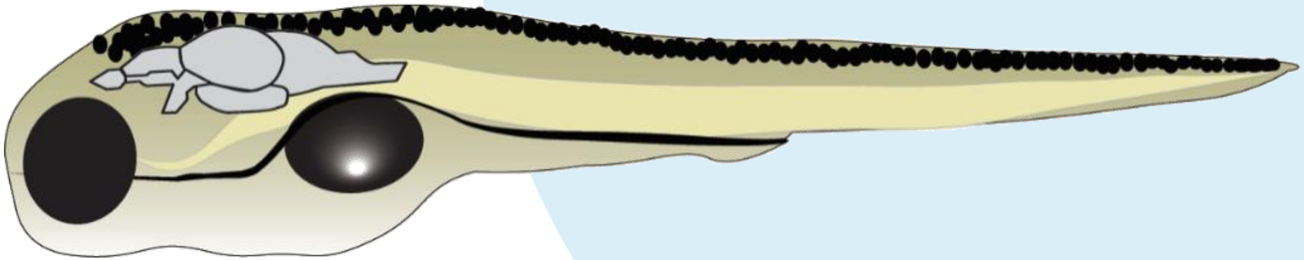
Building confidence in larval zebrafish behavior assays: From phenotypes to mechanisms



Prof. Dr. Tamara Tal
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01.30.2023

350.000

Source: Schmidt et al. 2016. EHP.

350.000

- <100 unique chemicals assessed for developmental neurotoxicity in ToxRef DB!

Source: Watford et al. 2019. PMID: 31340180

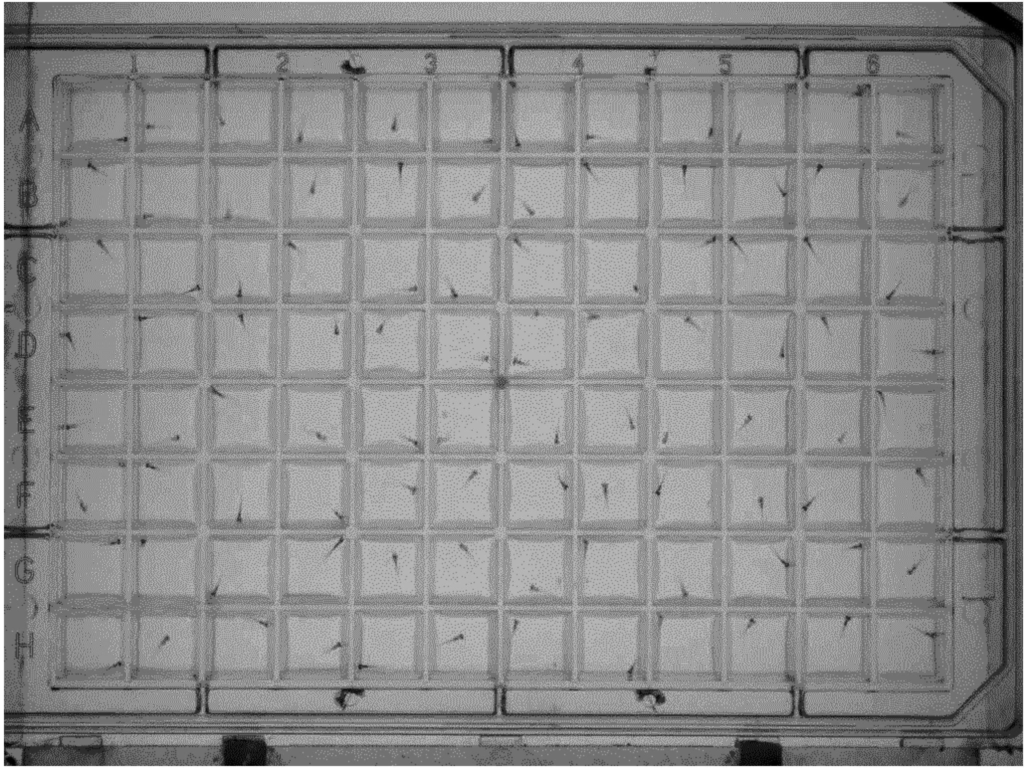
- 1 chemical
- >€1.000.000
- >1 year

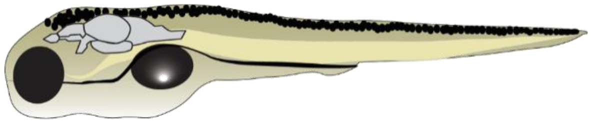
350.000

- <100 unique chemicals assessed for developmental neurotoxicity in ToxRef DB!

Source: Tsuji and Crofton. 2012. PMID: 22925212.

Motivation: How can we build confidence in zebrafish behavior tests for the detection of neurotoxicity?





- Alternative system
- Translational model
- Inexpensive
- Fast
- Easy to assess neurodevelopment
- Metabolically competent
- 3R compliant

State of the science

Light-dark transition test under review by OECD-DNT-Expert group

N. Klüver

1. Determine the **critical points** of zebrafish behavioral test
2. Design and execute a **harmonized protocol** to test critical points
3. Detect the **variables** affecting the **reproducibility** (by *Data Analysis*)
4. Establish a definitive and effective **testing methodology** for **DNT assessment**

Inter-laboratory Verification

Goal

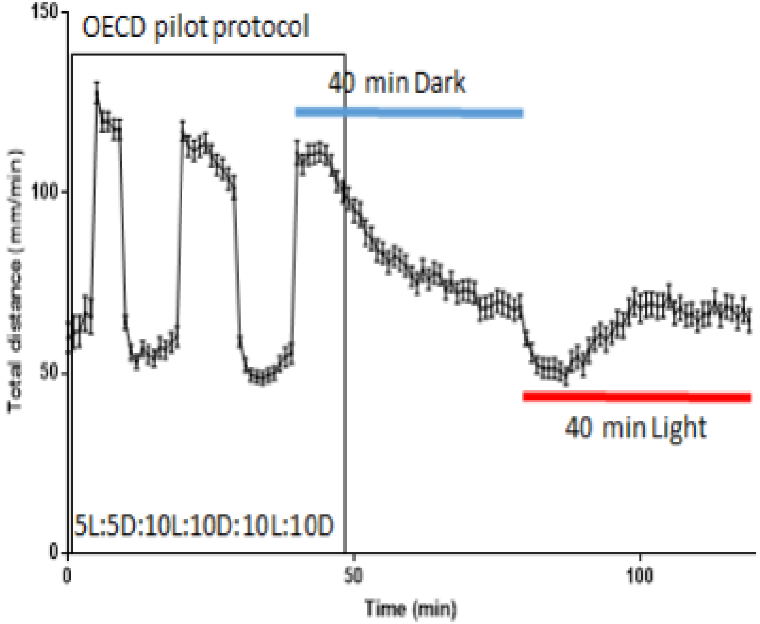
Determine the added value of the zebrafish behavioral assay for DNT

Discussion

Potential incorporation of Zebrafish behavioral assays into the **IVB** or **tiered testing for DNT**

State of the science
Light-dark transition test

N. Klüver



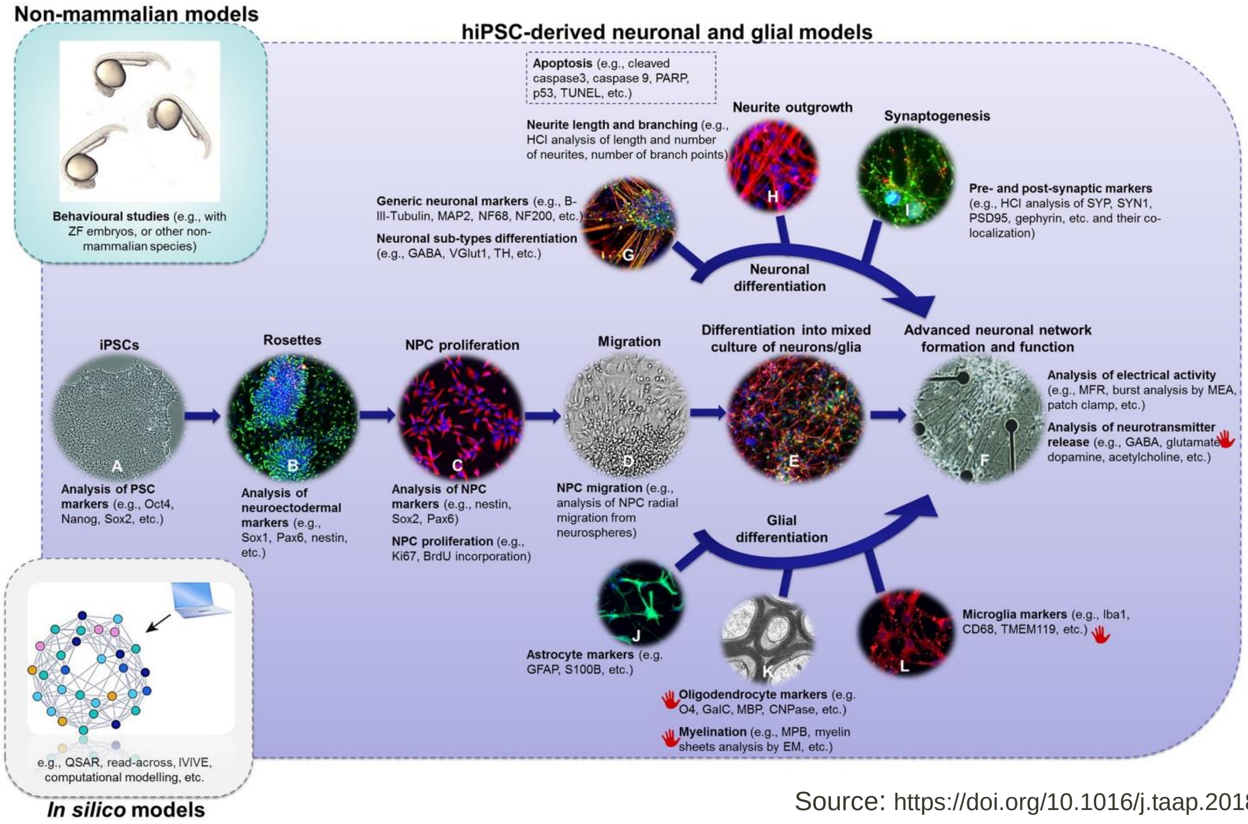
Rat OECD TG426
Roadmap for zebrafish assay design

Rat offspring endpoint	Larval zebrafish test	State of the test
Motor activity	Light-dark transition test	OECD working group; NIEHS SEAZIT Evaluation
	VAMR	PARC
Motor and sensory function	**VAMR**	PARC
Learning and memory	**VAMR**	PARC
Brain weight	NA	NA
Histopathological evaluation	NA	NA
Morphometric (quantitative) analysis of the brain	Fish Inspector, post-behavior	Developed, v2 release 2021

PARC (European Partnership for the Assessment of Risks from Chemicals); **SEAZIT** (Systematic Evaluation of the Application of Zebrafish in Toxicology); **VAMR** (Visual and Acoustic Motor Response NAM)

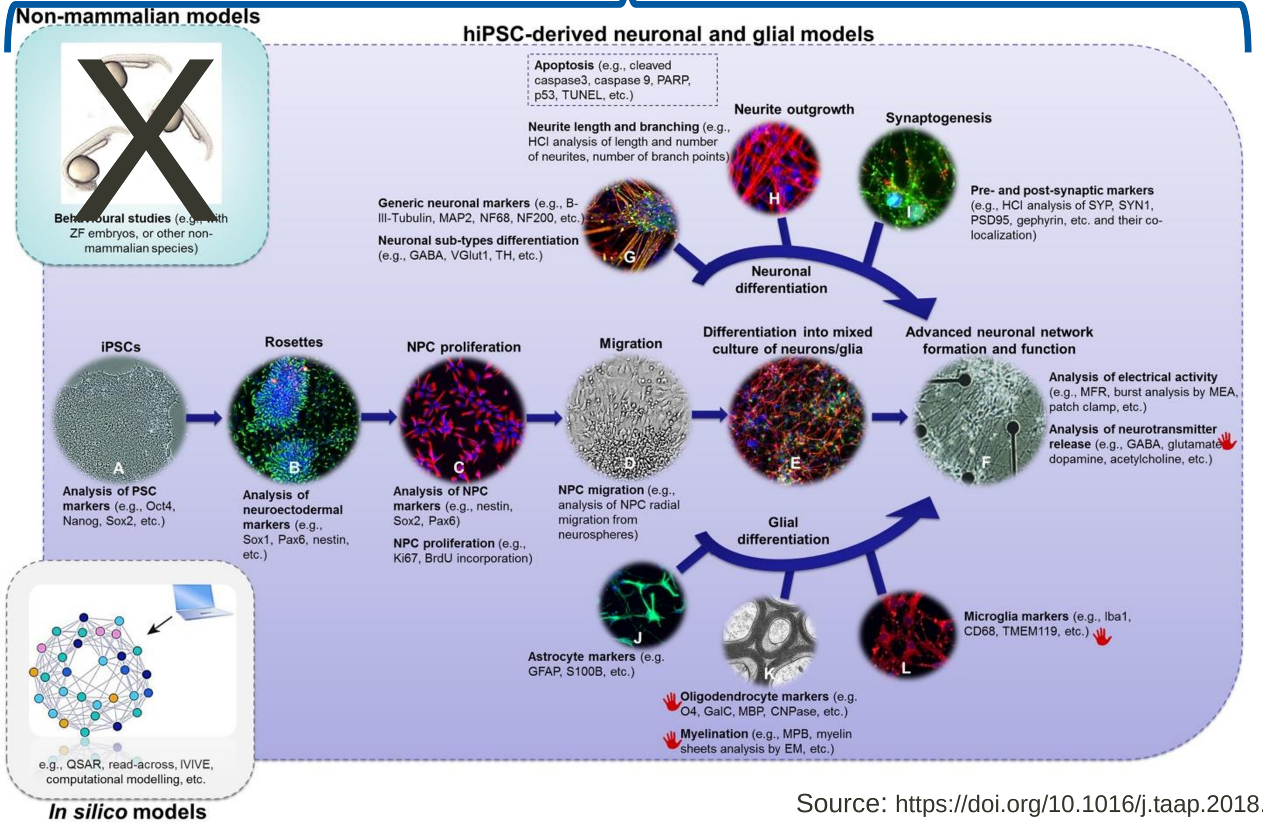
Roadmap for zebrafish developmental and acute neurotoxicity testing

Human *in vitro* developmental neurotoxicity test battery



Source: https://doi.org/10.1016/j.taap.2018.02.008

Roadmap for zebrafish developmental and acute neurotoxicity testing



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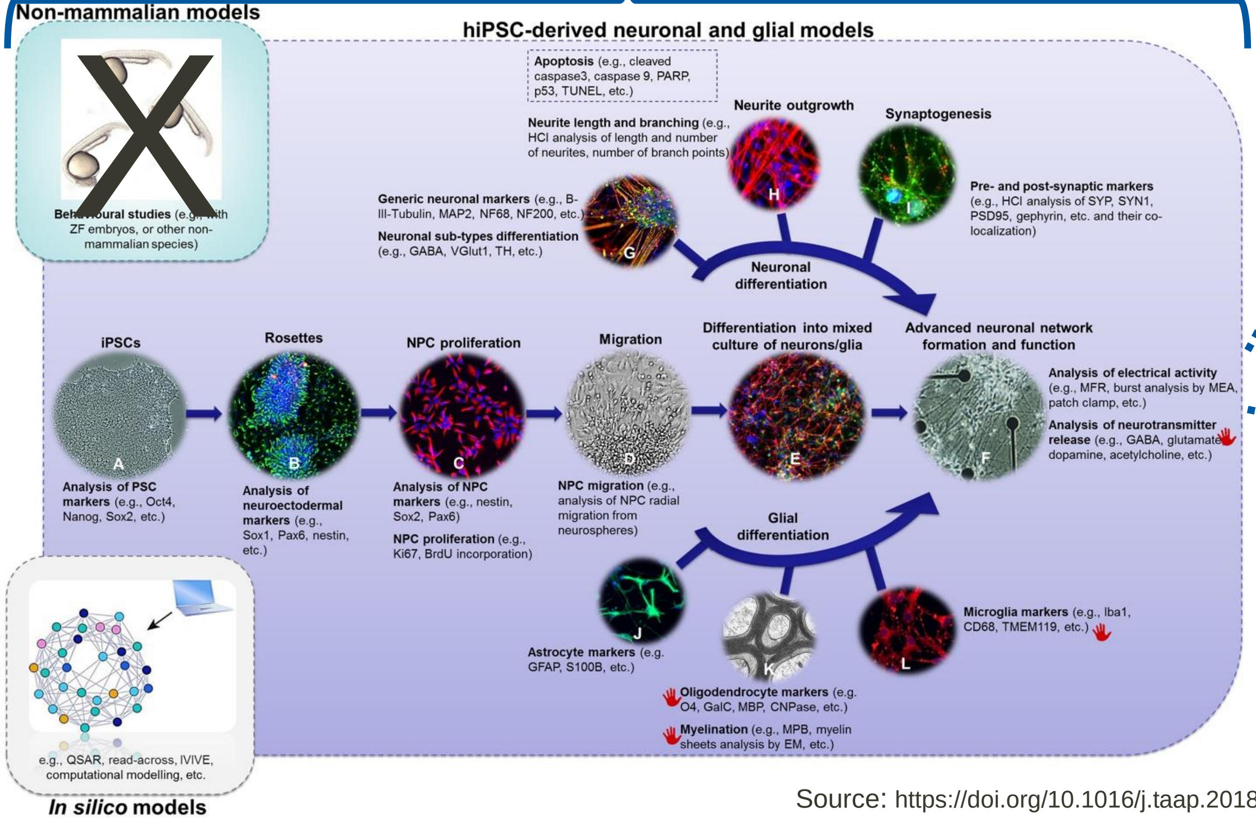
Roadmap for zebrafish developmental and acute neurotoxicity testing



Extend functional analysis

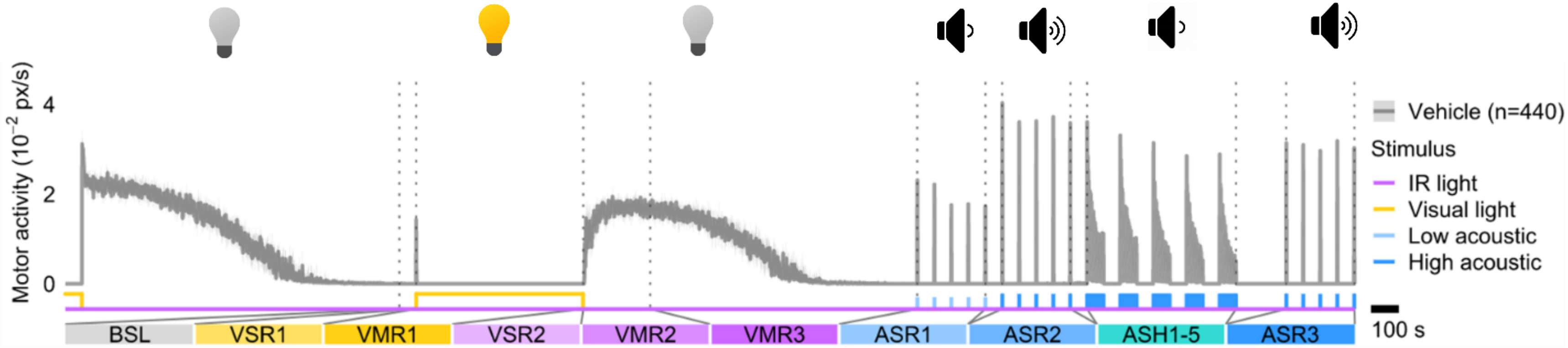
- Motor activity
- Motor and sensory function
- Learning and memory
- Brain morphometrics

Source: https://doi.org/10.1016/j.taap.2018.02.008

Case study: Expansion to a battery of visual and acoustic stimuli

Visual and Acoustic Motor Response (VAMR) New Approach Method (NAM)

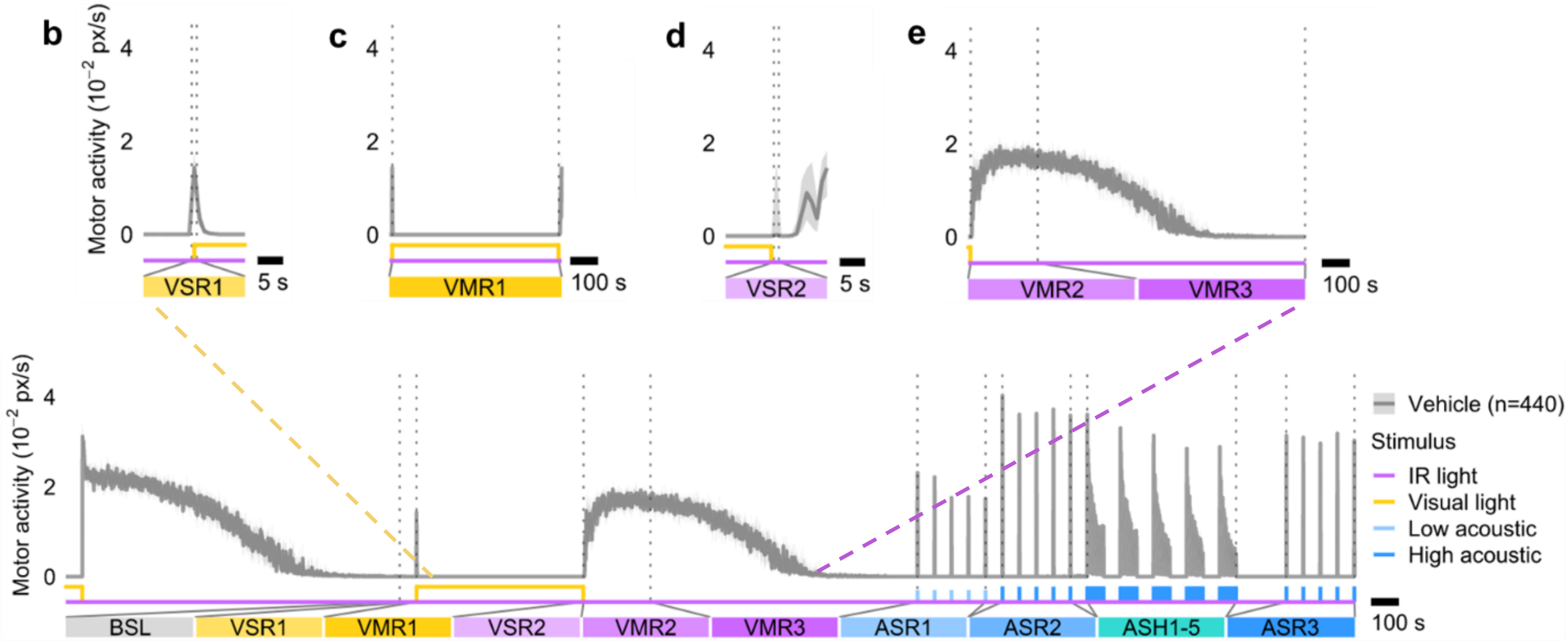
David Leuthold



BSL (Baseline); VSR (Visual Startle Response), VMR (Visual Motor Response);
ASR (Acoustic Startle Response); ASH (Acoustic Startle Habituation)

Added novel endpoints
Visual startle response

David Leuthold 

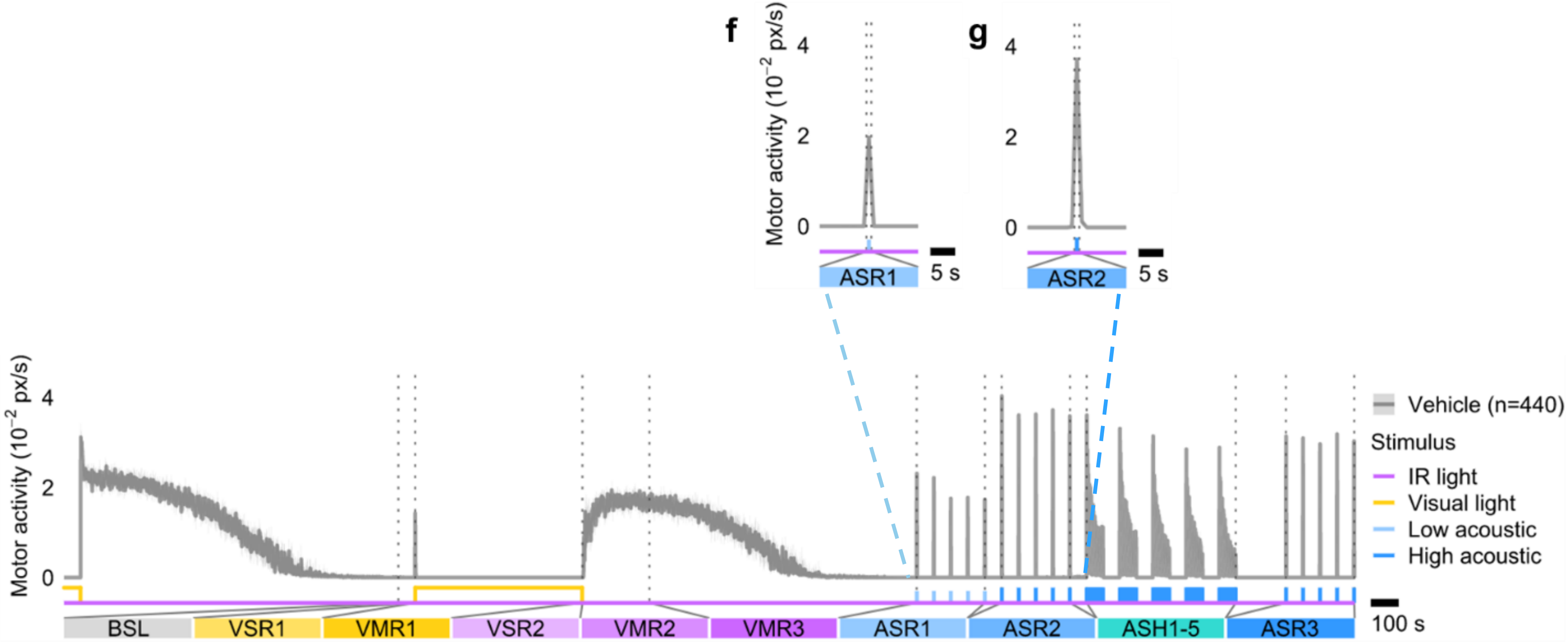


BSL (Baseline); VSR (Visual Startle Response), VMR (Visual Motor Response);
ASR (Acoustic Startle Response); ASH (Acoustic Startle Habituation)

Added novel endpoints

Acoustic startle response

David Leuthold

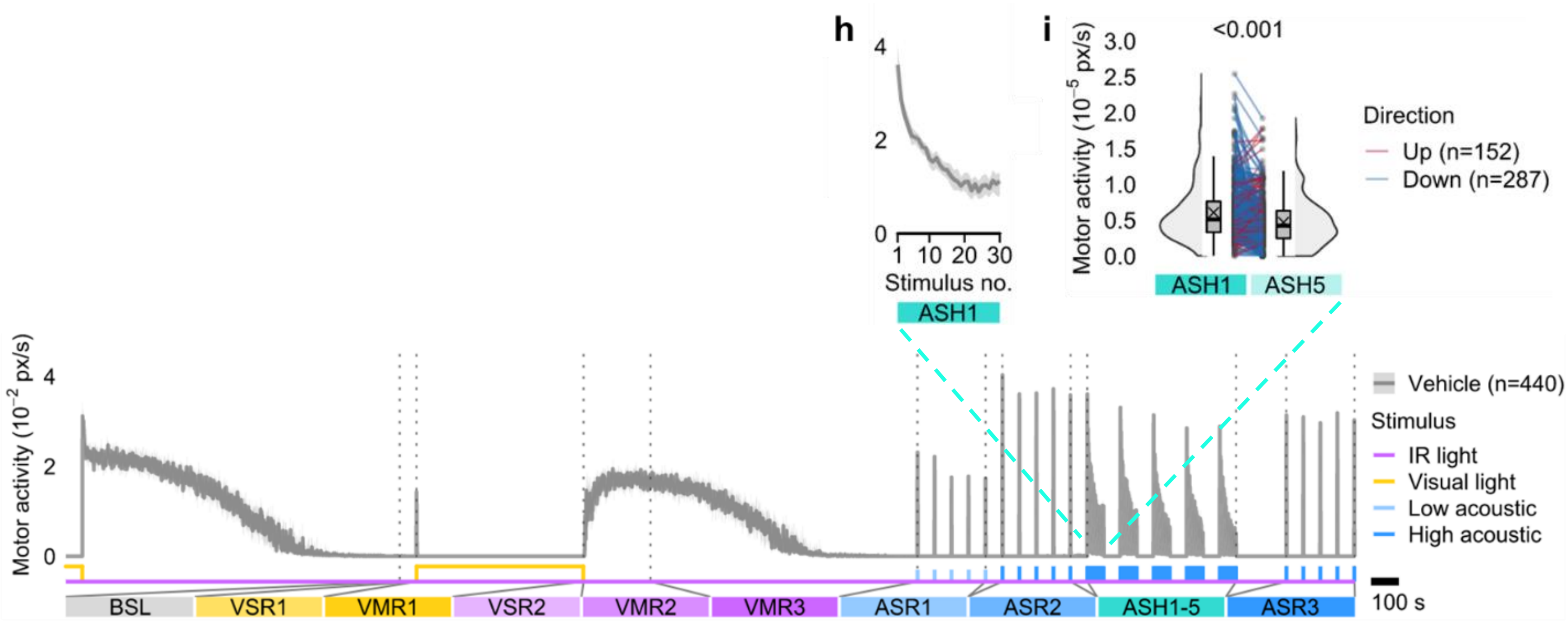


BSL (Baseline); VSR (Visual Startle Response), VMR (Visual Motor Response);
ASR (Acoustic Startle Response); ASH (Acoustic Startle Habituation)

Added novel endpoints

Non-associative habituation learning

David Leuthold



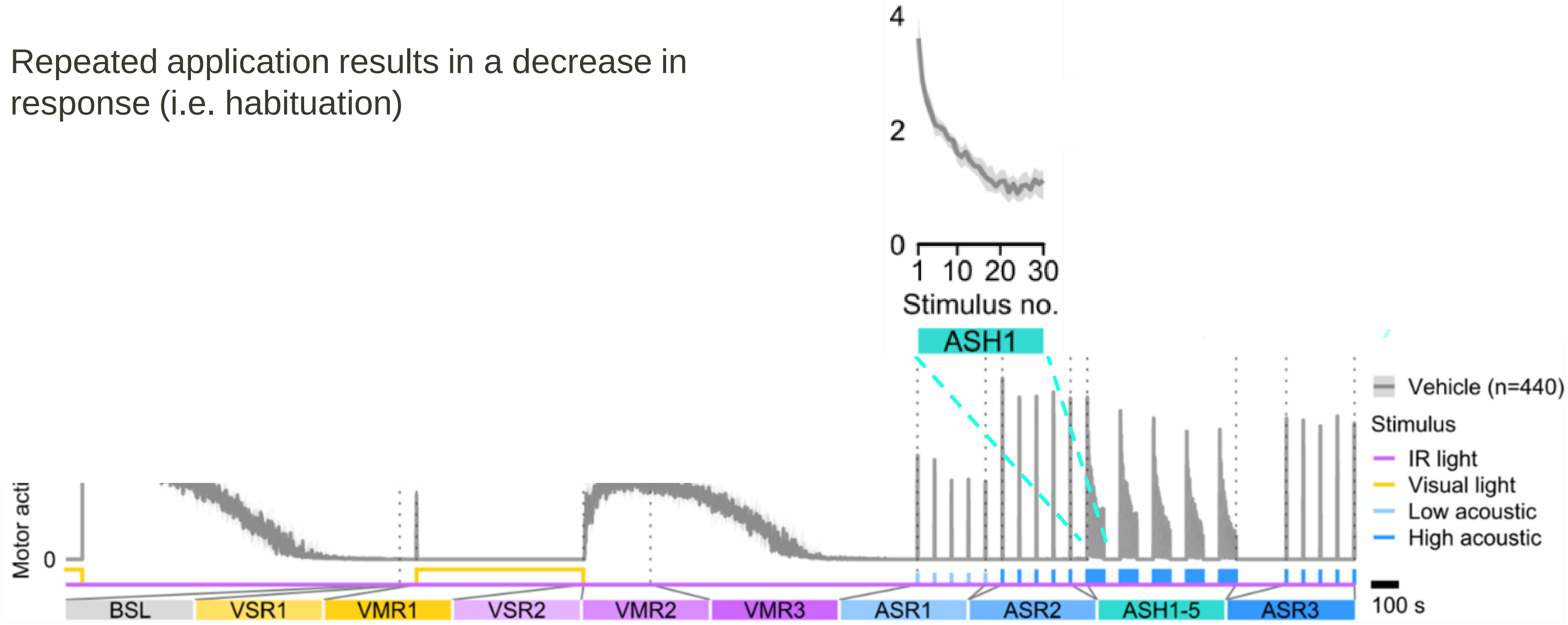
BSL (Baseline); VSR (Visual Startle Response), VMR (Visual Motor Response);
ASR (Acoustic Startle Response); ASH (Acoustic Startle Habituation)

10 criteria to demonstrate habituation

Non-associative habituation learning

David Leuthold

- Repeated application results in a decrease in response (i.e. habituation)



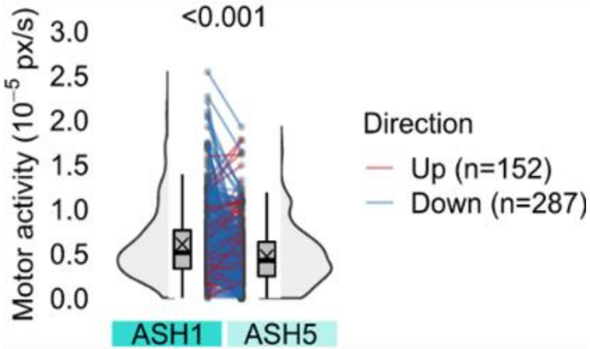
Source: Rankin et al. 2009. doi: 10.1016/j.nlm.2008.09.012.

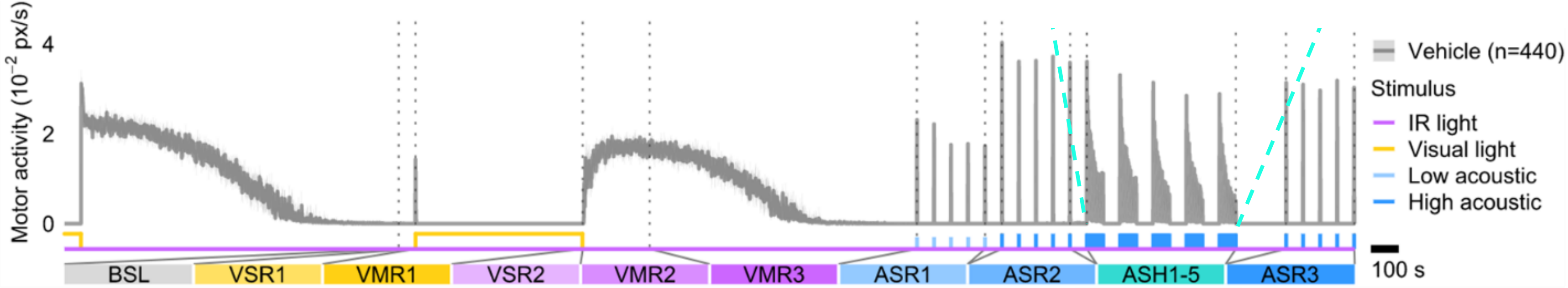
10 criteria to demonstrate habituation

Non-associative habituation learning

David Leuthold

- Repeated application results in a decrease in response (i.e. habituation)
- With repeated series of habituation training and recovery, decrease in response is more rapid or pronounced (i.e. potentiation of habituation)



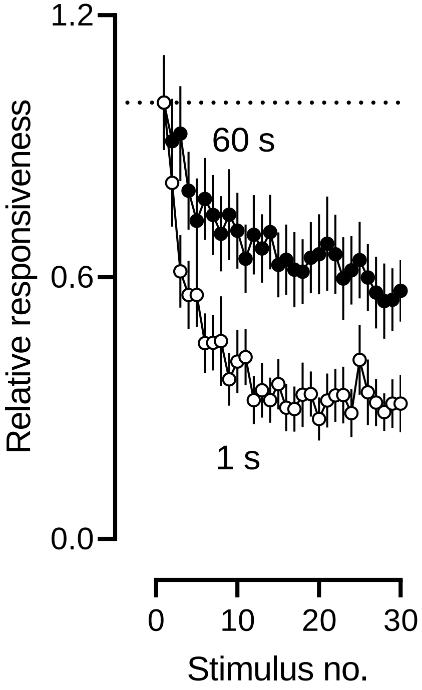


Source: Rankin et al. 2009. doi: 10.1016/j.nlm.2008.09.012.

10 criteria to demonstrate habituation
Non-associative habituation learning

David Leuthold

- Repeated application results in a decrease in response (i.e. habituation)
- With repeated series of habituation training and recovery, decrease in response is more rapid or pronounced (i.e. potentiation of habituation)
- More rapid the frequency of the stimulus, the more rapid and/or pronounced is the habituation

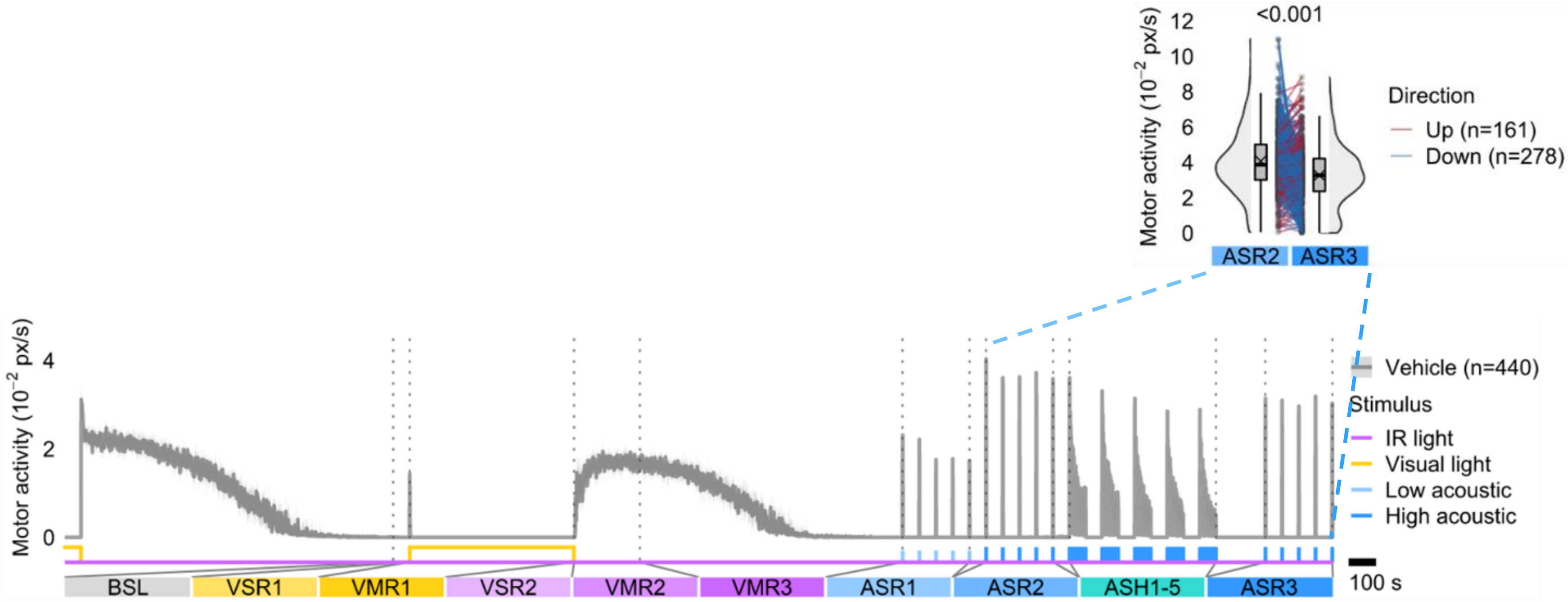


Source: Rankin et al. 2009. doi: 10.1016/j.nlm.2008.09.012.

Added novel endpoints

Memory retention

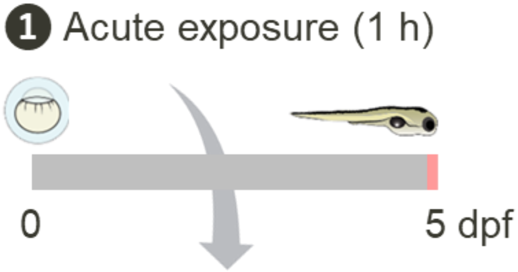
David Leuthold



BSL (Baseline); VSR (Visual Startle Response), VMR (Visual Motor Response);
ASR (Acoustic Startle Response); ASH (Acoustic Startle Habituation)

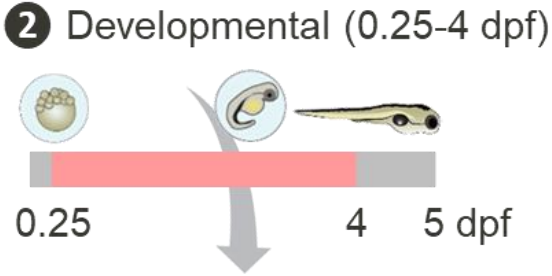
Multiple potential adverse outcomes
Exposure paradigm hacks

Acute neurotoxicity (ANT)



Receptor-mediated interactions

Developmental neurotoxicity (DNT)



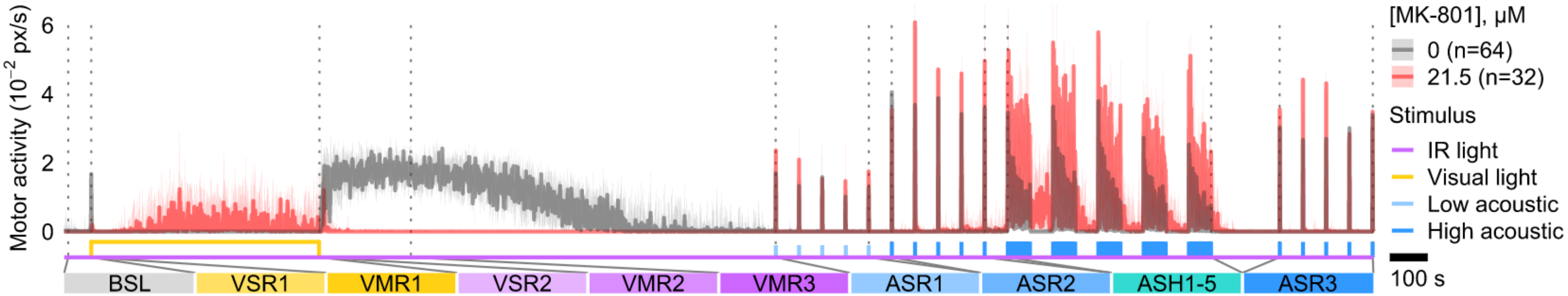
Receptor-mediated interactions that cause persistent structural OR functional effects

Build confidence: Replicate rat data

NMDA receptor antagonism (MK-801) blocks learning

David Leuthold

A.



B.

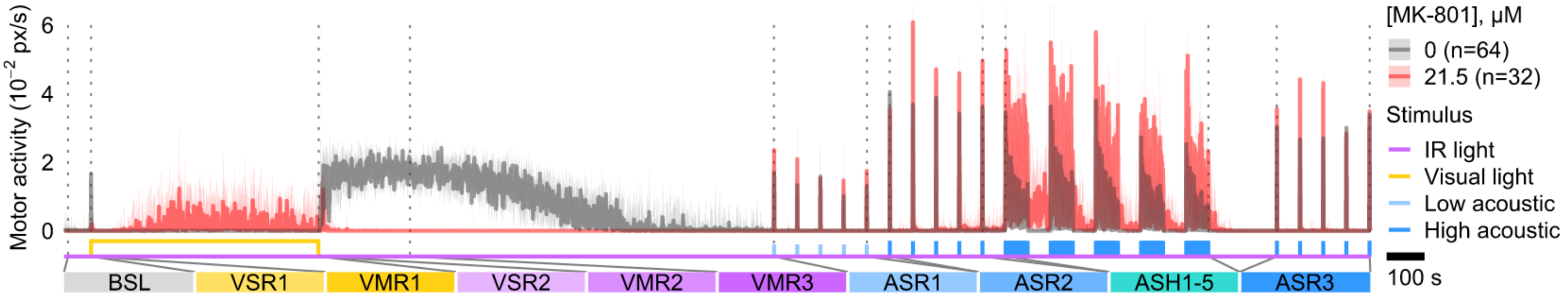
C.

Build confidence: Replicate rat data

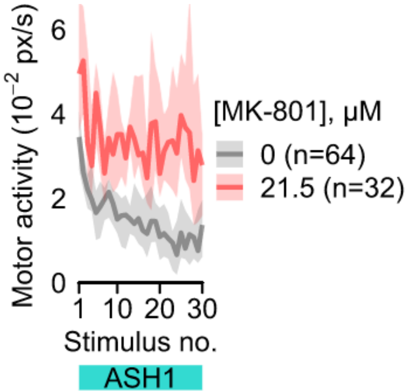
NMDA receptor antagonism (MK-801) blocks learning

David Leuthold

A.



B.



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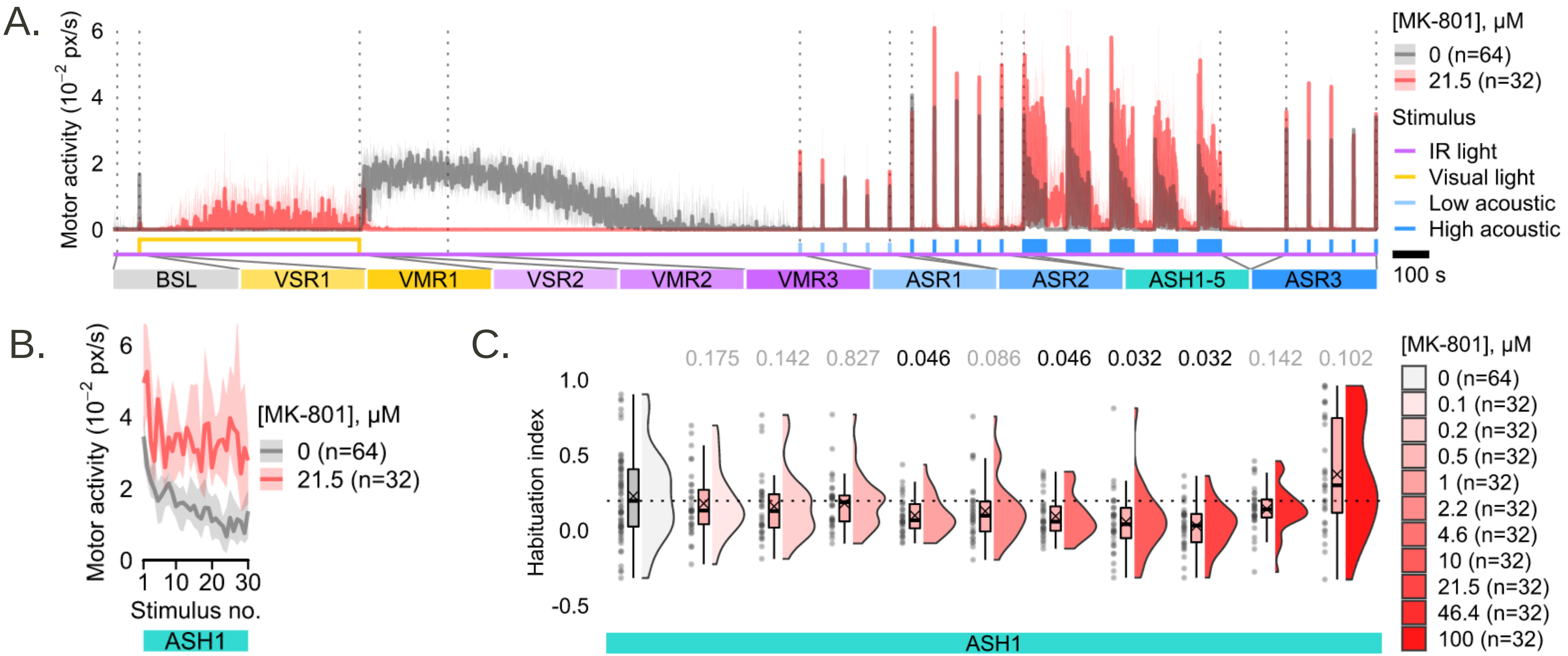
Build confidence: Replicate rat data

NMDA receptor antagonism (MK-801) blocks learning

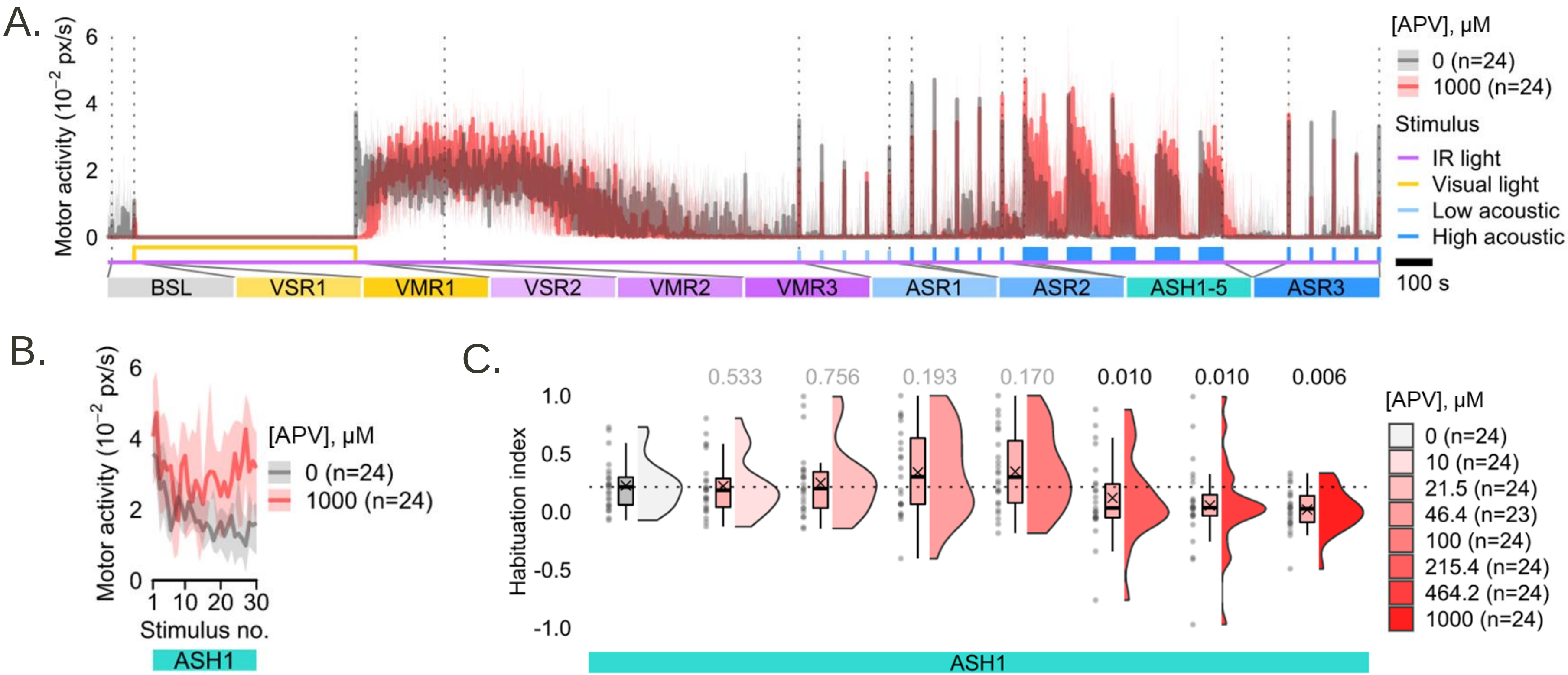
David Leuthold



Build confidence: Replicate rat data

NMDA receptor antagonism (APV) blocks learning

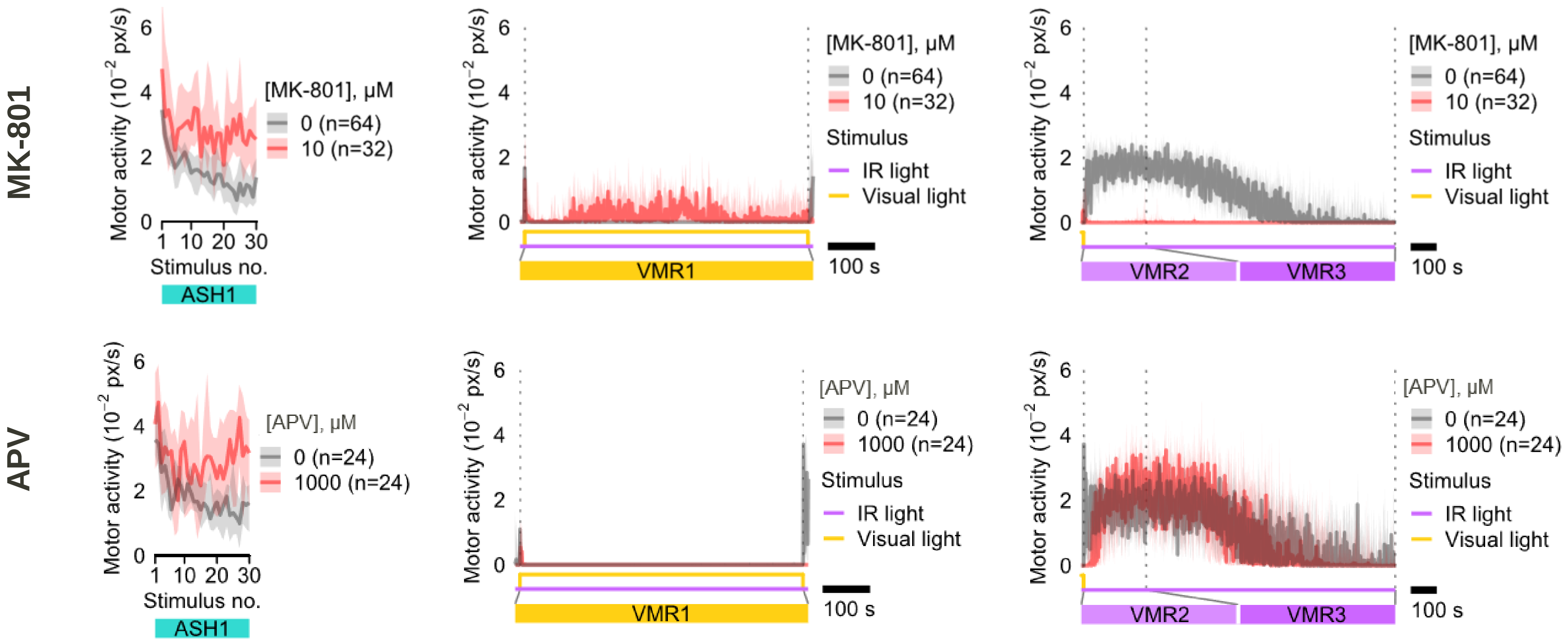
David Leuthold



MK-801 vs APV

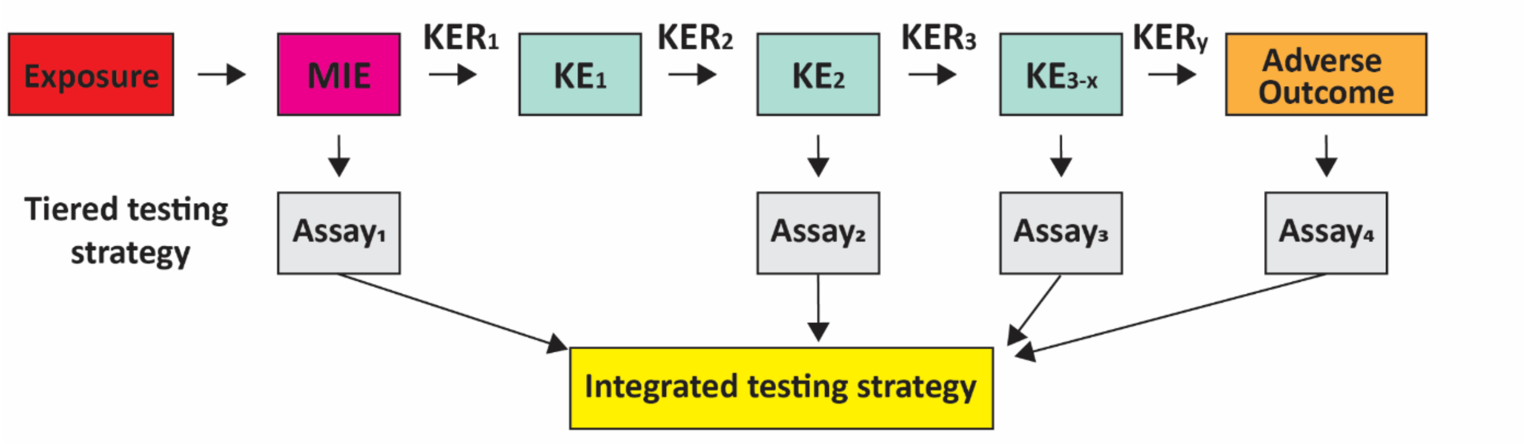
Two NMDA receptor antagonists yield different behavior patterns

David Leuthold



How can we build confidence in NAMs?

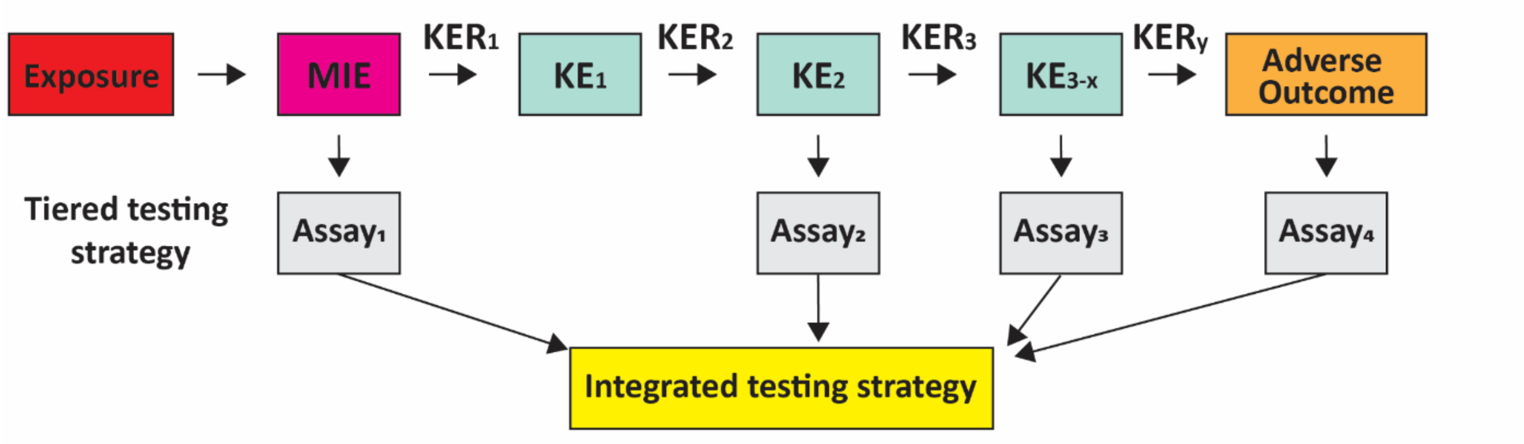
Anchored to AOPs, NAMs are the building blocks of Integrated Approaches to Testing and Assessment



$Assay_1 + Assay_2 + Assay_3 + Assay_x \overset{?}{\geq}$ OECD rat DNT test

MIE: Molecular Initiating Effect
KE: Key Event
KER: Key Event Relationship
AO: Adverse Outcome
AOP: Adverse Outcome Pathway

Key event <u>essentiality</u> represents a novel strategy to build confidence in NAMs



1. Is there a <u>causal link</u> between exposure, key events, and adverse outcomes?
2. Are key events conserved in humans?

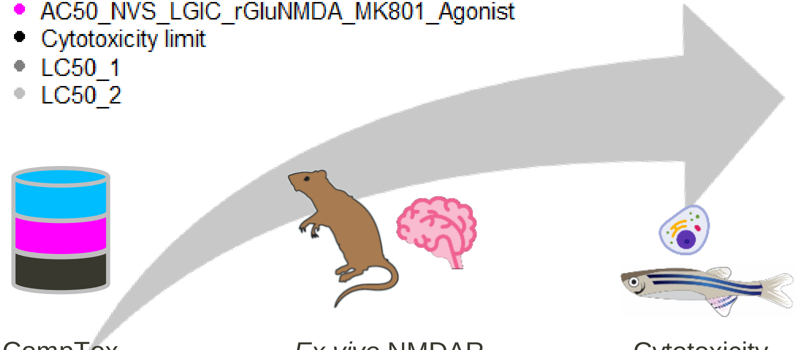
MIE: Molecular Initiating Effect
KE: Key Event
KER: Key Event Relationship
AO: Adverse Outcome
AOP: Adverse Outcome Pathway

Key event essentiality case study

Can we identify novel disruptors of habituation learning?

David Leuthold

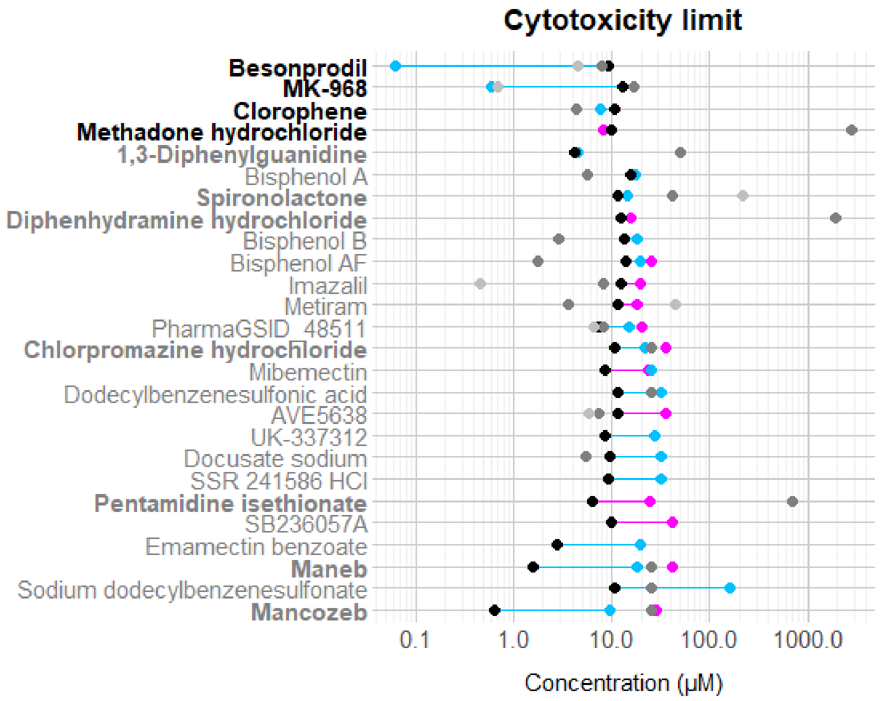
- AC50_NVS_LGIC_rGluNMDA_Agonist
- AC50_NVS_LGIC_rGluNMDA_MK801_Agonist
- Cytotoxicity limit
- LC50_1
- LC50_2



CompTox Chemicals Dashboard

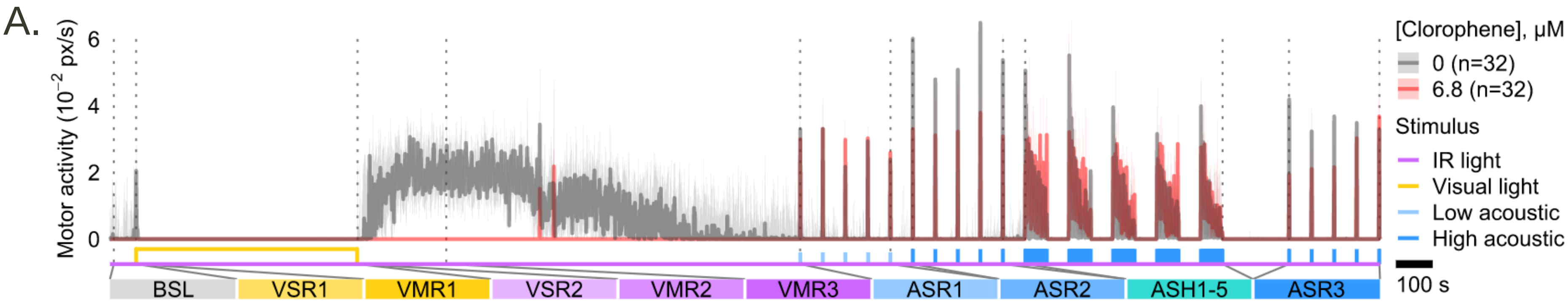
Ex vivo NMDAR ligand binding assays

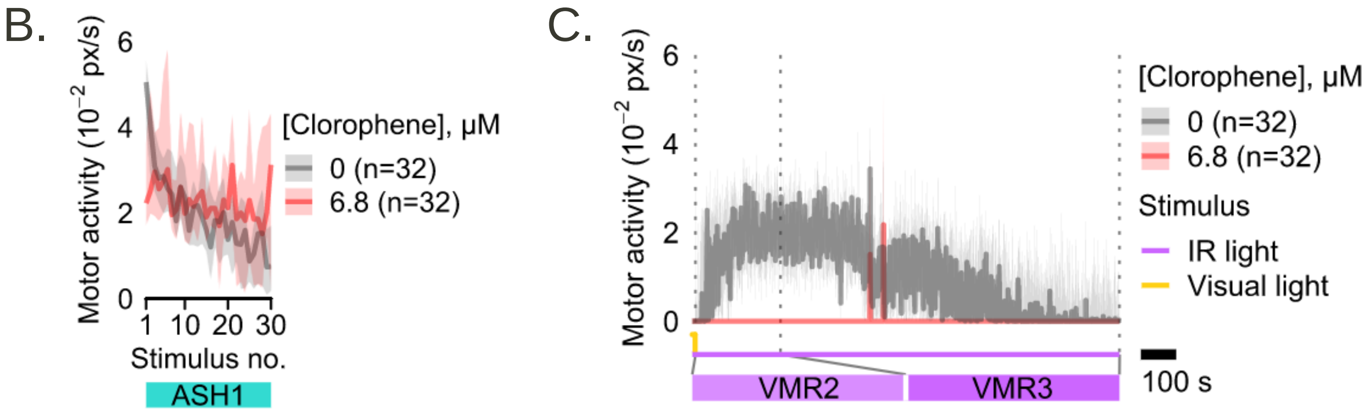
Cytotoxicity-, $LC50_{Fish}$-based selection



Unpredicted effects following exposure to clorophene
Multiple mechanisms?

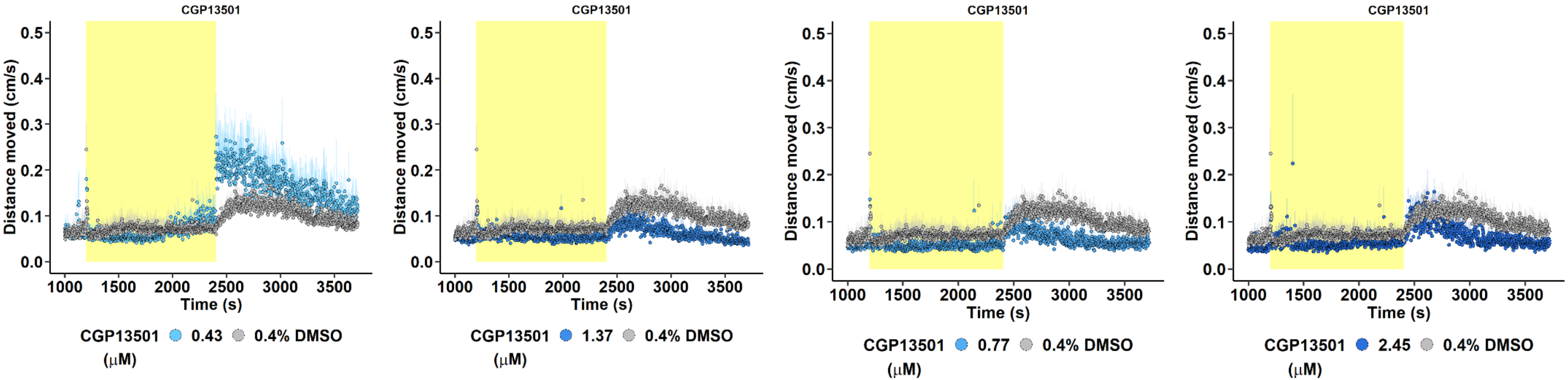
Nadia Herold
David Leuthold





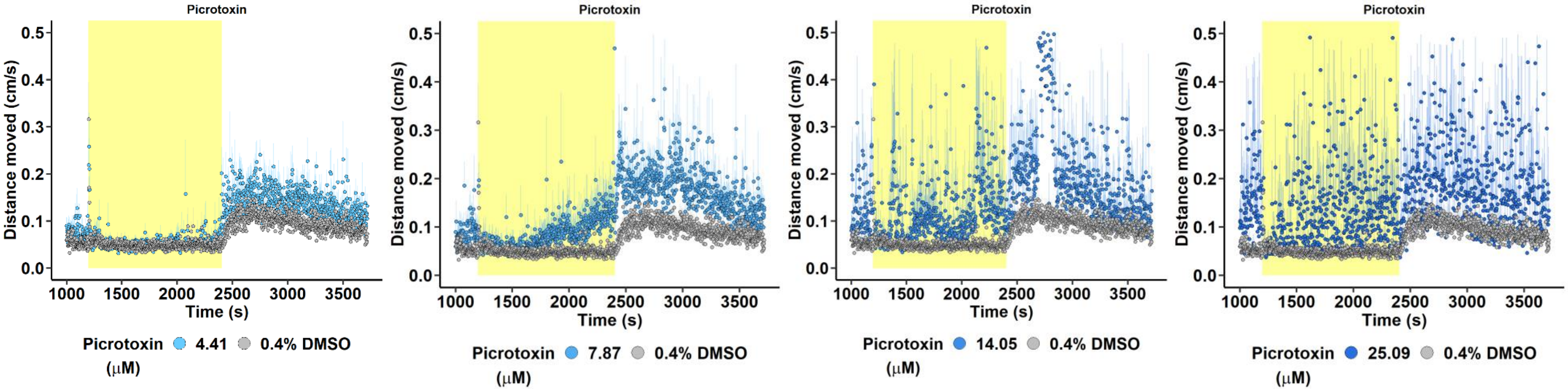
GABA$_B$ receptor agonism (CGP13501) modulates dark phase hyperactivity

Gabriel de Macedo



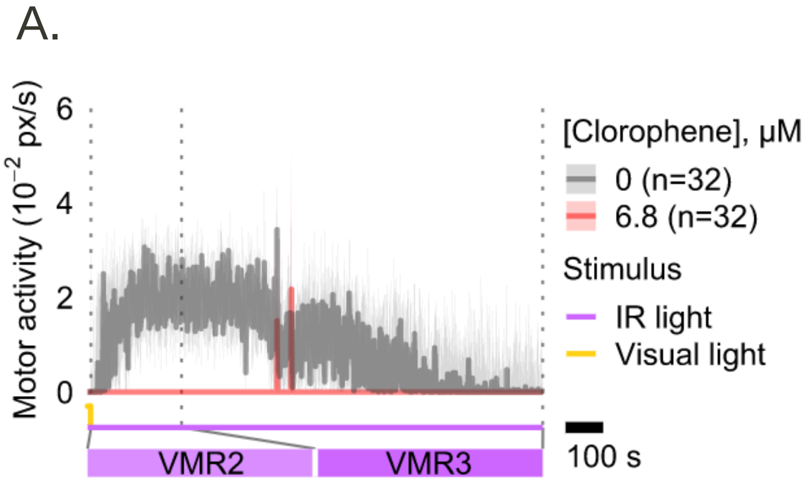
GABA$_A$ receptor antagonism (picrotoxin) causes dark phase hyperactivity

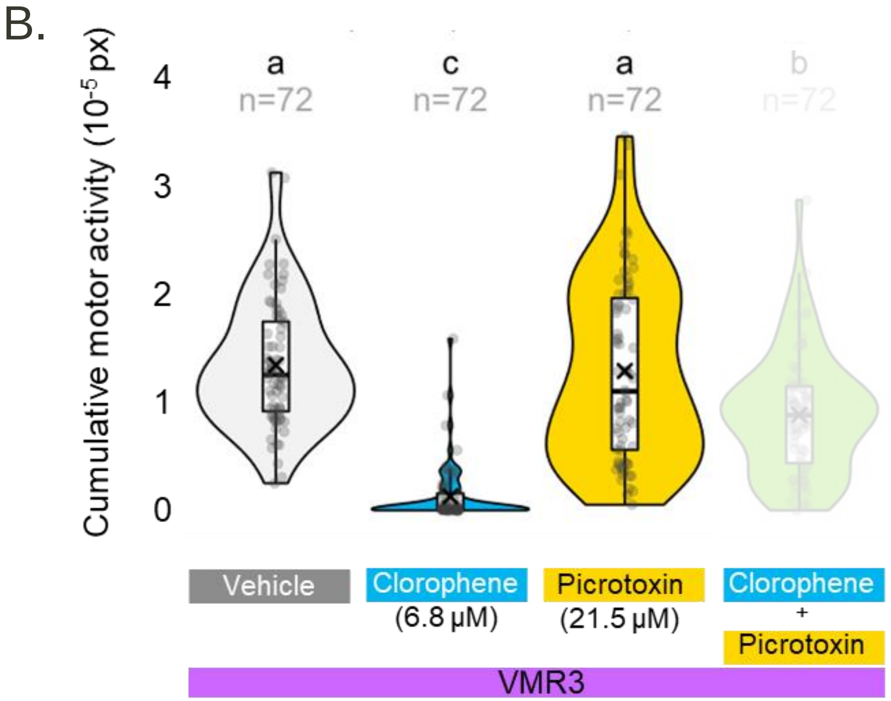
Gabriel de Macedo



Does clorophene exposure trigger GABA receptor-dependent VMR hypoactivity?

David Leuthold

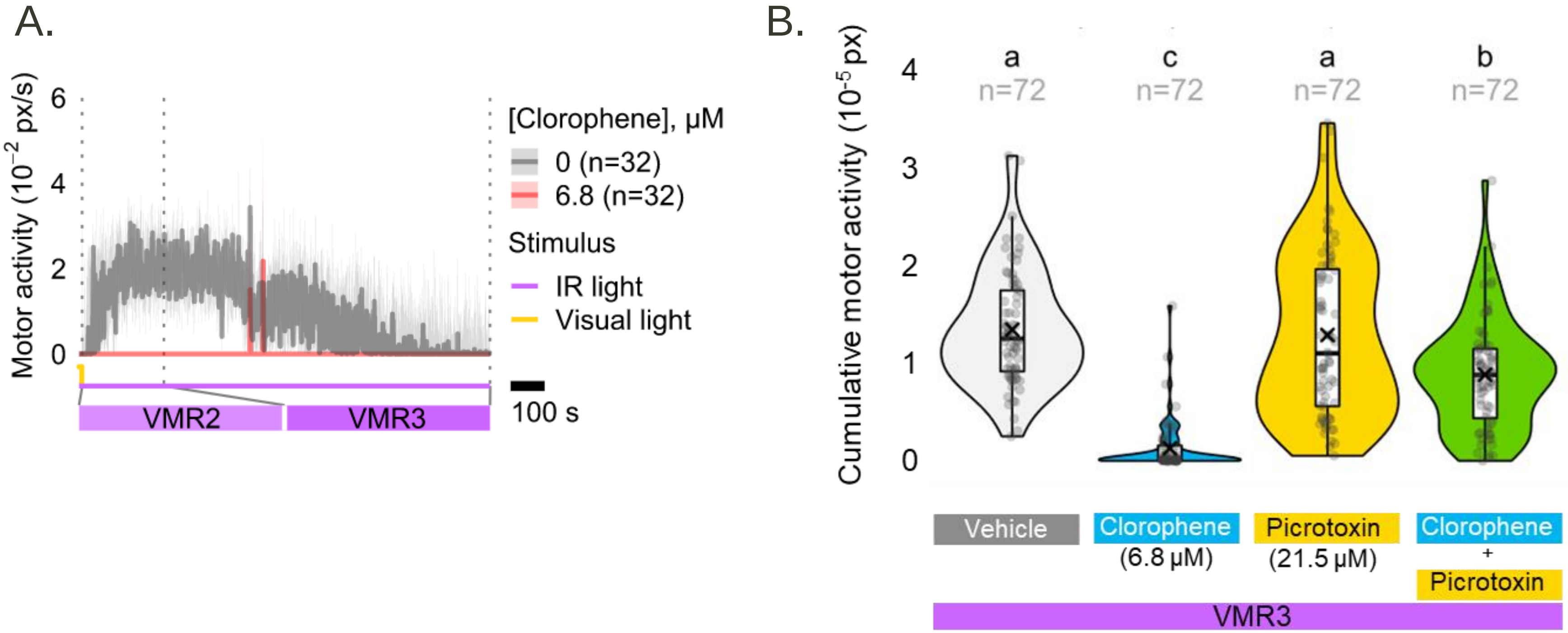




Agonism of GABA receptor by clorophene causes VMR hypoactivity

Mode of action 1

David Leuthold

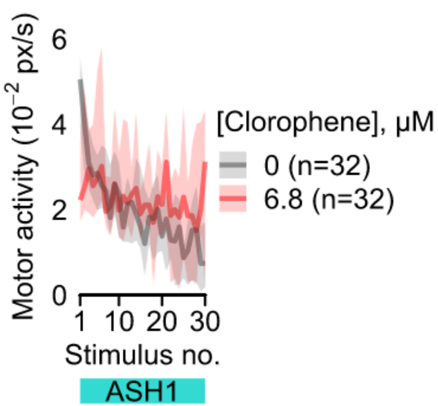


Clorophene impairs learning

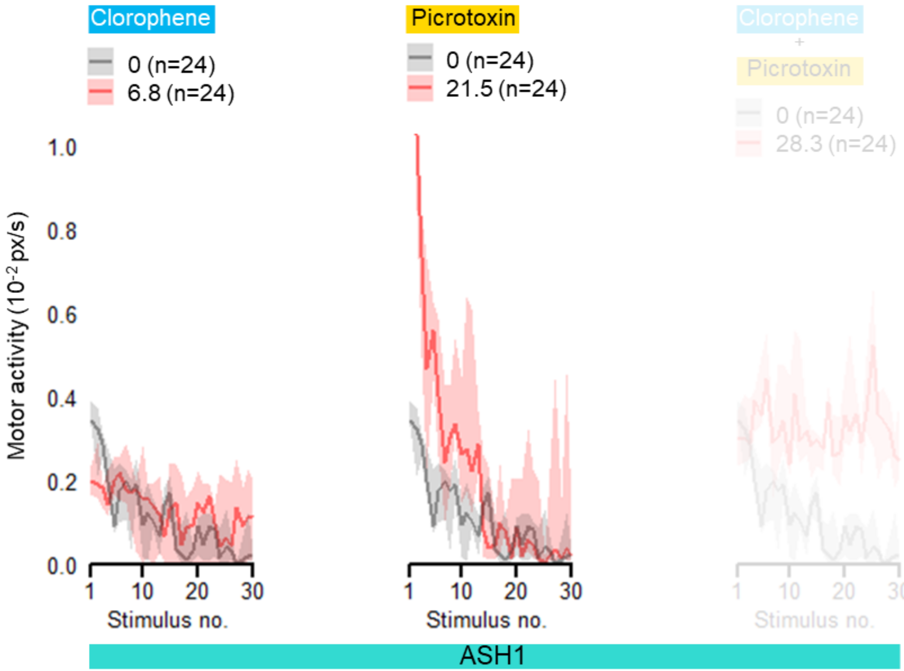
Mode of action 2

David Leuthold

A.

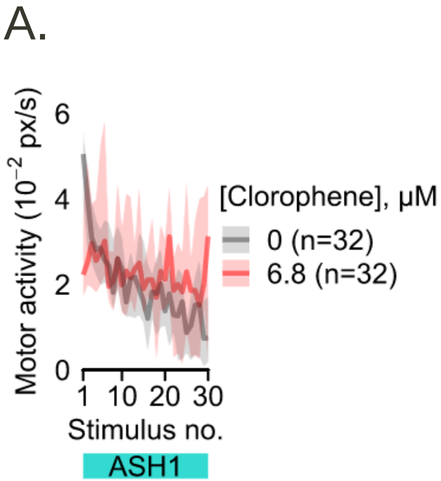


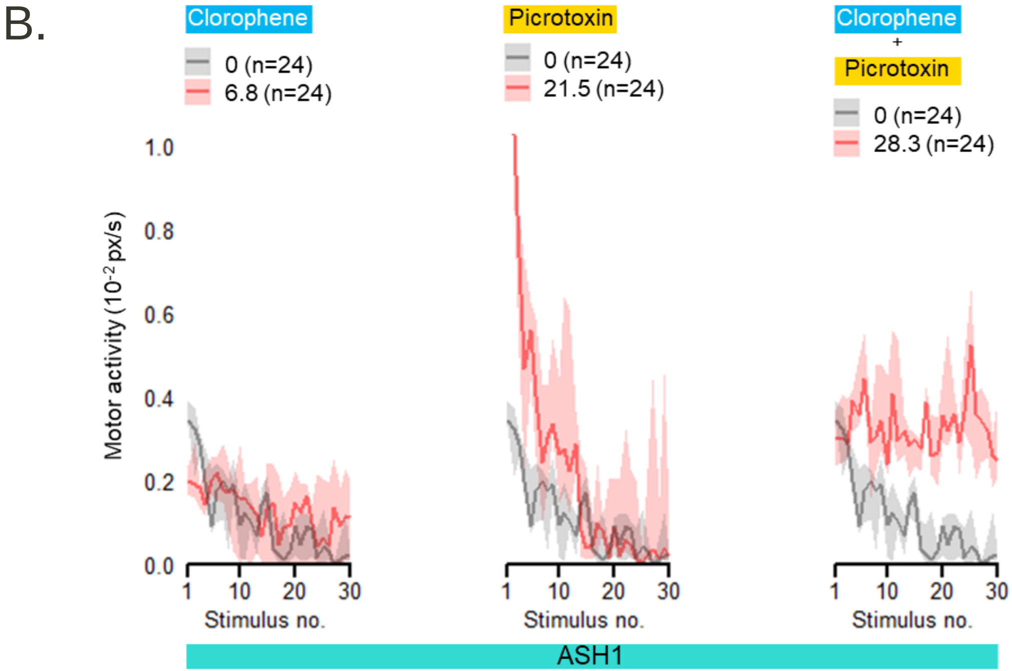
B.



Clorophene impairs learning
Multiple modes-of-action

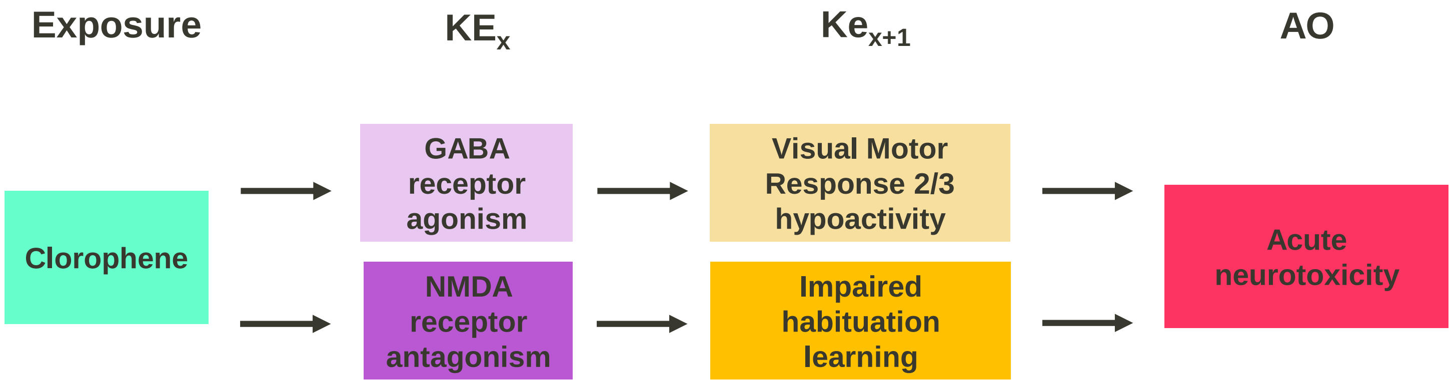
David Leuthold





Novel AOPs for acute neurotoxicity

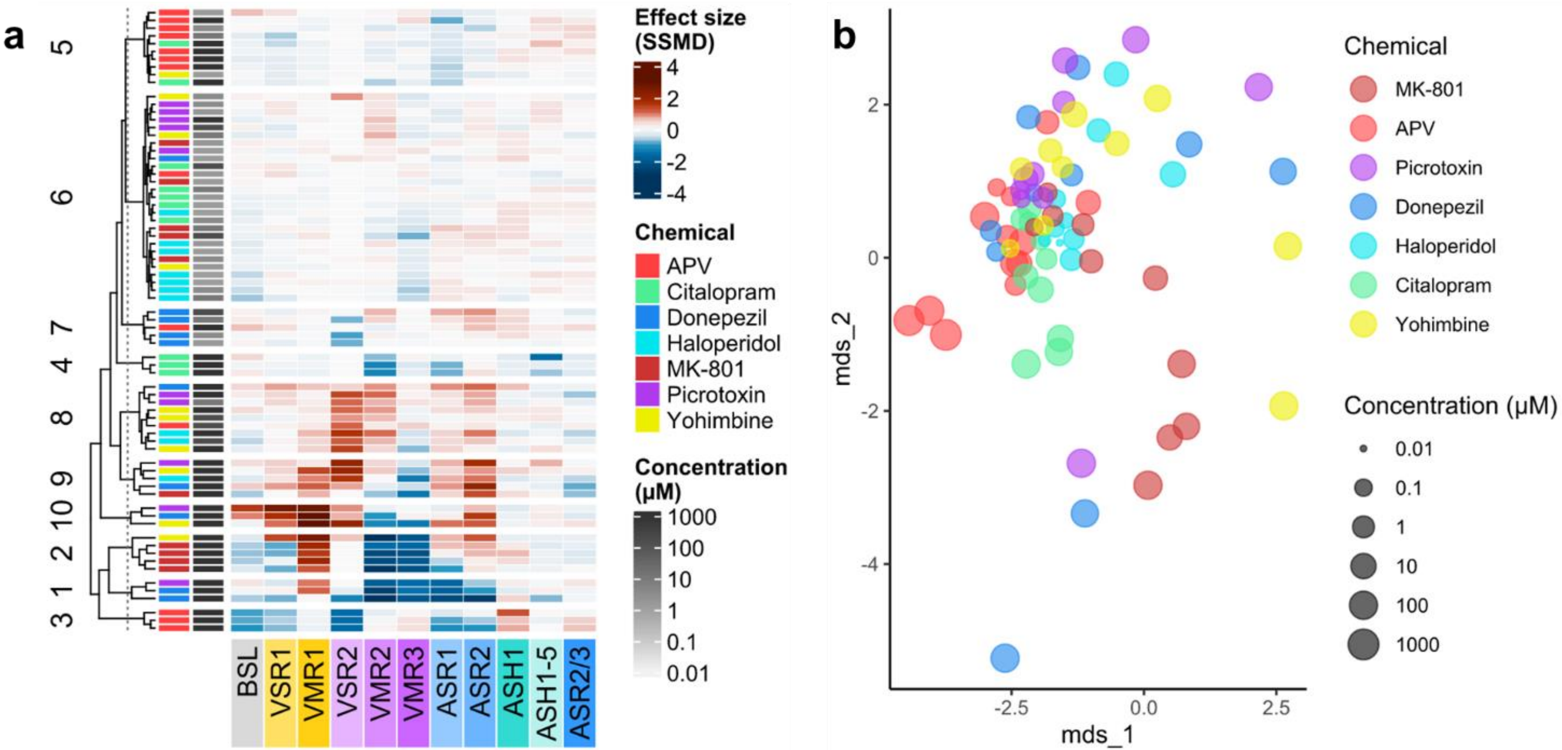
Nadia Herold
David Leuthold



Looking ahead: Acute neurotoxicity fingerprints

Classify hits and identify potential mode of action

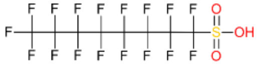
Nadia Herold
David Leuthold



What about developmental neurotoxicity?

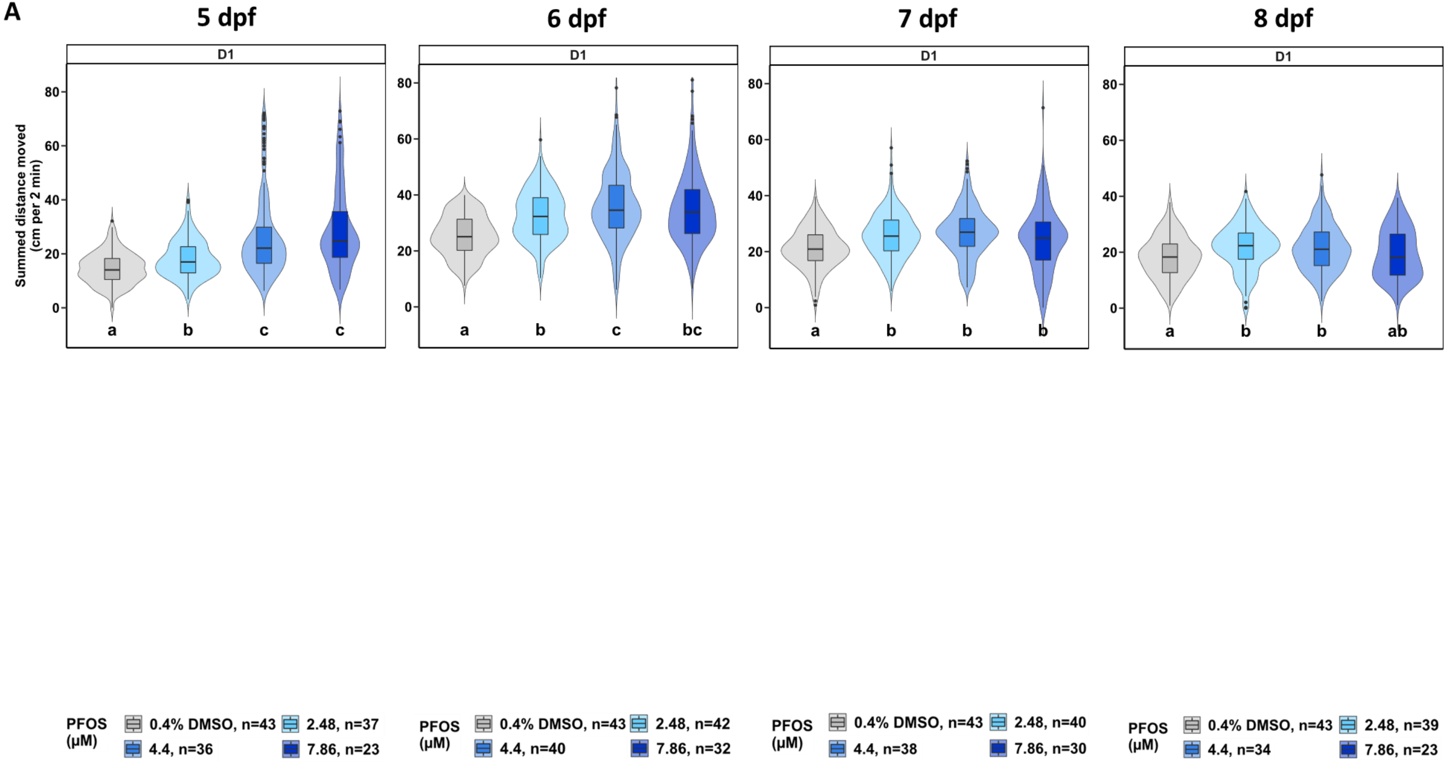
Chemical washout revealed distinct behavior phenotypes

Sebastian Gutsfeld
Ifeoluwa Omoyeni



Dark-phase hyperactivity

Visual startle response hyperactivity



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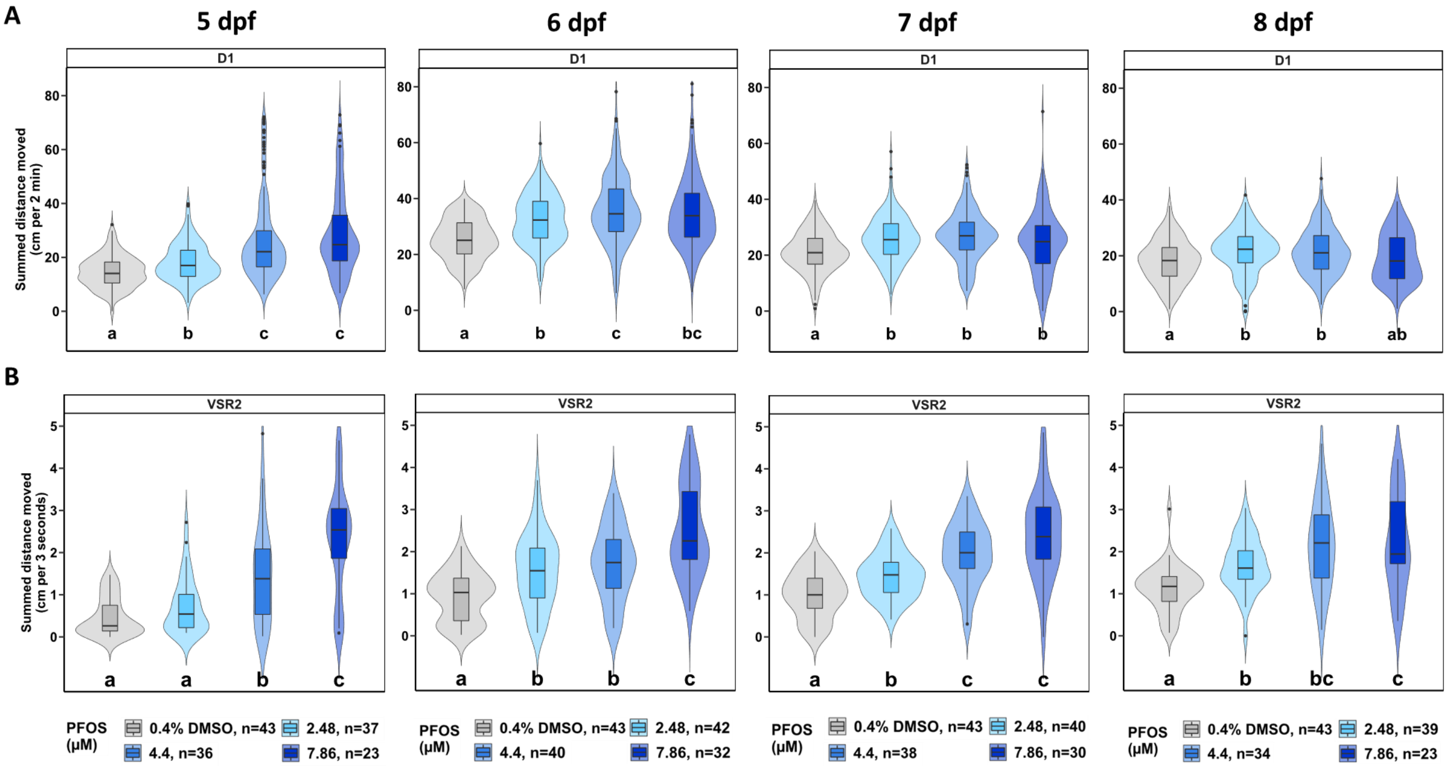
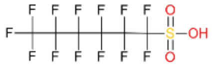


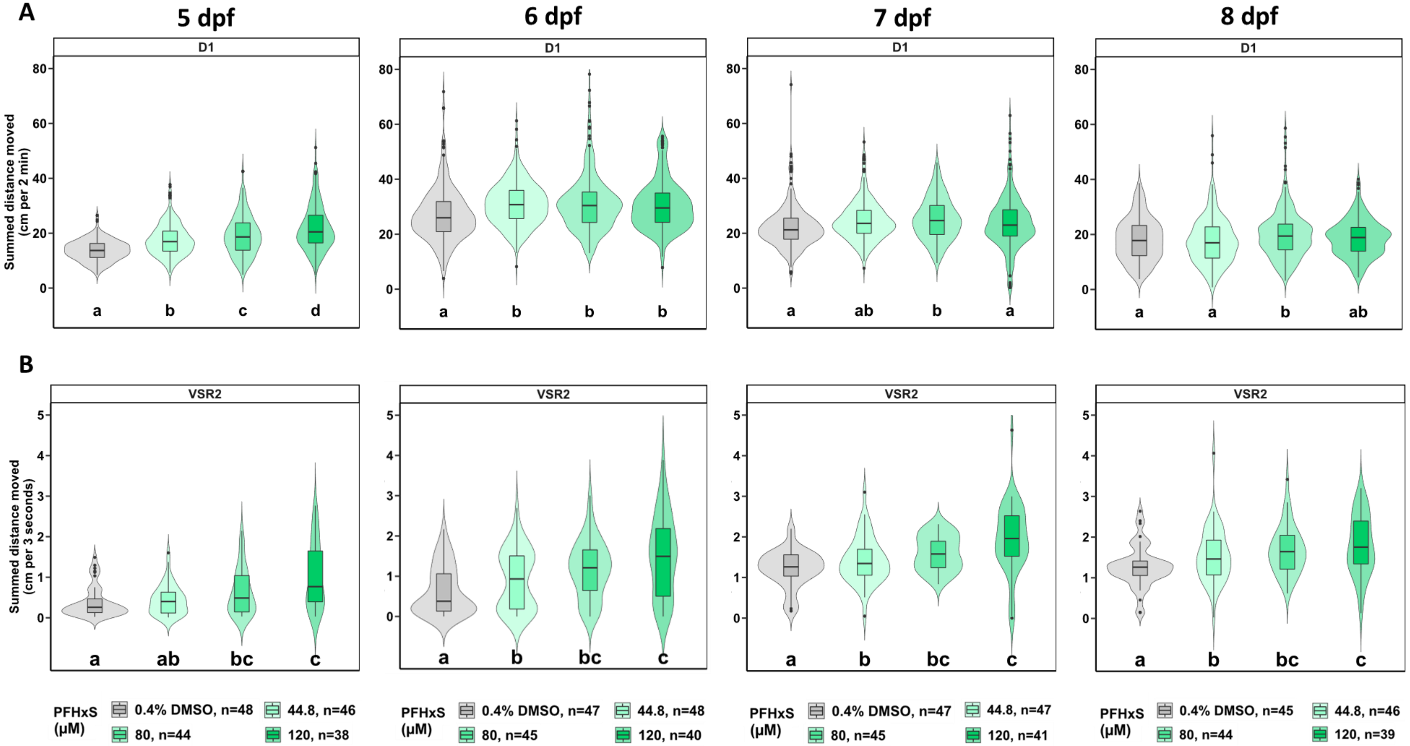
Exposure to structurally similar PFAS elicit same two phenotypes

Sebastian Gutsfeld
Ifeoluwa Omoyeni

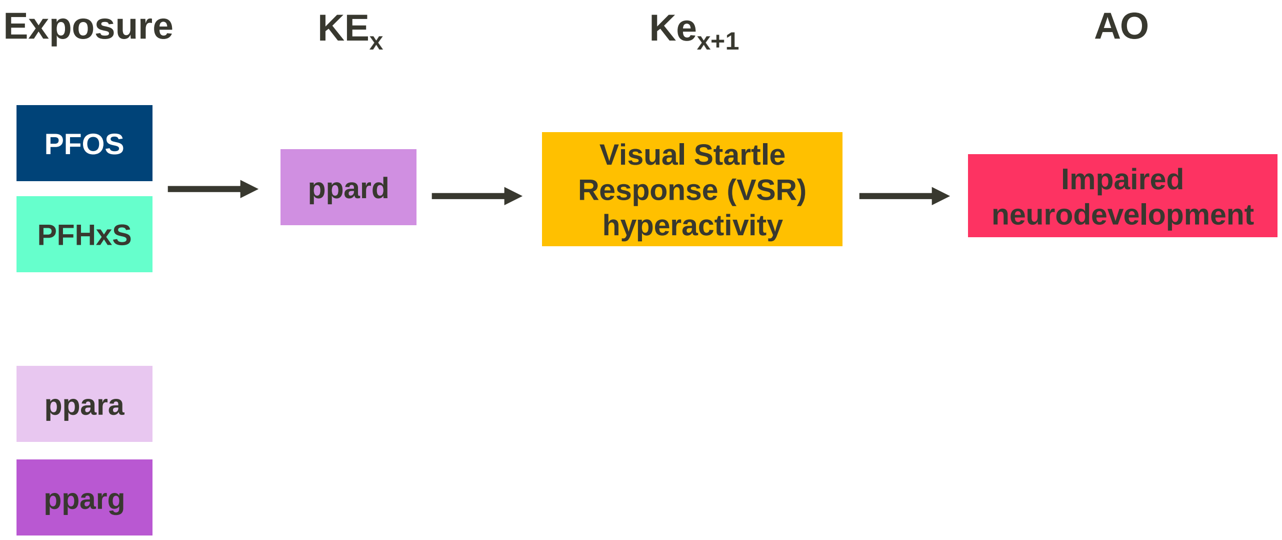


Dark-phase hyperactivity

Visual startle response hyperactivity



Novel AOP for developmental neurotoxicity



Summary

Building confidence in zebrafish DNT/ANT NAMs

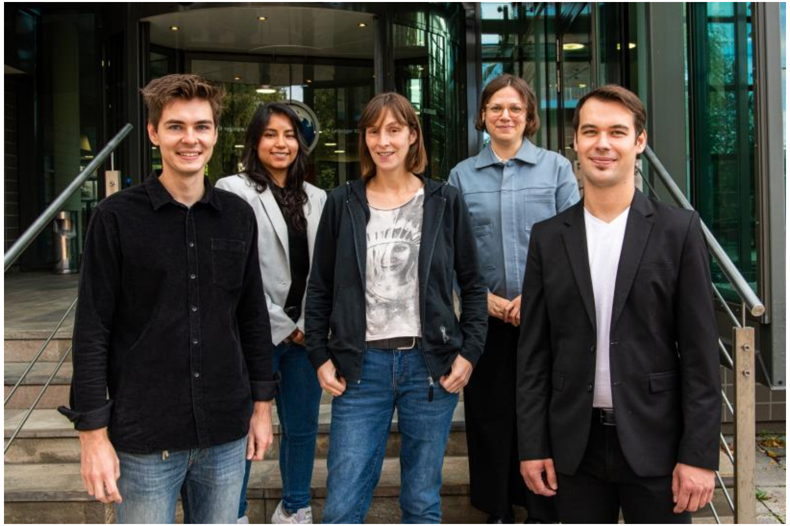
- Built an inexpensive screening tool in a translational model, relevant for human and ecological safety assessments

- Our work builds confidence in zebrafish NAM to identify chemicals with the potential to harm the developing nervous system:

1. Expands functionality aligns with OECD TG 426 endpoints
2. Enhances phenotypic resolution to identify putative mode-of-action (conserved in humans)
3. Applies pharmacological manipulation and gene editing to demonstrate causal relationships between key events and adverse outcomes

Our team

Molecular Tox Group



UFZ Molecular Toxicology Group
Gabriel de Macedo
Sebastian Gutsfeld
Nadia Herold
David Leuthold
Ifeoluwa Omoyeni
Nicole Schweiger
Tamara Tal

UFZ BIOTOX
Nils Klüver
Stefan Scholz
Wibke Busch

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