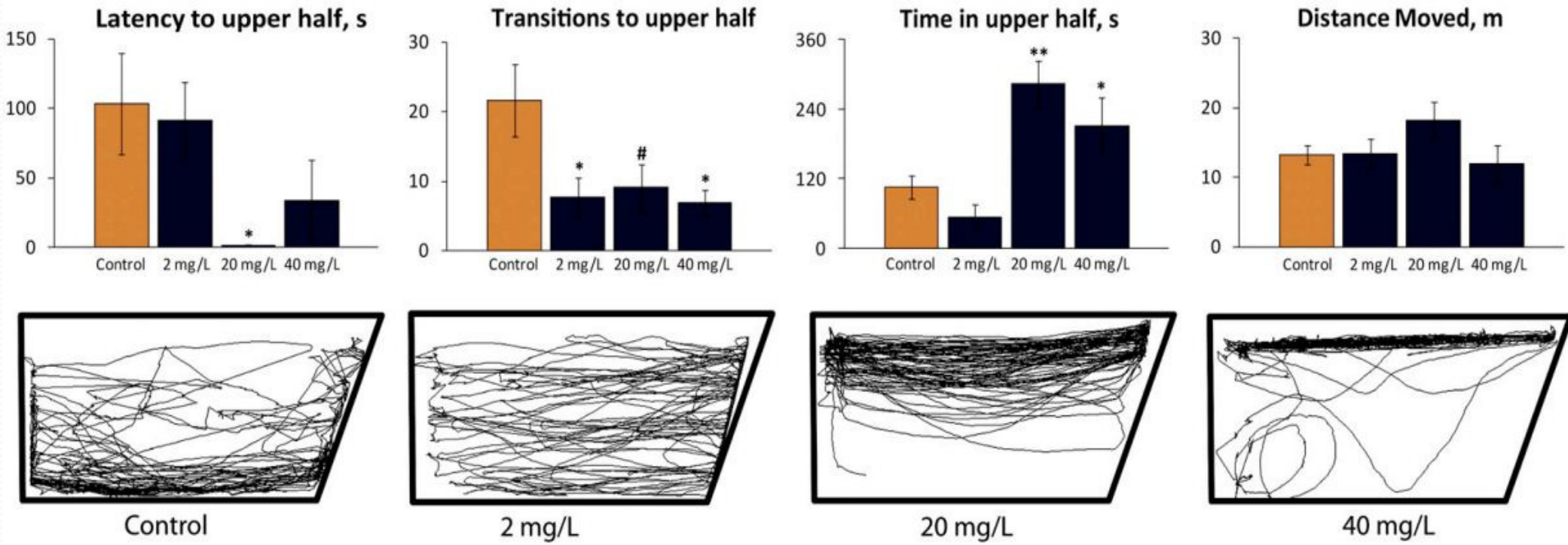
Pharmacological modulation of anxiety-like phenotypes in adult zebrafish behavioral models

LSD Light Dark Box test



Behavioral endpoints obtained in the 6-min light –dark box test (n = 12 per group; Experiment 2). *P b 0.05, **P b 0.01, #P = 0.05 –0.1 (trend) vs. controls; post-hoc Tukey test for significant ANOVA data.

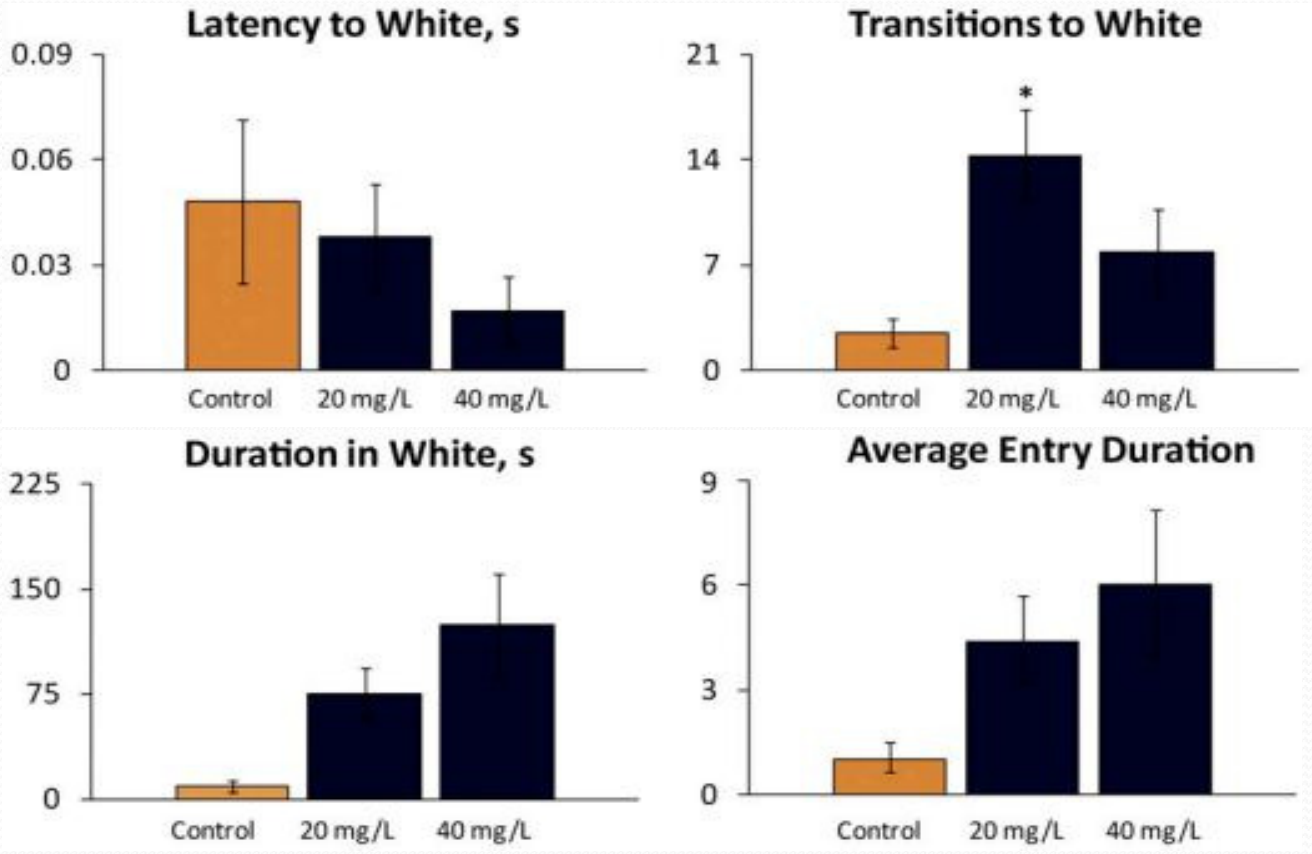
Ketamine: novel tank test



Behavioral effects of 20-min acute exposure to ketamine on zebrafish tested in the novel tank. Behavioral endpoints obtained in the 6-min novel tank test. In the bottom row of this panel, representative 2D traces were generated by Noldus Ethovision XT7 software using the side view video-recording. In all experiments, the traces were examined for each experimental cohort, rated from 1 to n (based on similarity to each other), and the middle trace was selected as representative, to illustrate the patterns of zebrafish locomotion

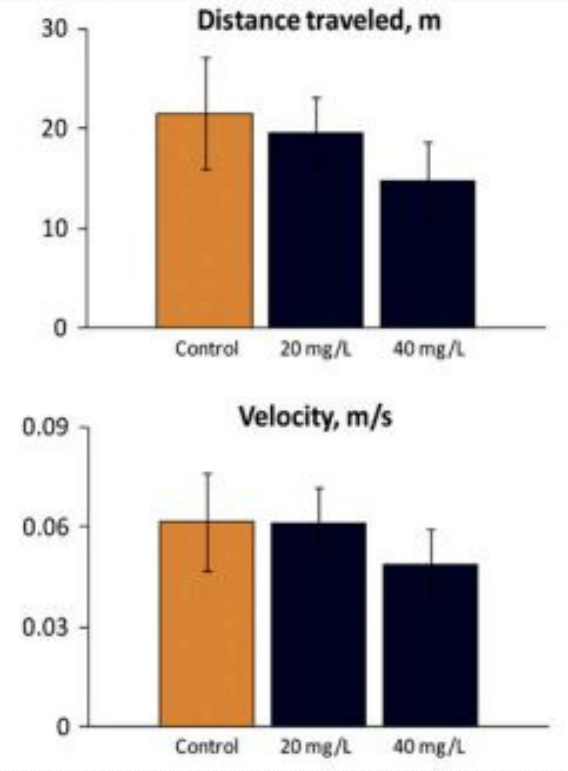
Behavioral and physiological effects of acute ketamine exposure in adult zebrafish

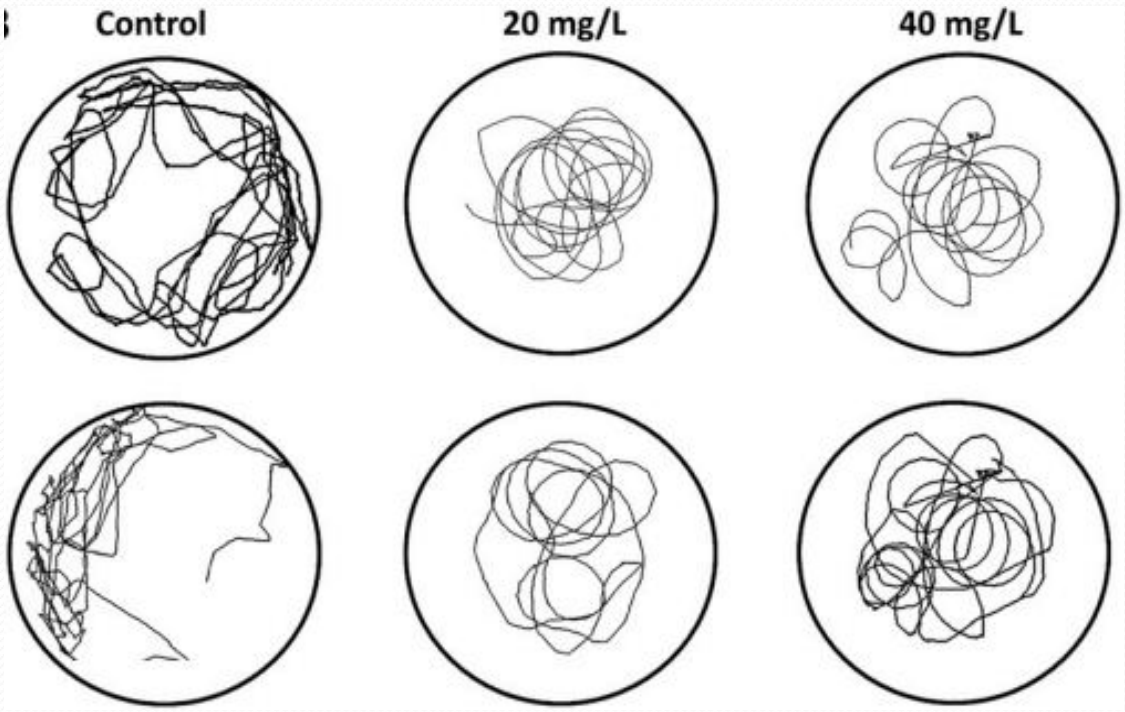
Ketamine Light Dark Box test



Behavioral endpoints obtained in the 6-min light –dark box test (n = 12 per group; Experiment 2). *P b 0.05, **P b 0.01, #P = 0.05 –0.1 (trend) vs. controls; post-hoc Tukey test for significant ANOVA data.

Behavioral effects of Ketamine

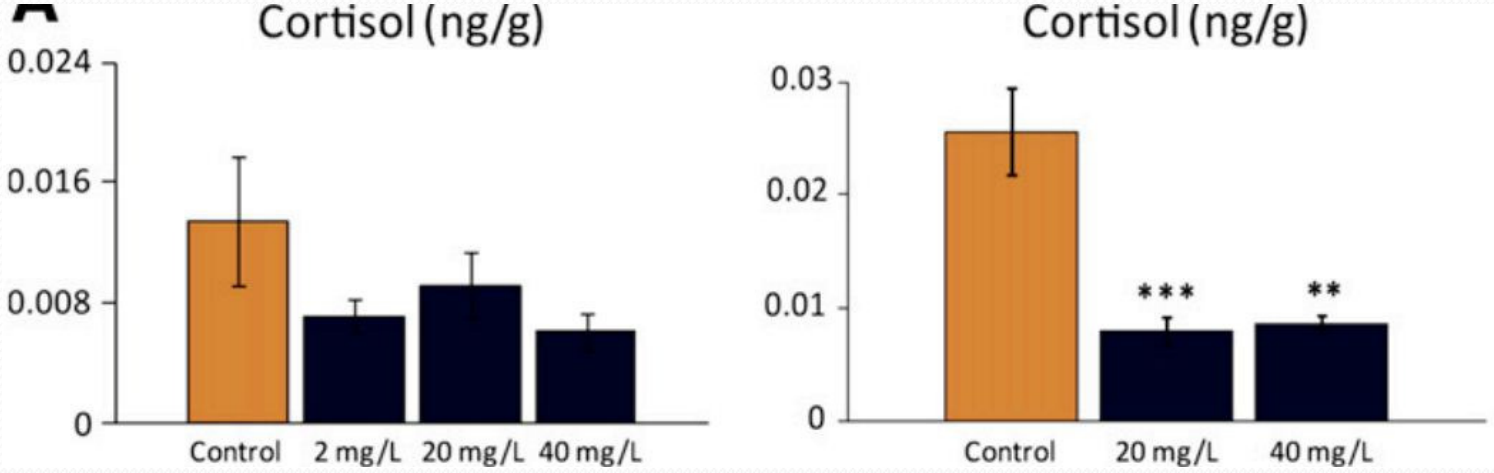




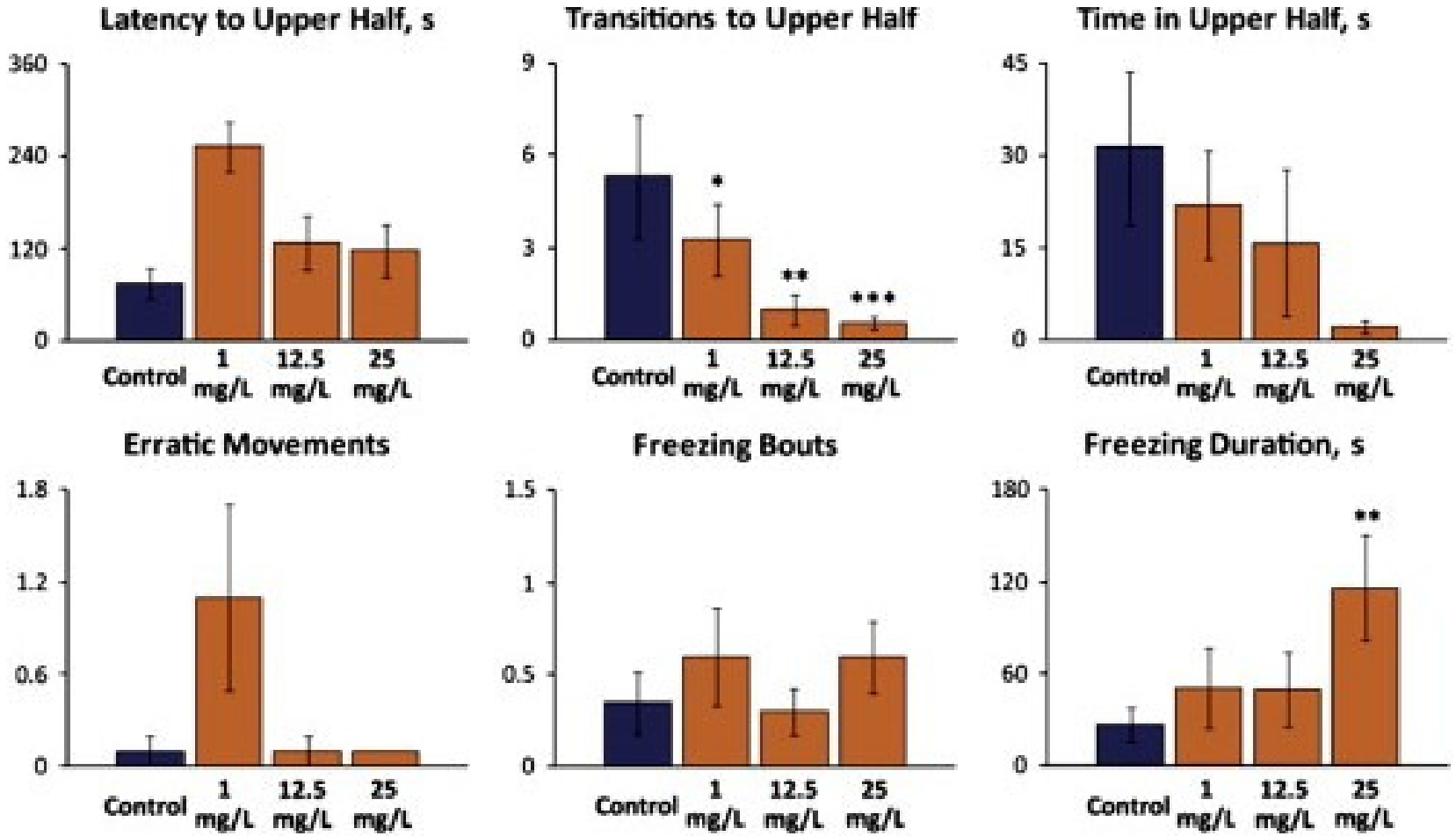
Behavioral effects of 20-min acute exposure to ketamine on zebrafish tested in the open field test (Experiment 3; n = 10 –12 per group). Behavioral data and representative traces were generated by Noldus Ethovision XT7 software using the side view video-recording. In all experiments, the traces were examined for each experimental cohort, rated from 1 to n (based on similarity to each other), and the middle trace was selected as representative, to illustrate the patterns of zebrafish locomotion.

Cortisol: ketamine effects

Whole-body cortisol expression levels in zebra fish exposed to ketamine for 20 min. A: Whole-body cortisol (ng/g body weight for each individual fish)

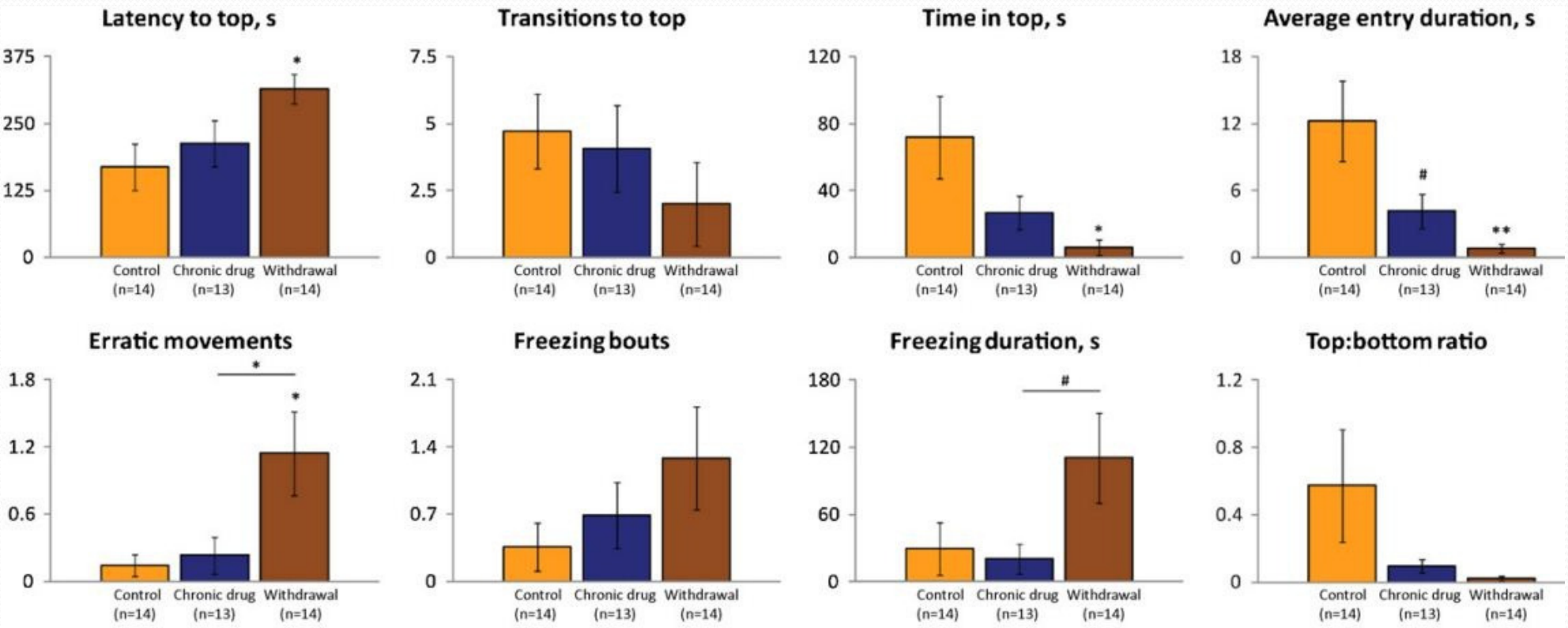


Behavioral effects of Cocaine



Cocaine (1–25 mg/L) significantly affects the number of top transitions ($F(3, 39) = 5.9$, $P < 0.005$) and freezing duration ($F(3, 39) = 5.7$, $P < 0.005$). Data are presented as mean ±SEM (n=10–14 per group), *P 0.05, **P 0.01, ***P0.005 vs. control; post-hoc Tukey test for significant ANOVA data.

Anxiety: Morphine withdrawal



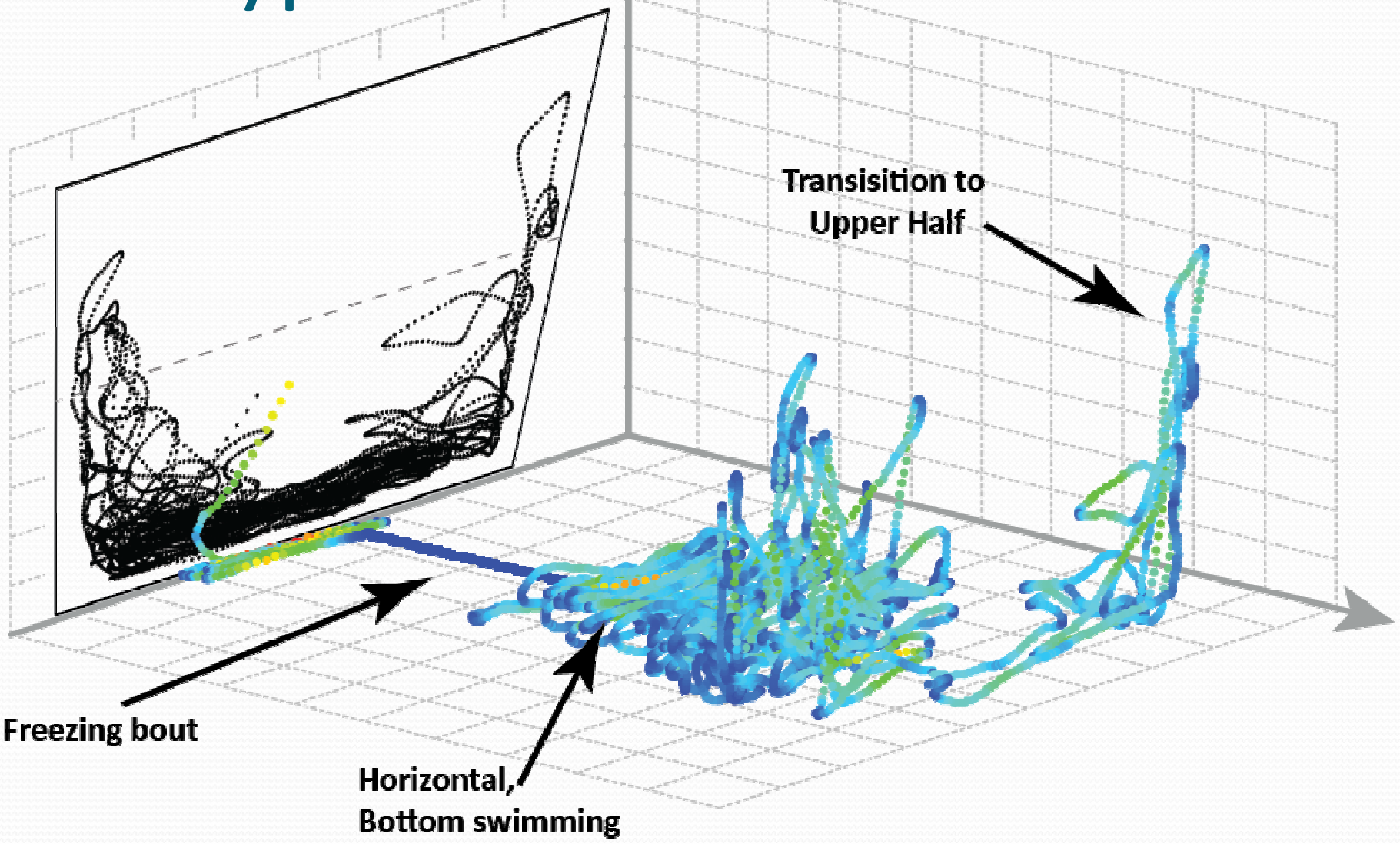
Anxiogenic effects of repeated withdrawal (two 3-h withdrawal periods daily for 1 week) from chronic 1-week morphine in adult zebrafish tested in the novel tank diving test. Data are presented as mean ± SEM, *P < 0.05, **P < 0.005, # P = 0.05–0.1 (trend); post-hoc Tukey test for significant Kruskal–Wallis data. Signs above data bars indicate significance/trends vs. control group, signs above horizontal lines indicate significance/trends between the respective experimental groups.

Summary of anxiogenic and anxiolytic modulation of adult zebrafish

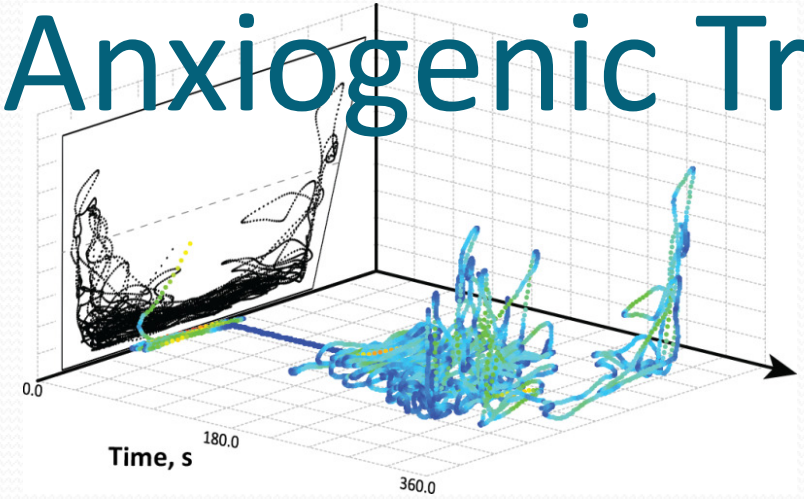
	Anxiogenic manipulations				Anxiolytic manipulations			
	Alarm Pheromone (n= 60)	Rpt Morphine Withdrawal (n= 30)	Leopard strain (n= 13)	Caffeine (n= 14)	Chronic Fluoxeine (n= 28)	Chronic Ethanol (n= 35)	Chronic Morphine (n= 35)	Nicotine (n= 40)
Behavioral parameters								
Latency to upper half, s	↑	↑	↑	—	↓	↓	↓	↓
Transitions to upper half	↓	↓	↓	↓	↑	↑	↑	↓
Time in upper half, s	↓	↓	↓	—	↑	↑	↑	↑
Erratic movements	↑	↑	—	—	↓	↓	—	↓
Freezing bouts	↑	↑	⇧	↑	↓	↓	—	↓
Freezing duration, s	↑	↑	⇧	↑	↓	↓	↓	↓
Endocrine endpoints								
Whole-body [cortisol], ng/g	↑	↑	N/A	↑	↓	↓	↓	↑

Three-Dimensional Neurophenotyping of Adult Zebrafish Behavior

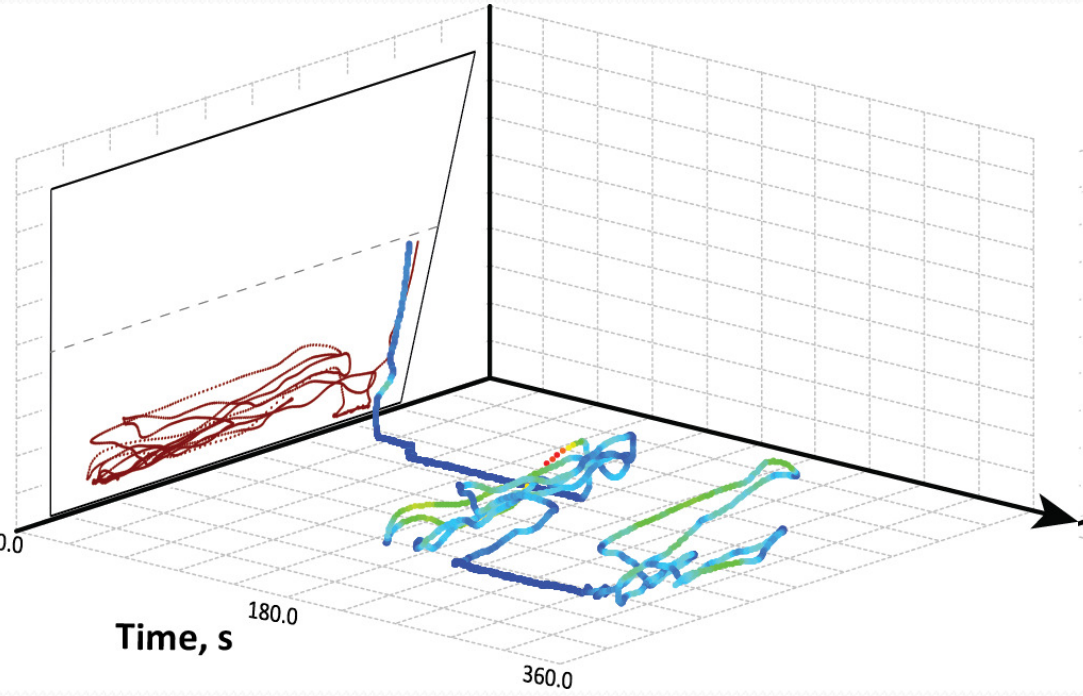
3D modeling: XY-Time Wild-Type Controls



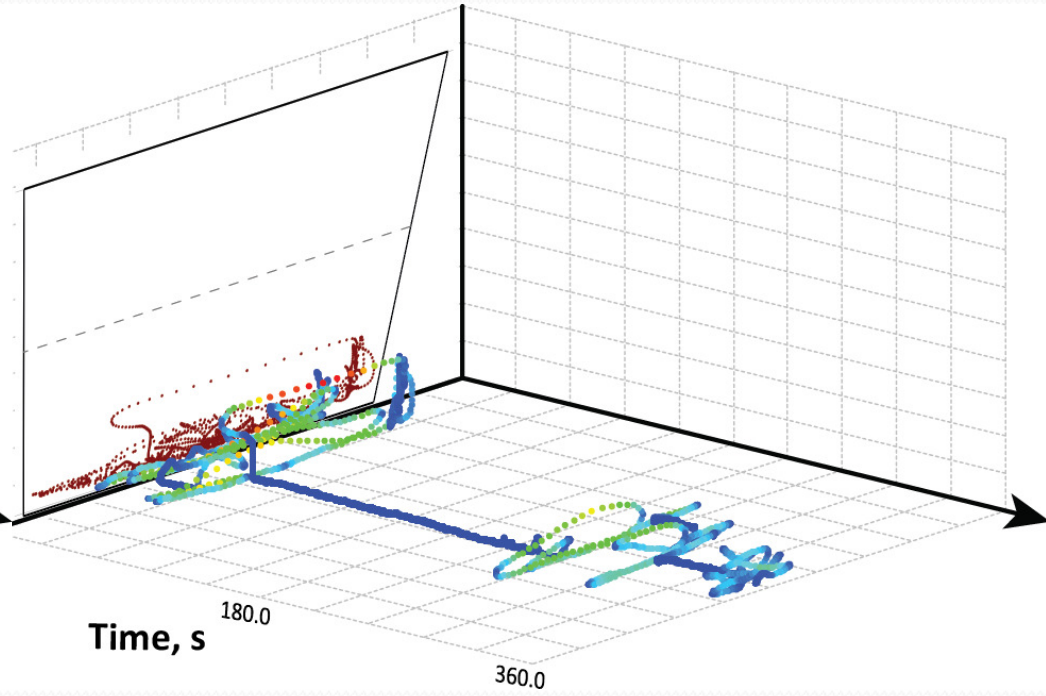
Anxiogenic Treatments



Wild-type Control

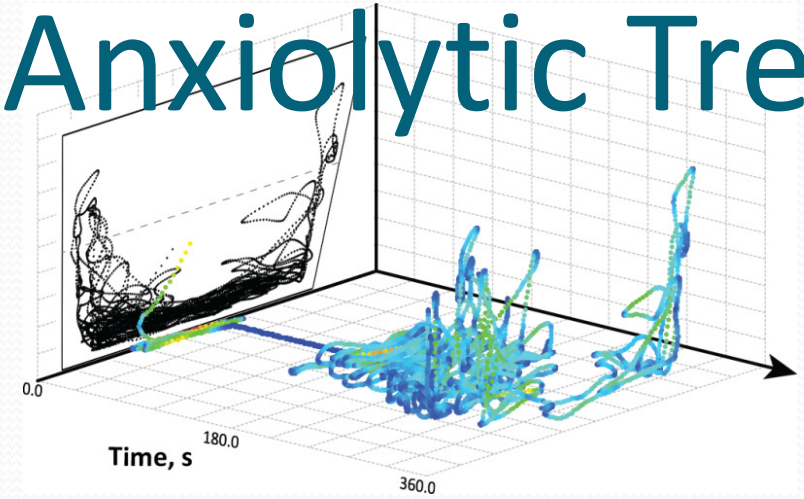


Caffeine (250mg/L , 20mins)

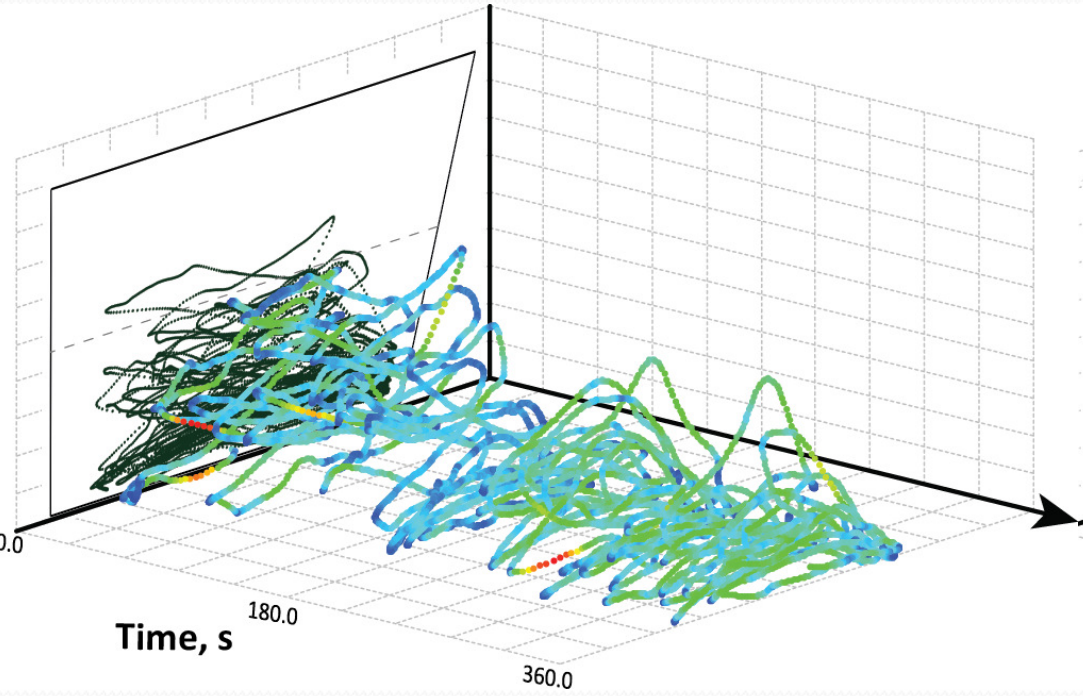


Alarm Pheromone (7mL)

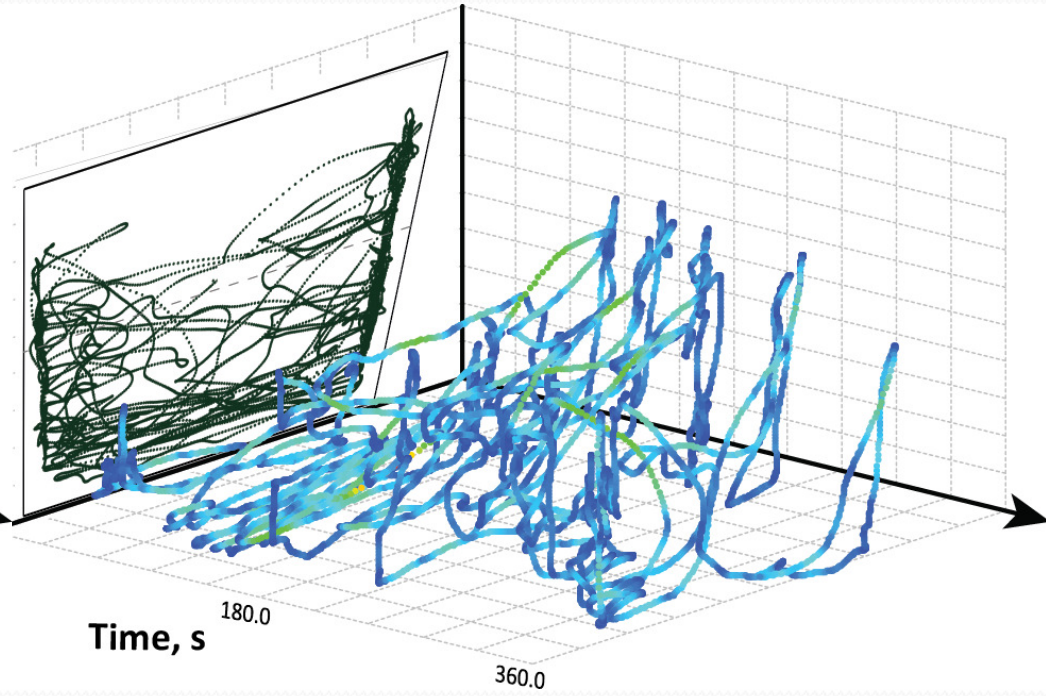
Anxiolytic Treatments



Wild-type Control

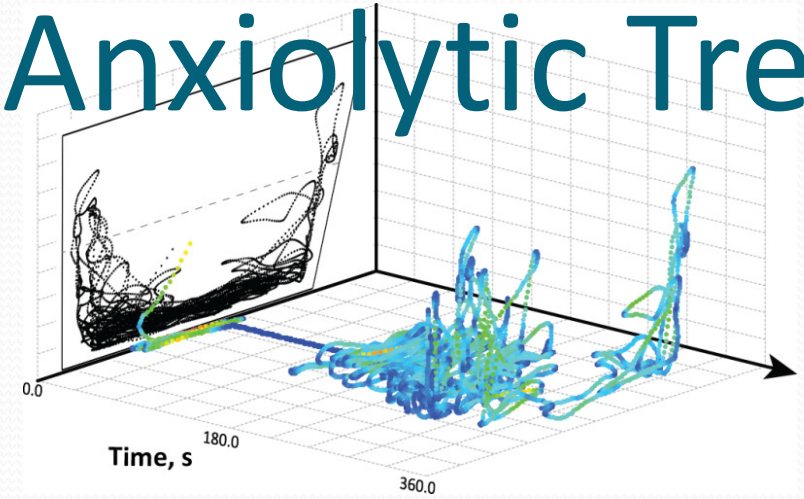


Chronic Morphine (1.5mg/L, 2 wks)



Chronic Ethanol (0.3%, 2 wks)

Anxiolytic Treatments



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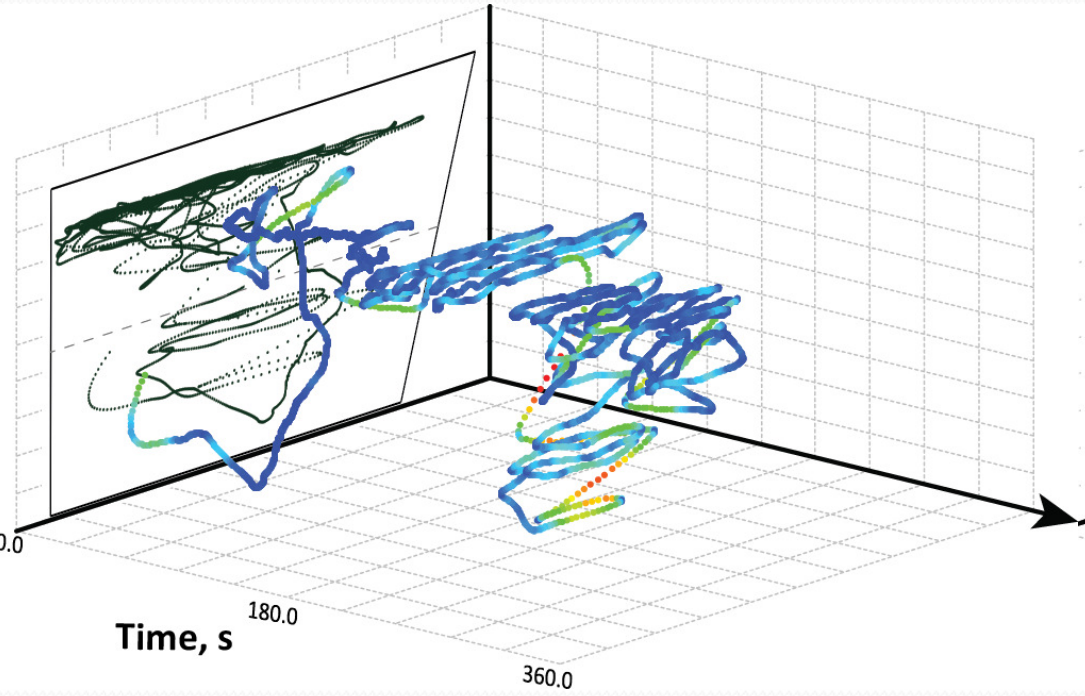
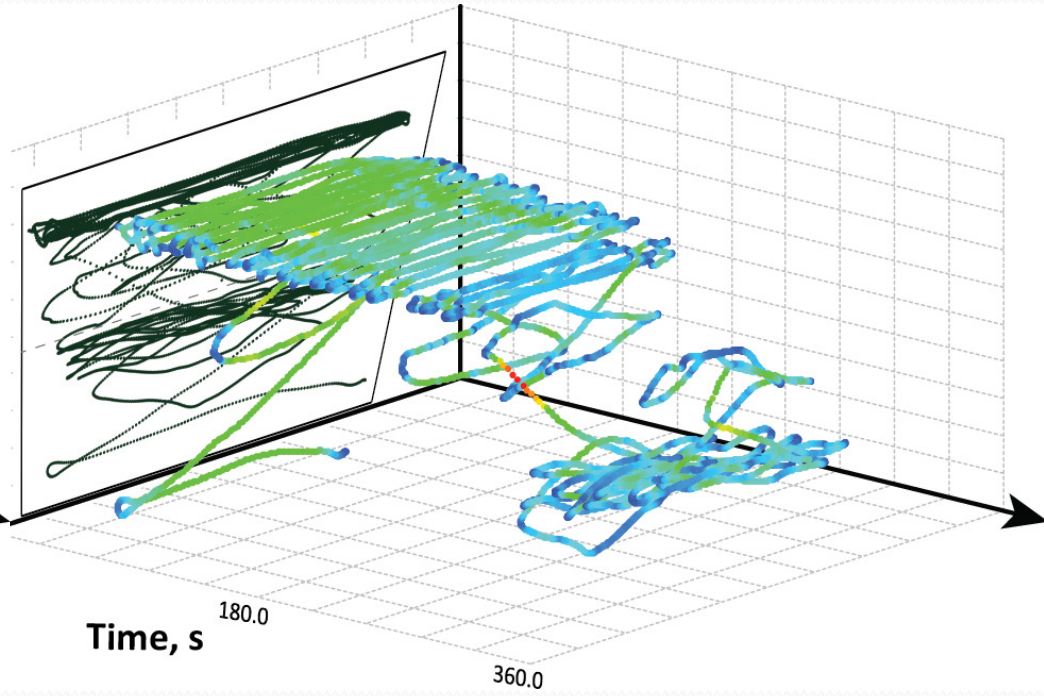
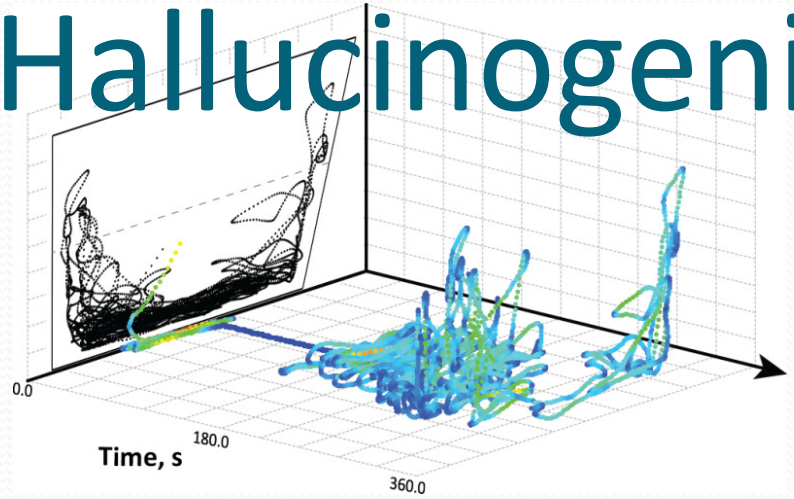


Chronic Fluoxetine (100 µg/L, 2 wks)

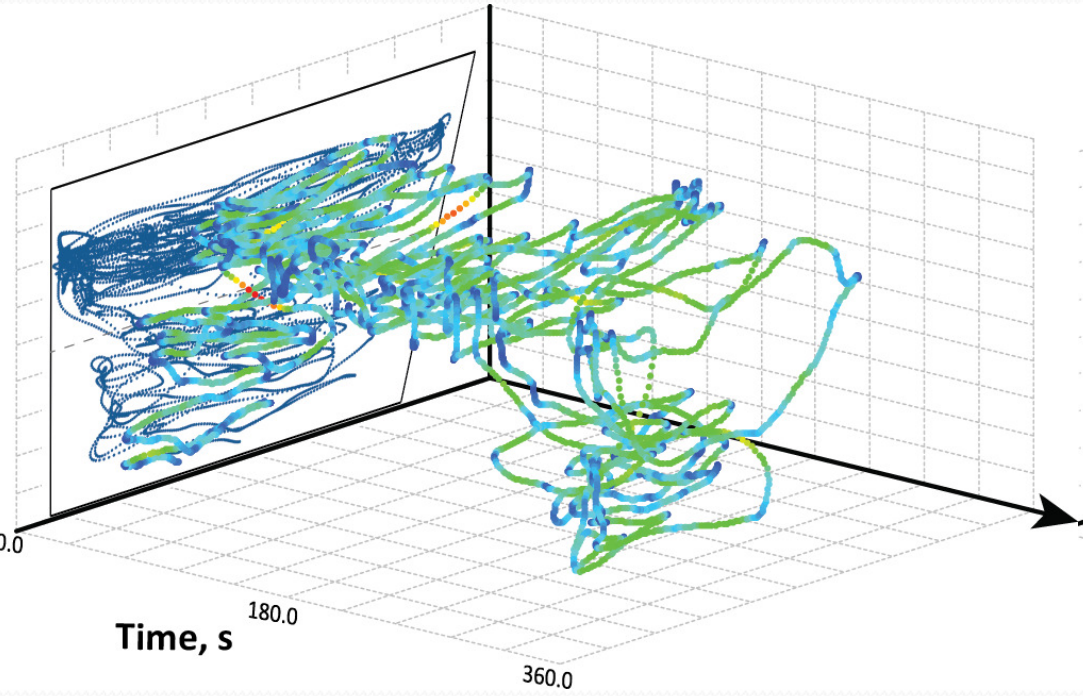


Nicotine (10 mg/L, 2 wks)

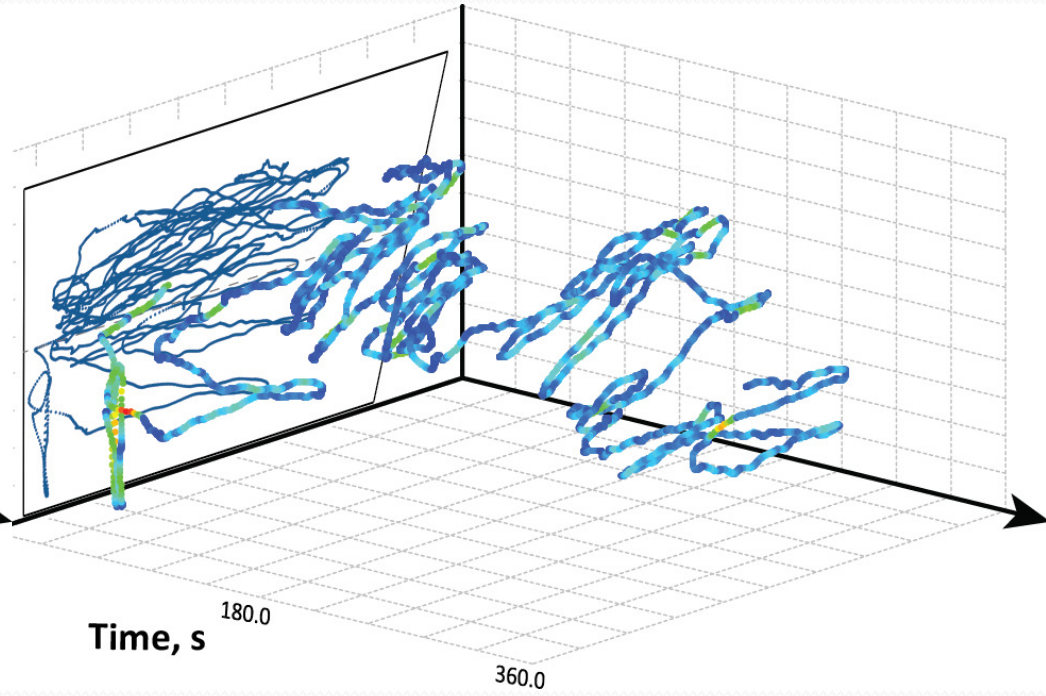
Hallucinogenic Treatments



Wild-type Control



LSD (250 µg/L, 20 mins)



MDMA (10 mg/L, 30 mins)

Conclusion

- Zebrafish models strongly parallel animal and clinical evidence, further supporting their validity and translatability for identifying mechanisms for anxiety regulation, and discovering potential new classes of anxiolytic drugs.

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Welcome to the Zebrafish Neurophenome Database!

Click here to enter ZND

We are currently collecting data - please submit!

ZNP is a new bioinformatics database established by the ZNRC, to provide a comprehensive resource for neurobehavioral and physiological data of adult zebrafish models.

ZNP incorporates validated and curated data from work published in this field, to improve the accessibility of current knowledge to researchers interested in using adult zebrafish models. Overall, this program will allow investigators to rapidly review data, to direct their research using these models.

If your lab is interested in contributing or submitting data to the ZNP, please contact ZNRC coordinator Dr. Allan V Kalueff (Tulane Medical School) via email.