The International STRESS AND BEHAVIOR Society (ISBS)
Yamaguchi University, Yamaguchi, Japan

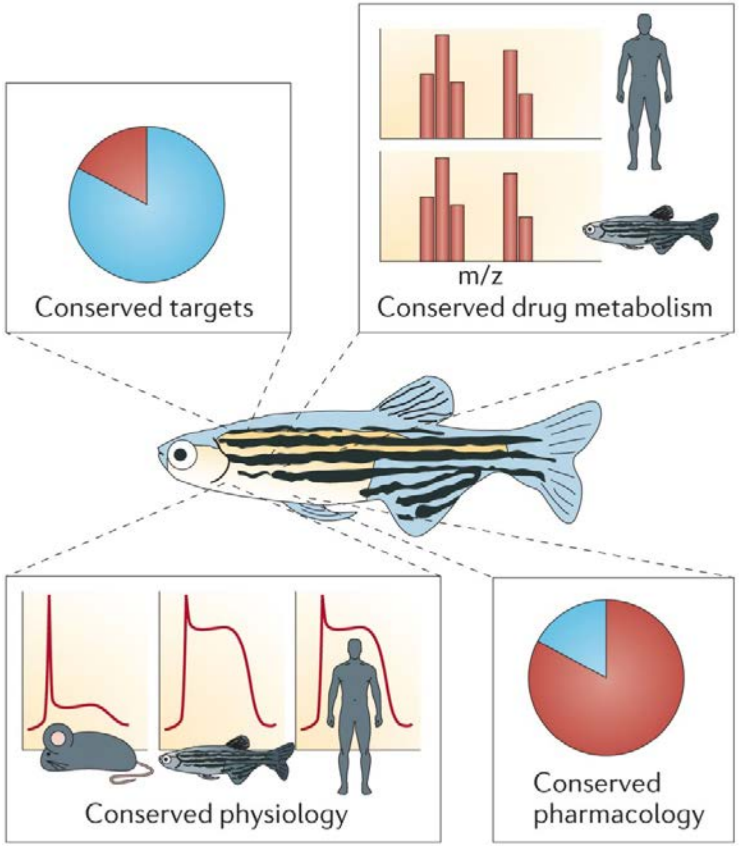
Zebrafish Brain and Behavior

Professor Allan V. Kalueff, PhD, DBiol
St. Petersburg State University, Russia
ISBS President

16th International Regional (Asia) ISBS Neuroscience and Biological Psychiatry “Stress and Behavior” Conference

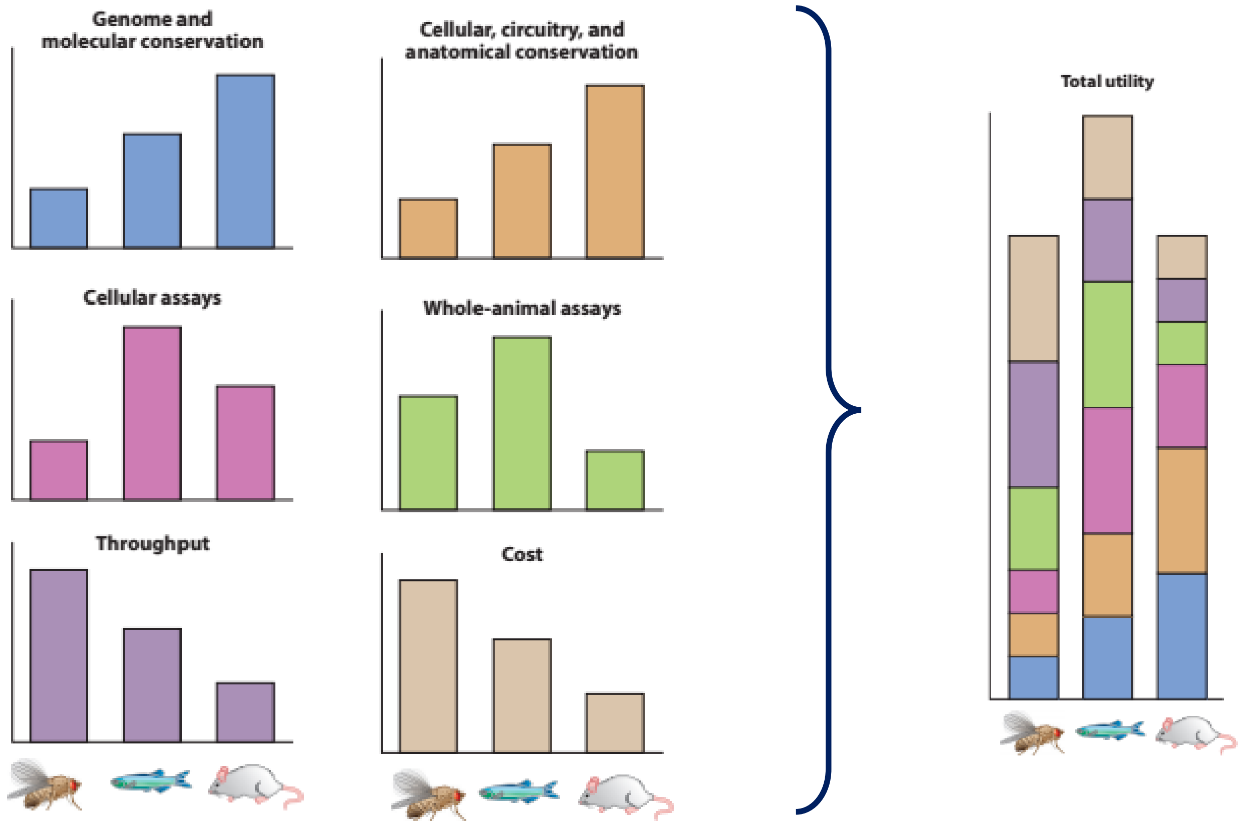
September 3-5, 2022
Yamaguchi University, Japan

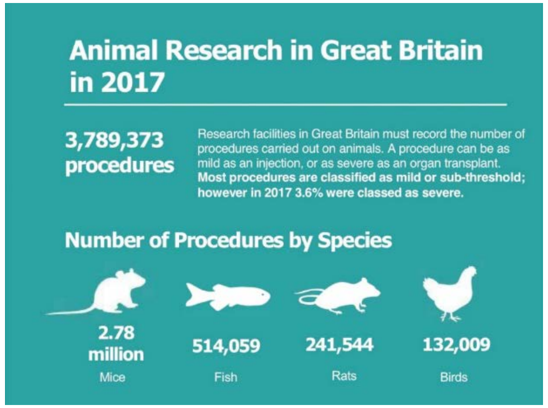
Zebrafish as a model



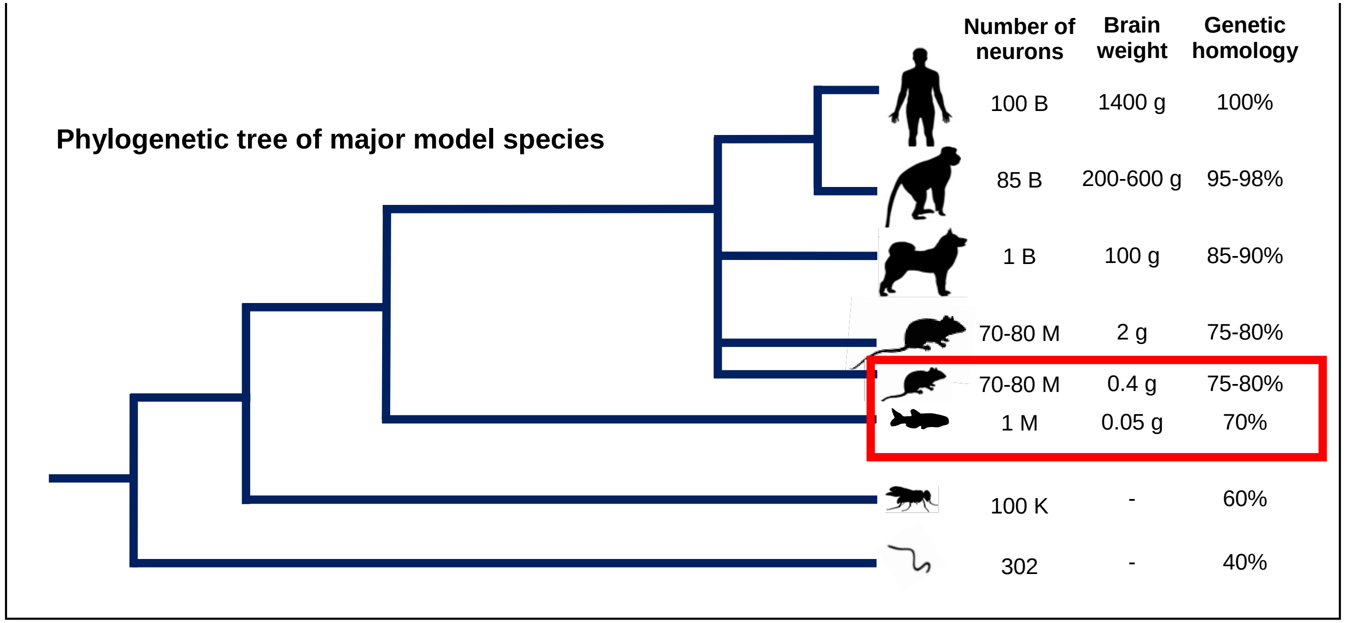
Nature Reviews | Drug Discovery

MacRae & Peterson, 2015



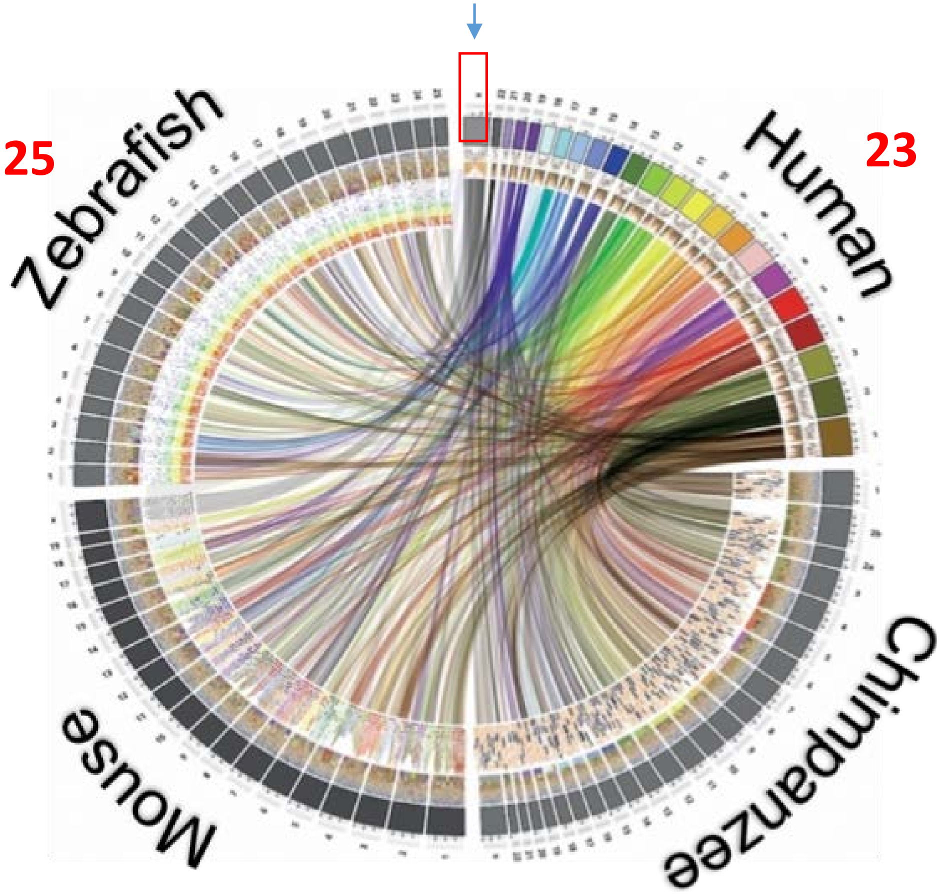


Zebrafish as a genetic machine



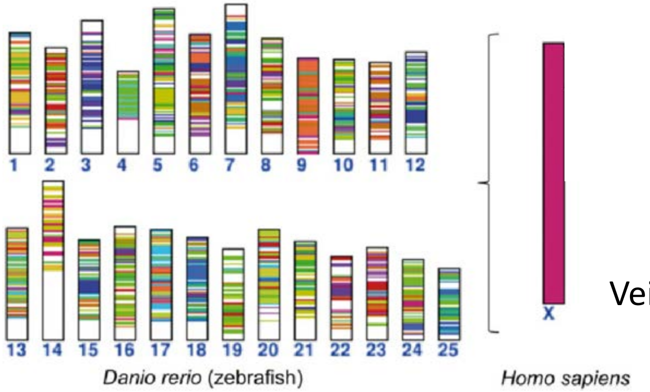
Number of neurons	Brain weight	Genetic homology
100 B	1400 g	100%
85 B	200-600 g	95-98%
1 B	100 g	85-90%
70-80 M	2 g	75-80%
70-80 M	0.4 g	75-80%
1 M	0.05 g	70%
100 K	-	60%
302	-	40%

Stewart et al 2014



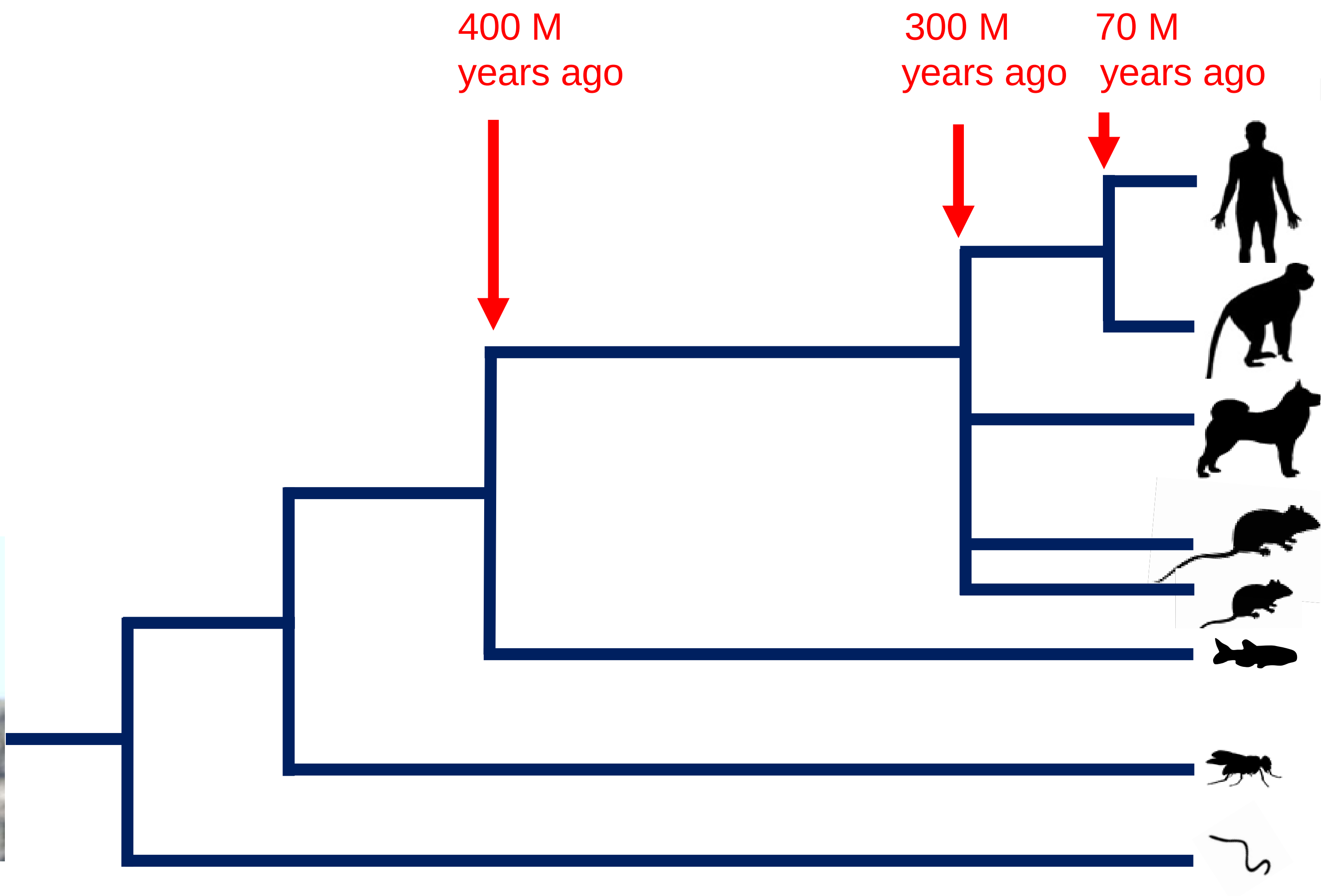
NIH, 2016

Genetic homology



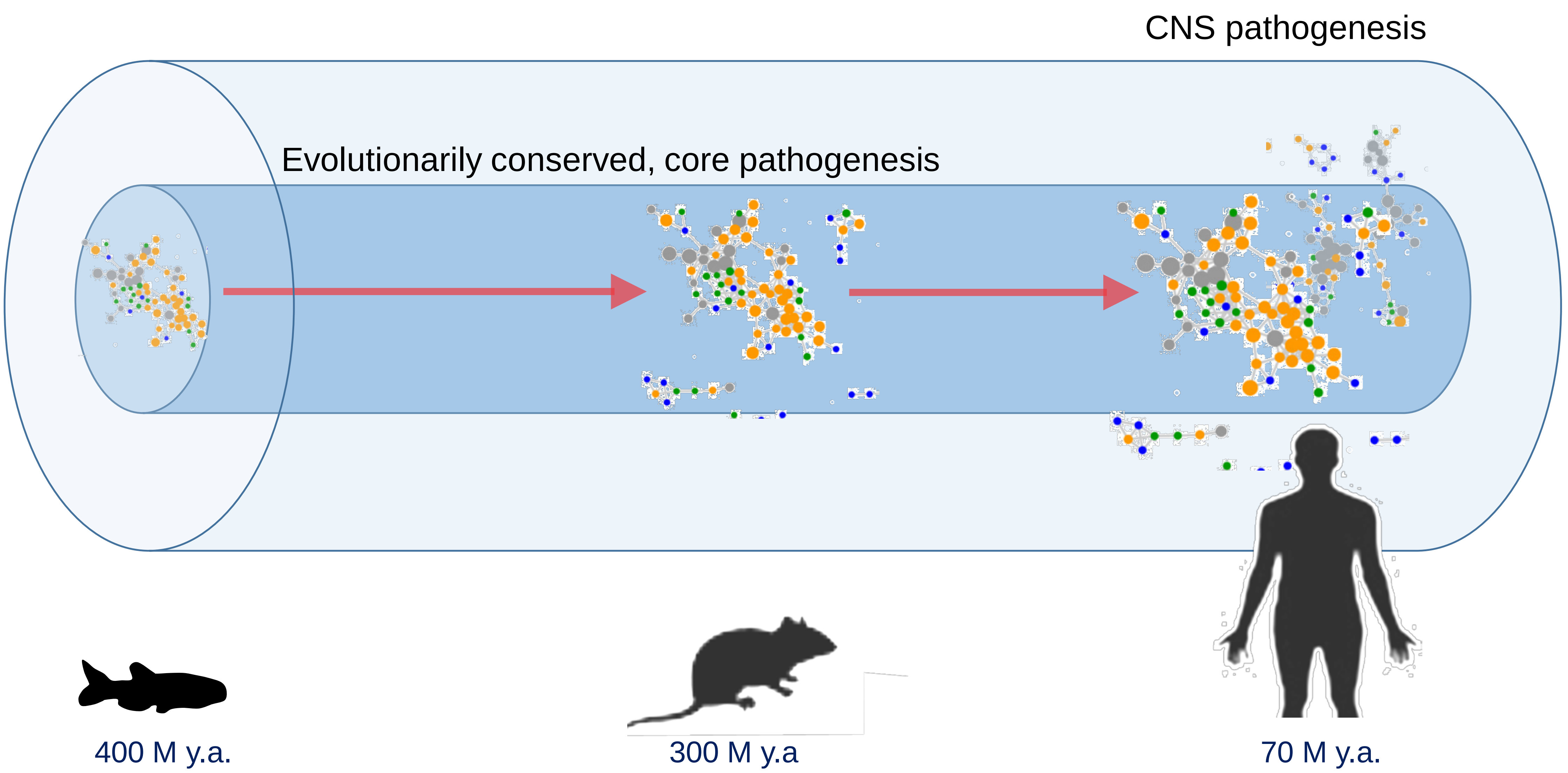
Veitia et al. 2015

Zebrafish as a time machine

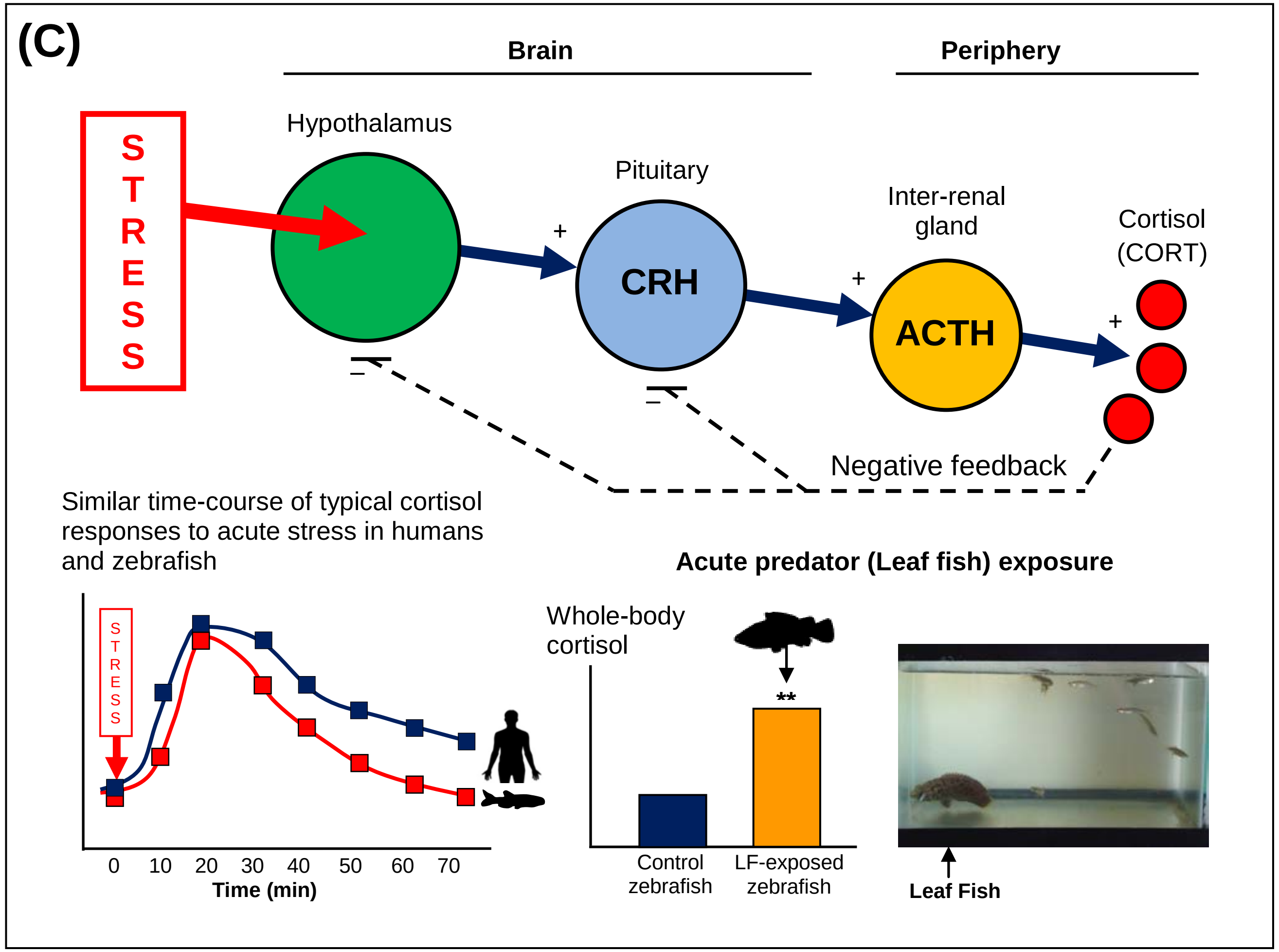


Stewart et al 2014

Zebrafish as a translational machine

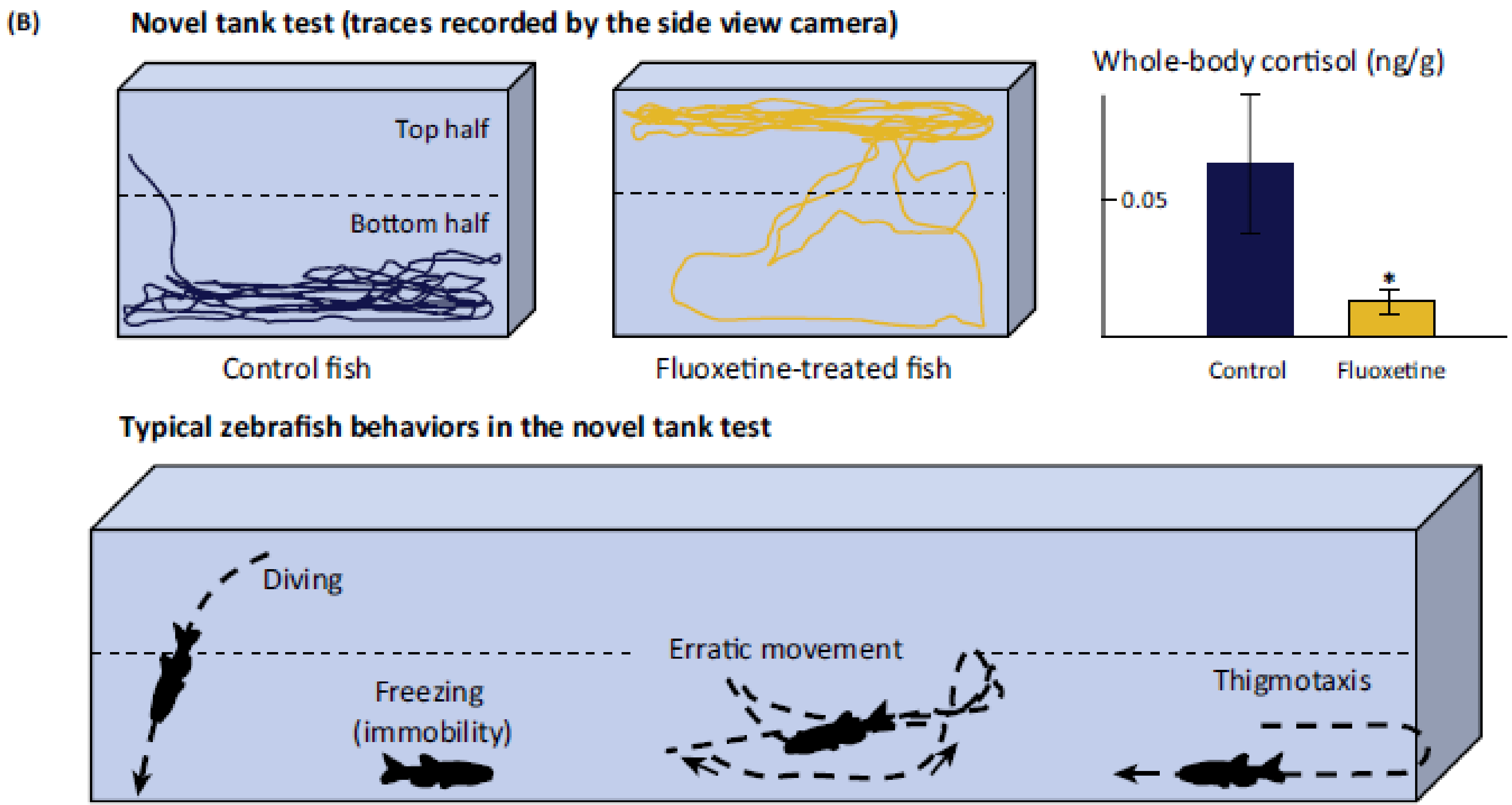


Zebrafish as a translational machine



Stewart et al 2014

CNS diseases: Anxiety

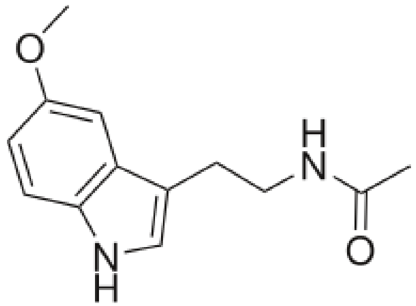


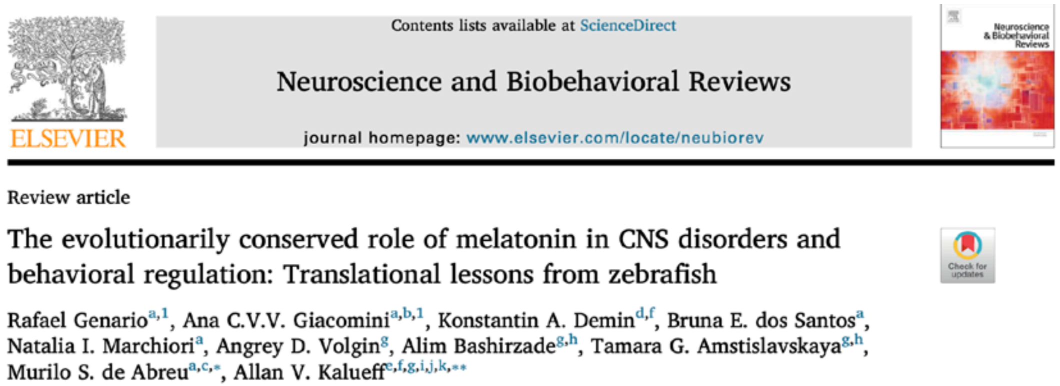
Stewart et al 2014

How can we use this powerful biological machine in neuroscience research?

Aim: To understand neuroendocrine mechanisms and identify novel drug targets

Melatonin CNS effects




Contents lists available at ScienceDirect

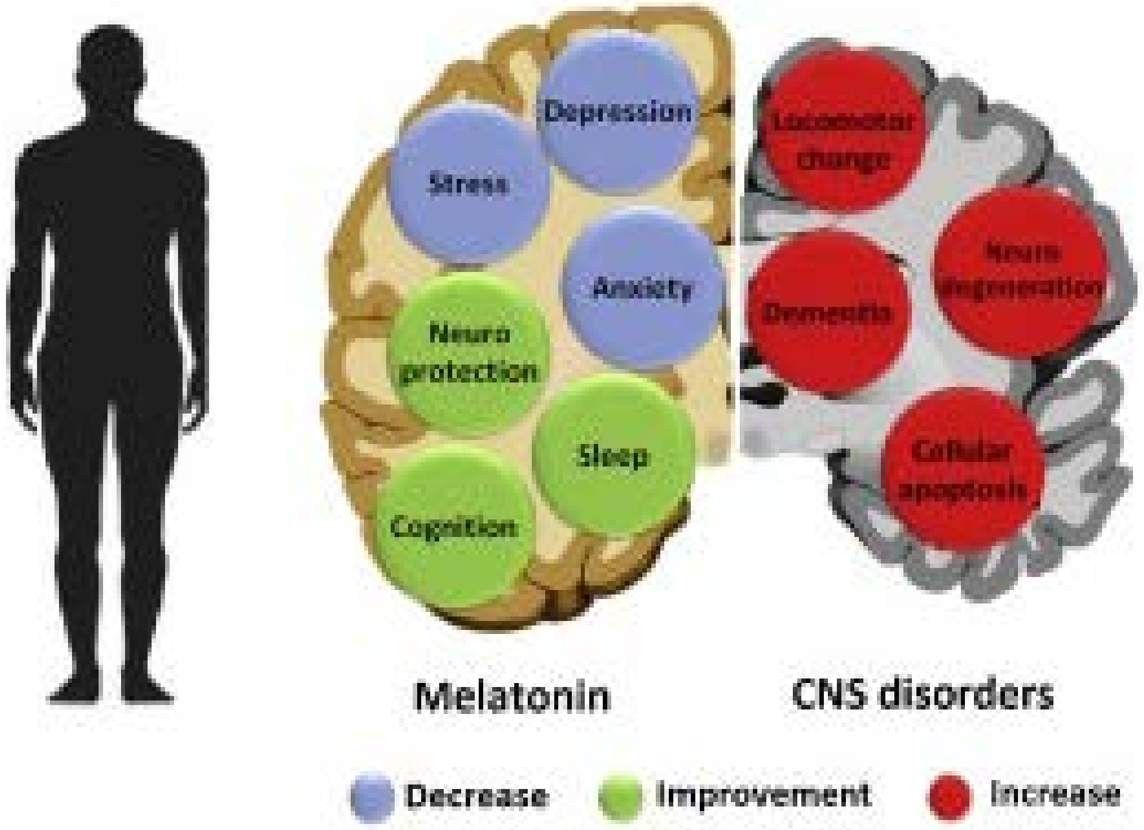
Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com/locate/neubiorev

Review article

The evolutionarily conserved role of melatonin in CNS disorders and behavioral regulation: Translational lessons from zebrafish

Rafael Genario[a,1], Ana C.V.V. Giacomini[a,b,1], Konstantin A. Demin[d,f], Bruna E. dos Santos[a], Natalia I. Marchiori[a], Angrey D. Volgin[g], Alim Bashirzade[g,h], Tamara G. Amstislavskaya[g,h], Murilo S. de Abreu[a,c,*], Allan V. Kalueff[e,f,g,i,j,k,**]



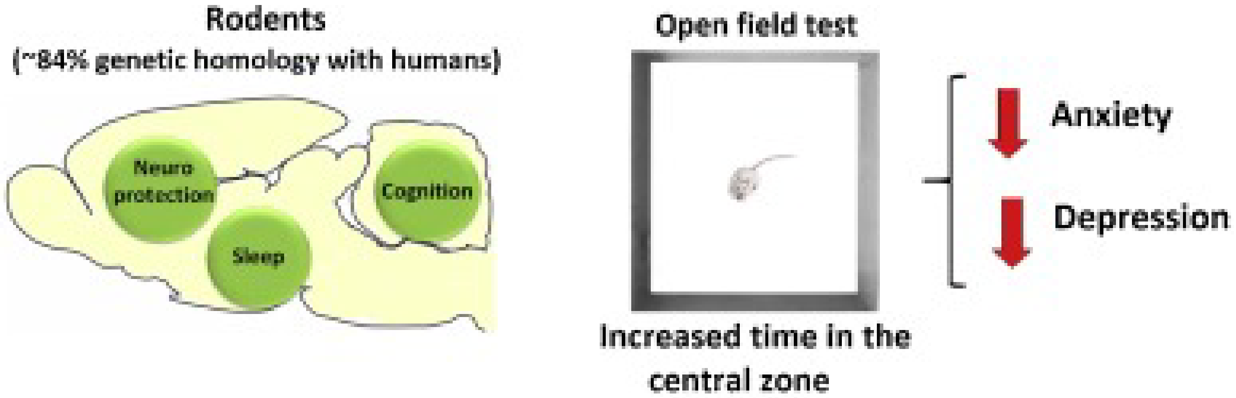
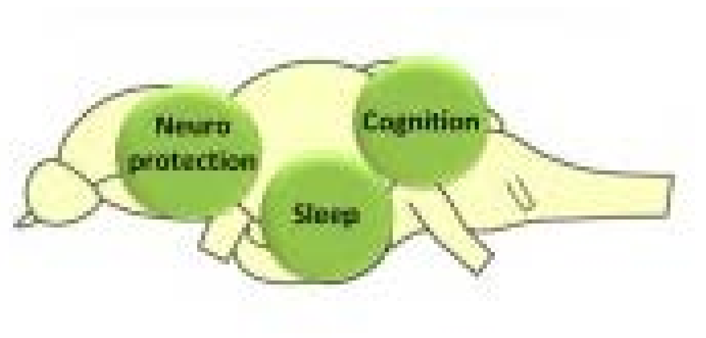


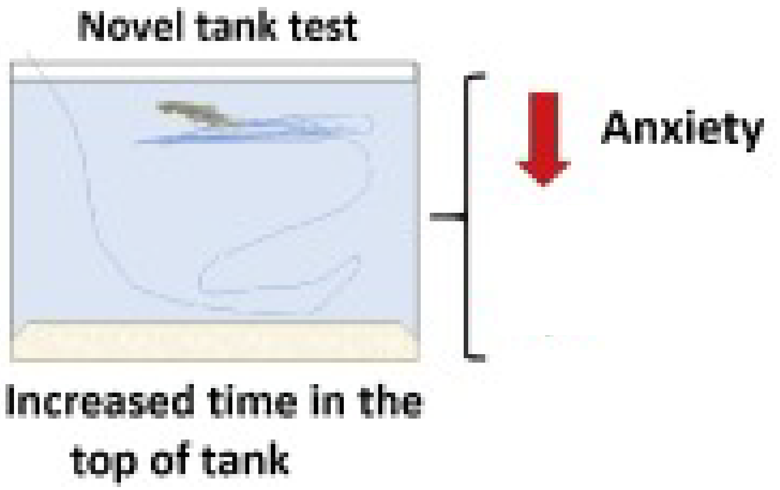
Table 2

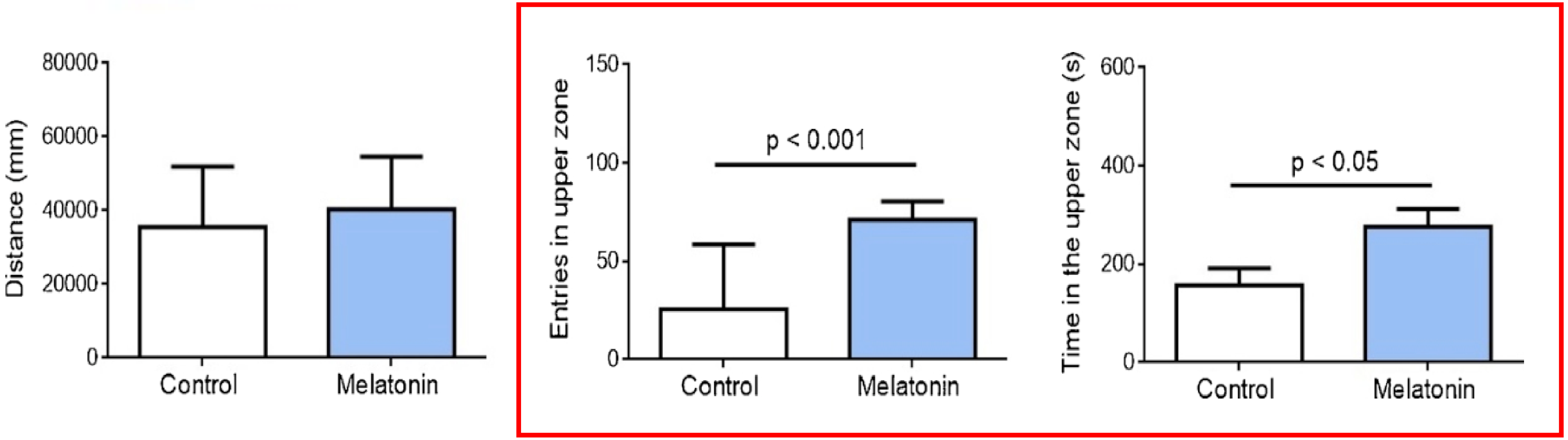
Melatonin-related genes in zebrafish (accessed in www.ensembl.org, searching for melatonin-related genes in zebrafish) and their human and mouse orthologues, based on the National Center for Biotechnology Information (NCBI) Genetic Testing Registry (GTR) database with % homology calculated based on protein identity using the HomoloGene database (www.ncbi.nlm.nih.gov/homologene).

Genes	Symbols	Main neurobiological functions	% Homology		
			Zebrafish *vs* Human	Zebrafish *vs* Mouse	Human *vs* Mouse
Melatonin receptor 1 A (a and b)	*Mtnr1aa, Mtnr1ab*	Control of reproductive and circadian actions of melatonin	75	74	84
Melatonin receptor 1 A-like*	*Mtnr1 Al*	Control of reproductive and circadian actions of melatonin	77	74	–
Melatonin receptor 1Ba**	*Mtnr1Ba*	Control of reproductive and circadian actions of melatonin	60	59	81
Melatonin receptor 1Bb	*Mtnr1Bb*	Control of reproductive and circadian actions of melatonin	69	62	
Melatonin receptor 1C***	*Mtnr1C*	Control of reproductive and circadian actions of melatonin	–	–	–
Arylalkylamine *N*-acetyltransferase 1	*Aanat1*	Biosynthesis of melatonin	67	71	85
Arylalkylamine *N*-acetyltransferase 2	*Aanat2*	Biosynthesis of melatonin	62	64	85
Dopa decarboxylase	*Ddc*	Biosynthesis of dopamine	72	74	89
Acetylserotonin *O*-methyltransferase	*Asmt*	Biosynthesis of melatonin	47	36	48
Hypocretin/orexin receptor 2	*Hcrtr2*	Regulation of feeding behavior	71	72	94
Tryptophan hydroxylase 1a	*Tph1a*	Biosynthesis of serotonin	80	78	89
Tryptophan hydroxylase 1b	*Tph1b*	Biosynthesis of serotonin	77	75	89
Tryptophan hydroxylase 2	*Tph2*	Biosynthesis of serotonin	74	77	93
Average homology rate, %			**69.25**	**68**	**83.7**

Anxiolytic effects of melatonin in zebrafish



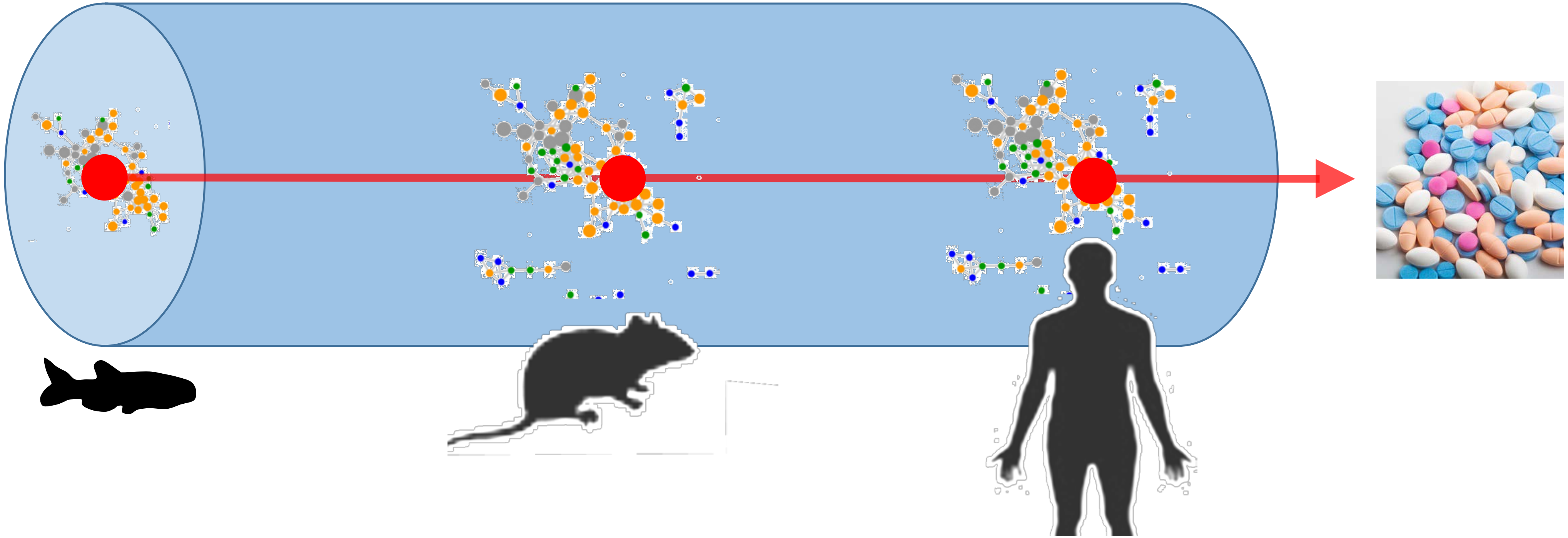




Effects of melatonin 24-h treatment (0.232 mg/L) in adult zebrafish under light housing, tested in the 15-min novel tank test. U-test (n = 20) vs. controls.

Genario et al., 2019

Melatonin-related molecules are an evolutionarily conserved target for anxiolytic effects across taxa



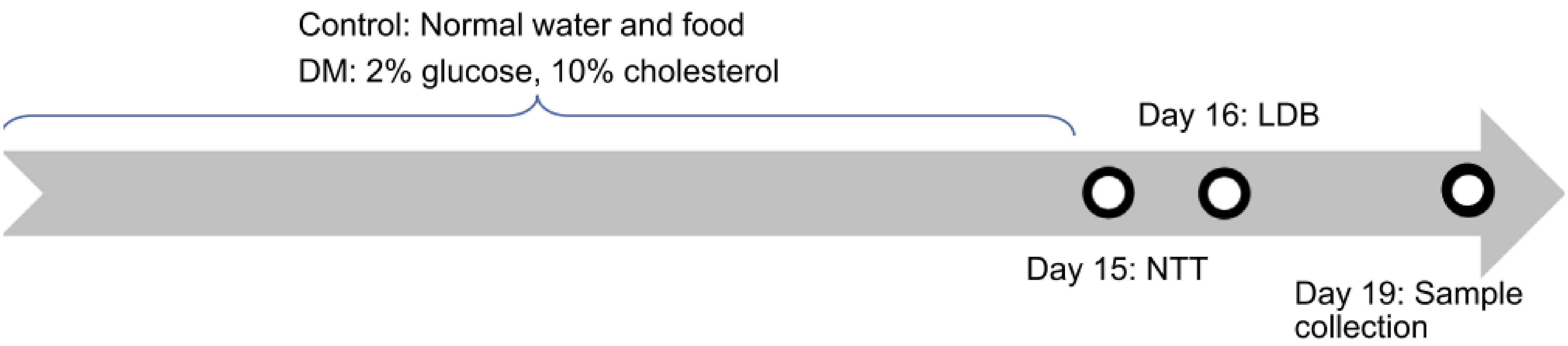
New anxiolytic drugs can be developed by targeting melatonin signaling pathways

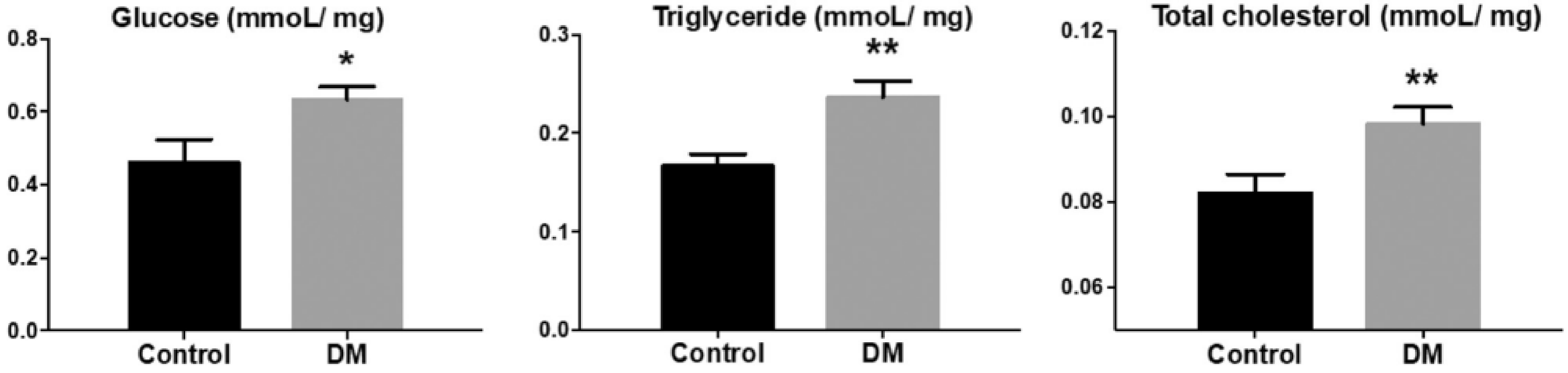
Zebrafish as molecular machines

Aim. To expand the range of molecular models using zebrafish behavioral research and chronic stress models

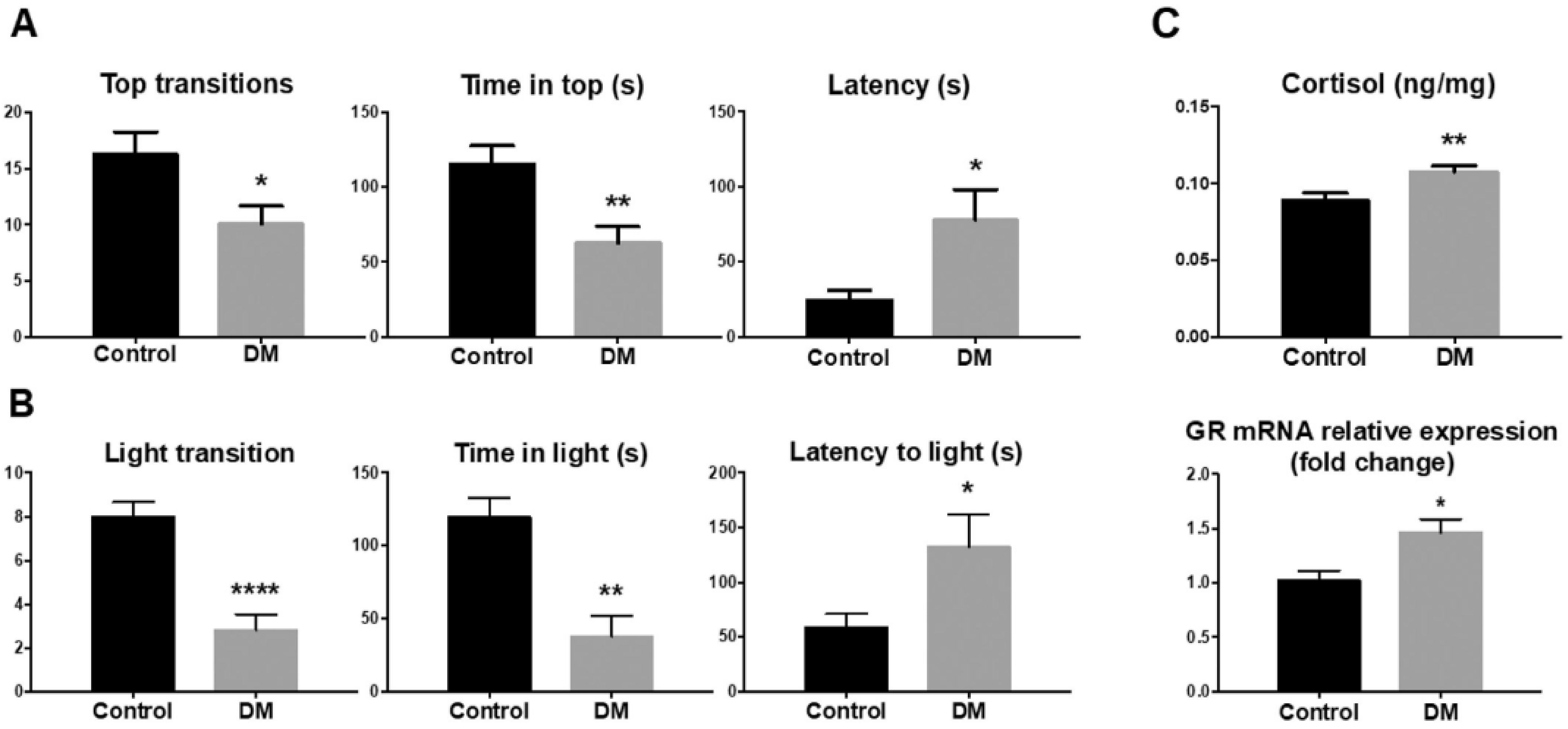
Q: Can we use zebrafish to model stress-related molecular processes?

Zebrafish models of diabetes DM type 2

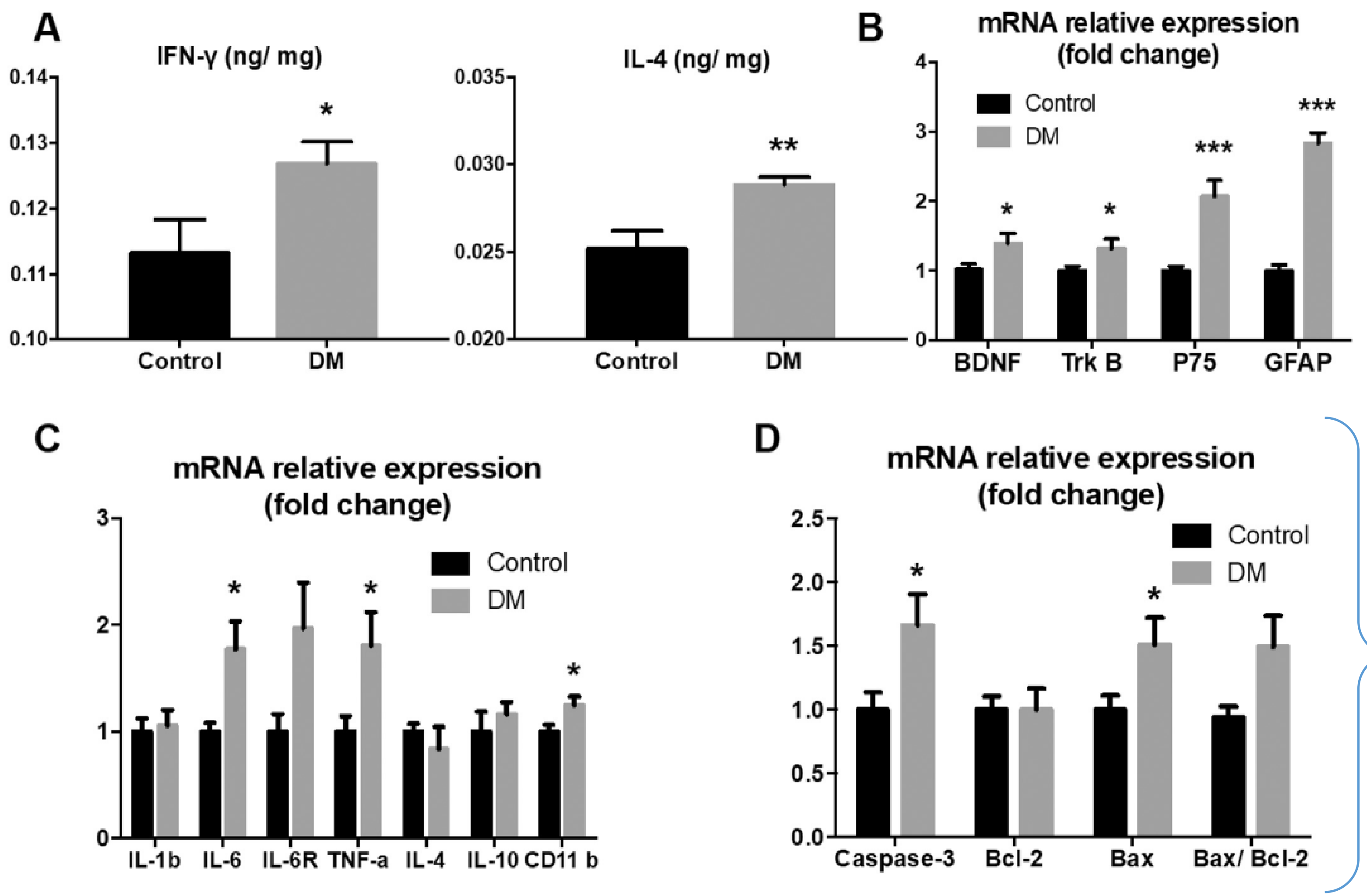


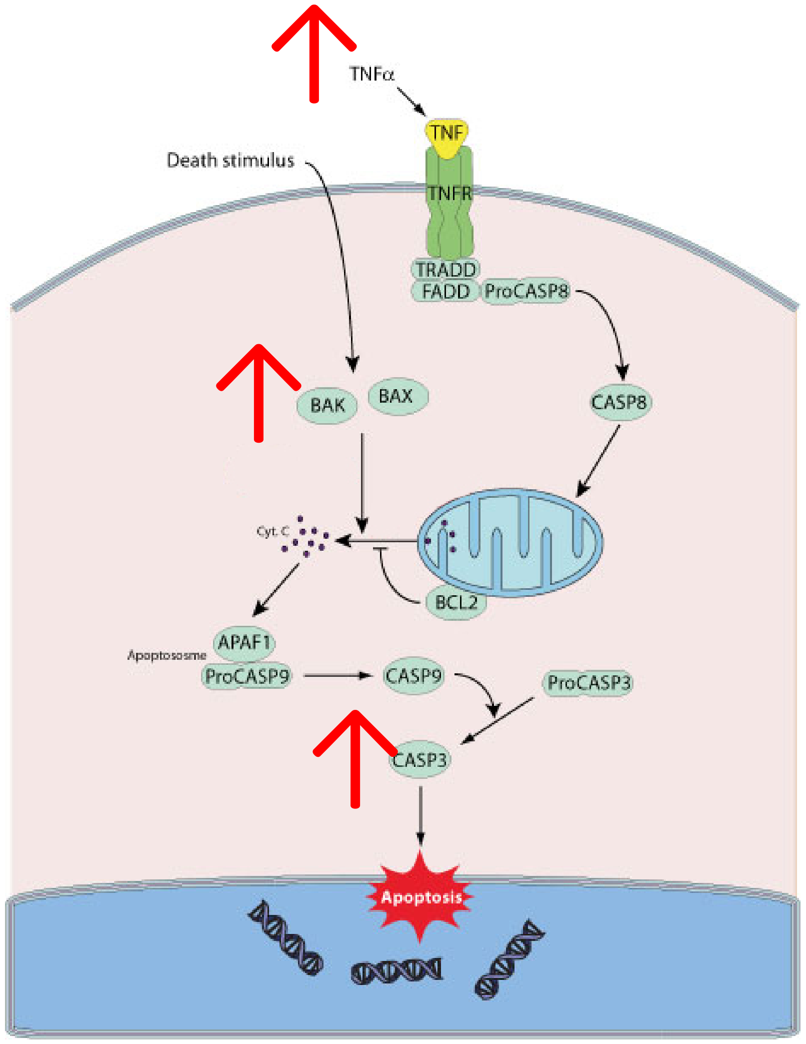


Robust DM-induced anxiety in zebrafish on day 15/16

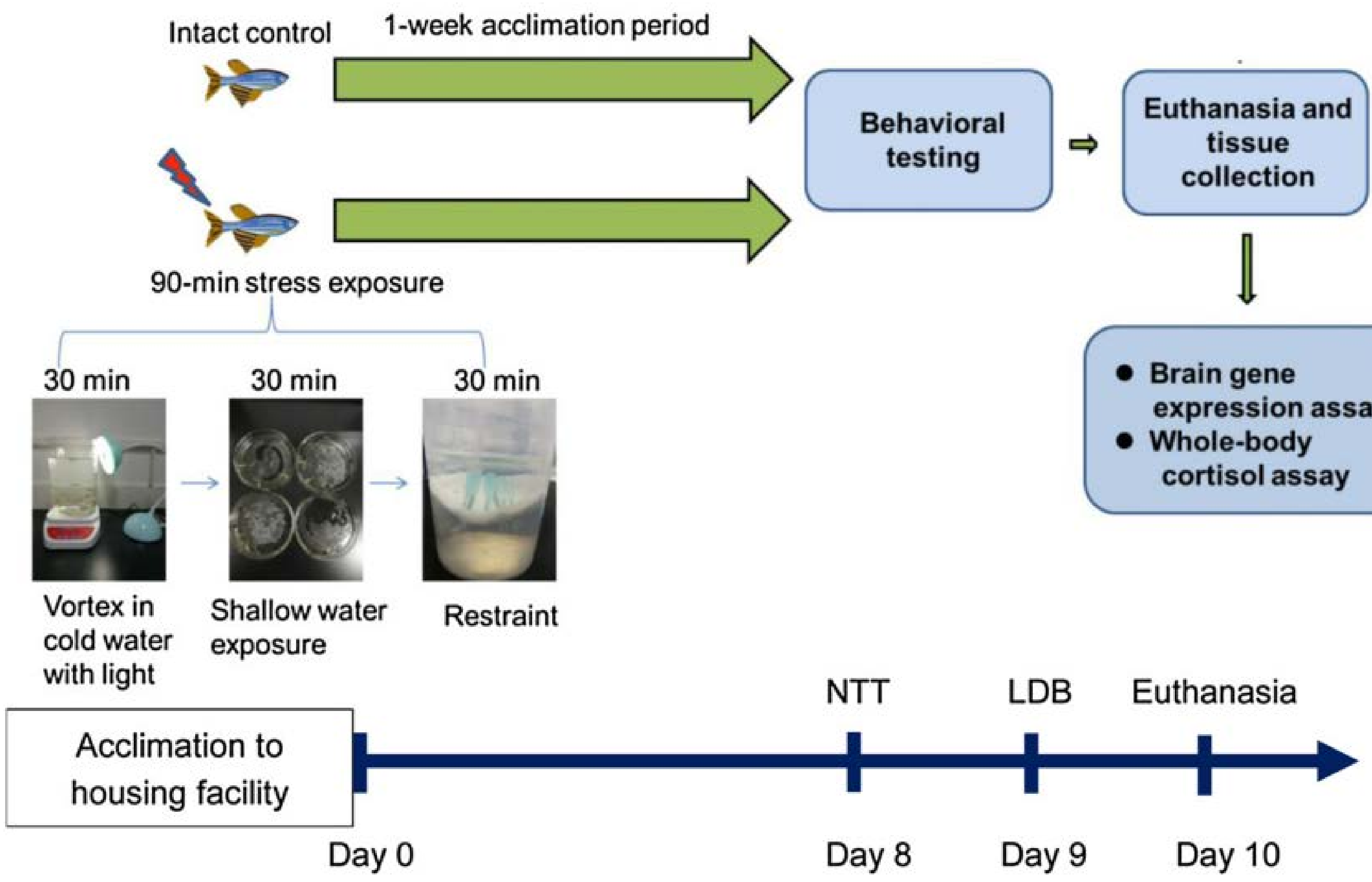


Molecular biomarkers in zebrafish brain

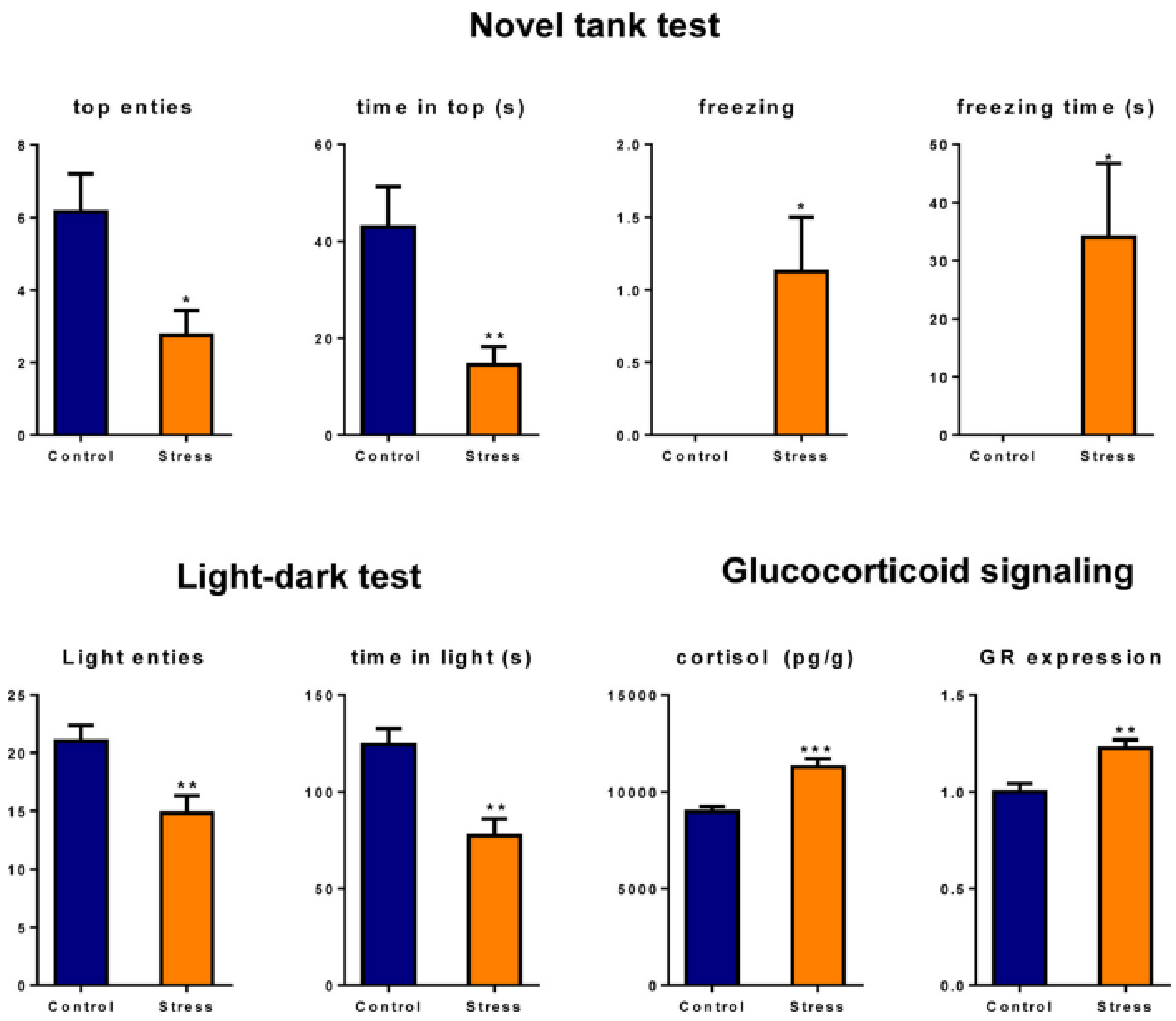




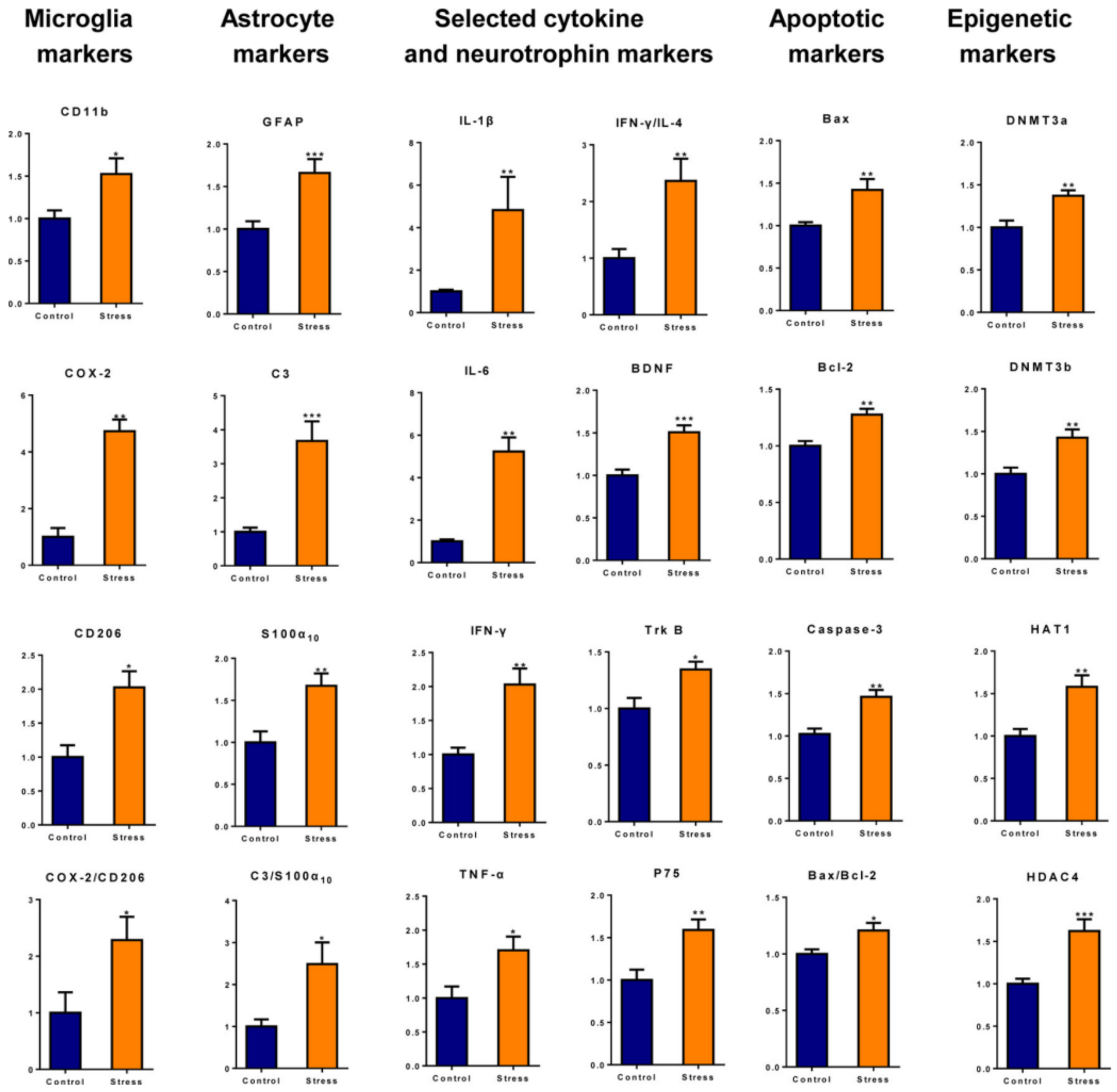
PTSD Modeling in zebrafish

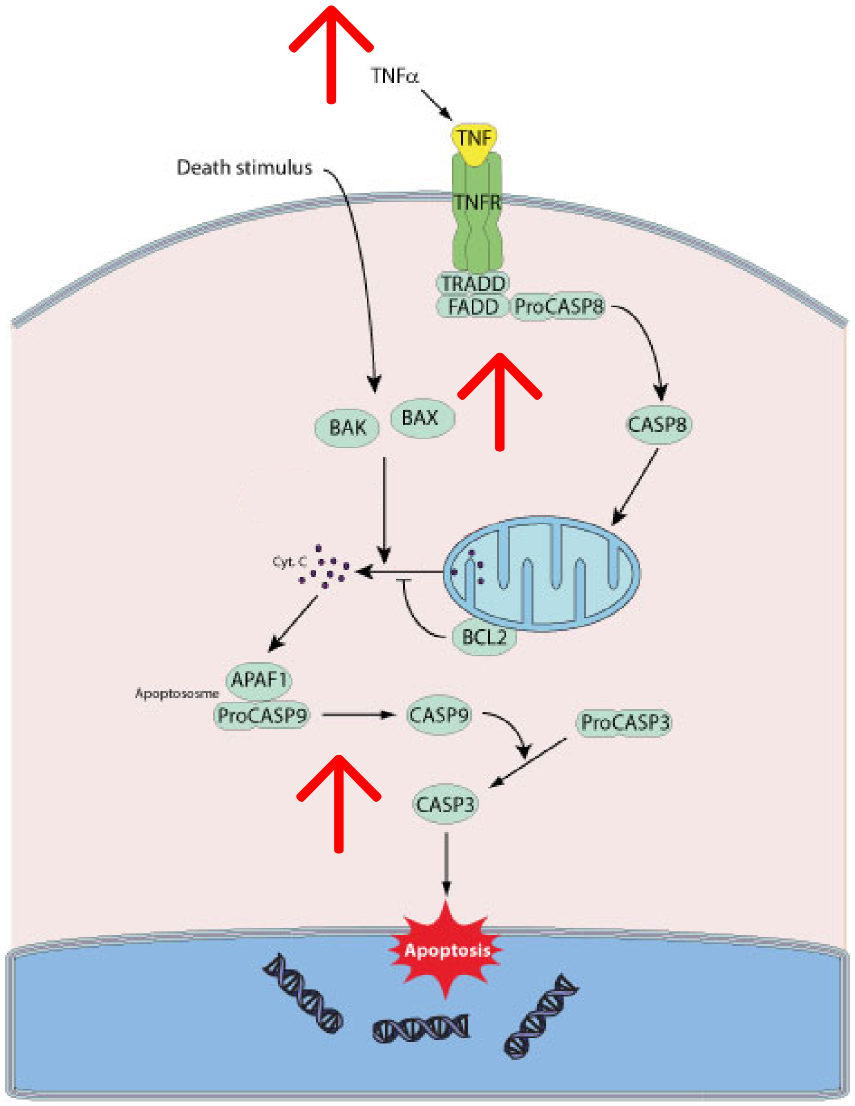


Model validation: robust PTSD-like phenotype

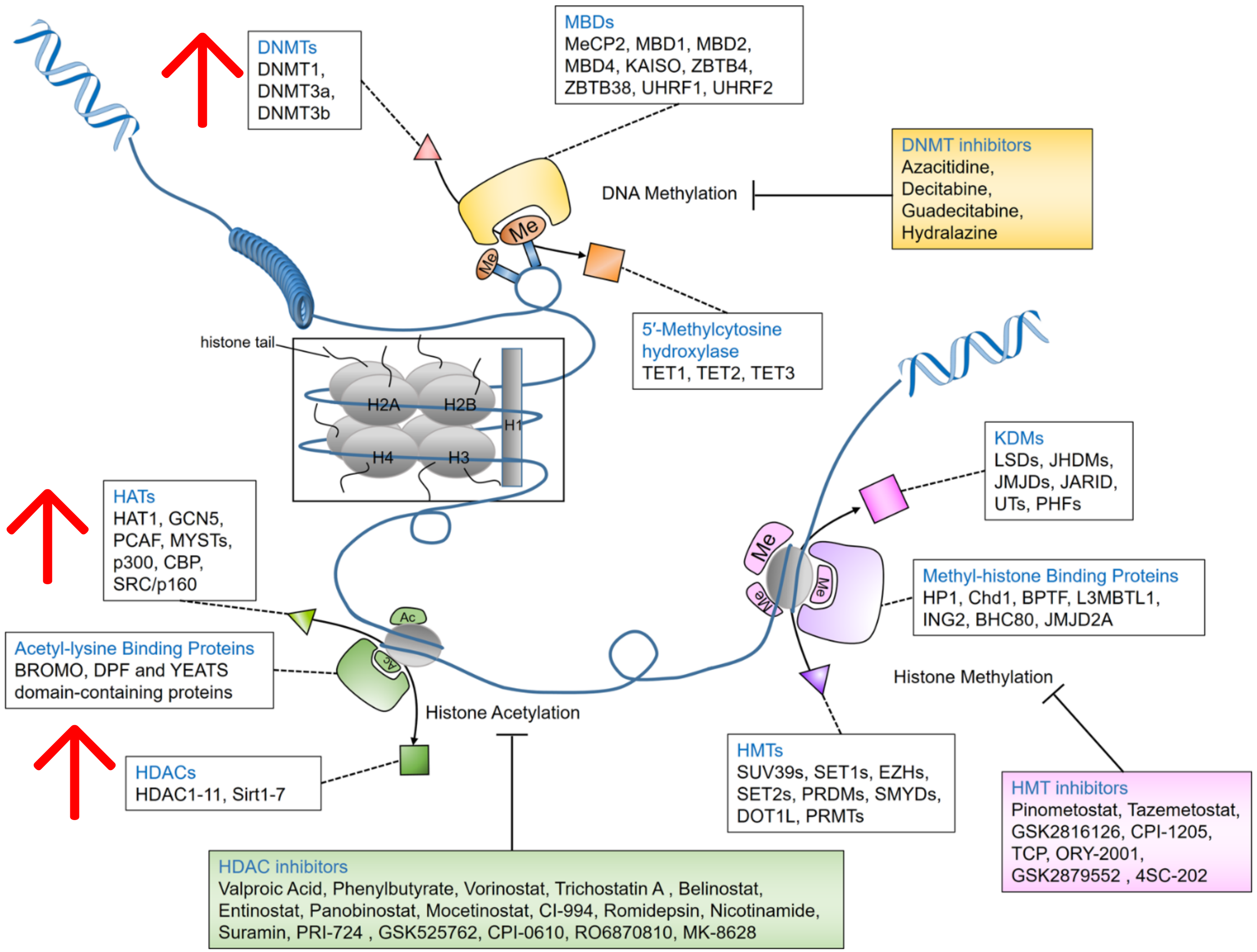


Molecular biomarkers in the brain

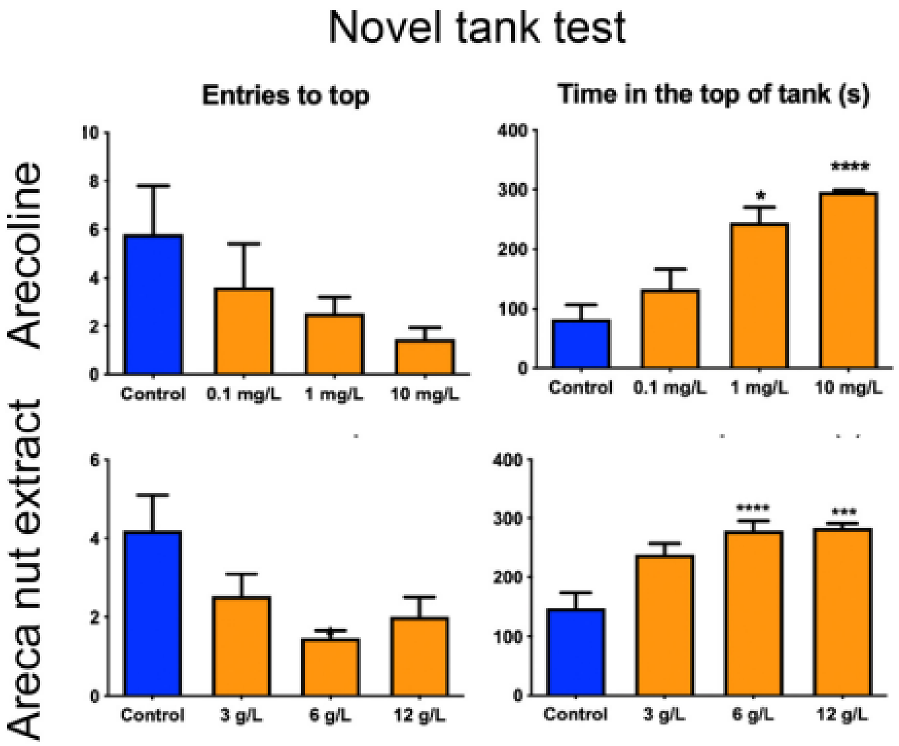




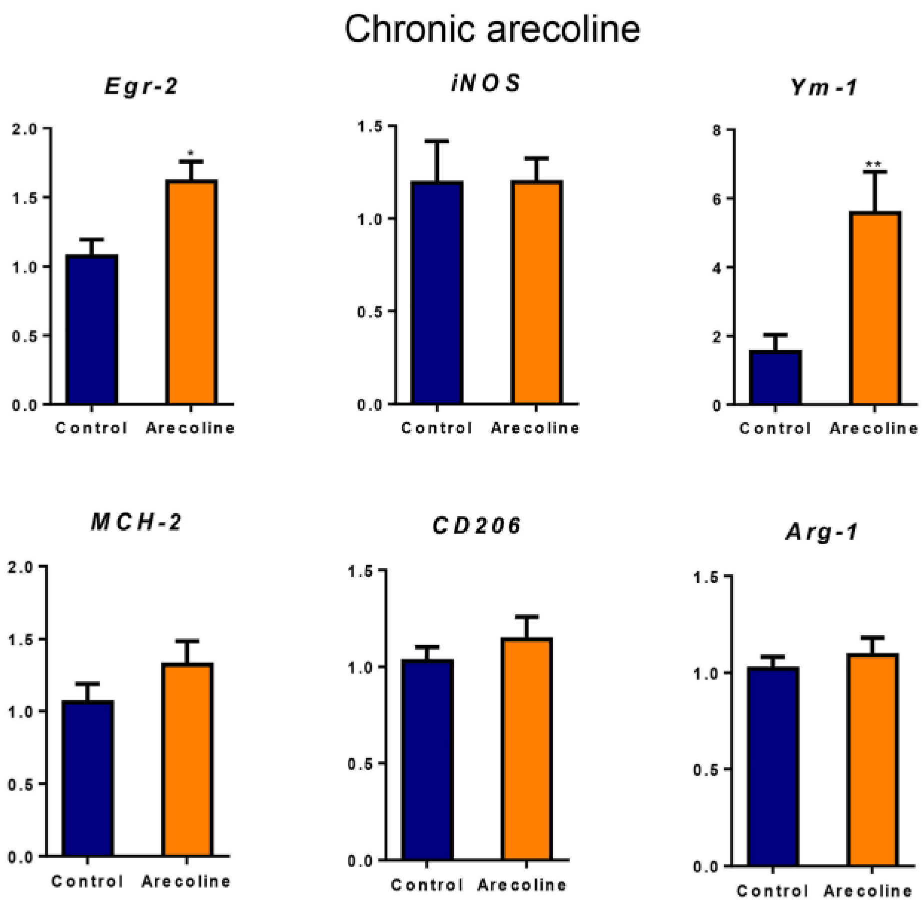
Altered epigenetic biomarkers

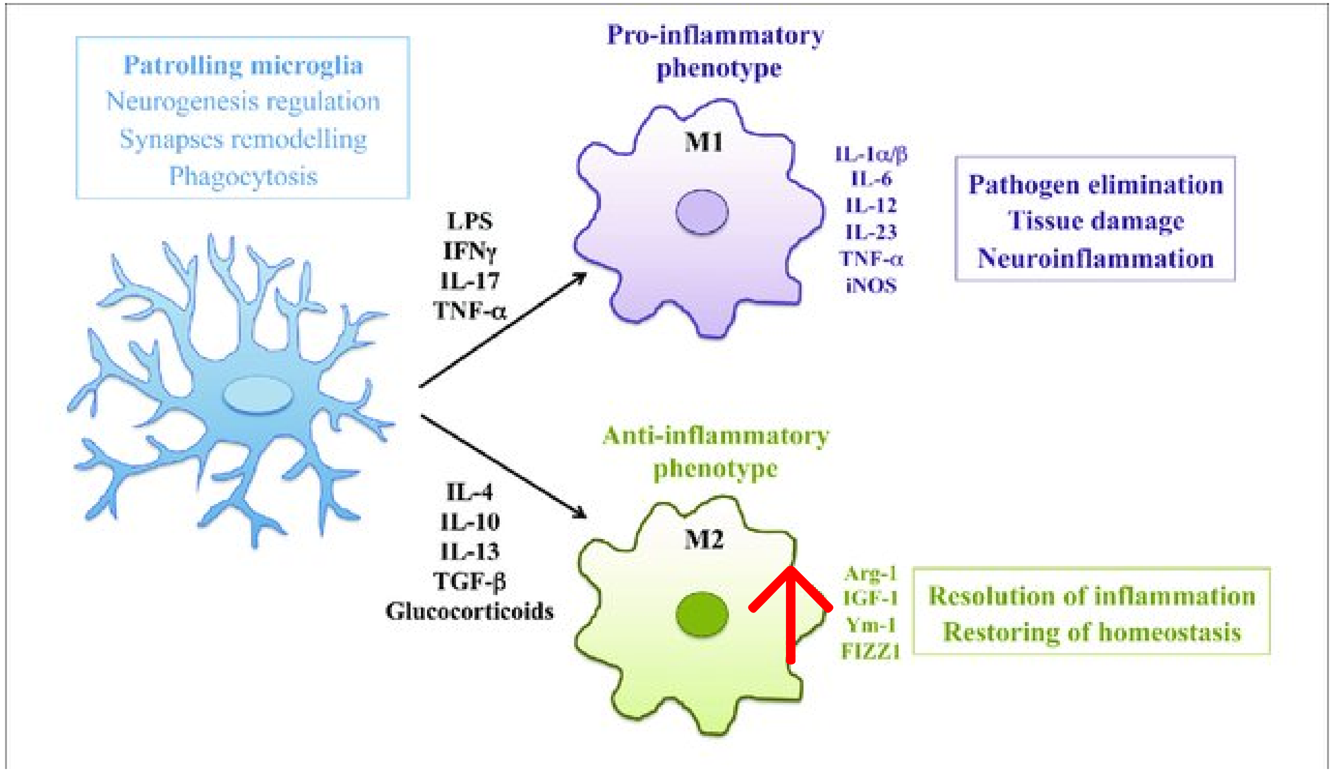


Chronic arecoline



Arecoline activates neuroprotective M2-microglia



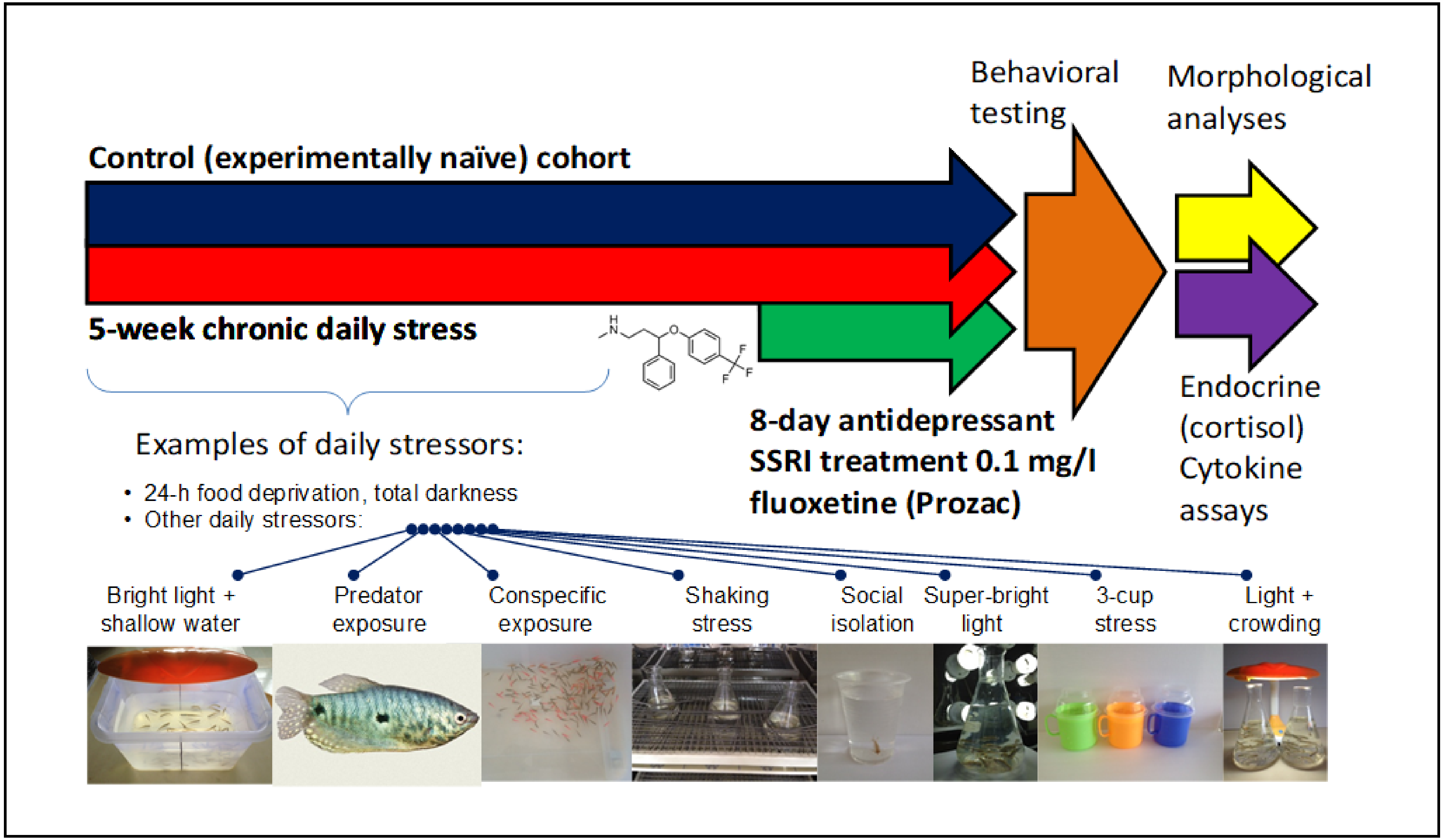


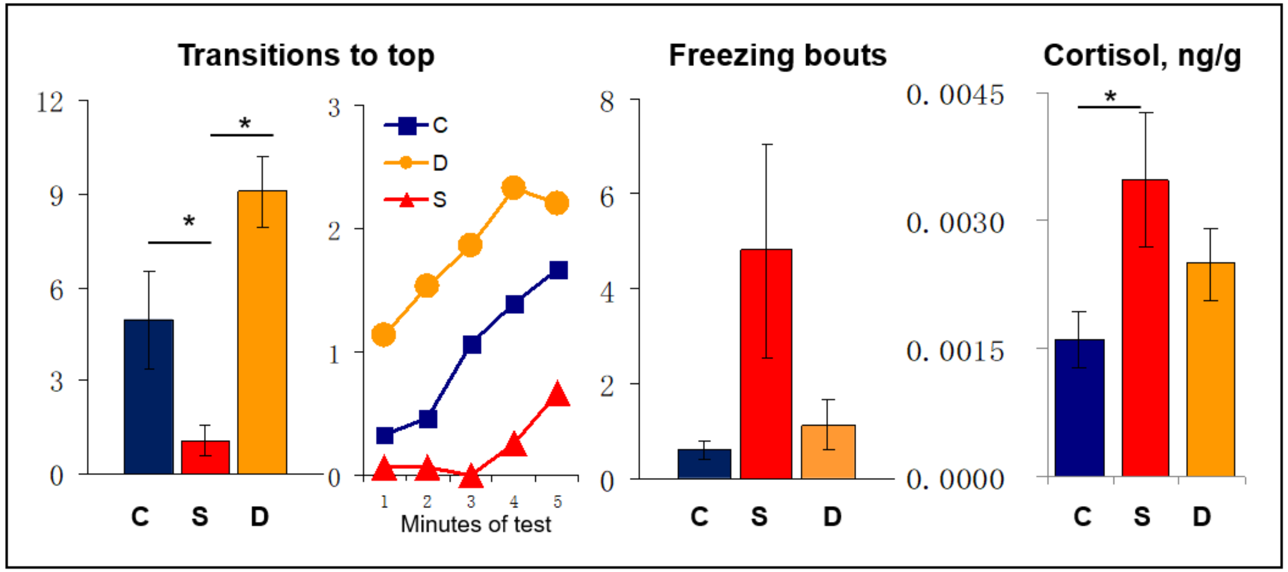
Zebrafish as molecular machines

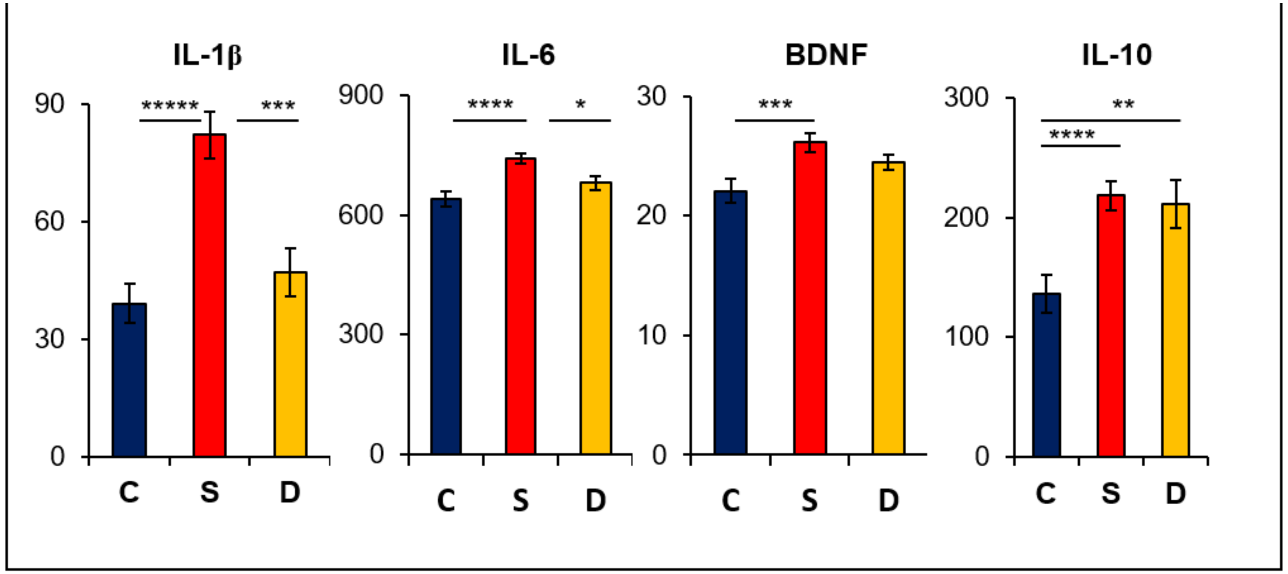
Aim. To expand the range of molecular models using zebrafish behavioral research and chronic stress models

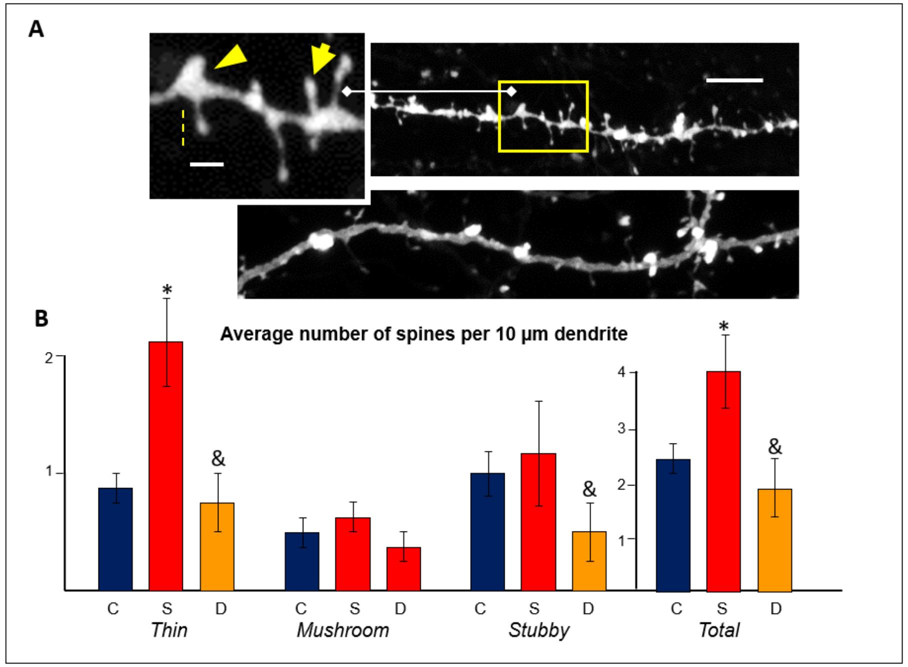
Q: How do zebrafish chronic stress responses develop over time?

Model of chronic stress in zebrafish

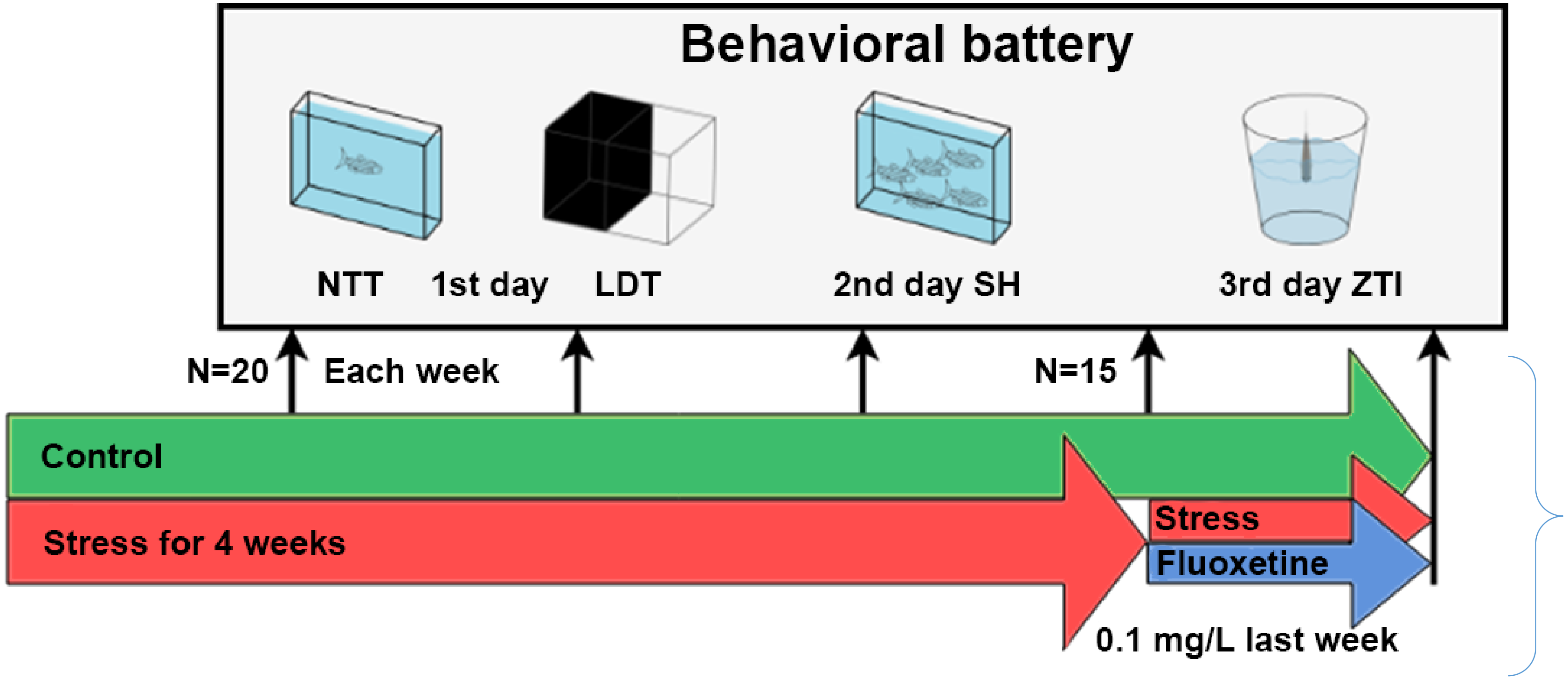



Transitions to top
12
9
6
3
0
*
*
C S D
3
2
1
0
C
D
S
1 2 3 4 5
Minutes of test
Freezing bouts
8
6
4
2
0
C S D
Cortisol, ng/g
0. 0045
0. 0030
0. 0015
0. 0000
*
C S D


IL-1β
90
60
30
0
*****
***
C S D
IL-6
900
600
300
0
****
*
C S D
BDNF
30
20
10
0
***
C S D
IL-10
300
200
100
0
**
****
C S D


A
B
Average number of spines per 10 µm dendrite
2
1
*
&
&
4
3
2
1
*
&
C S D
Thin
C S D
Mushroom
C S D
Stubby
C S D
Total

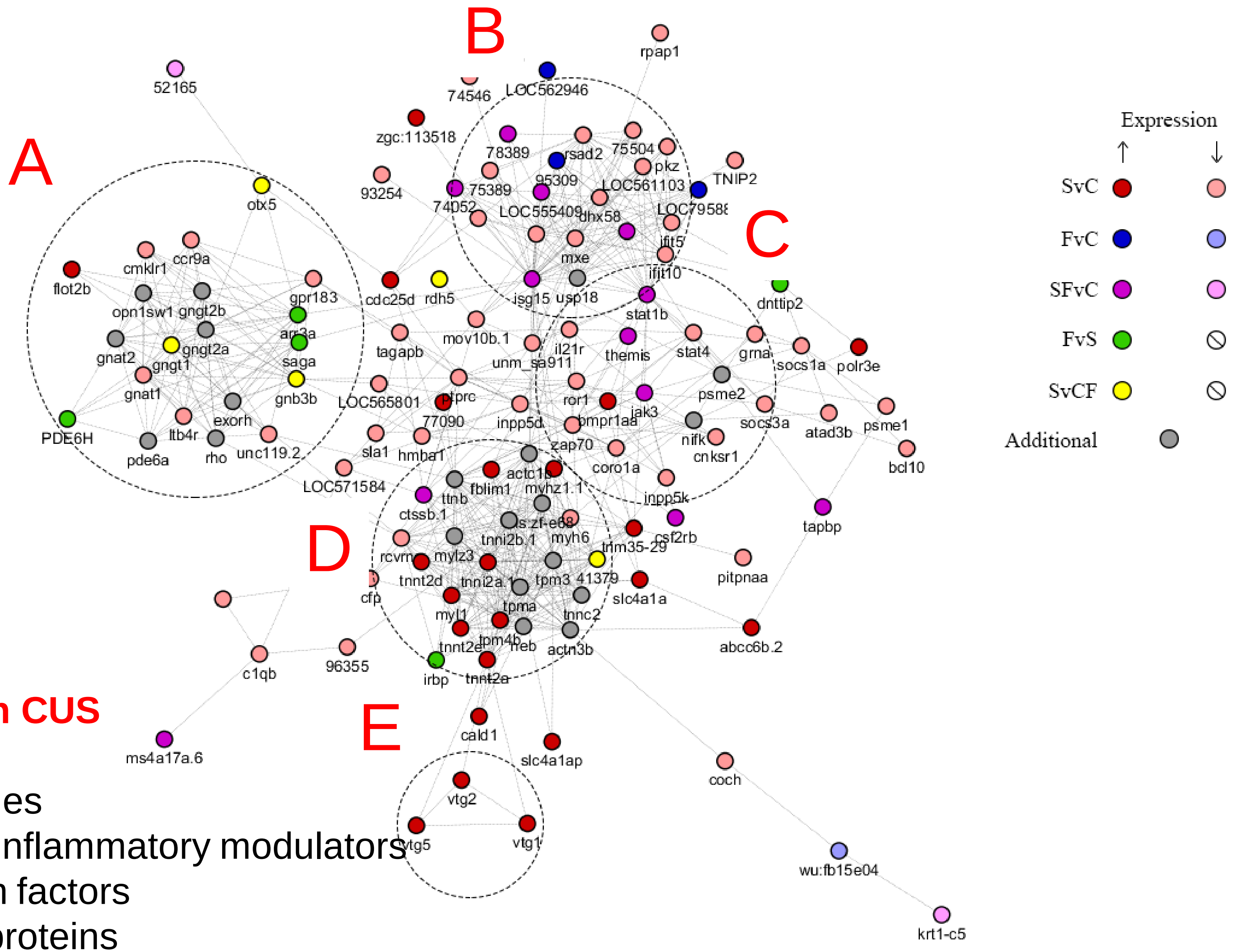
Chronic unpredictable stress (CUS)



RNAseq

Demin et al., 2020

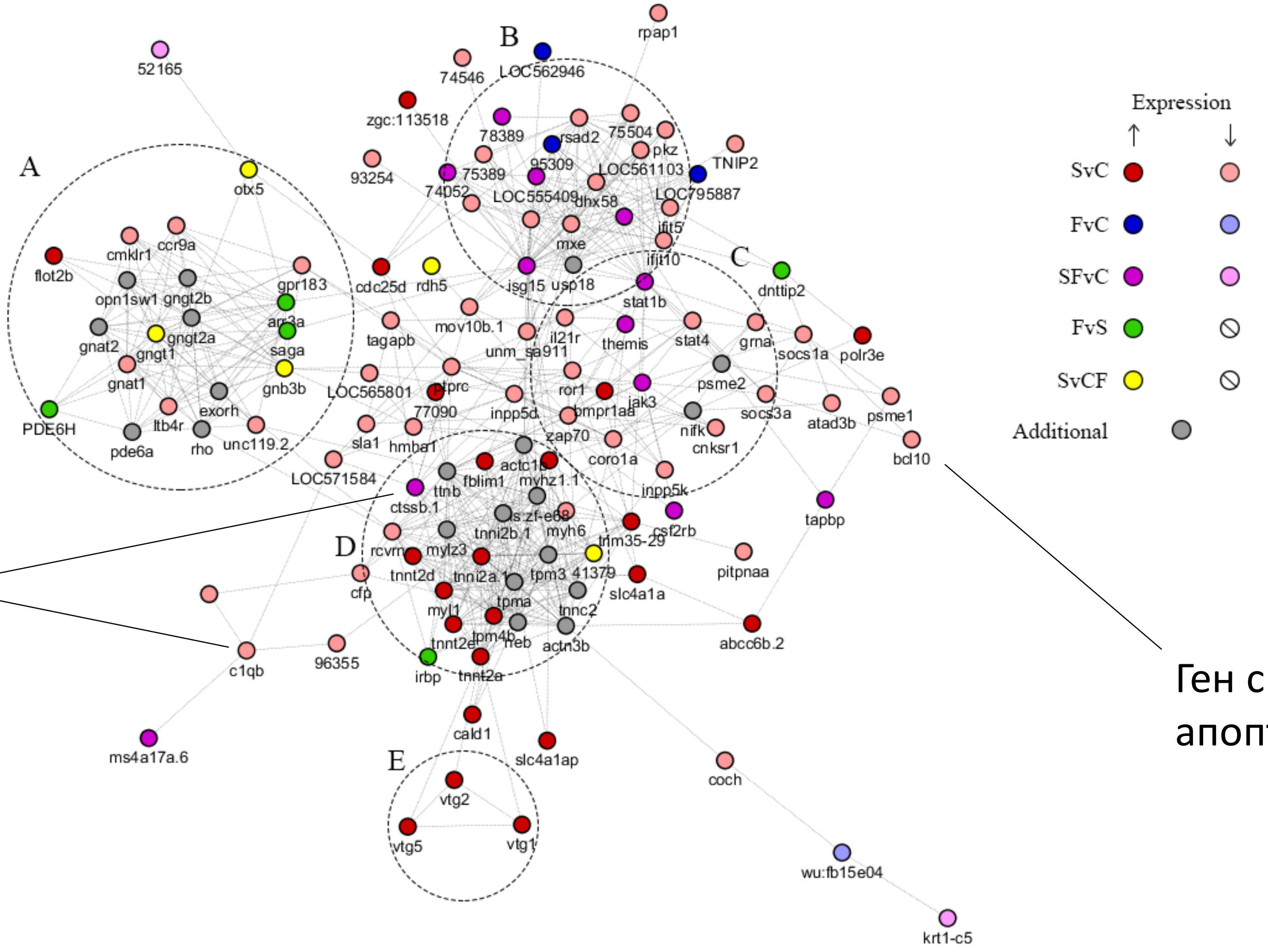
Molecular network modeling (STRING) of differentially expressed genes in zebrafish CUS



Molecular pathways implicated in CUS

A arrestins and GPCRs-related genes
B ubiquitin related genes and their inflammatory modulators
C inflammation-related transcription factors
D cytoskeletal and motility related proteins
E vitellogenins (developmental/estrogen-related hormones)

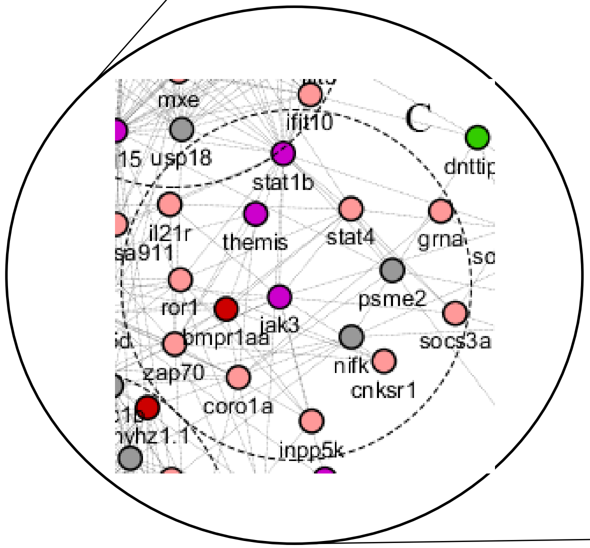
Транскриптомные эффекты хронического стресса

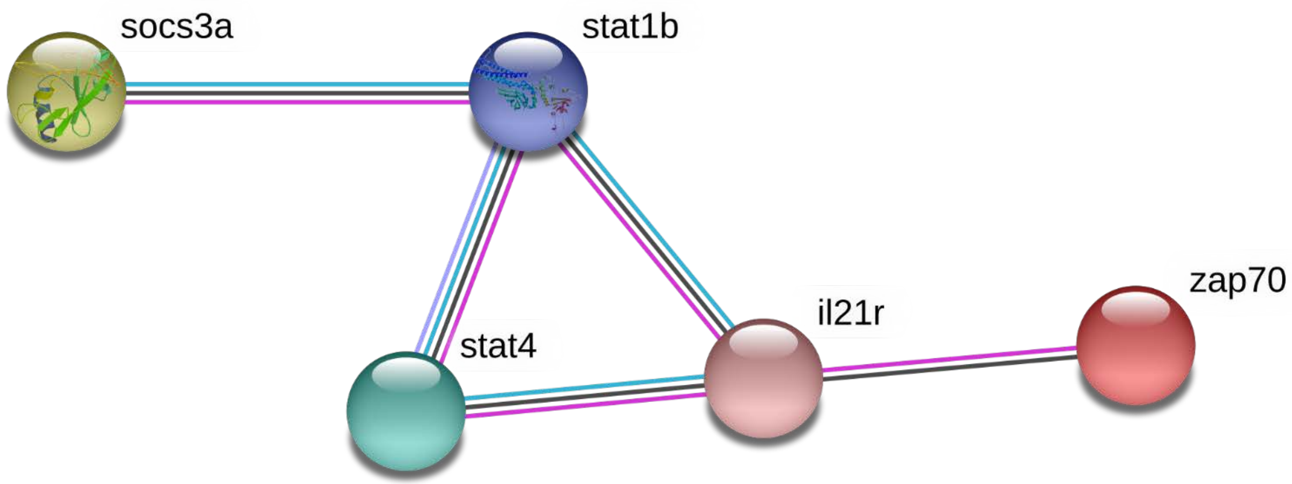


Гены связанные со старением

Ген связанный с апоптозом

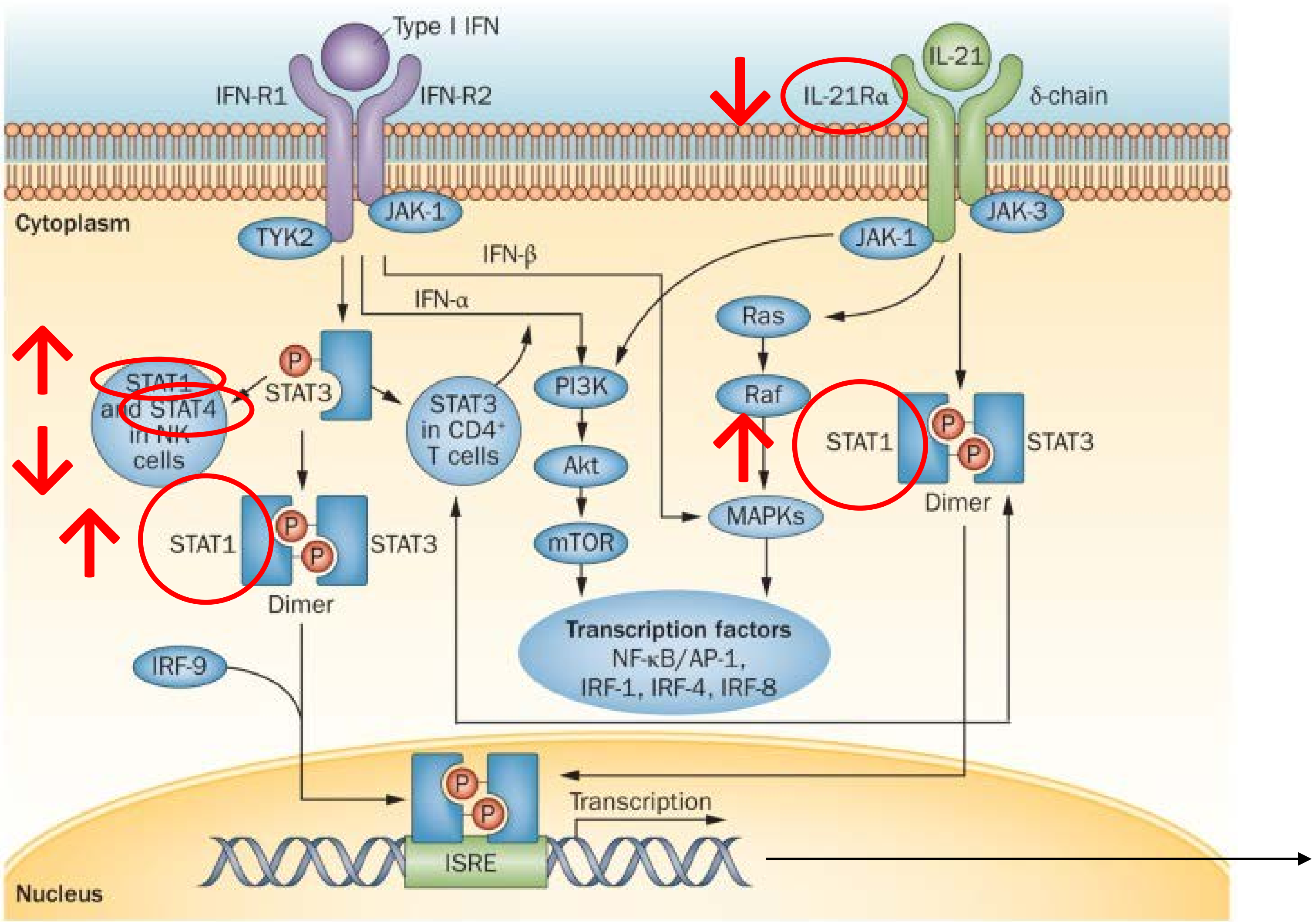
Inflammatory factors





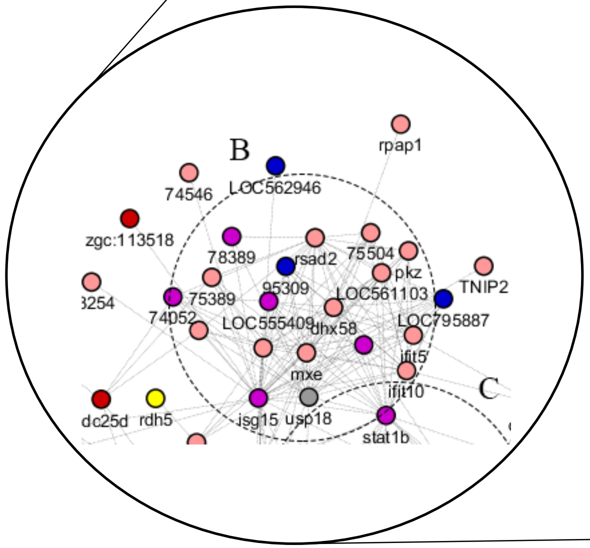
stat1b ↑ mediator of cytokine and hormone signaling
stat4 ↓(корр.) mediator of cytokine and hormone signaling
socs3a ↓(корр.) suppressor of cytokine signaling
il21r ↓ (корр.) IL receptor
zap70↓ (корр.) activator of T-cells

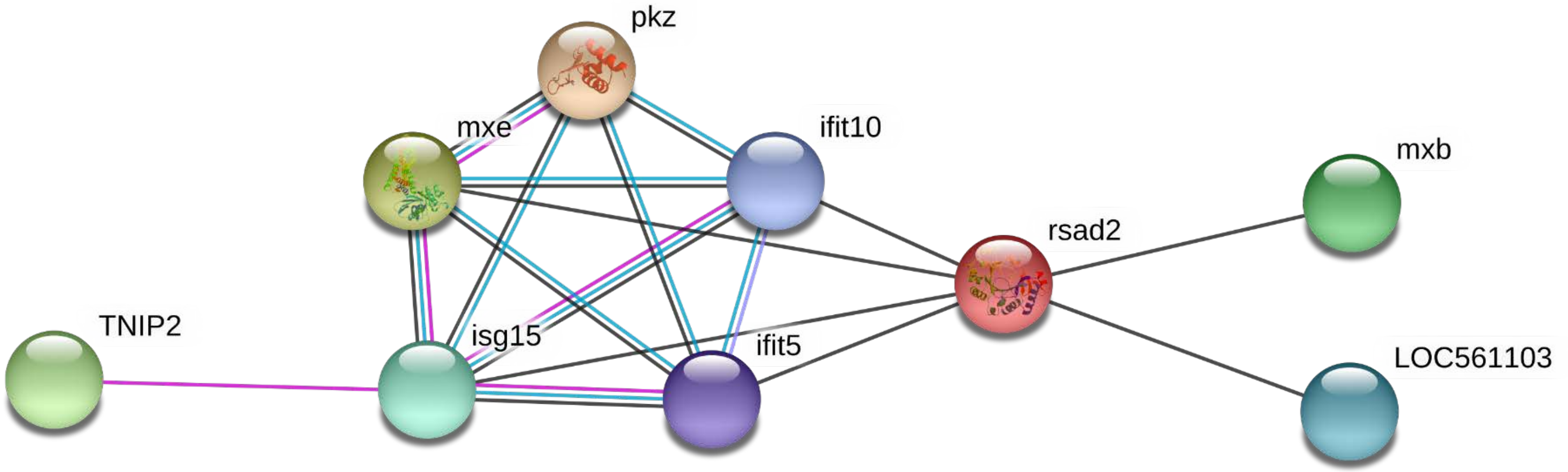
IL21R, STAT1, STAT4



Apoptosis and cytotoxicity
Транскрипция воспалительных генов (*isg15, ifit*)

Ubiqitinization-related proteins





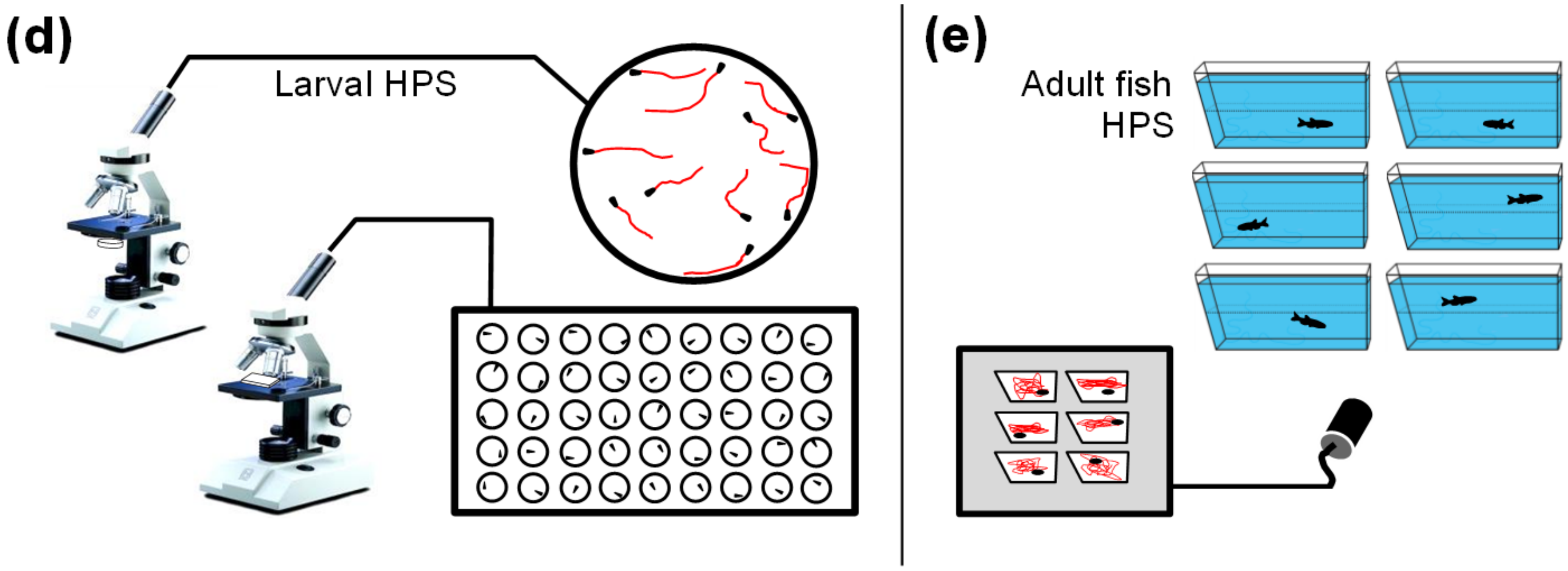
Isg15 **↑**
rsad2 **↓**
Ifit10 **↓**
Ifit5 **↓**
pkz **↓**

Smart Zebrafish

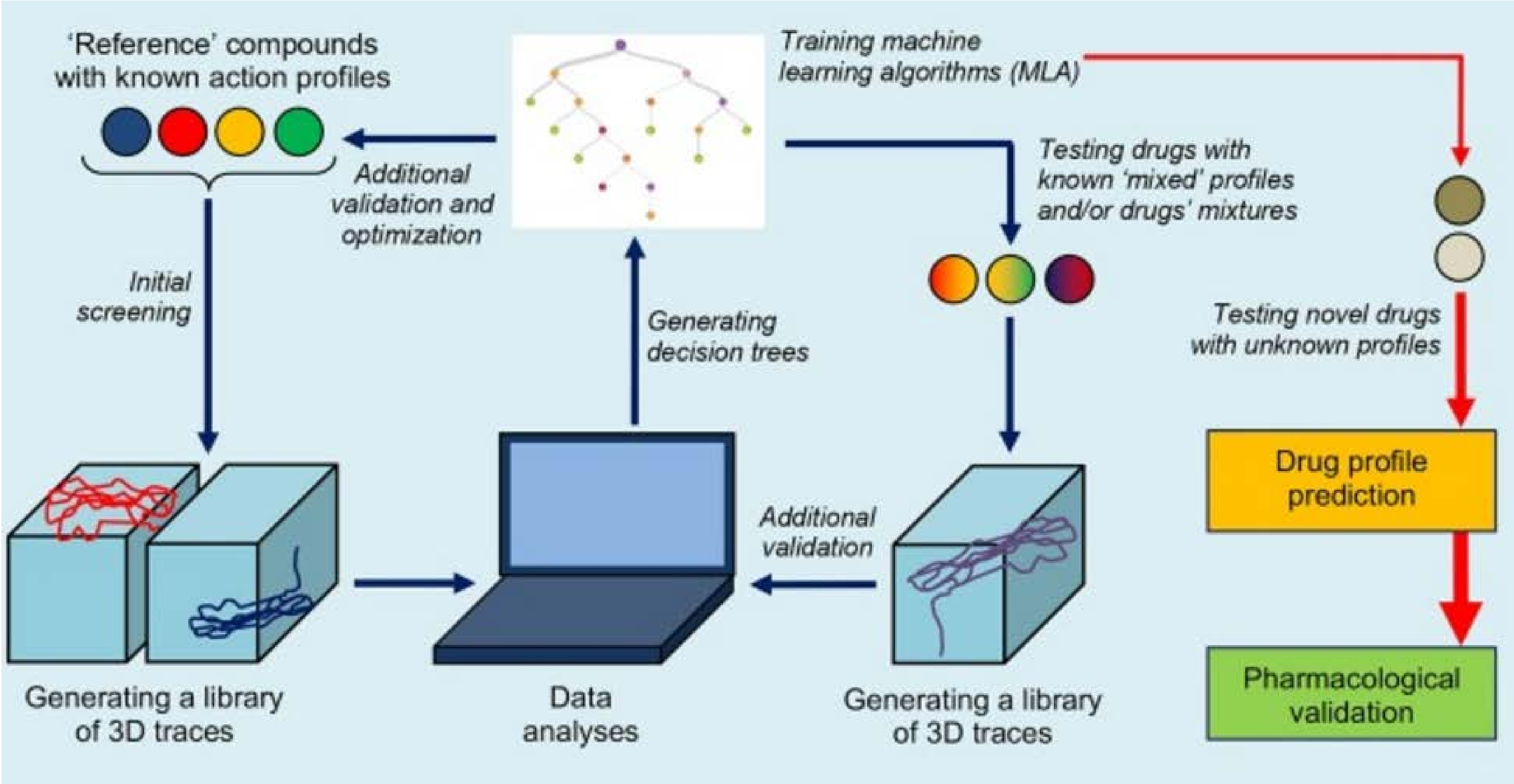
Aim. To apply artificial intelligence tools to understand and predict CNS drug properties

Q: Can we use zebrafish screens to study NOVEL CNS drugs?

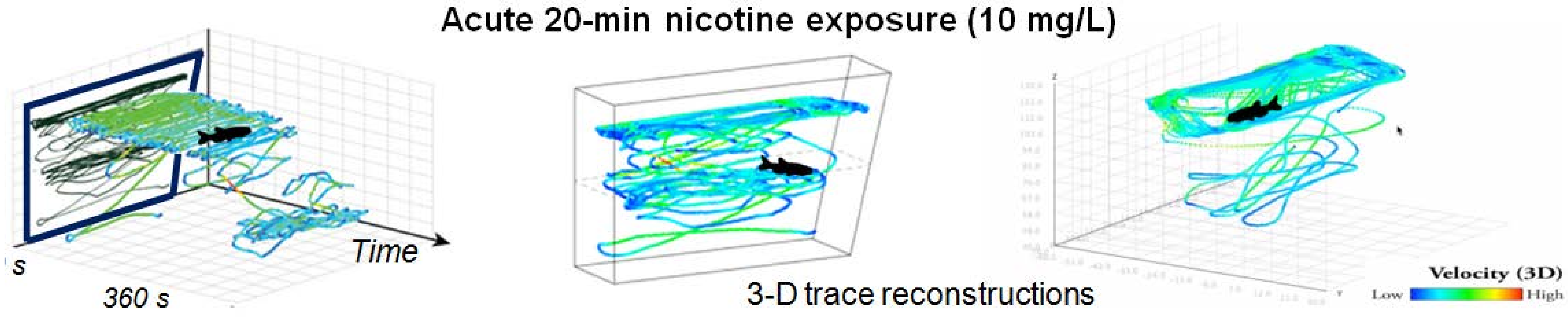
High-throughput drug screening
>10 000 drugs/day

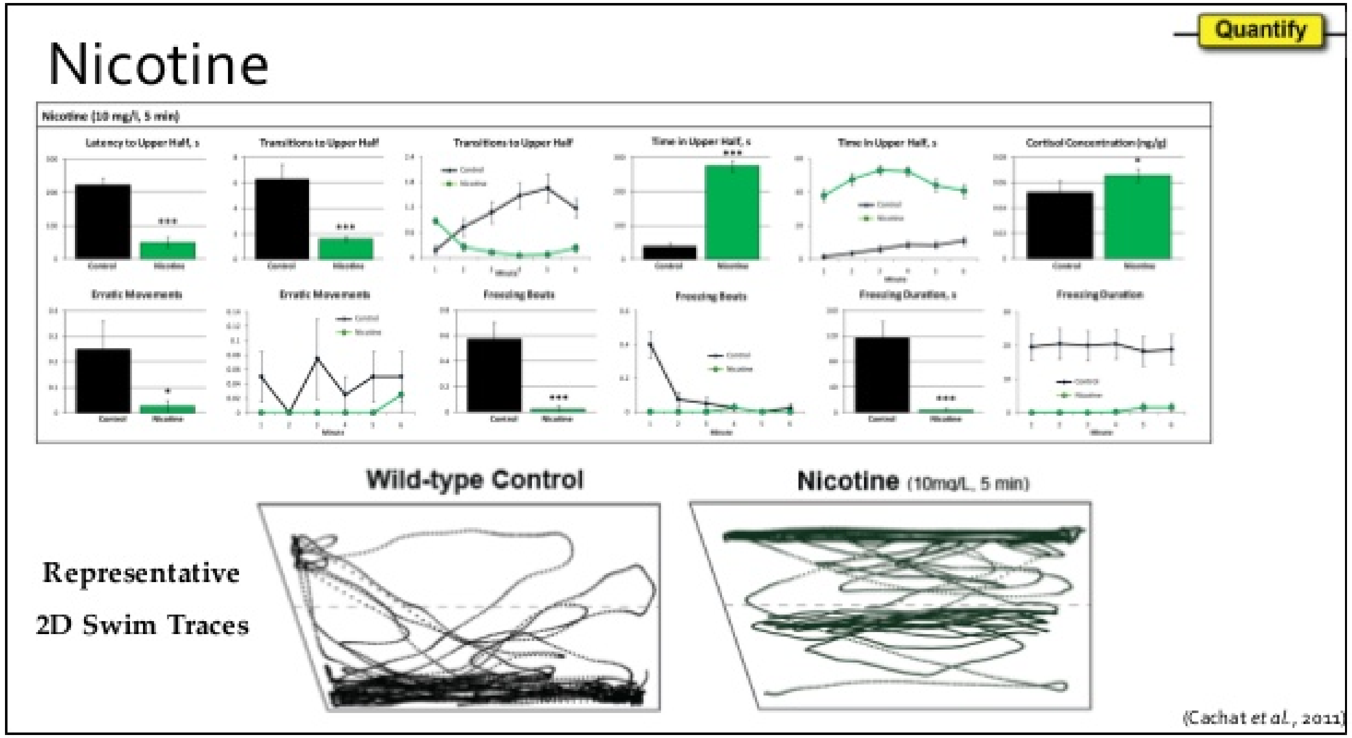


New strategies for CNS drug discovery

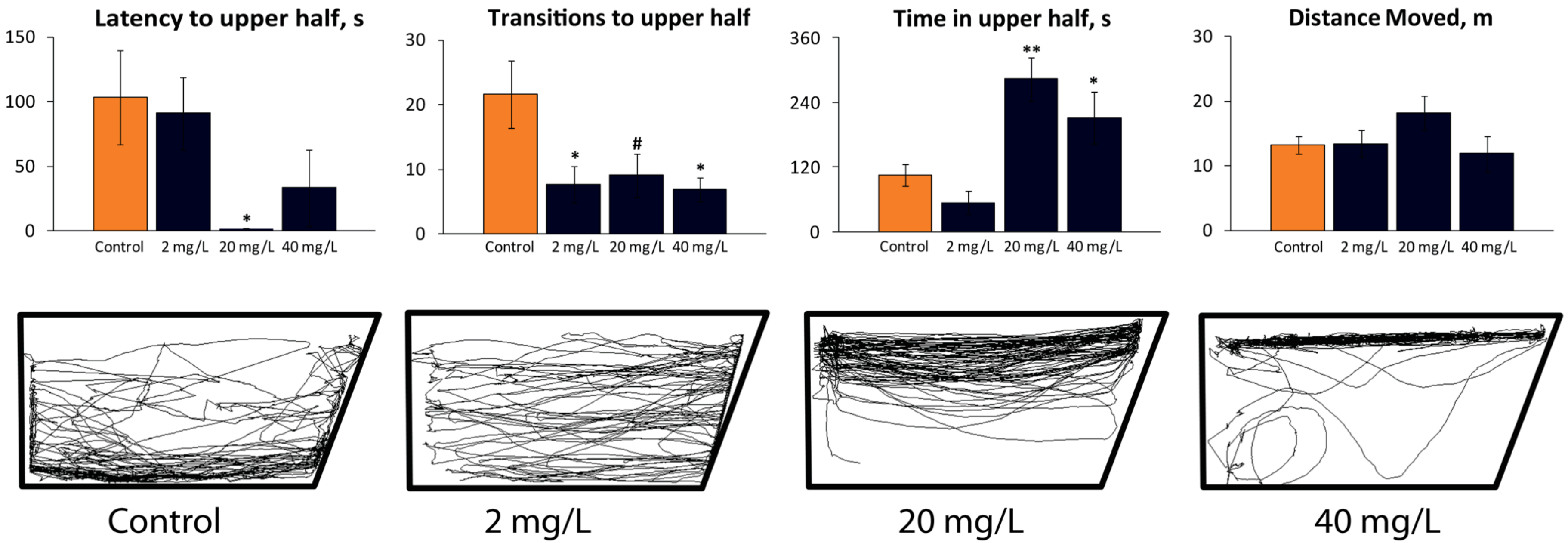


Acute micotine (10 mg/L) effects

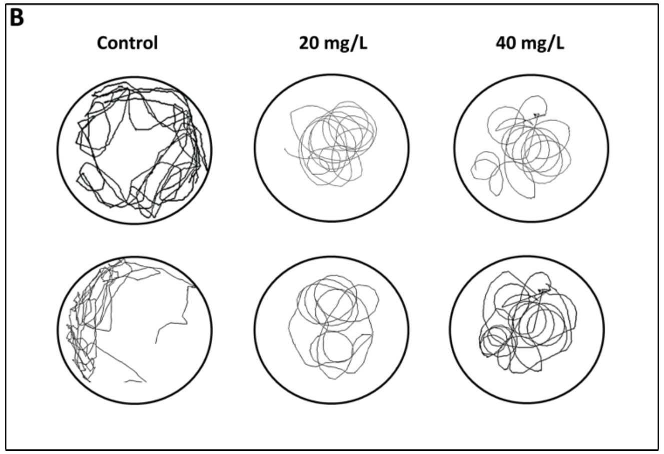




Acute ketamine effects



- Strong anxiolytic effect at 20 and 40 mgL
- Stereotyped circling behaviors at higher doses, on horizontal plane – not visible from front view (2D), requires 3D reconstruction

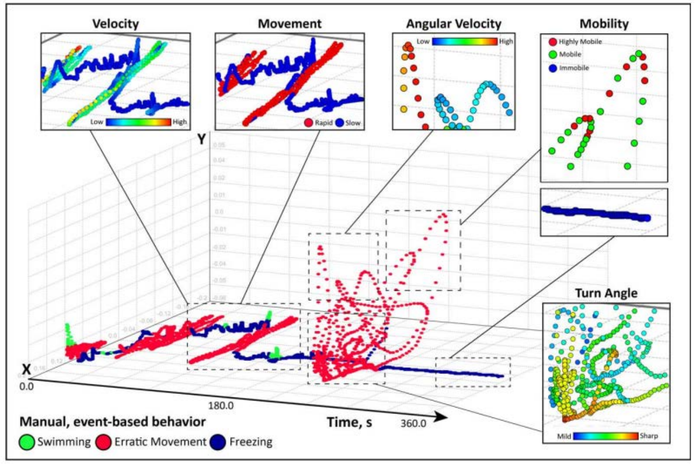


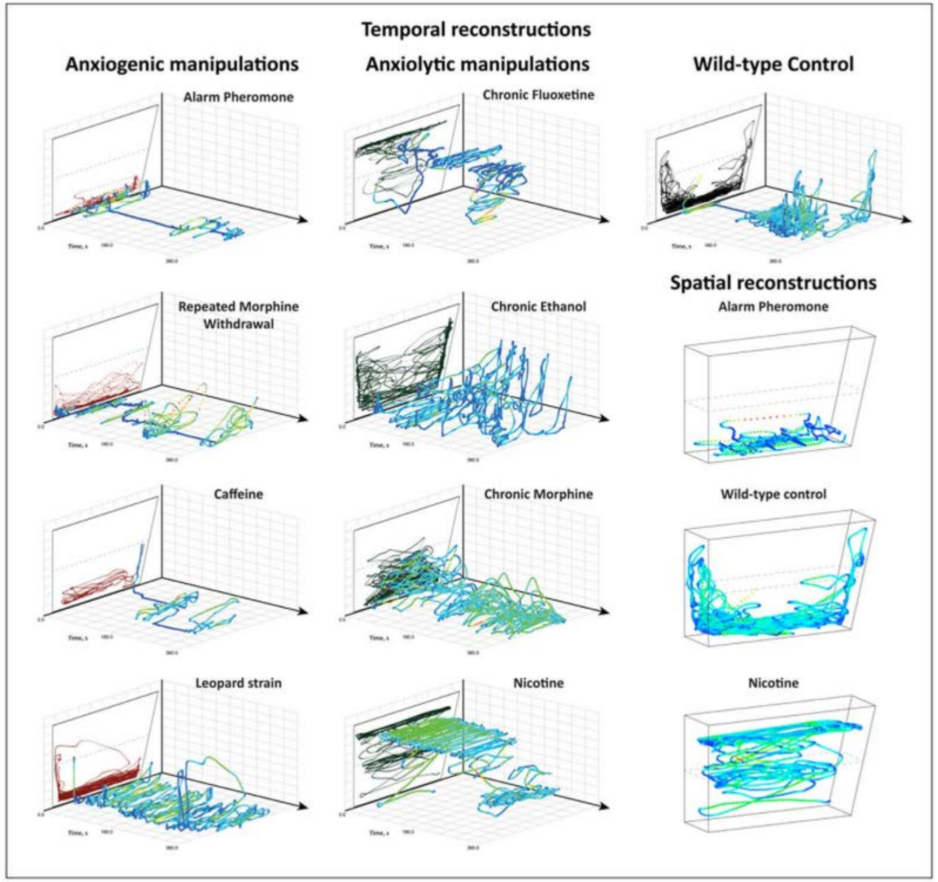
Kyzar et al. 2012

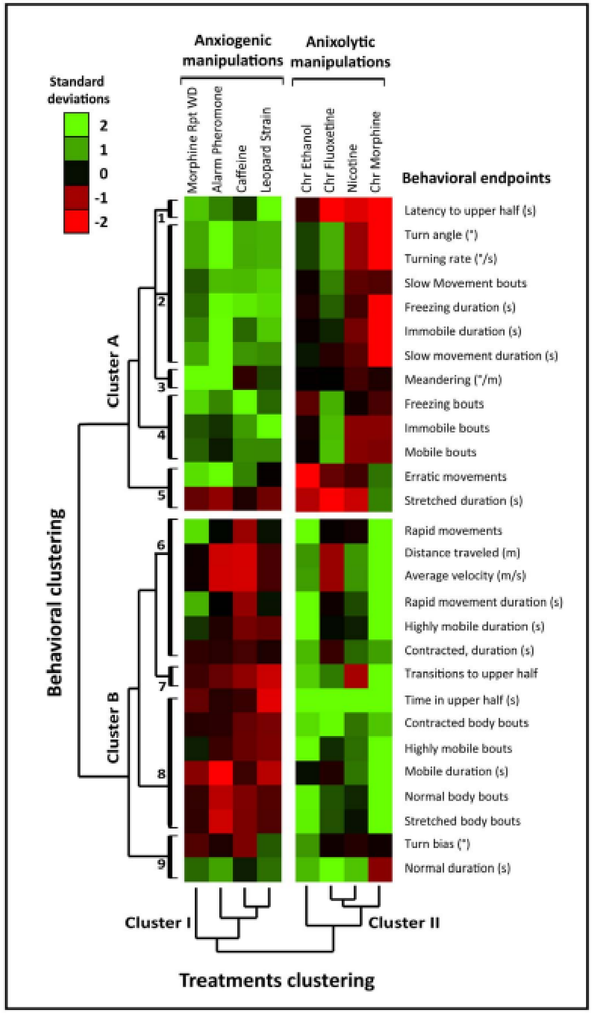
OPEN ACCESS Freely available online
PLoS one

Three-Dimensional Neurophenotyping of Adult Zebrafish Behavior

Jonathan Cachat, Adam Stewart, Eli Utterback, Peter Hart, Siddharth Gaikwad, Keith Wong, Evan Kyzar, Nadine Wu, Allan V. Kalueff*







First application of Artificial Intelligence (AI) to zebrafish CNS models

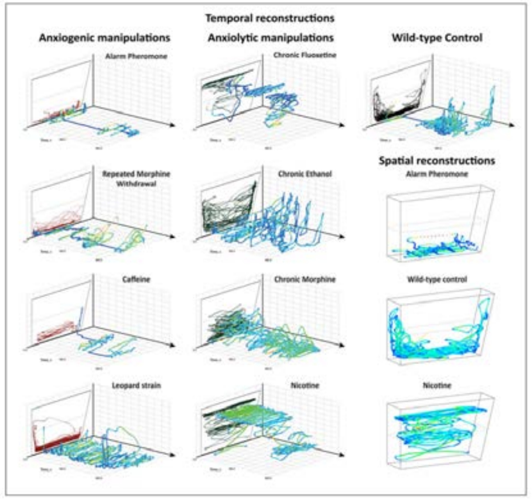
› Prog Neuropsychopharmacol Biol Psychiatry. 2022 Jan 10;112:110405.
doi: 10.1016/j.pnpbp.2021.110405. Epub 2021 Jul 25.

Artificial intelligence-driven phenotyping of zebrafish psychoactive drug responses

Dmitrii V Bozhko [1], Vladislav O Myrov [1], Sofia M Kolchanova [1], Aleksandr I Polovian [1], Georgii K Galumov [1], Konstantin A Demin [2], Konstantin N Zabegalov [3], Tatiana Strekalova Murilo S de Abreu [5], Elena V Petersen [6], Allan V Kalueff [7]

Affiliations + expand

PMID: 34320403 DOI: 10.1016/j.pnpbp.2021.110405



Here, we applied the artificial intelligence (AI) neural network-based algorithms to a large dataset of adult zebrafish locomotor tracks collected previously in a series of in vivo experiments with multiple established psychotropic drugs.

We first trained AI to recognize various drugs from a wide range of psychotropic agents tested, and then confirmed prediction accuracy of trained AI by comparing several agents with known similar behavioral and pharmacological profiles.

ACS Chemical Neuroscience

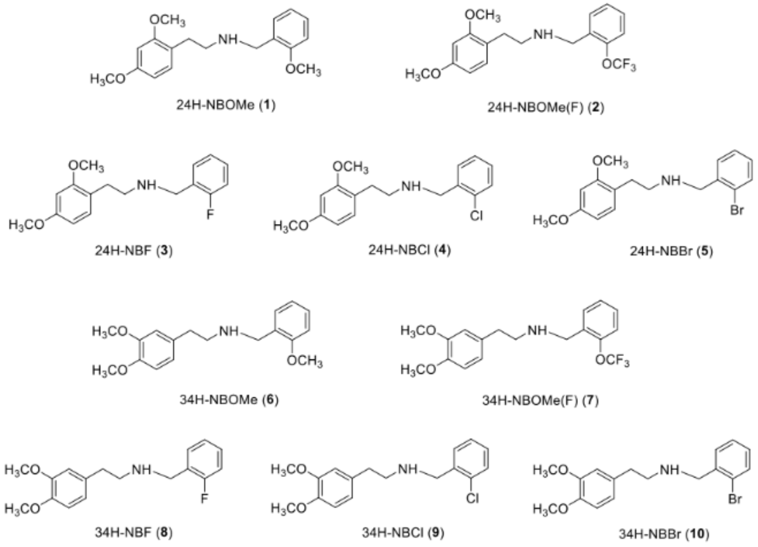
pubs.acs.org/chemneuro Research Article

Acute behavioral and Neurochemical Effects of Novel *N*-Benzyl-2-Phenylethylamine Derivatives in Adult Zebrafish

Konstantin A. Demin,* Olga V. Kupriyanova, Vadim A. Shevyrin,* Ksenia A. Derzhavina, Nataliya A. Krotova, Nikita P. Ilyin, Tatiana O. Kolesnikova, David S. Galstyan, Yurii M. Kositsyn, Abubakar-Askhab S. Khaybaev, Maria V. Seredinskaya, Yaroslav Dubrovskii, Raziya G. Sadykova, Maria O. Nerush, Mikael S. Mor, Elena V. Petersen, Tatyana Strekalova, Evgeniya V. Efimova, Savelii R. Kuvarzin, Konstantin B. Yenkoyan, Dmitrii V. Bozhko, Vladislav O. Myrov, Sofia M. Kolchanova, Aleksander I. Polovian, Georgii K. Galumov, and Allan V. Kalueff*

Cite This: https://doi.org/10.1021/acschemneuro.2c00123 Read Online

Here, we test a battery of ten novel N-benzyl-2-phenylethylamine (NBPEA) derivatives with the 2,4- and 3,4-dimethoxy substitutions in the phenethylamine moiety and the −OCH3, −OCF3, −F, −Cl, and −Br substitutions in the ortho position of the phenyl ring of the N-benzyl moiety, assessing their acute behavioral and neurochemical effects in 18 the adult zebrafish



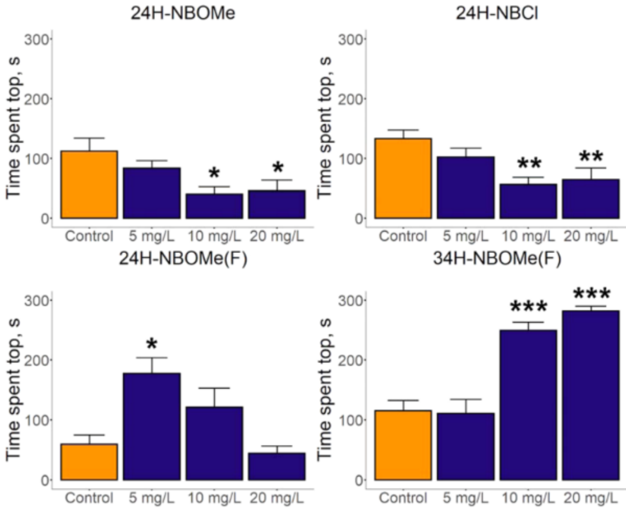


Figure 2. Behavioral effects of selected NBPEAs on the anxiety-related top time behavior assessed in the zebrafish NTT. The increased top time typically reflects an anxiolytic-like effect. Data are presented as mean ± S.E.M. (n = 15−17 per group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control, posthoc Dunn's test for significant Kruskal−Wallis data. Graphs were constructed using ggplot2 R package[139] (also see Table S1 for statistical details).

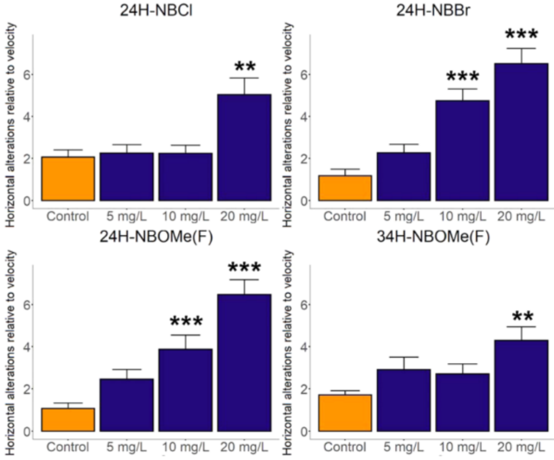


Figure 3. Behavioral effects of selected NBPEAs on the horizontal "shuttling" behavior in the zebrafish NTT. The endpoint was calculated as the total number of left-to-right or right-to-left horizontal transitions and normalized by dividing it by the distance traveled. The increased "shuttling" behavior likely reflects potential hallucinogenic-like properties. Data are presented as mean ± S.E.M. (n = 15−17 per group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control, posthoc Dunn's test for significant Kruskal−Wallis data. Graphs were constructed using ggplot2 R package[139] (also see Table S1 for statistical details).

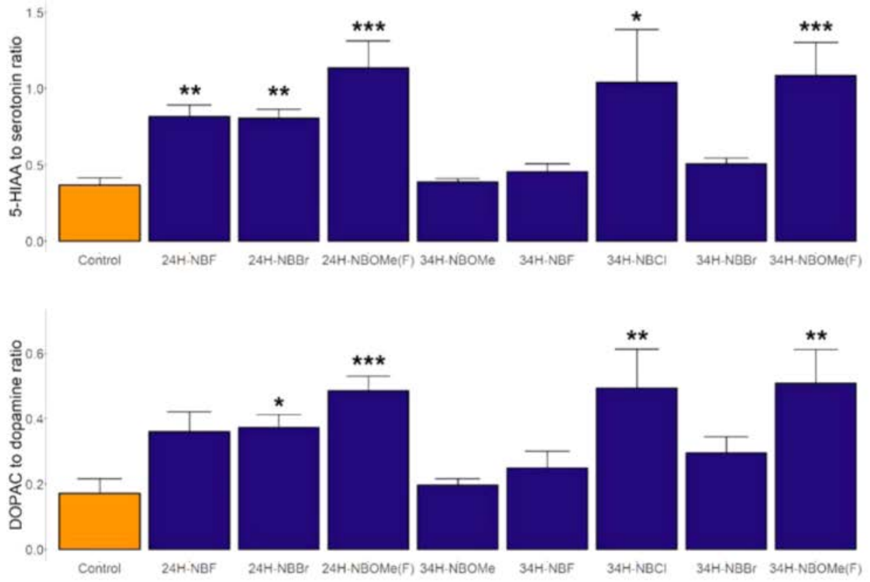


Figure 4. Altered brain SERT and DAT turnover (assessed as the corresponding metabolite/neurotransmitter ratios) by selected NBPEAs in the adult zebrafish. Data are presented as mean ± S.E.M. ($n = 8-10$ per group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control, posthoc Dunn's test for significant Kruskal–Wallis data. Graphs were constructed using ggplot2 R package[139] (also see Table S3 for statistical details).

- Overall, substitutions in the N-benzyl moiety modulate locomotion, and substitutions in the phenethylamine moiety alter zebrafish anxiety-like behavior, also affecting the brain serotonin and/or dopamine turnover.
- Computational analyses of zebrafish behavioral data by artificial intelligence identified several distinct clusters for these agents, including anxiogenic/hypolocomotor (24H−NBF, 24H−NBOMe, and 34H− NBF), behaviorally inert (34H−NBBr, 34H−NBCl, and 34H−NBOMe), anxiogenic/hallucinogenic-like (24H−NBBr, 24H−NBCl, 24 and 24H−NBOMe(F)), and anxiolytic/hallucinogenic-like (34H−NBOMe(F)) drugs.
- Our computational analyses also revealed phenotypic similarity of the behavioral activity of some NBPEAs to that of selected conventional serotonergic and antiglutamatergic hallucinogens.
- In silico functional molecular activity modeling further supported the overlap of the drug targets for NBPEAs tested here and the conventional serotonergic and antiglutamatergic hallucinogens.

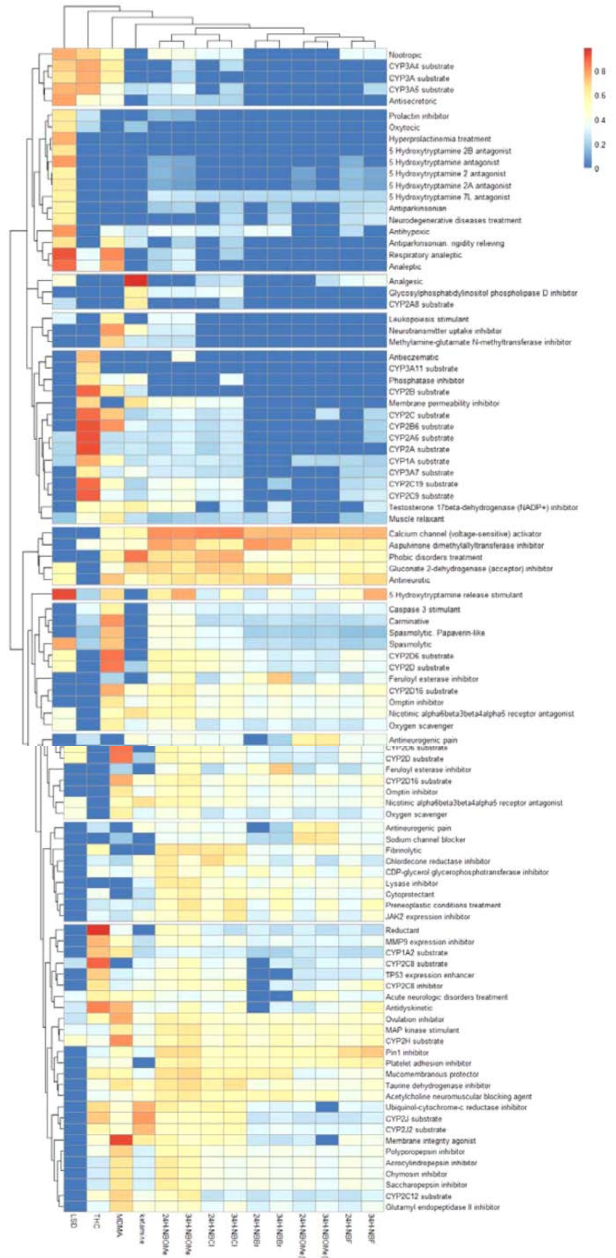
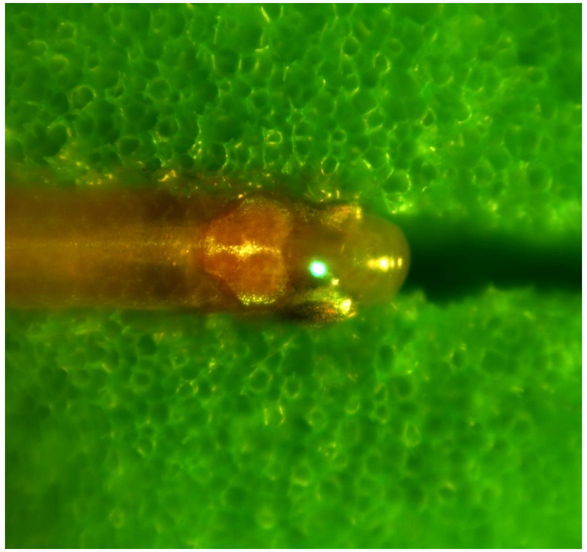


Figure 9. Heatmap diagram showing the probability of functional activities (Pa, %) of the drugs assessed using PASSonline software[129] with hierarchical clustering. Note the tight clustering of novel NBPEAs with MDMA and ketamine (see main functional activity clusters summarized in Table 4).

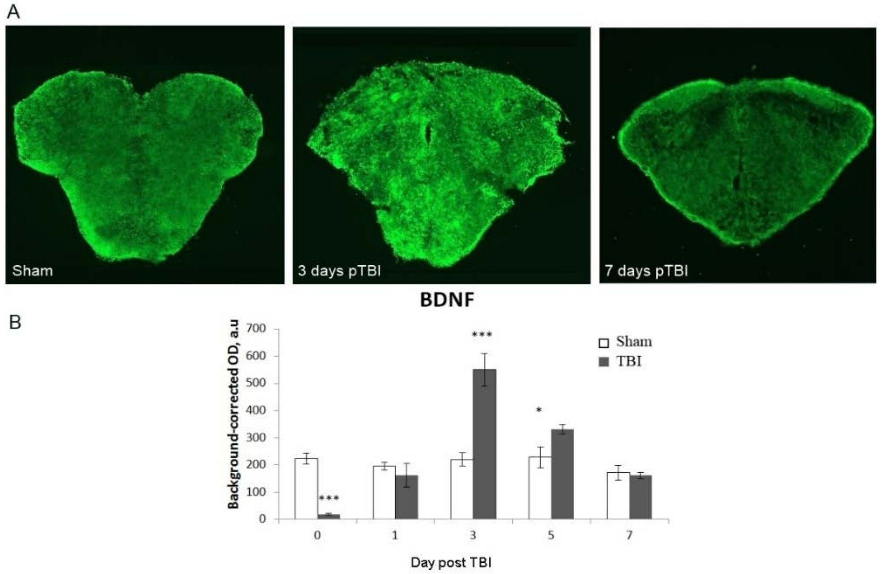
Selected other cool applications of zebrafish CNS models

Laser-induced TBI model

Научно-исследовательский институт
нейронаук и медицины



BDNF expression in telecephalon



BDNF – as a potential important target in TBI therapy

Conclusions

Zebrafish are powerful biological machines for neuroscience research

These models allow us:

1) to measure (=objectively quantify) behavioral deficits
2) to parallel behavioral data with endocrine profiling (e.g., cortisol, melatonin)
3) to develop models based on newly recognized behavioral domains
4) to identify ‘core’ (evolutionarily conserved) candidate molecular targets
5) to assess biological consequences of chronic stress
7) to parallel phenotypic data with neurochemical alterations (e.g., serotonin and NE)
8) to identify novel potential molecular cascades (e.g., omics data)
9) to screen novel CNS drugs and predict their properties
11) to develop novel AI-based models and tools to identify novel drugs
12) to have fun all the way as we do research!

THANK YOU!