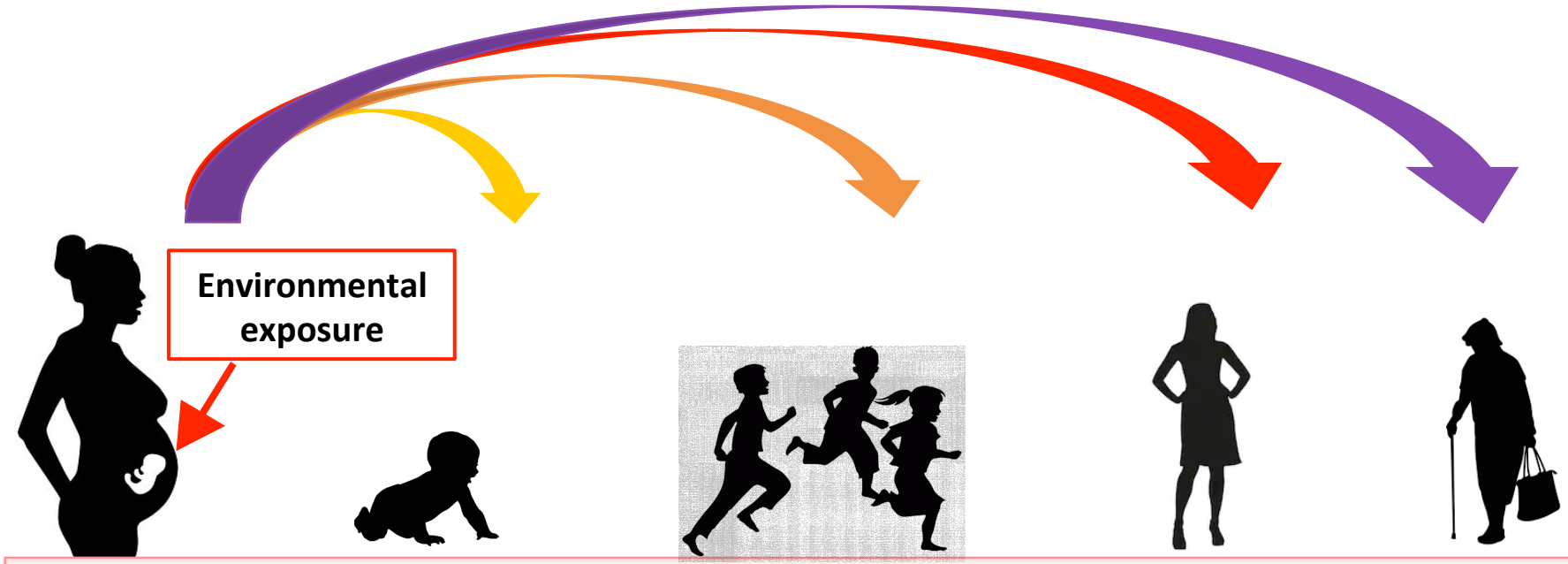
Effects of Developmental Exposures can be lifelong

- Critical windows of vulnerability
- DoHaD – Developmental Origins of Health and Disease



+2000 mothers

In utero **Birth** **Childhood** **Adolescence**

SELMA cohort

Examination of health of offspring

Urine Plasma/Serum

Mixture of chemicals in fluids of pregnant mothers ⟺ **Adverse health outcome**

Mixture of chemicals with so-called ‘bad-actors’



In utero **Birth** **Childhood** **Adolescence**

SELMA cohort

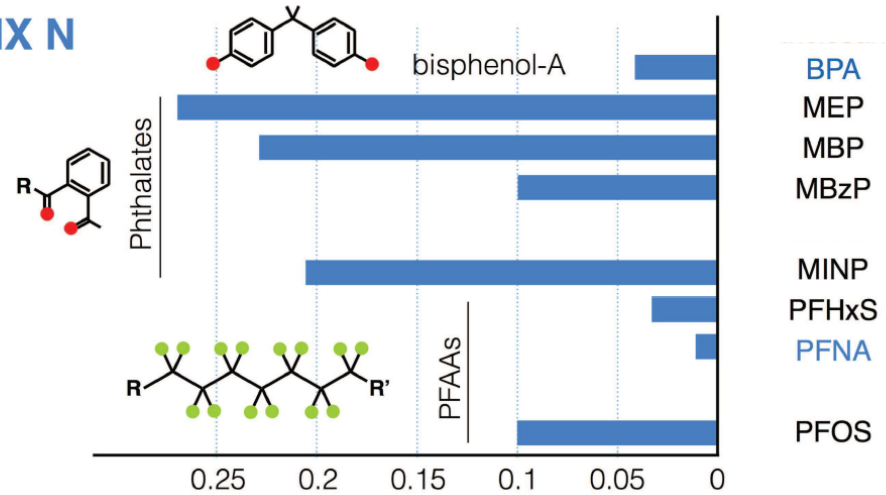
Urine   Plasma/Serum

Examination **language delay**
(number spoken words at 30 months)
= biomarker of neurodevelopmental delay

Mixture of chemicals in fluids of pregnant mothers ⟺ **Adverse health outcome**

Mix N0 Neuro-development

MIX N



relative concentration



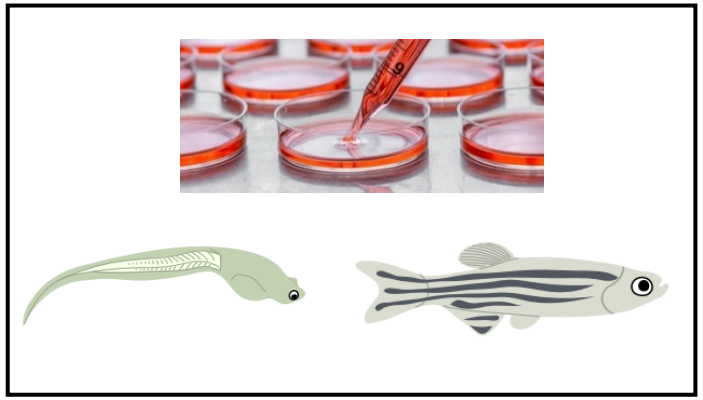

In utero **Birth** **Childhood** **Adolescence**

SELMA cohort

Examination of health

Urine   Plasma/Serum

Mixture of chemicals in fluids of pregnant mothers ⟺ **Adverse health outcome**



Mixture of chemicals with so-called 'bad-actors'




In utero | **Birth** | **Childhood** | **Adolescence**

SELMA cohort

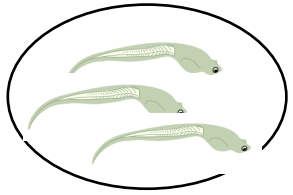
Examination of health

Urine   Plasma/Serum

Mixture of chemicals in fluids of pregnant mothers ⟺ **Adverse health outcome**

A. Does the mixture have thyroid hormone **disruptive** potential?
B. Can the mixture **interfere** with normal **brain development**?

3-day exposure



Xenopus tadpoles

→ 1. Fluorescent reporter assay (XETA)

→ 2. Gene expression – thyroid hormone signalling pathway

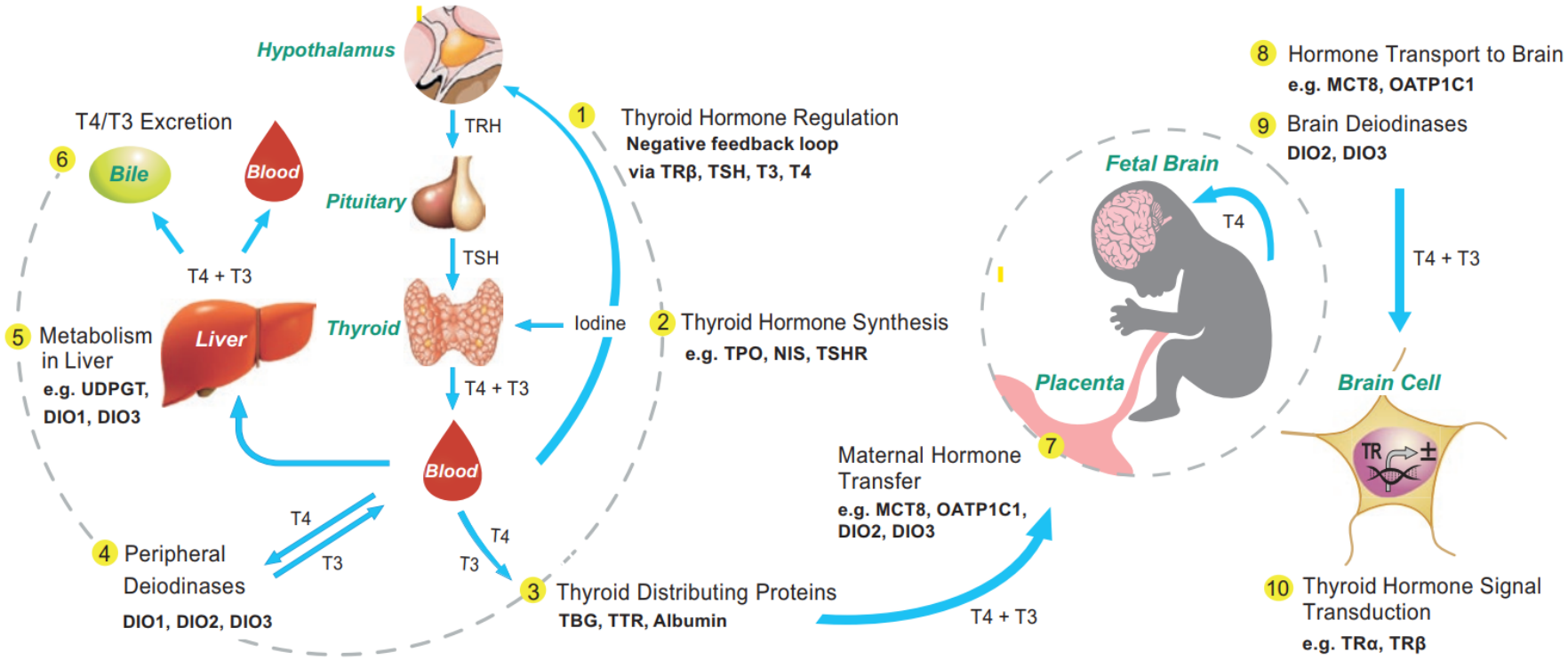
→ 3. Behaviour analysis

Mix N0

Mix N0

A. Does the mixture N0 have thyroid hormone **disruptive** potential?
B. Can the mixture N0 **interfere** with normal **brain development**?

Sites of Interference for Thyroid Disrupting Chemicals



Gilbert et al. 2020

Thyroid hormones induce physiological changes in all vertebrates

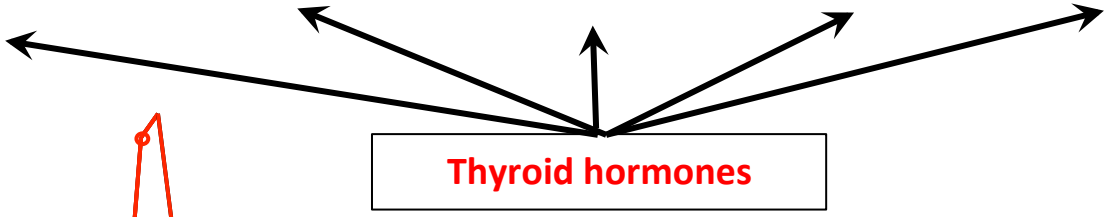
Scophthalmus maximus

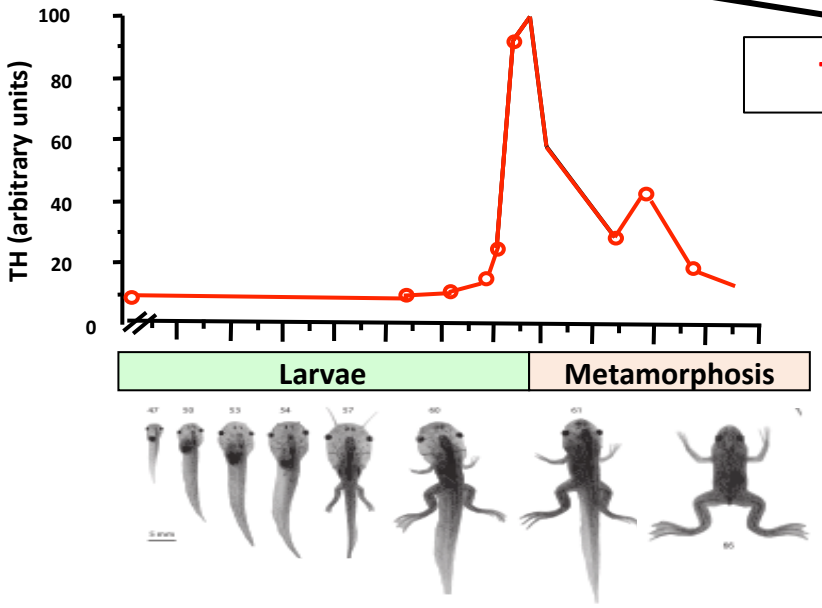
Xenopus laevis

Serinus canaria

Mus musculus

Homo sapiens





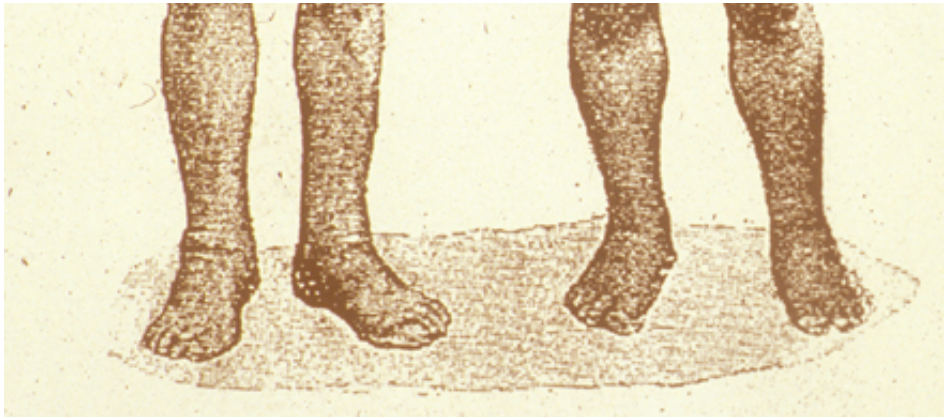
Leloup and Buscaglia, 1977

Xenopus laevis - advantages

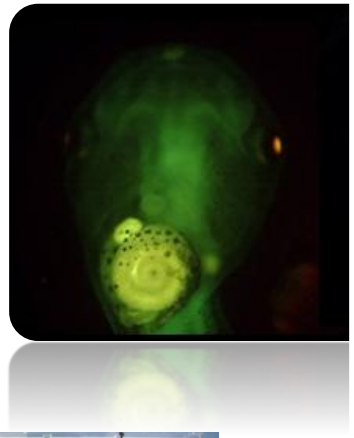
- Synchronized and external embryo development
- Easy access to embryos / exposure to mixtures
- Metabolically competent embryos

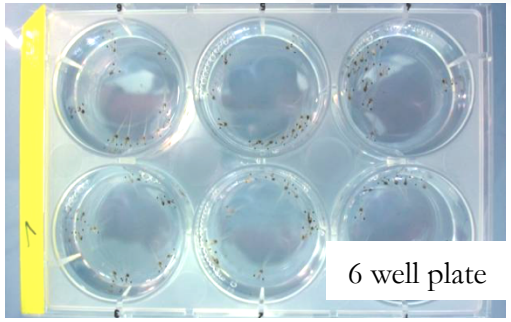
Without a minimum of thyroid hormone, at the right time, a tadpole fails to become a frog and a human baby becomes a cretin.

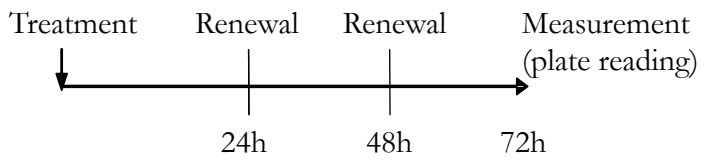
Jacques Legrand 1983



Xenopus eleutheroembryo thyroid assay (XETA OECD TG 248)



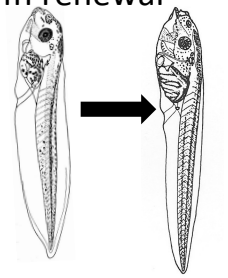




Fini et al., 2007 Env Sci Tech

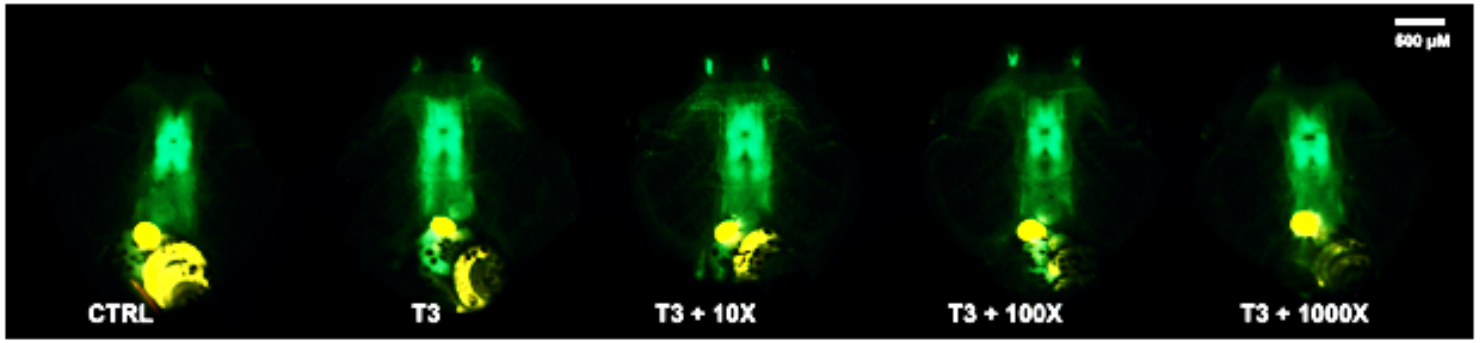
Does the mixture N0 have thyroid hormone disruptive potential?

3-day treatment
24h renewal

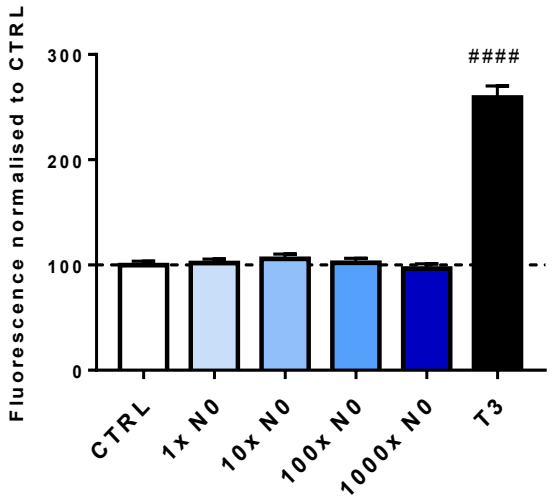


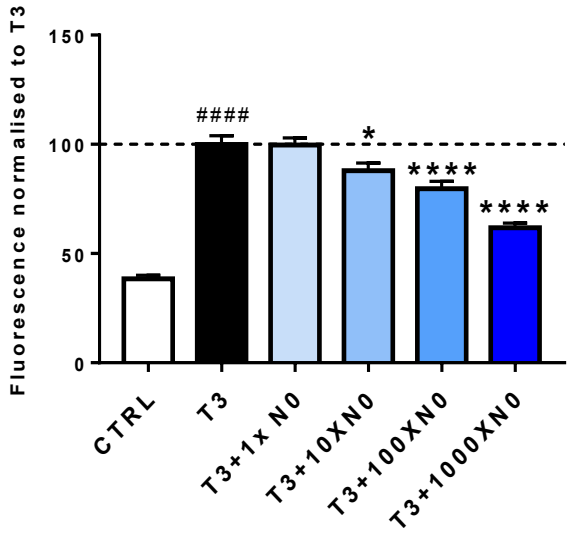
NF45 NF47

GFP quantification



	1x N (mol/L)	10x N (mol/L)	100x N (mol/L)	1000x N (mol/L)
MEP	2.7E-08	2.7E-07	2.7E-06	2.7E-05
MBP	2.26E-08	2.26E-07	2.26E-06	2.26E-05
MBzP	1.05E-08	1.05E-07	1.05E-06	1.05E-05
MINP	2.06E-08	2.06E-07	2.06E-06	2.06E-05
BPA	4.0E-09	4.0E-08	4.0E-07	4.0E-06
PFHxS	3.20E-09	3.20E-08	3.20E-07	3.20E-06
PFNA	1.10E-09	1.10E-08	1.10E-07	1.10E-06
PFOS	1.03E-08	1.03E-07	1.03E-06	1.03E-05



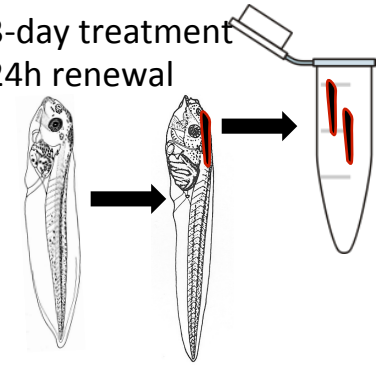


- Parametric one-way ANOVA
- Pool 3 independent experiments with each n=15 tadpoles

Mix N0 dose-dependently alters thyroid hormone availability after only 72 hours of exposure in *Xenopus*

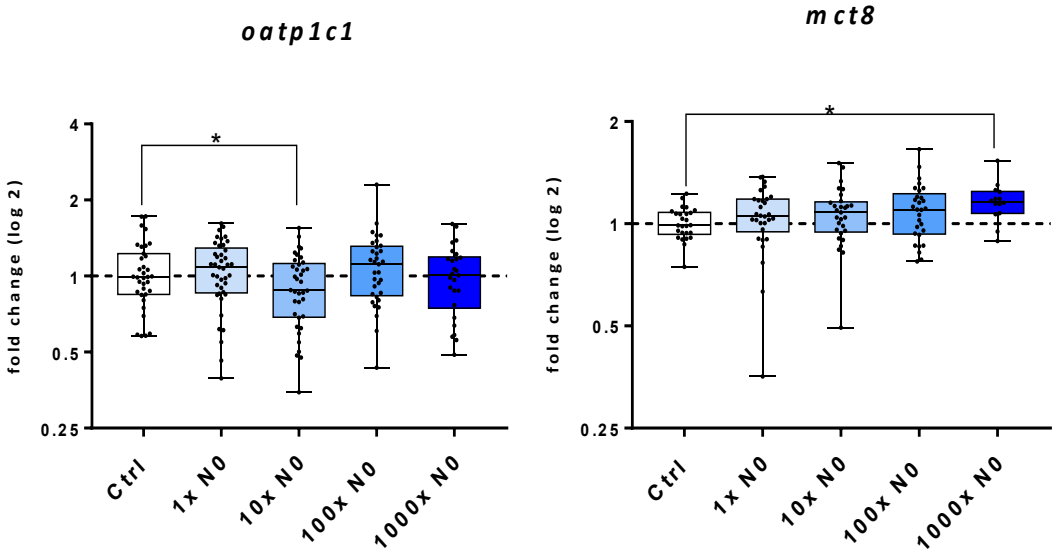
Does the mixture N0 affect brain gene expression?

3-day treatment
24h renewal



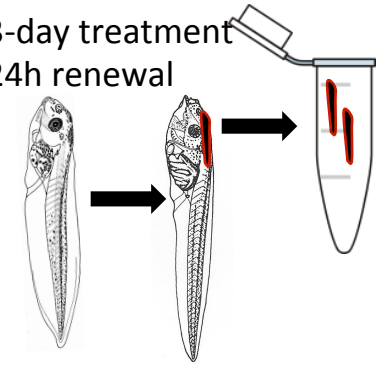
NF45 NF47

RT-qPCR



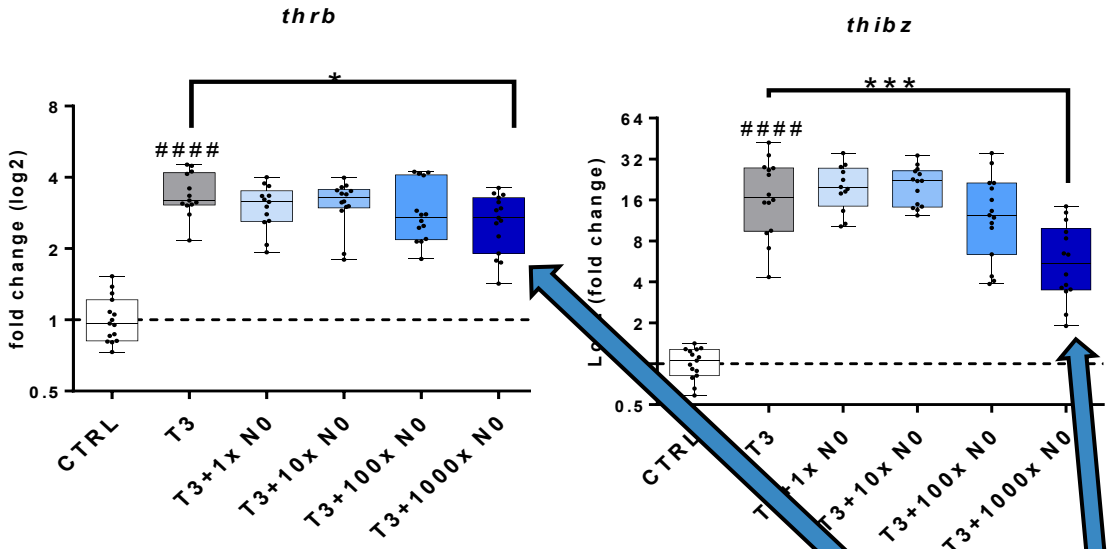
Does the mixture N0 affect brain gene expression?

3-day treatment
24h renewal

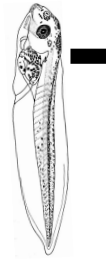


RT-qPCR

NF45 NF47

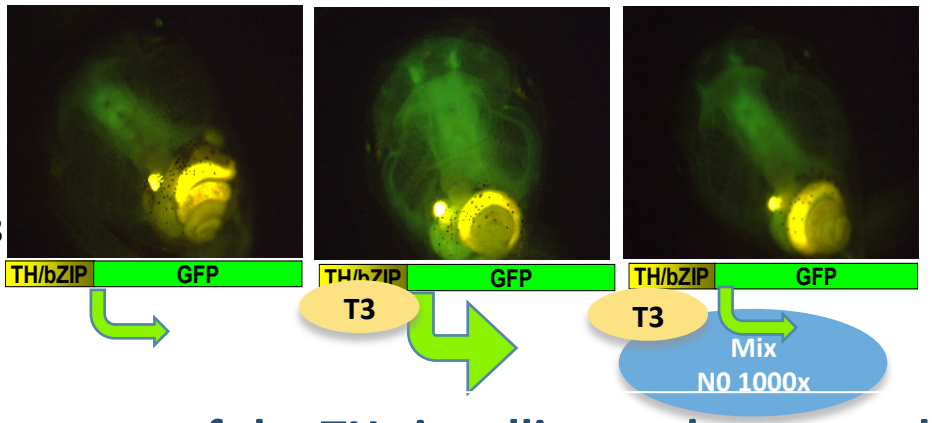


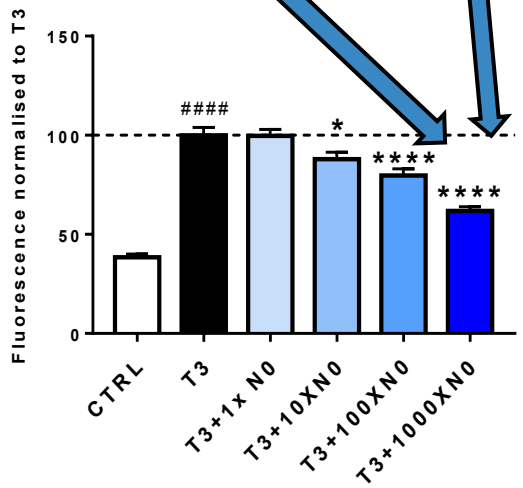
3 d treatment/24h renewal



NF45 NF48

GFP quantification



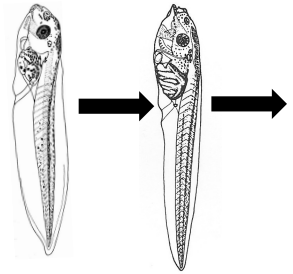


Key genes of the TH signalling pathway are dysregulated in brains of exposed tadpoles to mixture N0

13

Does the mixture N0 alter behaviour?

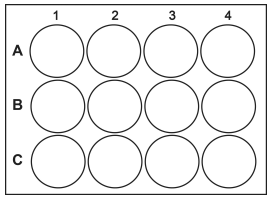
3-day treatment
24h renewal



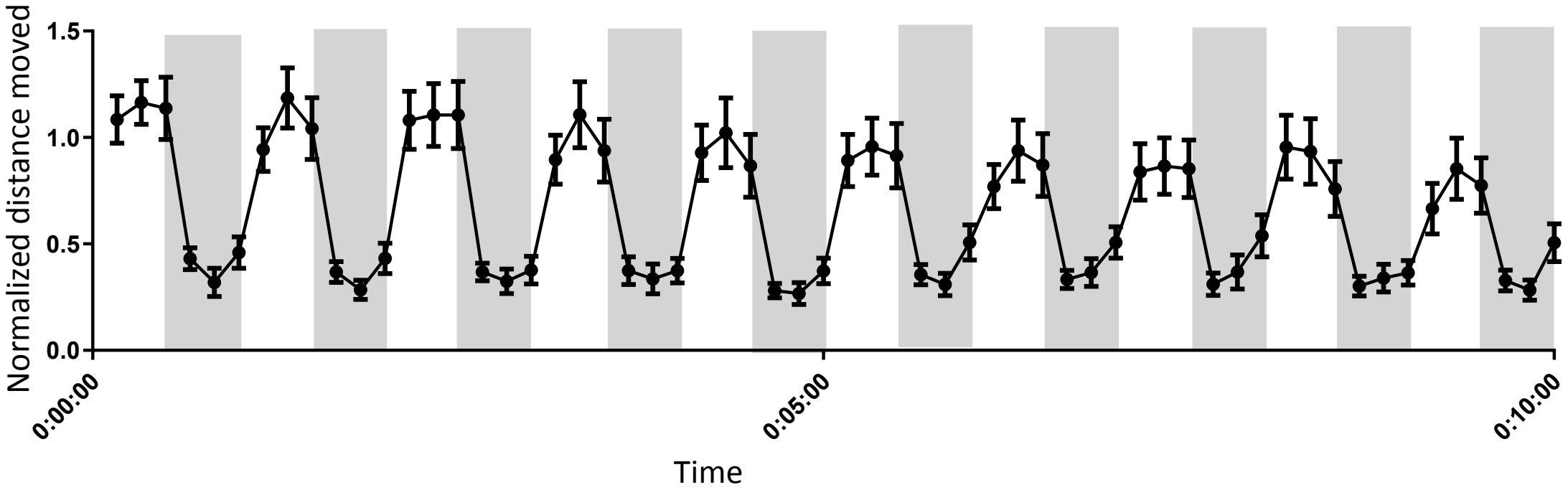
NF45 NF47

Mobility tracking

1 tadpole/well

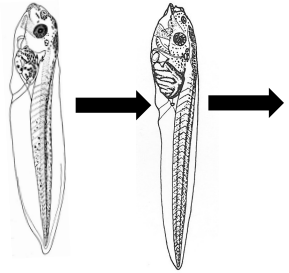


Video tracking for 10 min
(30 sec light on/ 30 sec light off)



Does the mixture N0 alter behaviour?

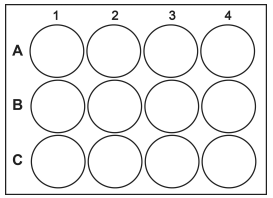
3-day treatment
24h renewal



NF45 NF47

Mobility tracking

1 tadpole/well



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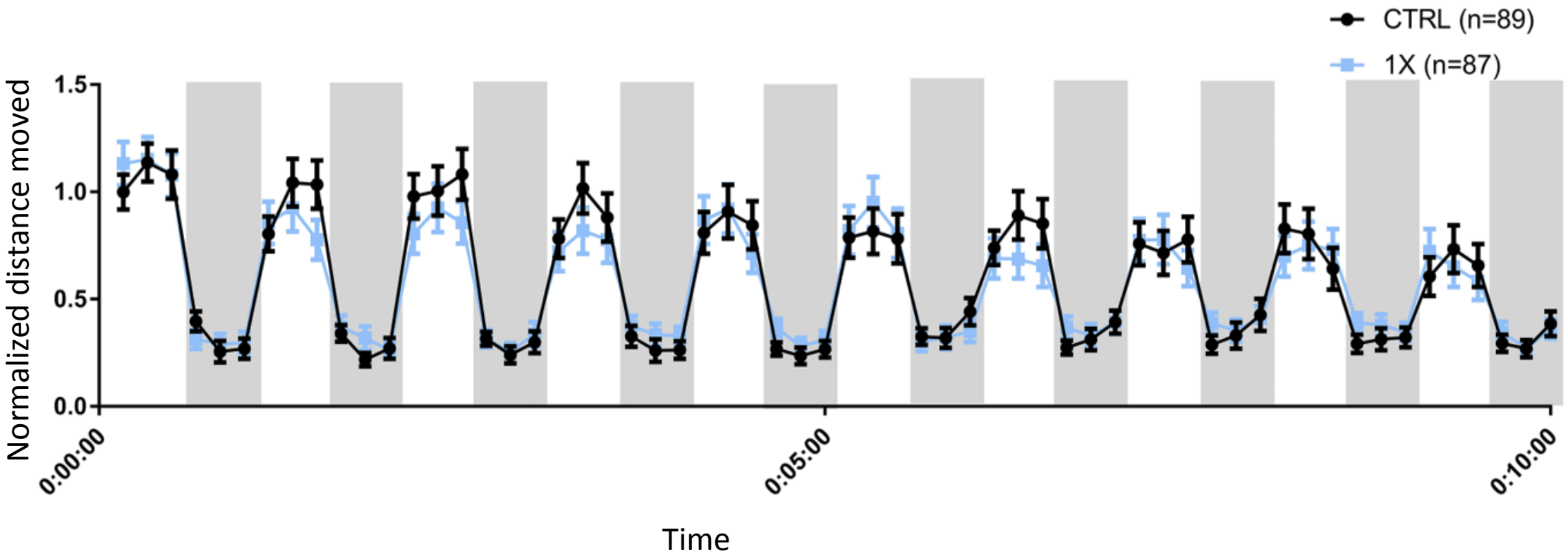
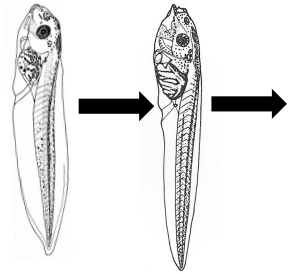


Does the mixture N0 alter behaviour?

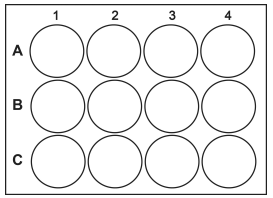
3-day treatment
24h renewal



NF45 NF47

Mobility tracking

1 tadpole/well



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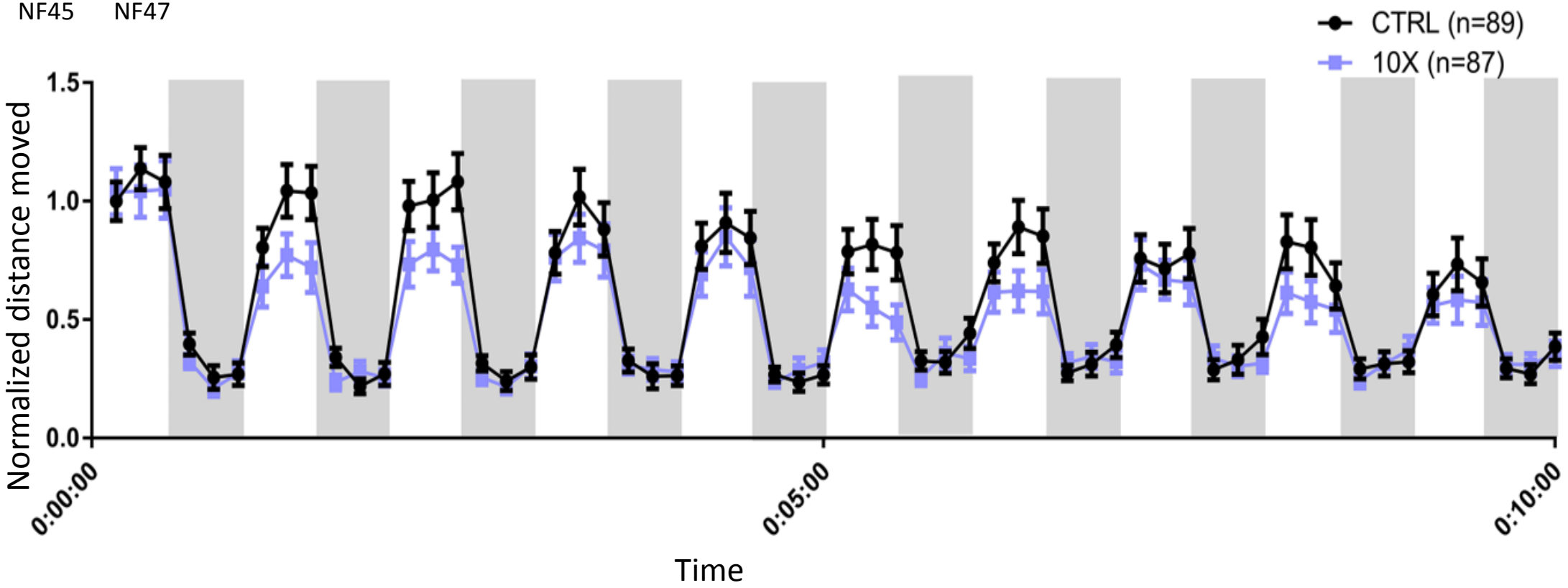
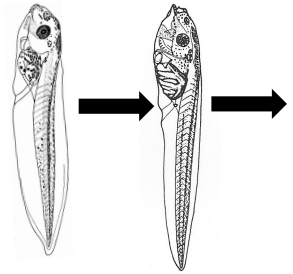


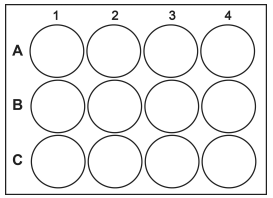
Does the mixture N0 alter behaviour?

3-day treatment
24h renewal



Mobility tracking

1 tadpole/well



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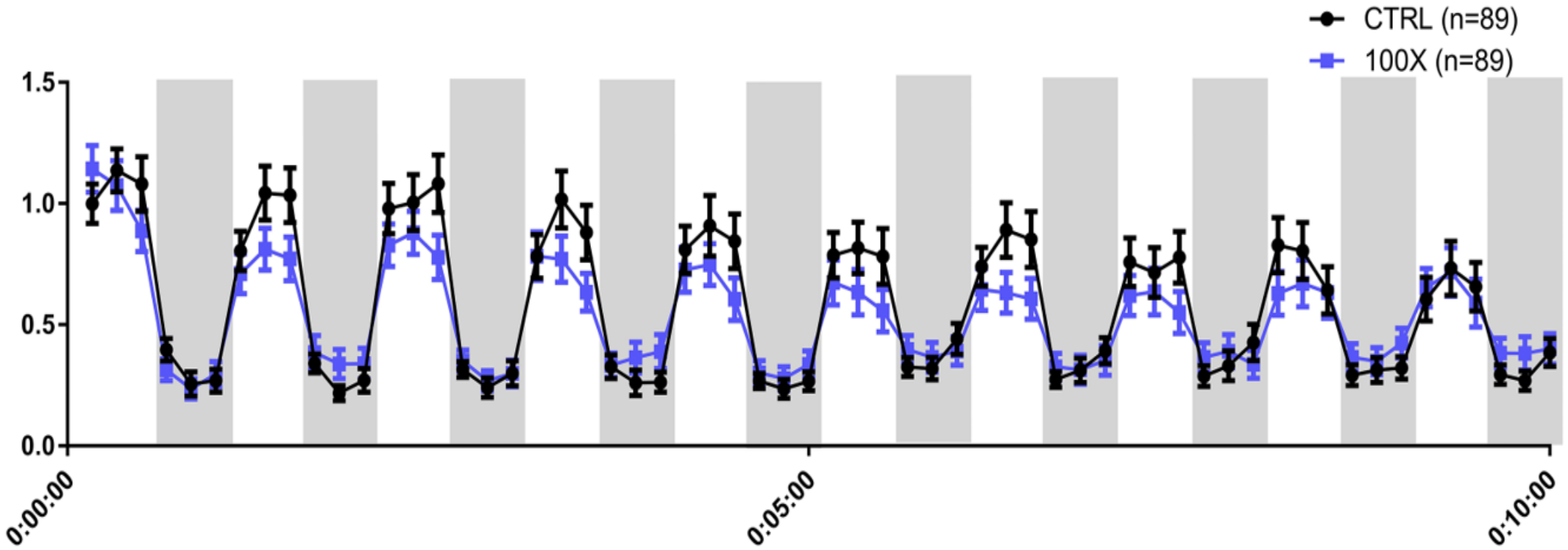
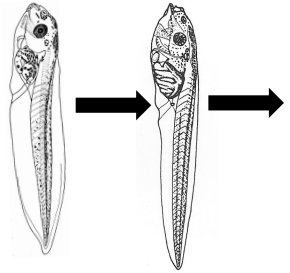


Does the mixture N0 alter behaviour?

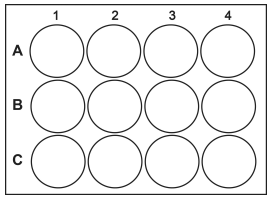
3-day treatment
24h renewal



NF45 NF47

Mobility tracking

1 tadpole/well



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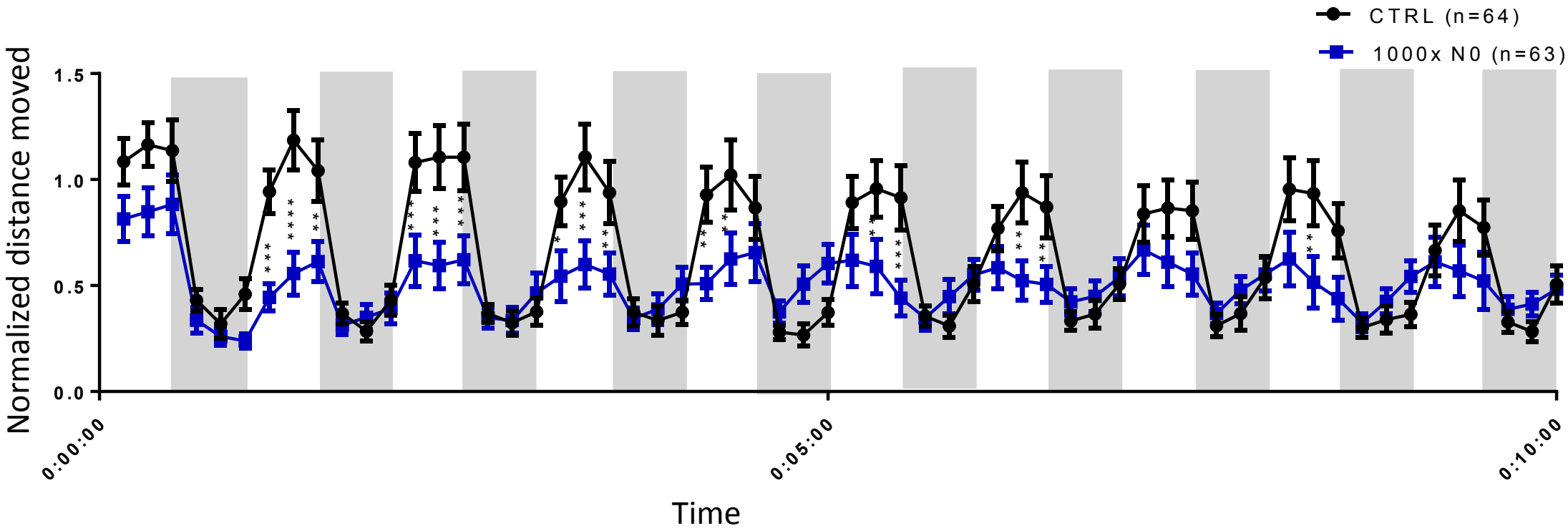
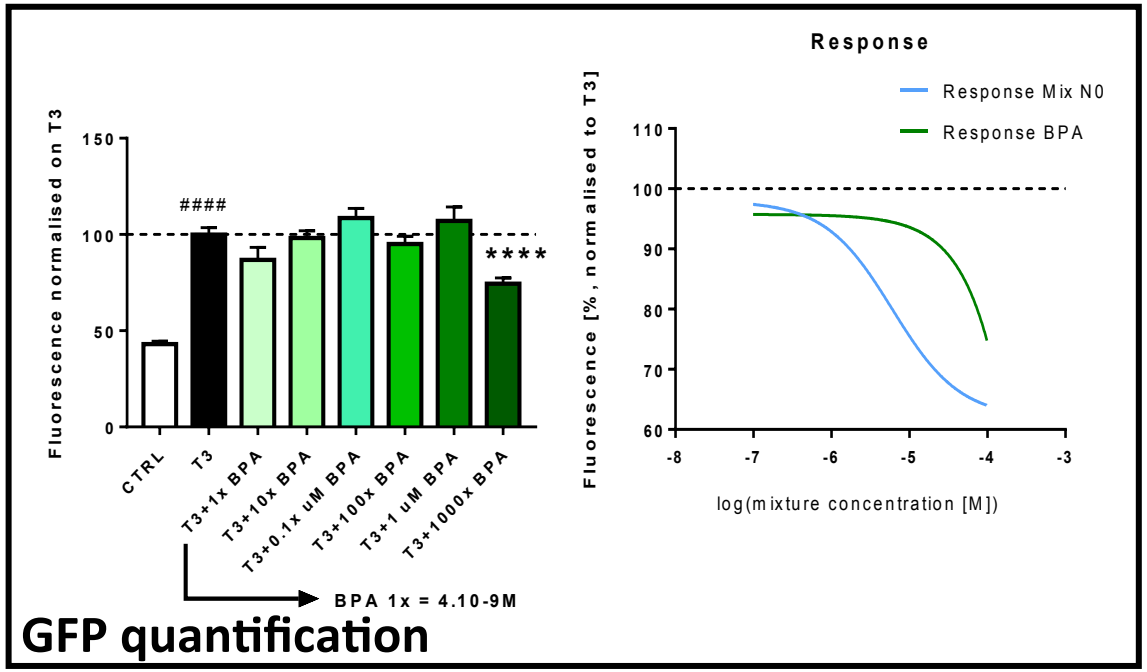
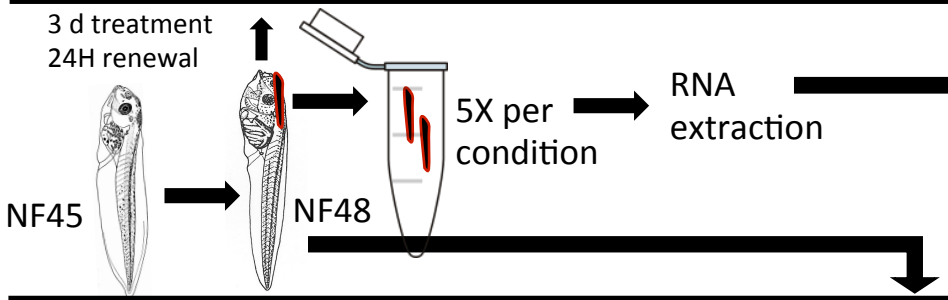


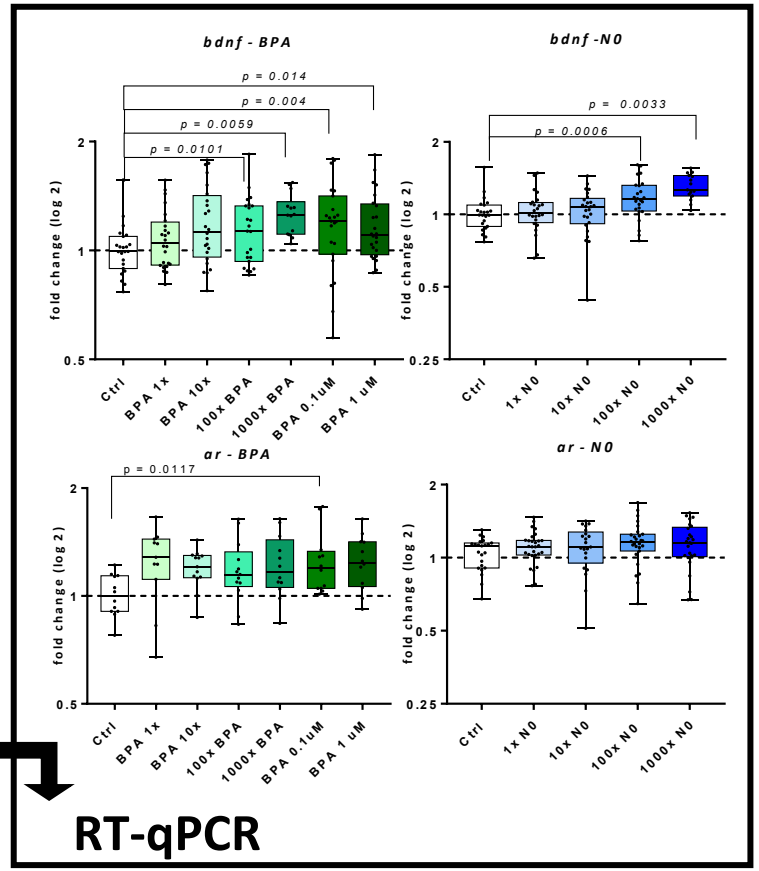
Mix N0 alters mobility at high dose

Supplementary information: Differential effects of BPA vs Mix N

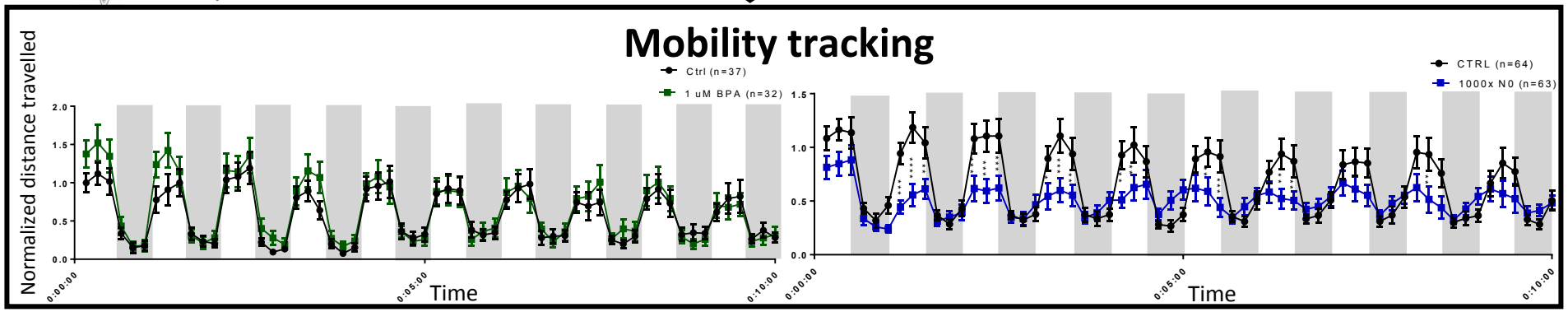


GFP quantification





RT-qPCR



From cohorts to molecules: adverse impacts of endocrine disrupting mixtures

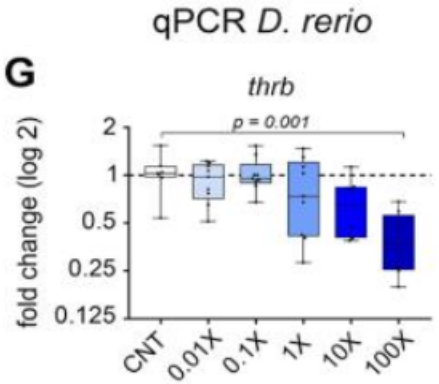
The mixture associated with neurodevelopmental delay in children:

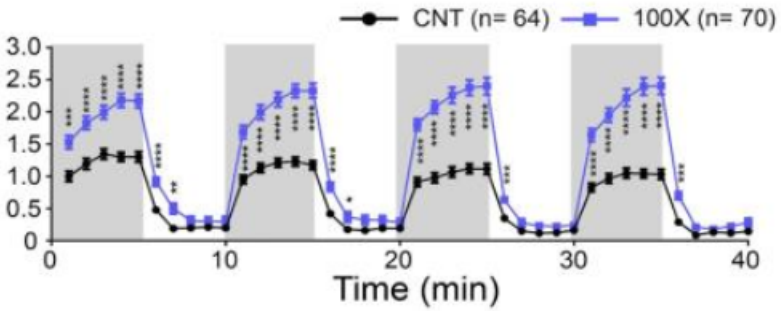
1. Disrupts TH signalling (XETA)

2. Affects brain gene expression in Xenopus and Zebrafish

3. has potential to impede behaviour
   = possible indication of aberrant brain development

Corroborates results found in Human fetal neuronal progenitors

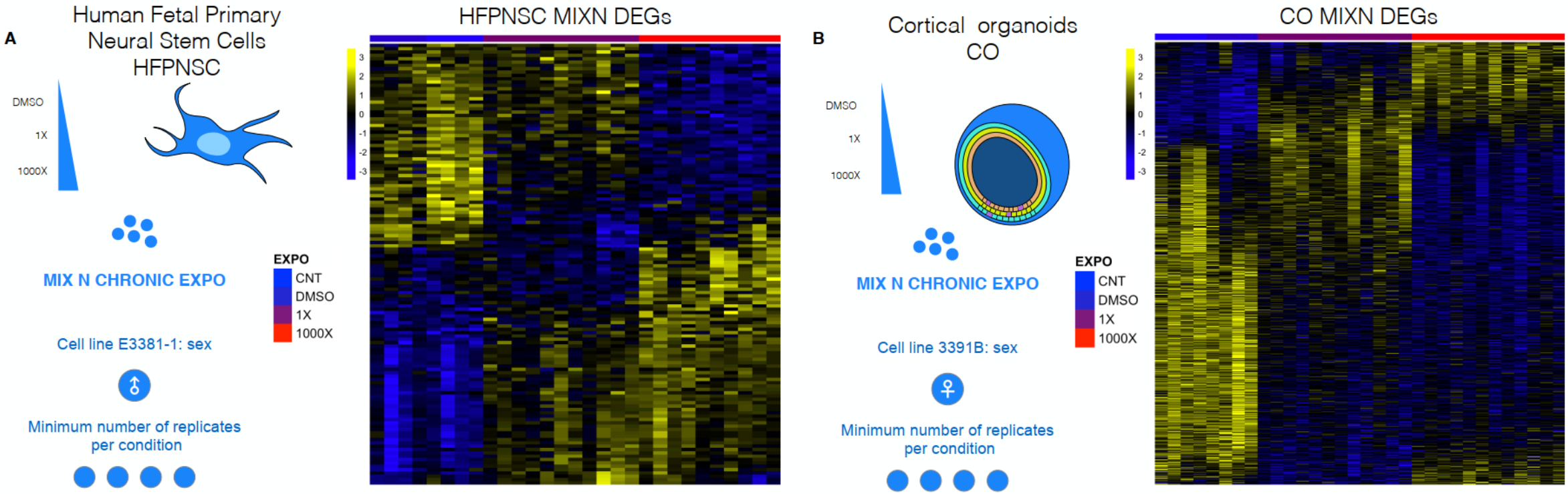




Are the DEGs identified in the *in vitro* neuronal systems implicated in hormonal pathways? If yes, which ones?

Mix N0

Mixture N alters gene expression in neural stem cells and human organoids

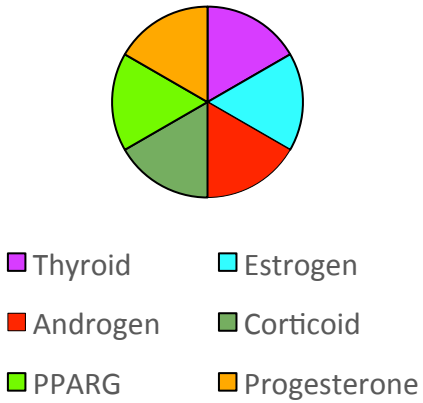


Caporale, Leemans et al, Science Feb 2022

Are the DEGs identified in the *in vitro* neuronal systems implicated in hormonal pathways? If yes, which ones?

1. Gene set enrichment analysis (R)

= focusing on gene sets that share a common biological function – in our case hormonal function



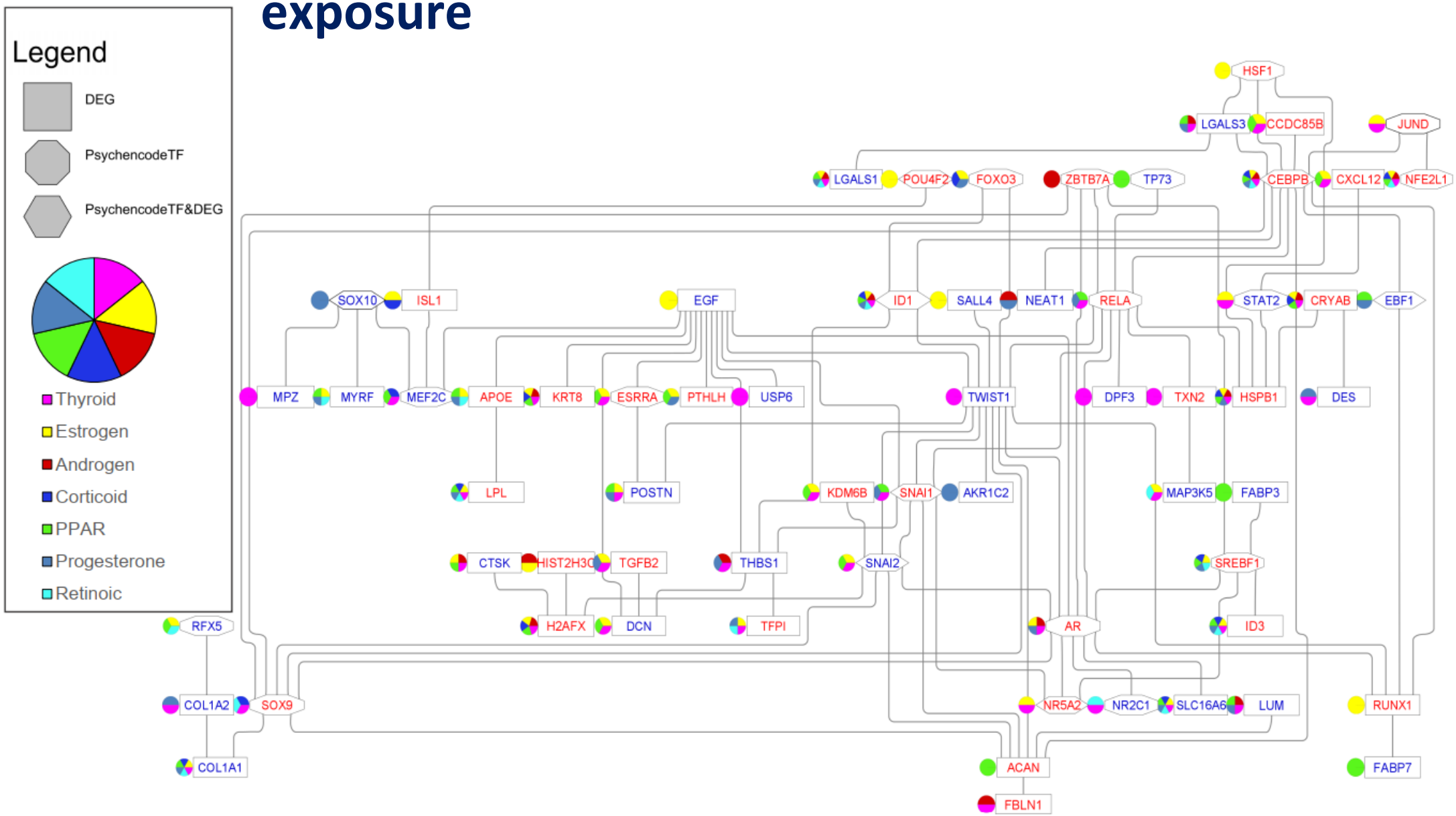
2. Application of threshold

3. Network generation (Genomatix/Cytoscape)

a) Import list into Genomatix Pathway System – networks are formed based on extracted information both from public- and proprietary databases

b) Import network information and expression data into cytoscape

Multiple hormonal axes are disrupted by the mixture exposure



Caporale, Leemans et al, Science Feb 2022

Conclusions

- Fine tuning of hormonal signaling is crucial for proper brain development
- Chemicals cross the placenta barrier and can interfere with hormonal pathways

Adverse effects of a mixture «neurodevelopment » identified from epidemiological data were validated in experimental models especially in modifying TH signaling in vivo (xenopus and zebrafishes)

How use these data on mixtures in innovative risk assessment? _Chris Gennings

& beyond

- No legislation on combined cocktail effects. Shall we think about a « legacy chemical mixture » in our experiment as we cannot avoid it in human?
- Starting from cohorts to molecules makes possible to build an AOP from molecules to population

From Experimental methods to risk assessment - Adverse Outcome Pathways