Novel approaches using the microelectrode array to measure network function in larval zebrafish

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Disclaimer

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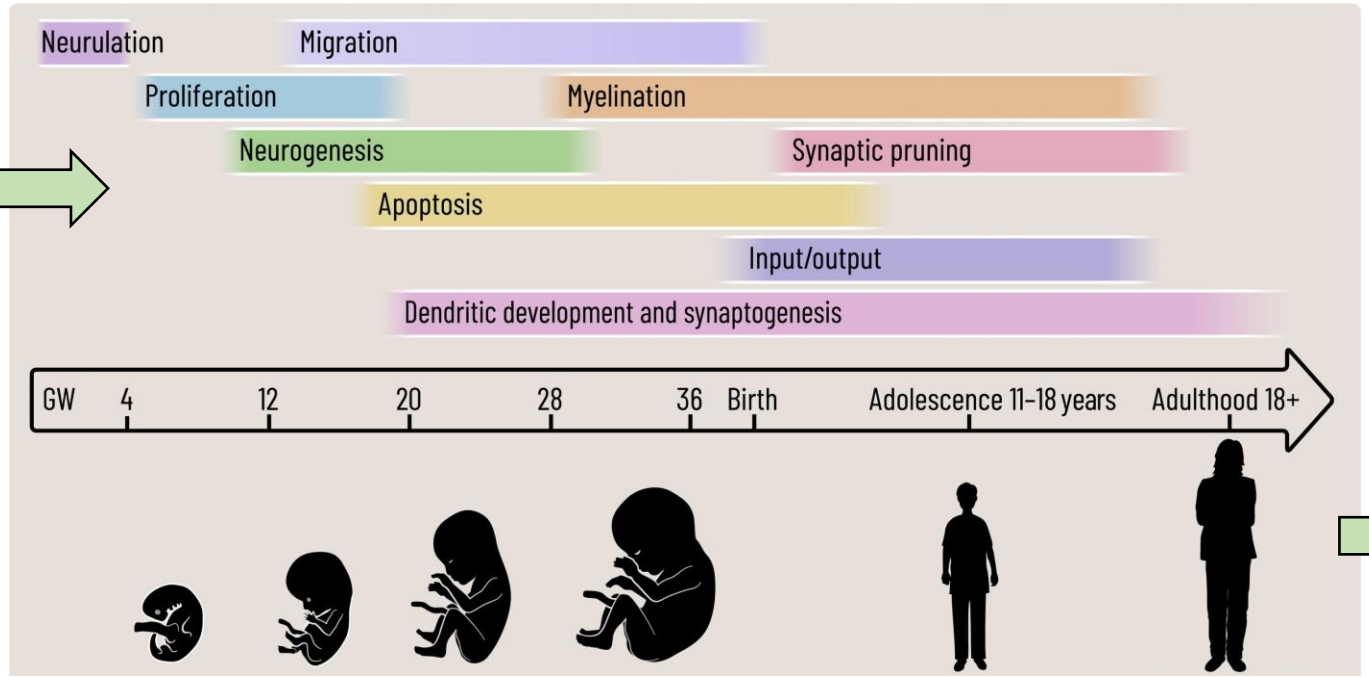
Developmental neurotoxicity (DNT) testing

- **Developmental neurotoxicity (DNT)** refers to any adverse outcome on the normal development of the nervous system structures and/or functions that results from exposure to a substance (US EPA, 1998)
  - To protect the developing brain from potentially hazardous chemicals, DNT testing guidelines were established by the US Environmental Protection Agency (EPA) and the Organization for Economic Co-operation and Development (OECD).
  - However, these guideline DNT studies use large numbers of animals, are costly, and evaluate one chemical at a time.

Chemical exposure

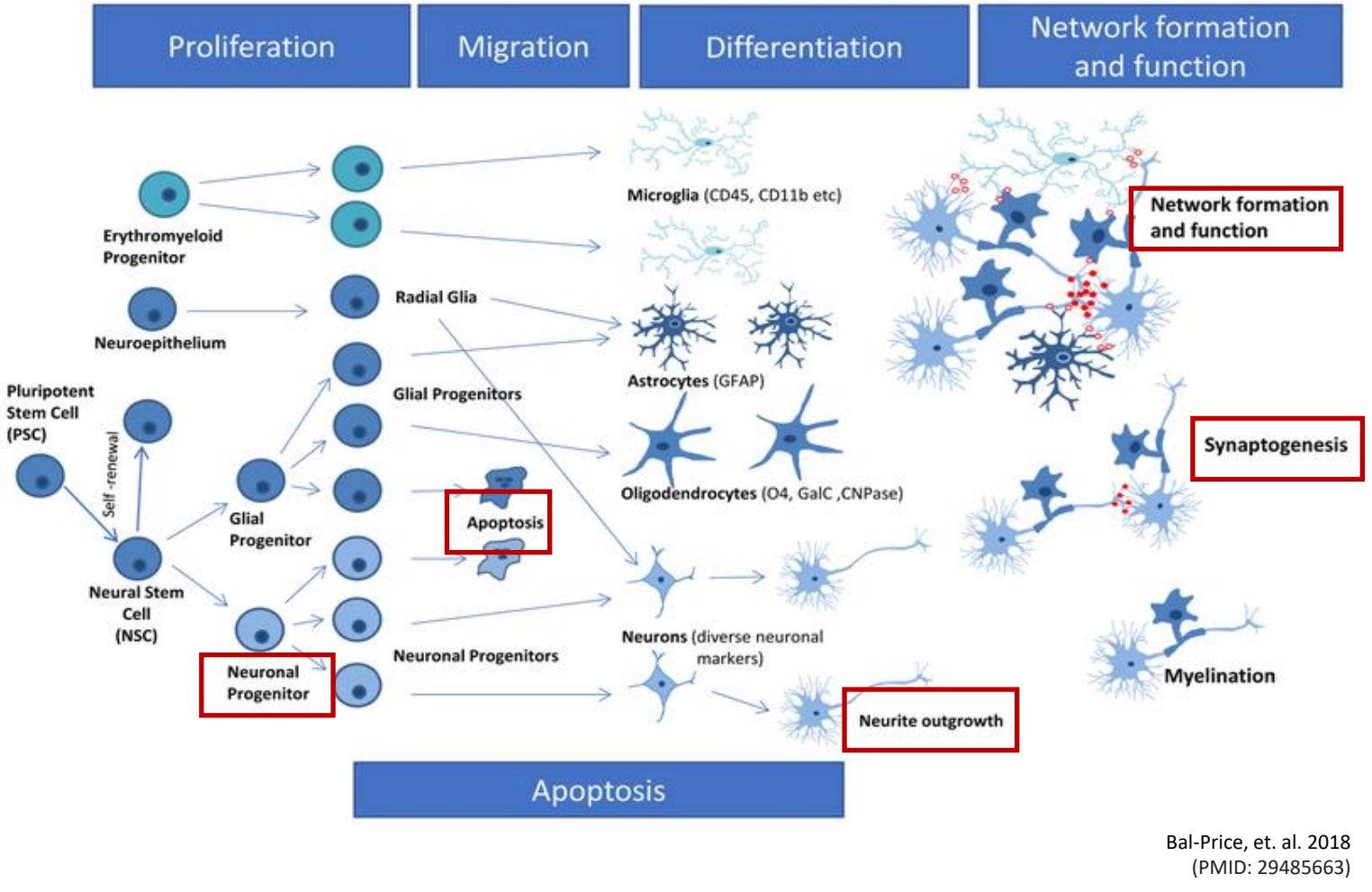
86,000 registered chemicals



Adverse neurodevelopmental Outcomes: Long-lasting changes in the brain and on behavior

Chini and Hanganu-Opatz, 2021
(PMID: 33246578)

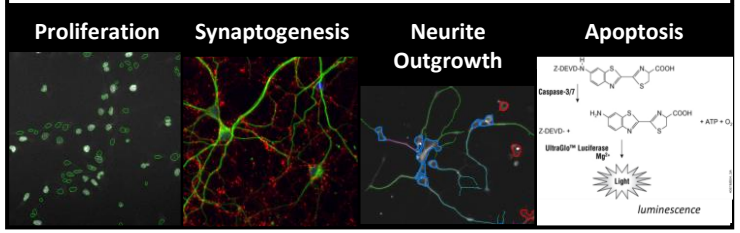
Developmental neurotoxicity (DNT) new approach methodologies (NAMs)



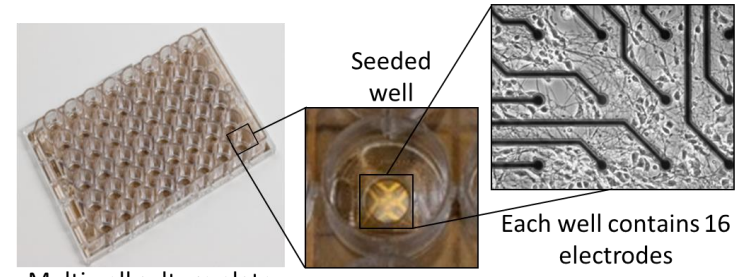
DNT *in vitro* battery

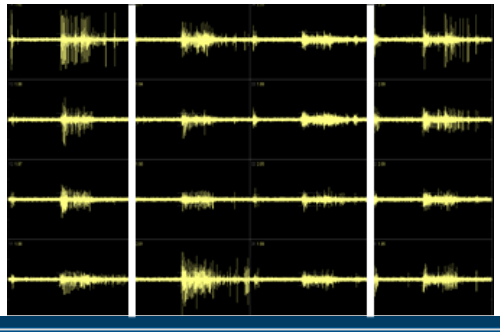
Bal-Price, et. al. 2018
(PMID: 29485663)

High-content imaging

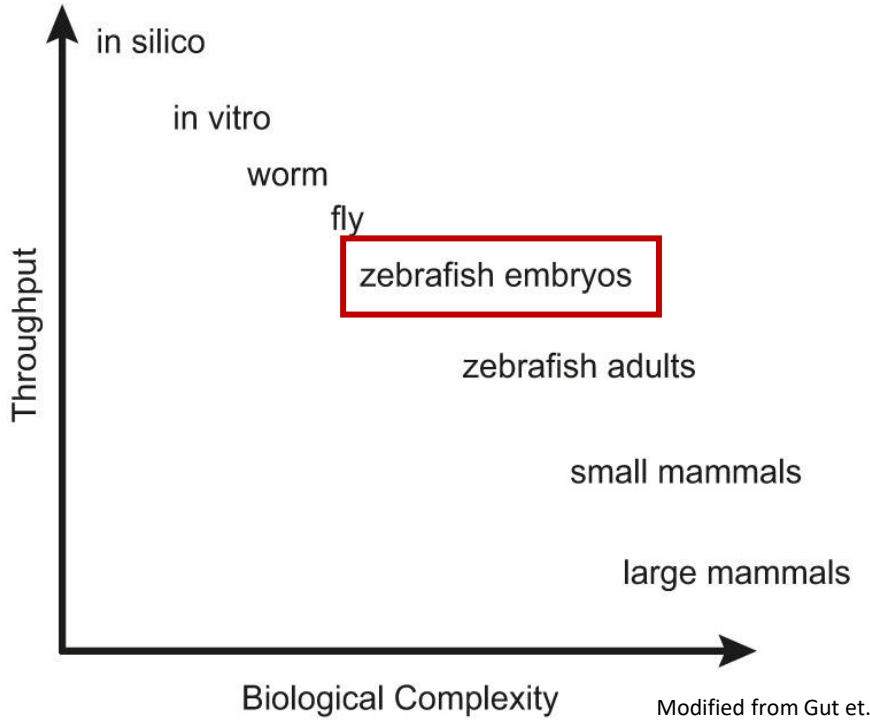


The network formation assay

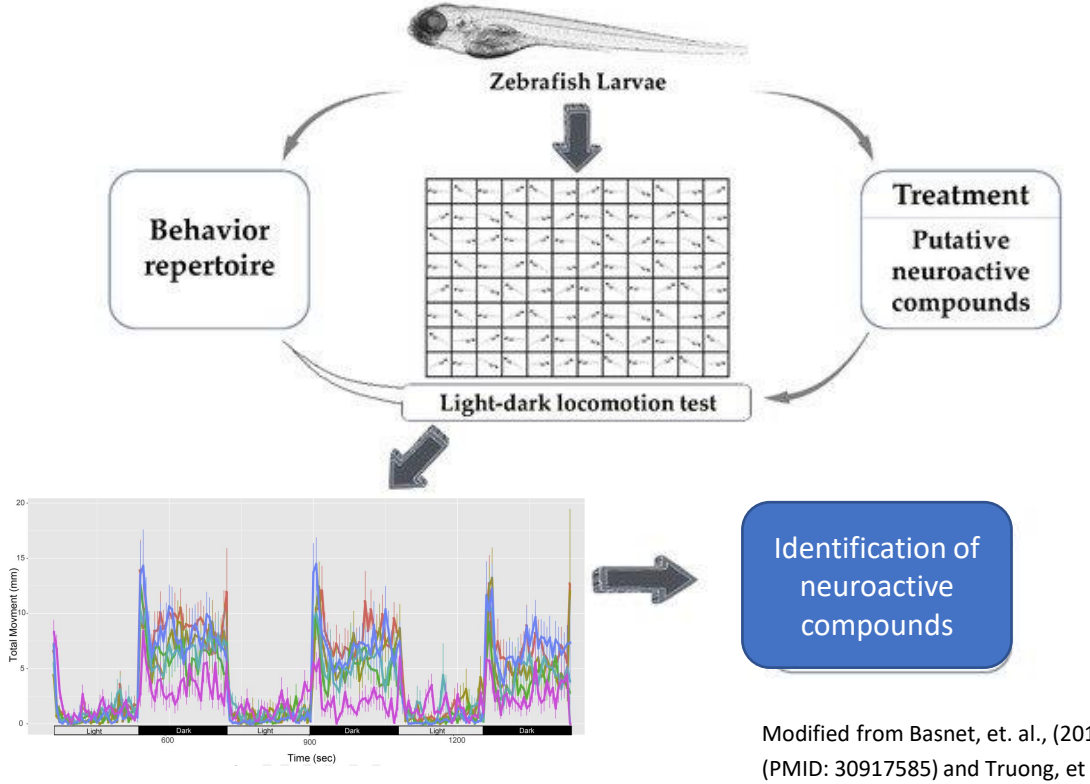




Zebrafish have emerged as an alternative species model for the *in vivo* assessment of DNT.



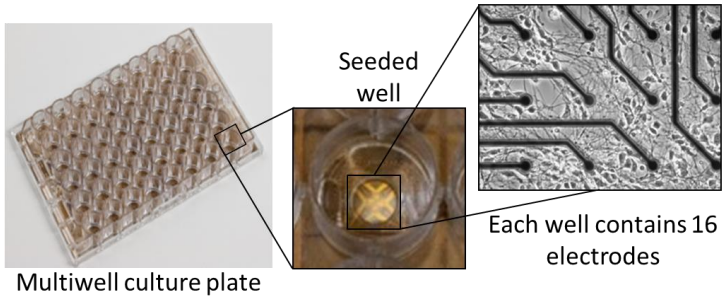
Modified from Gut et. al., (2017) (PMID: 28468832)

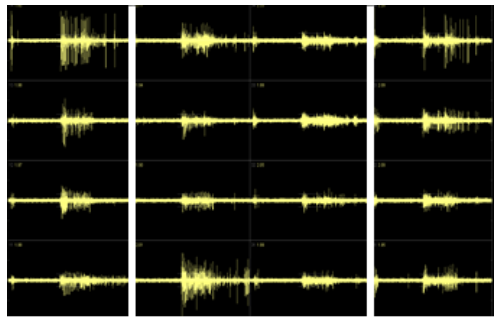


Modified from Basnet, et. al., (2019) (PMID: 30917585) and Truong, et al., (2016) (PMID: 27593350)

How do we bridge the gap between in vitro and in vivo DNT screening methods?

The network formation assay





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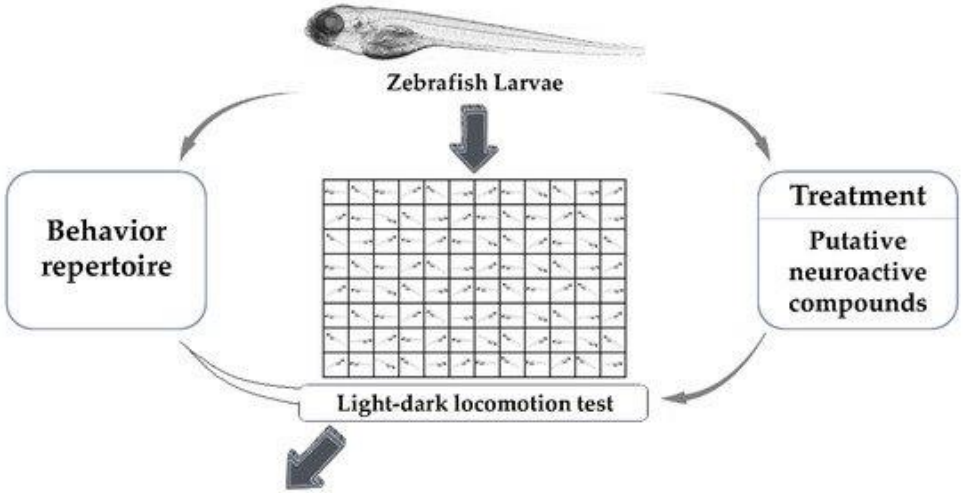
Pathfinder Innovation Projects

The larval zebrafish light-dark behavioral assay

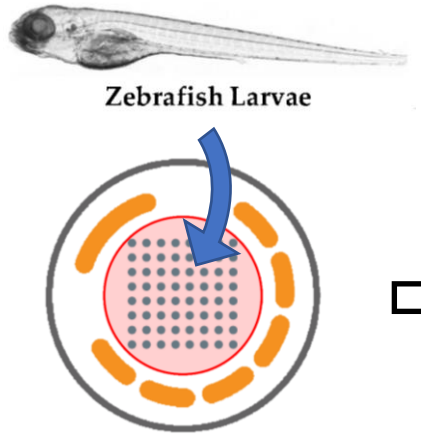


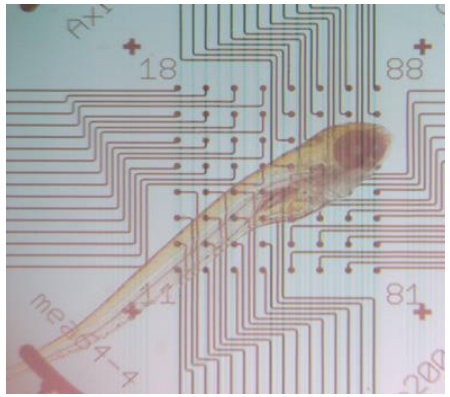
Identification of neuroactive compounds

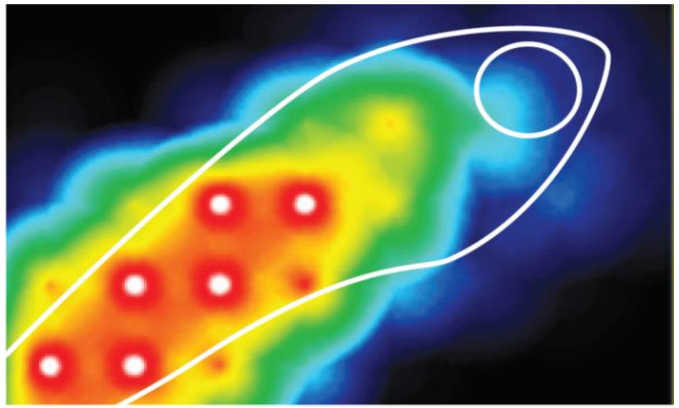
Modified from Basnet, et. al., (2019) (PMID: 30917585) and Truong, et al., (2016) (PMID: 27593350)

“Fish on a Chip”: Linking network function and behavior

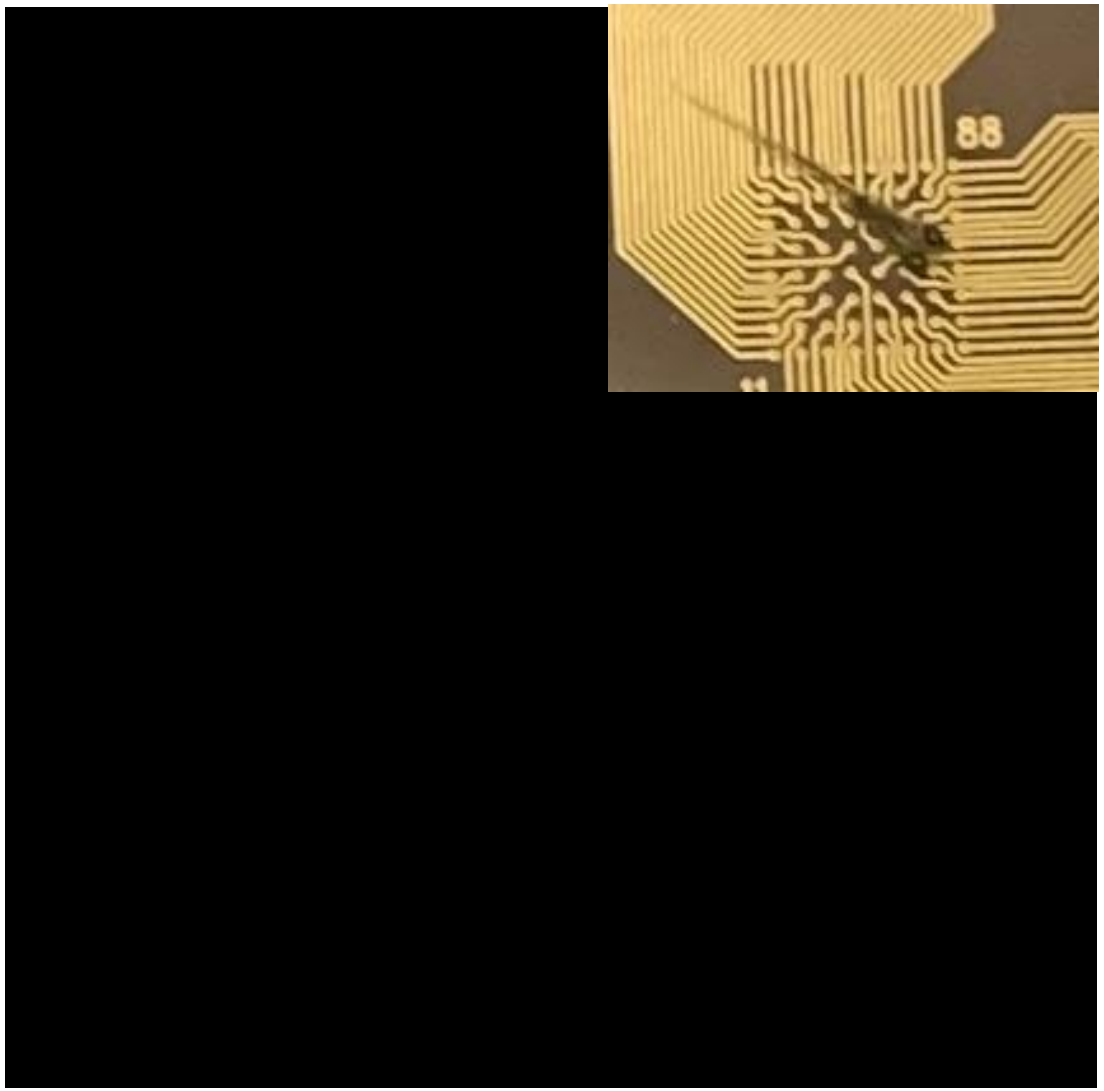
Phase 1: Establish protocol for larval zebrafish MEA assay

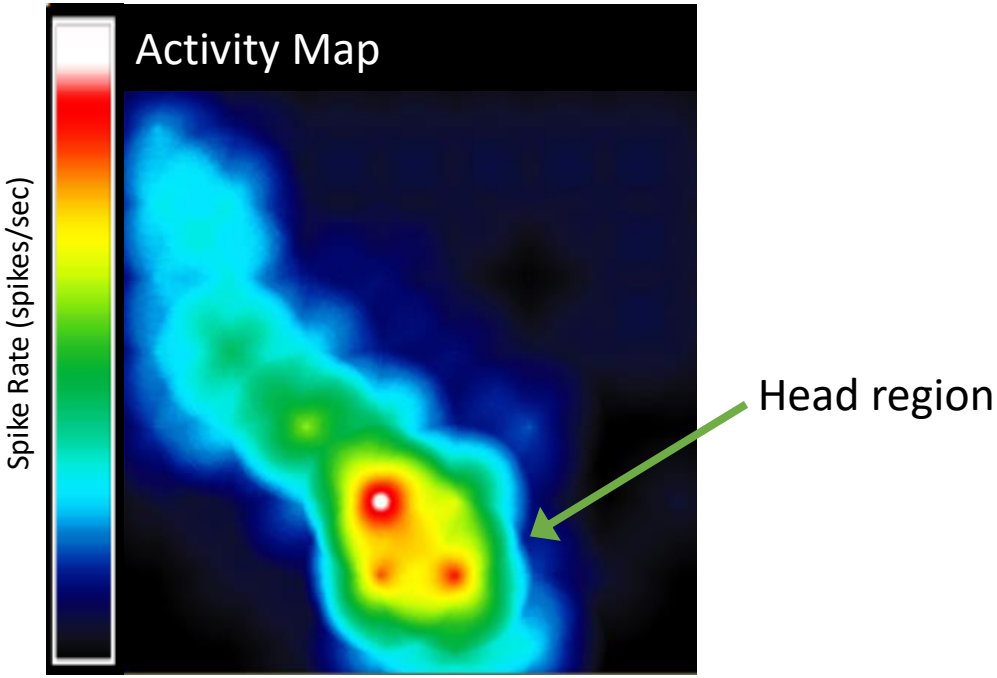




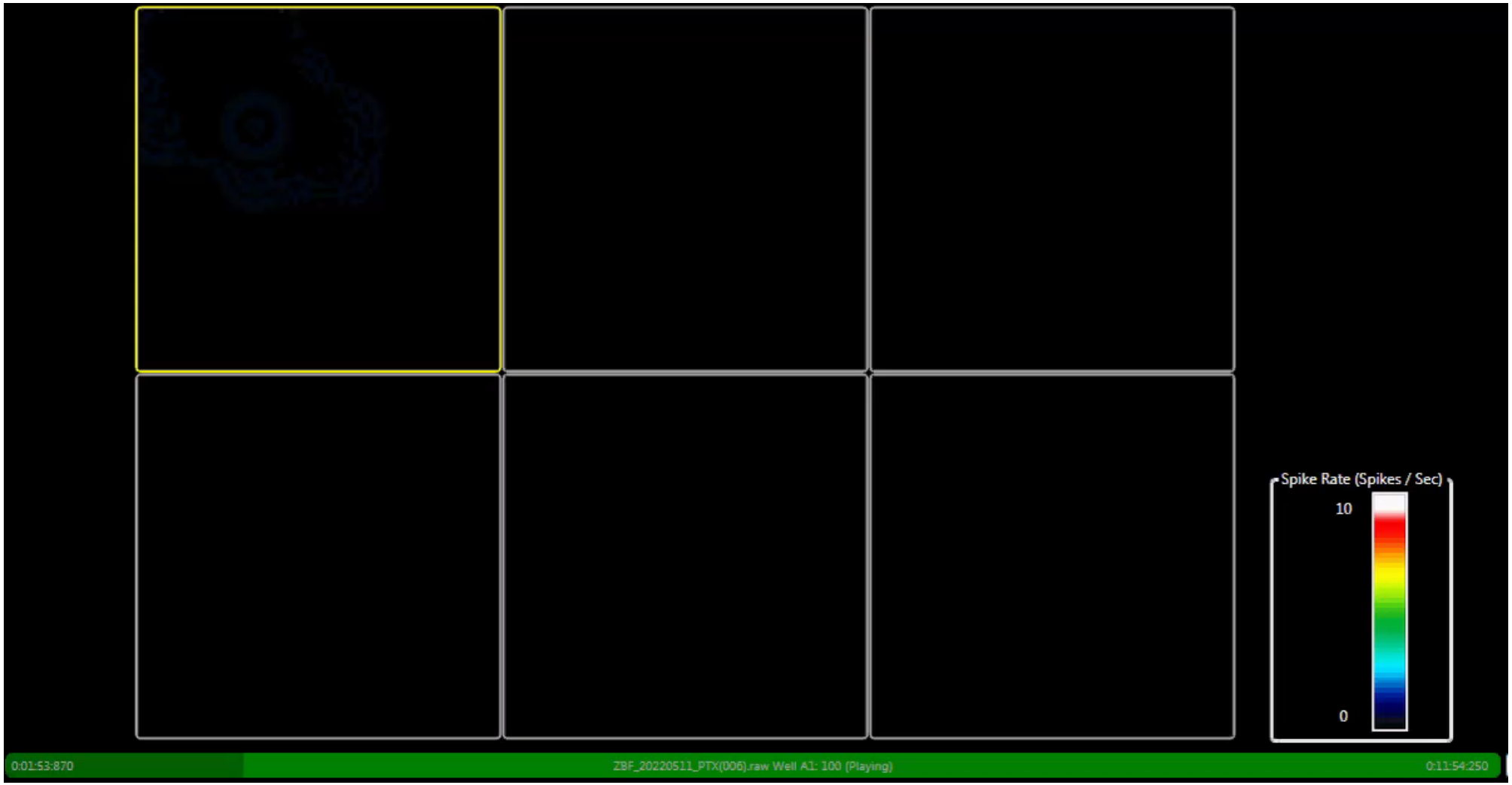


Heatmap from a 5 dpf zebrafish placed on a microelectrode array

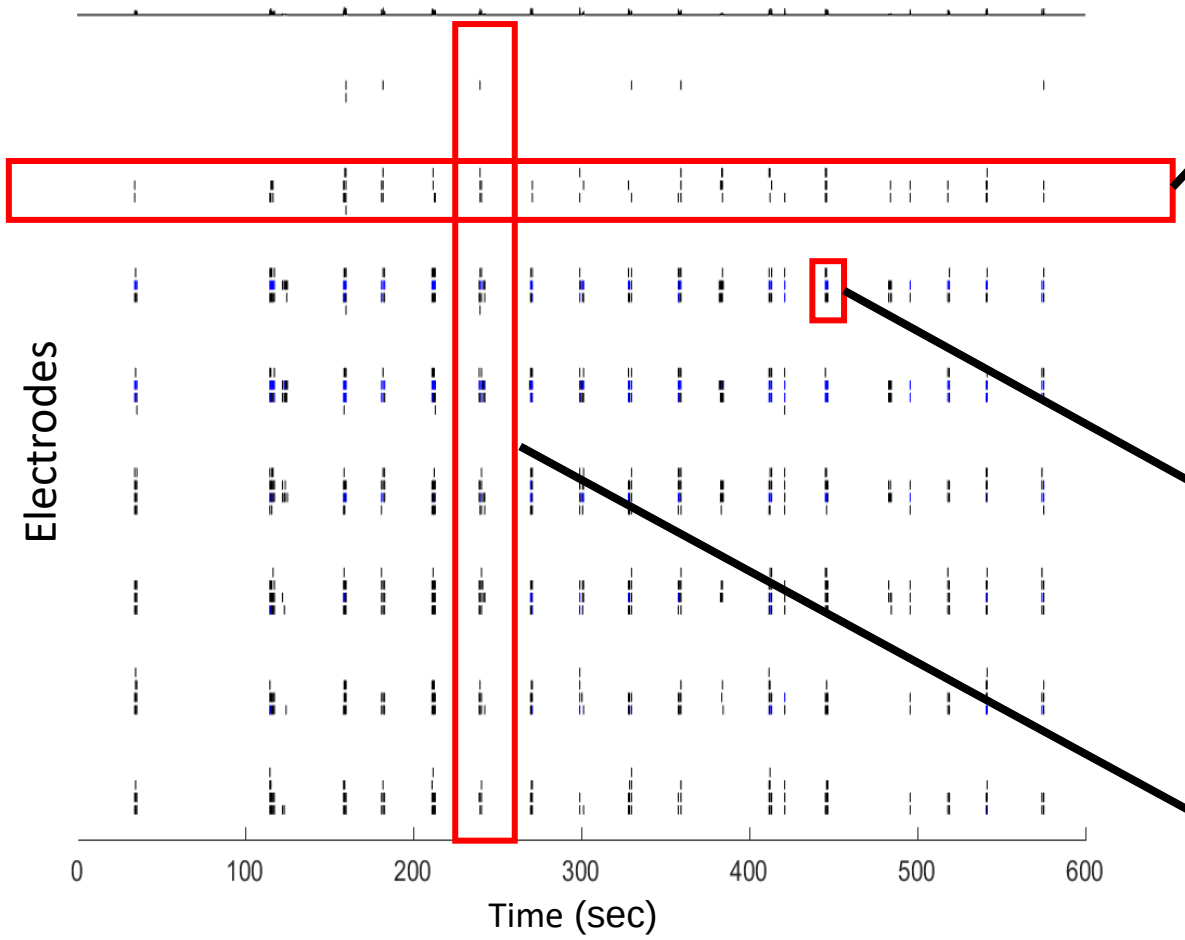




We can record brain activity from multiple zebrafish at a time

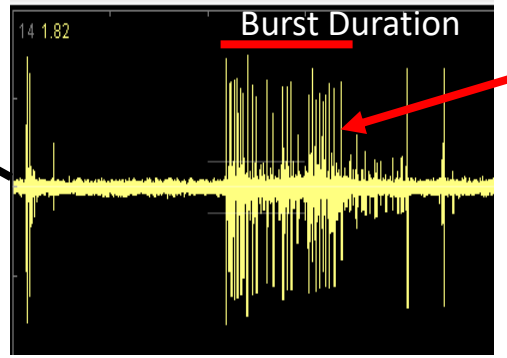


The MEA measures multiple characteristics of network function



General Activity- overall rate of firing or bursting; measured on each electrode and averaged across the well.

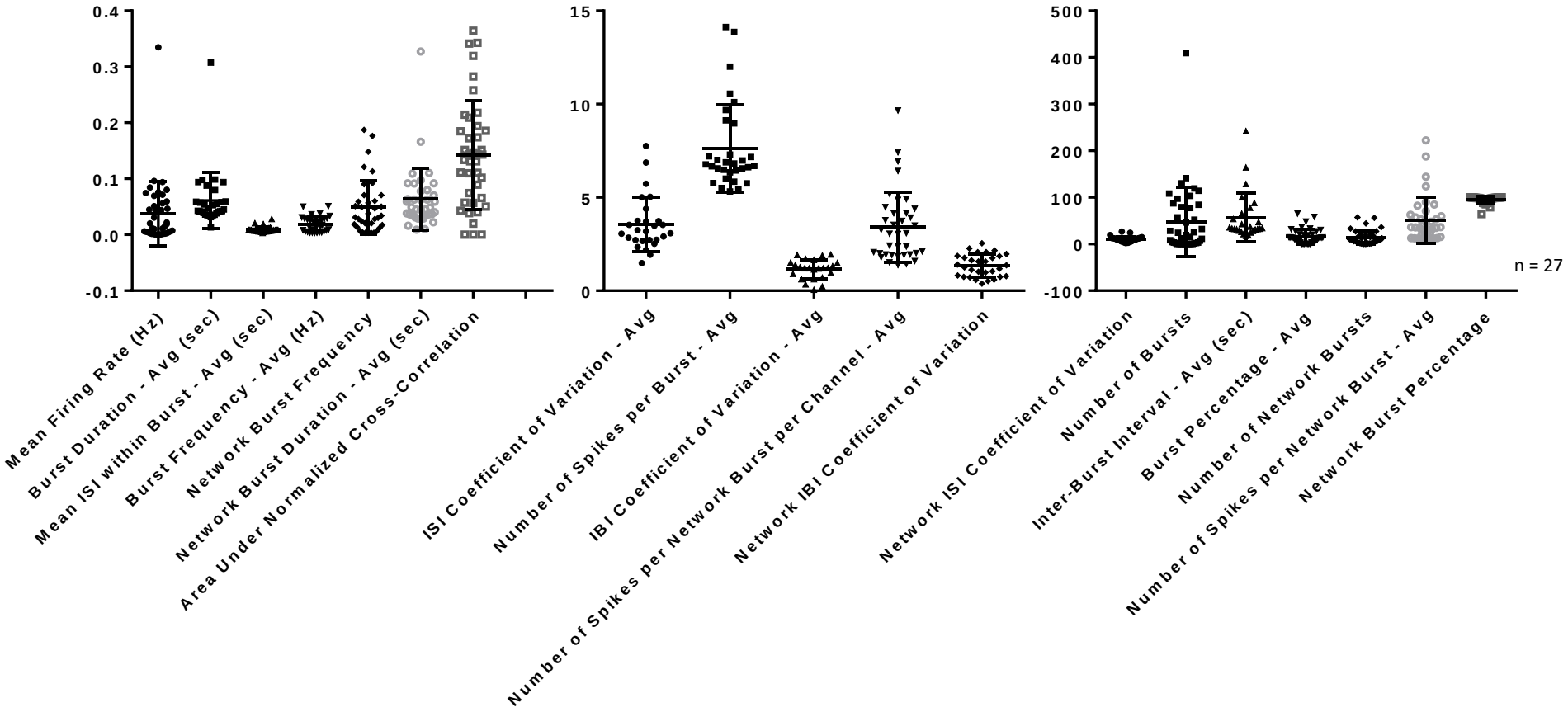
Bursting Structure- the length and number of events in a burst; measured on each electrode and averaged across the well.



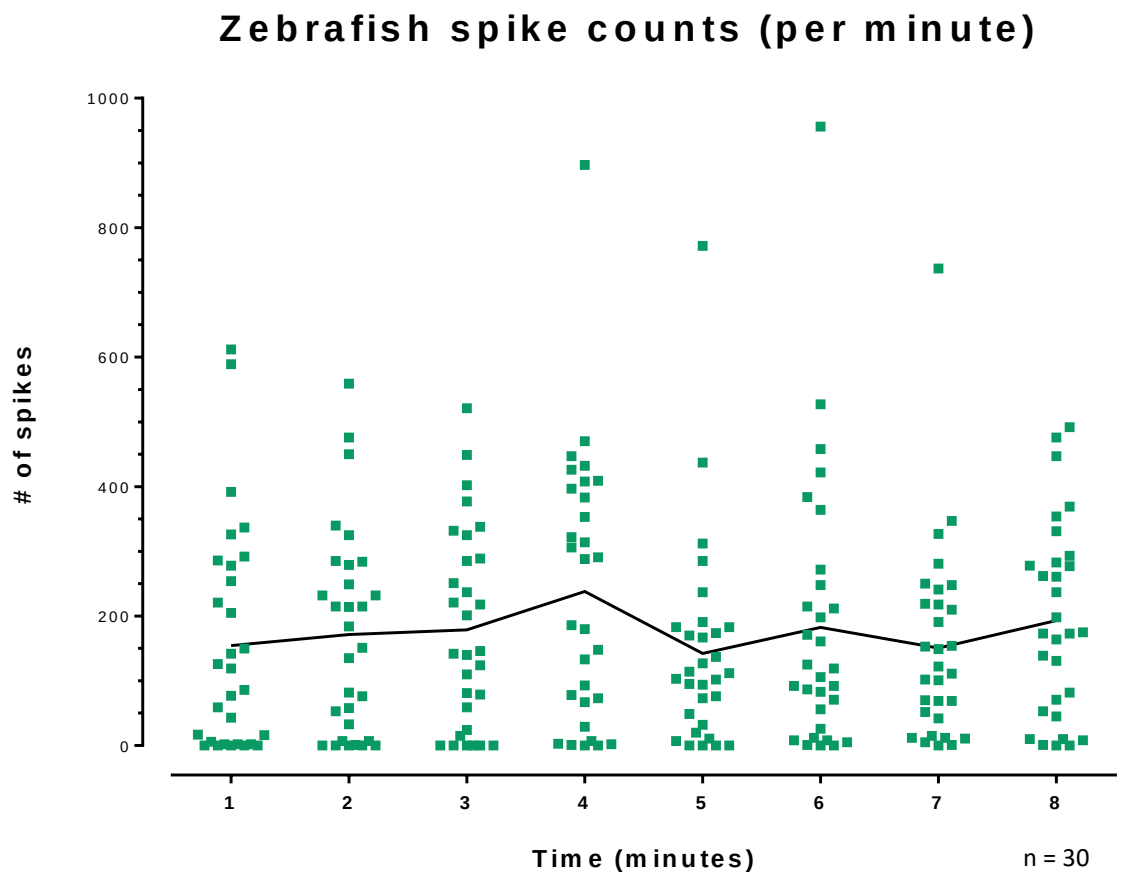
Number of Action Potential "Spikes"/burst

Connectivity- communication of information across electrodes (Correlation coefficients, Network Spikes, Mutual Information); averaged for the well.

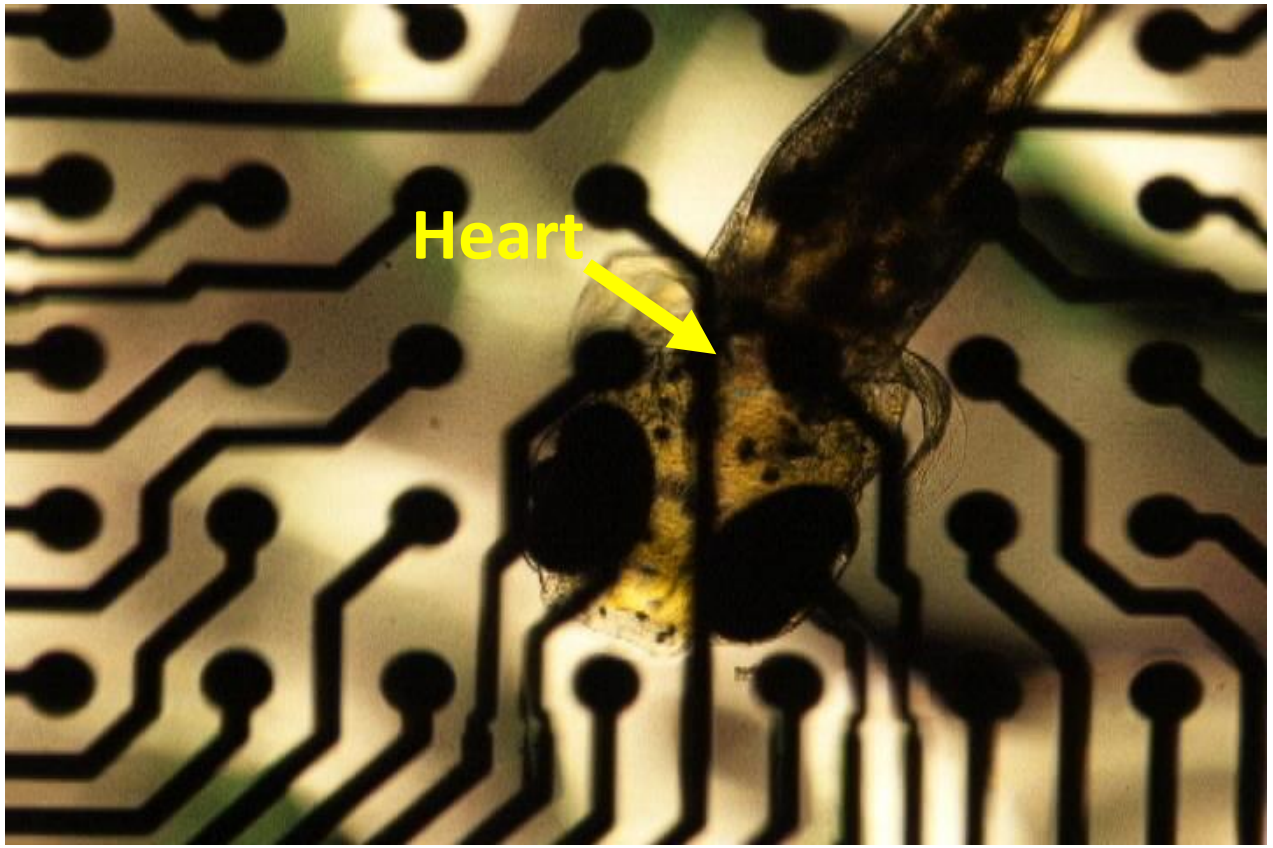
Multiple endpoints can be measured using the MEA system



Embedding in agarose does not alter zebrafish brain activity over the course of the experiment



Zebrafish are viable when embedded in agarose

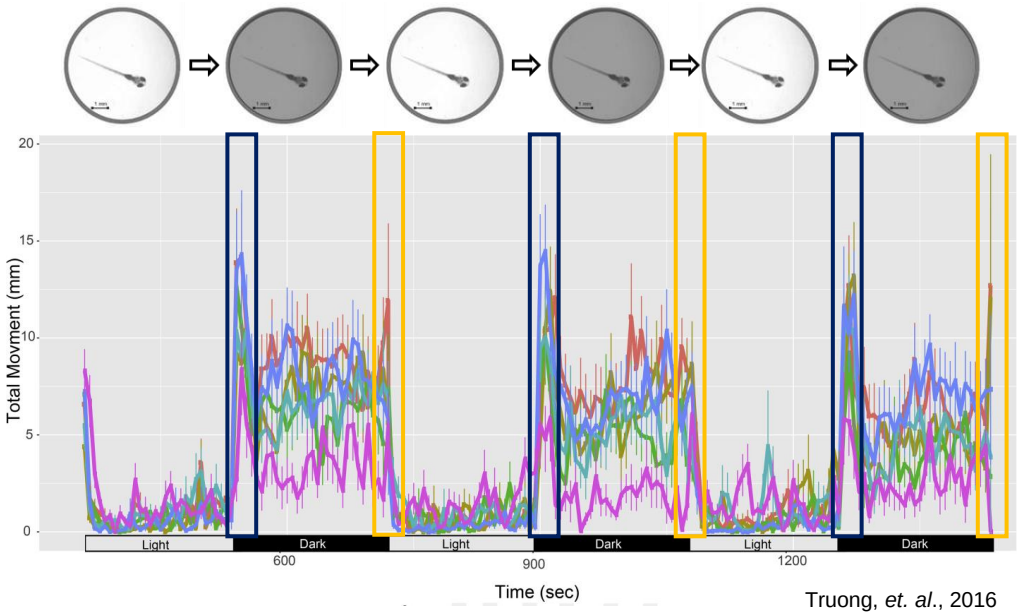


“Fish on a Chip”: Linking network function and behavior

Phase 2: Develop a novel zebrafish light-dark assay using MEA technologies in order to link the in vivo zebrafish behavioral and in vitro MEA data.

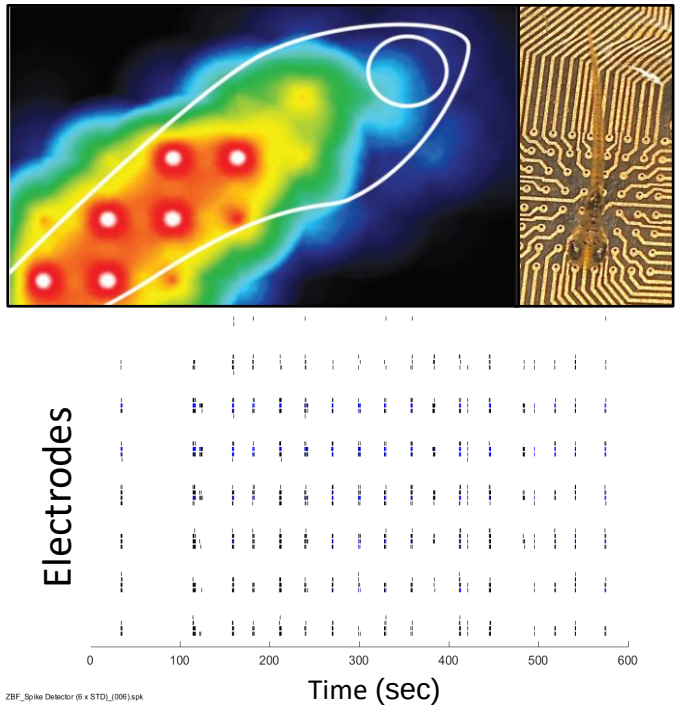
The larval zebrafish light-dark behavioral assay

Alternating light-dark photoperiods correspond to changes in larval zebrafish locomotor activity



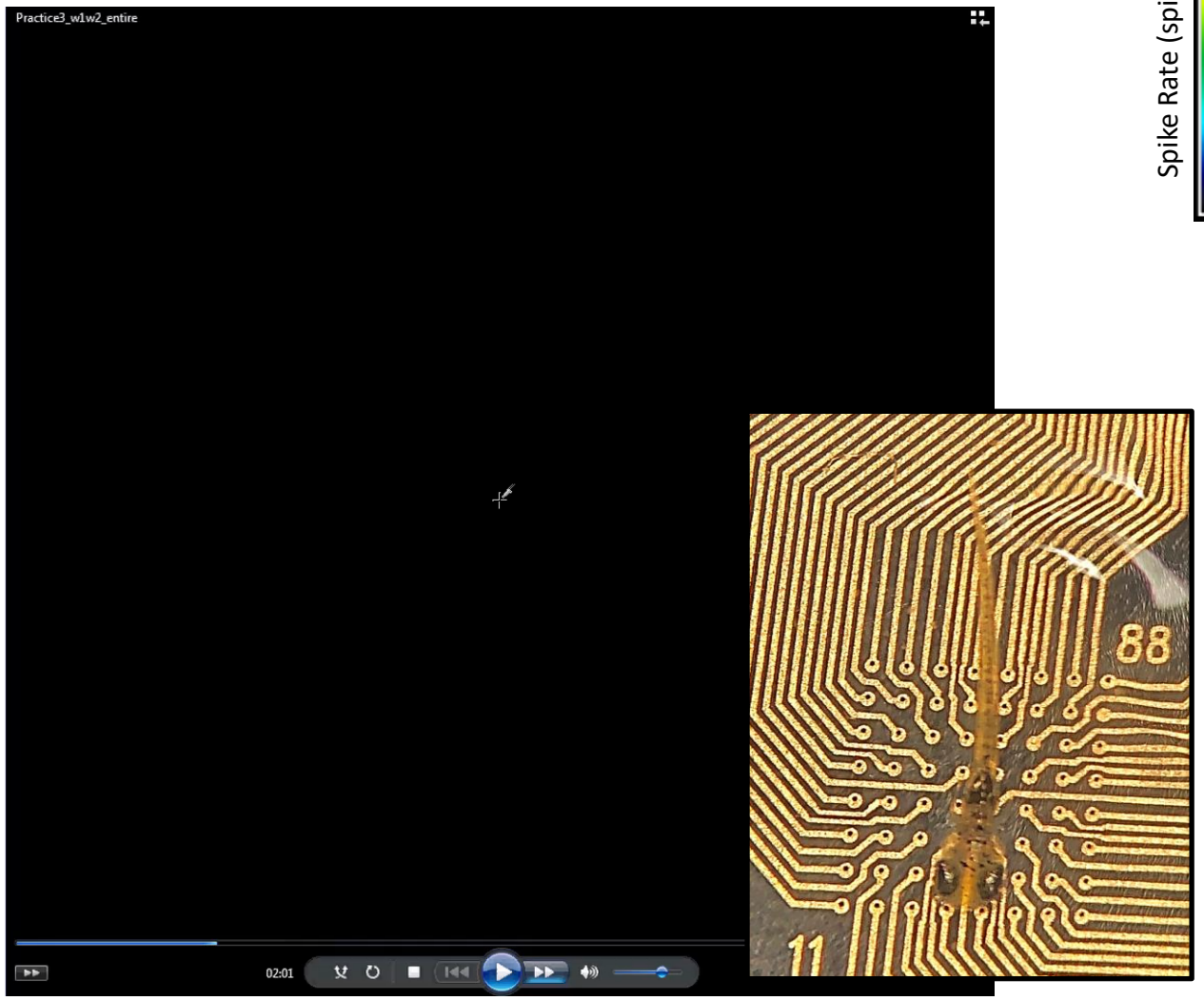
Truong, *et. al.*, 2016

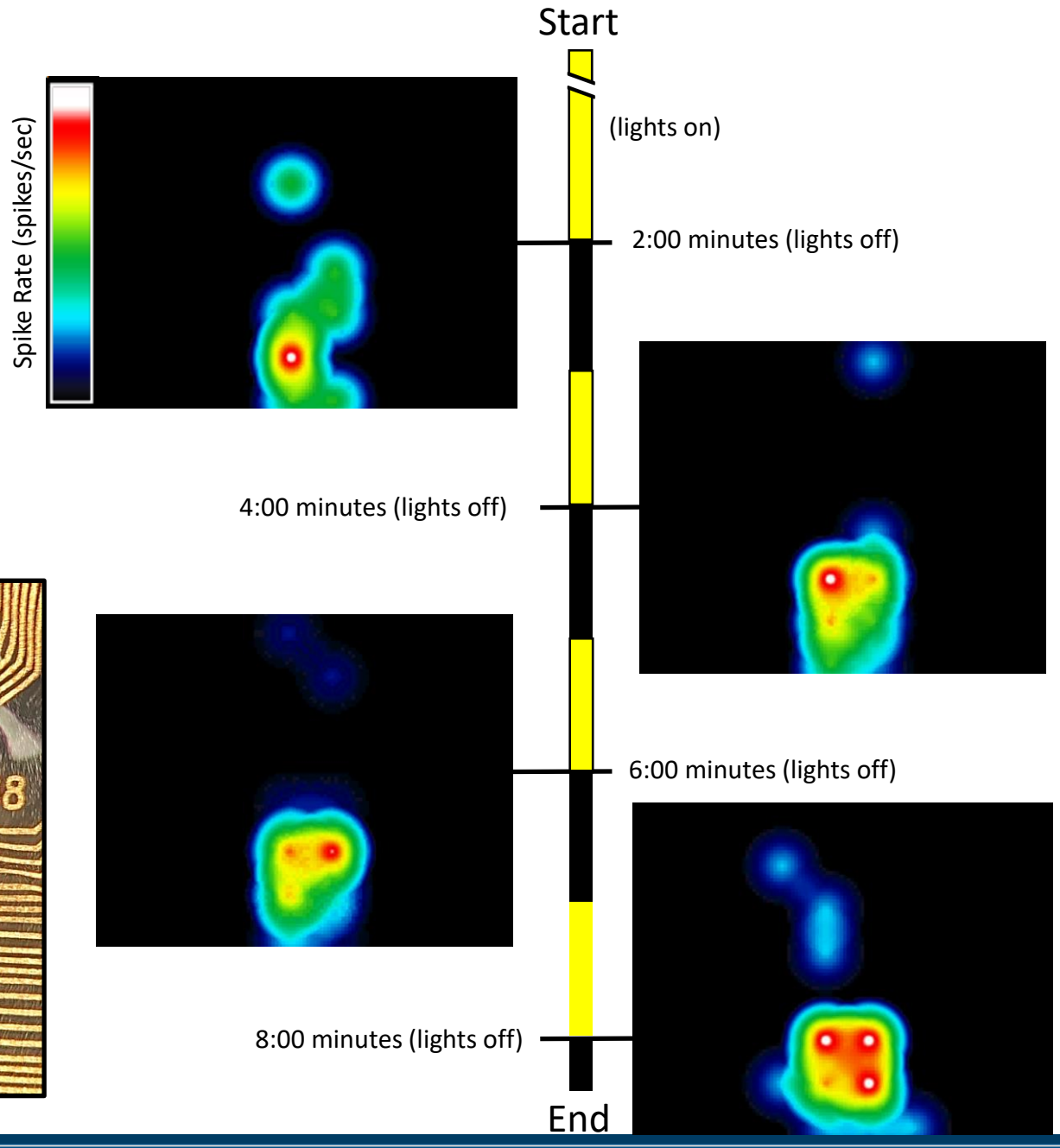
The larval zebrafish MEA assay



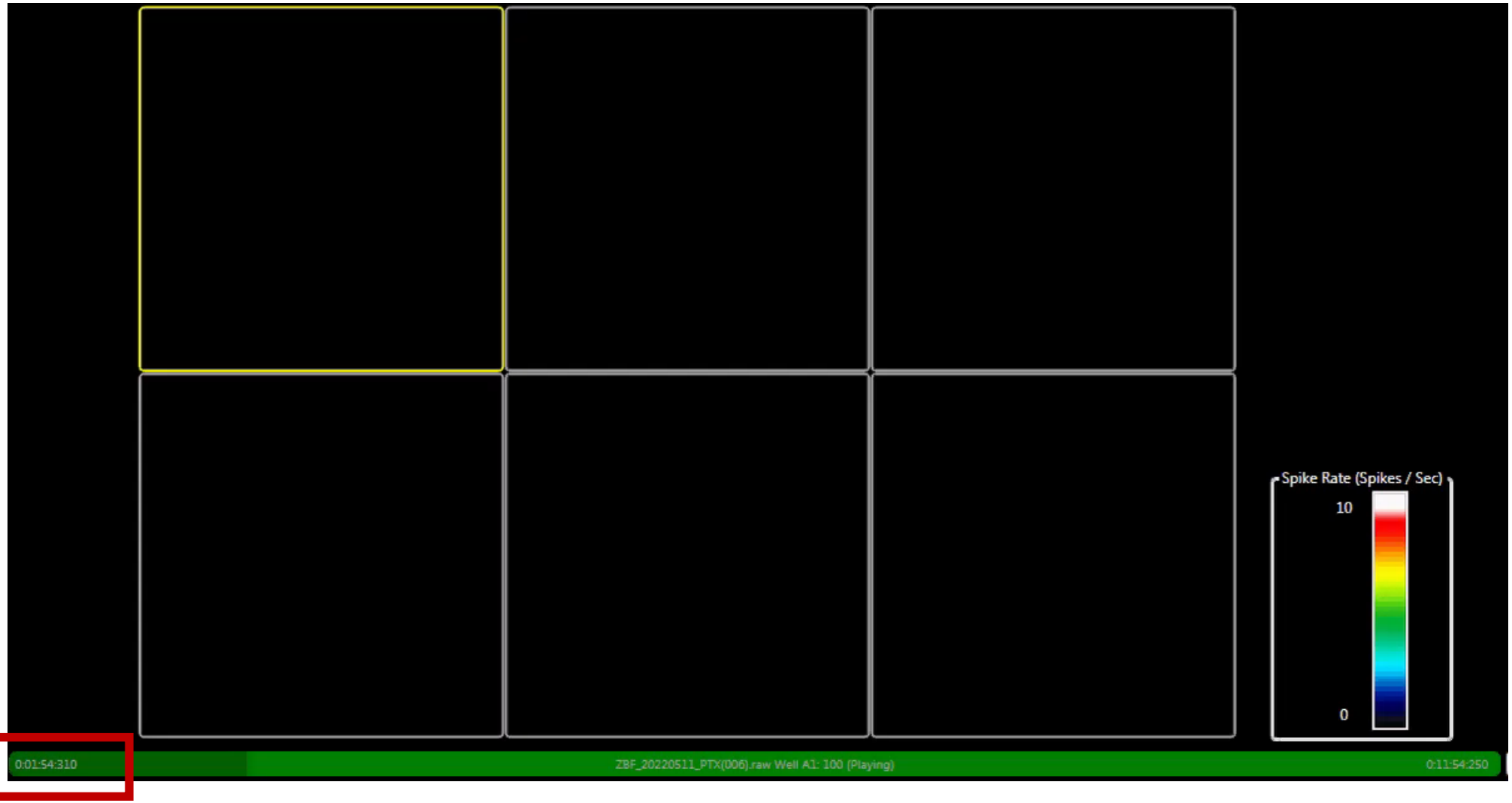
Novel larval zebrafish light-dark MEA assay

Heatmaps of zebrafish placed on an MEA with alternating light-dark transitions



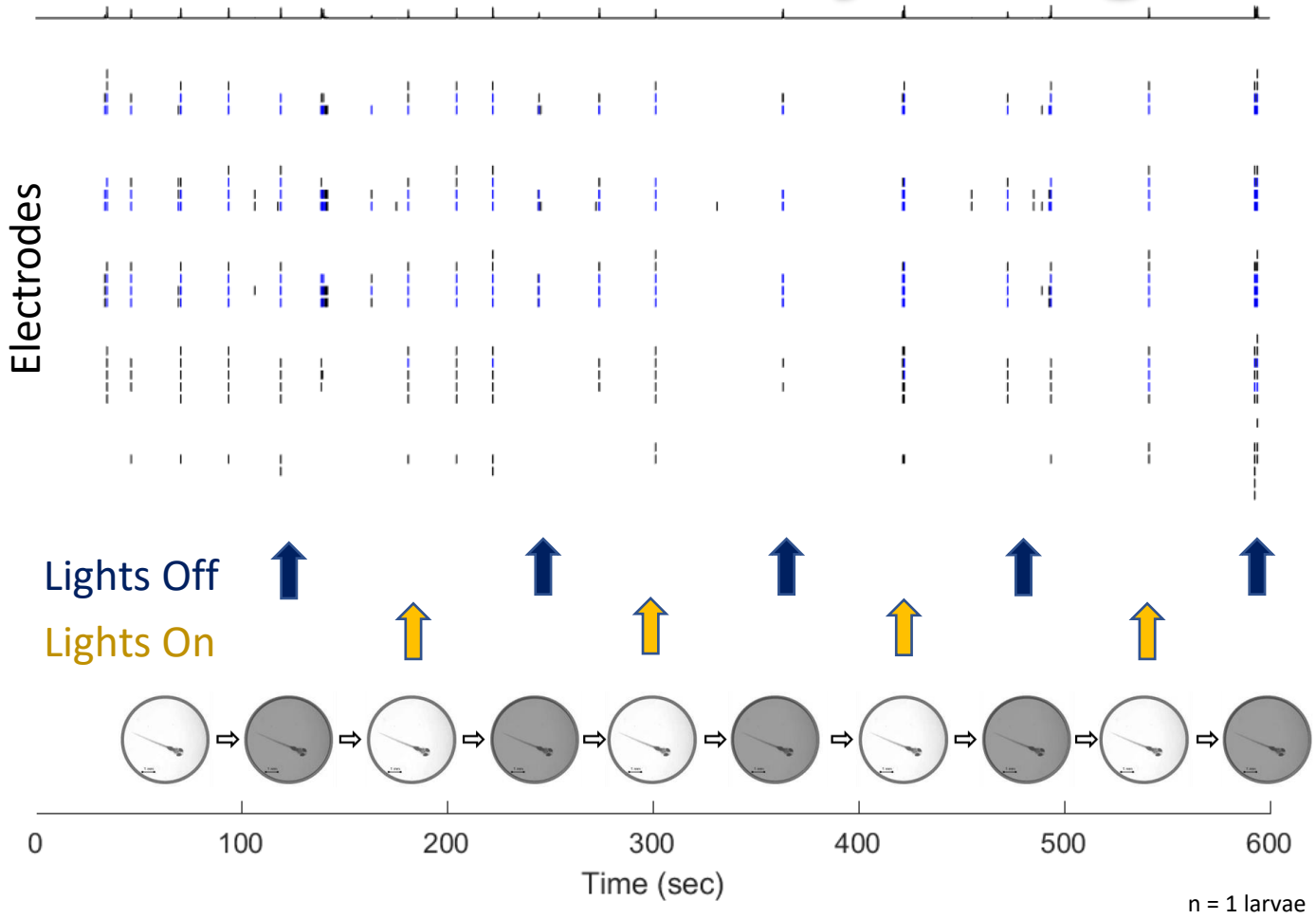


During the light to dark transition (at 2:00 min) - brain activity appears in all larval zebrafish

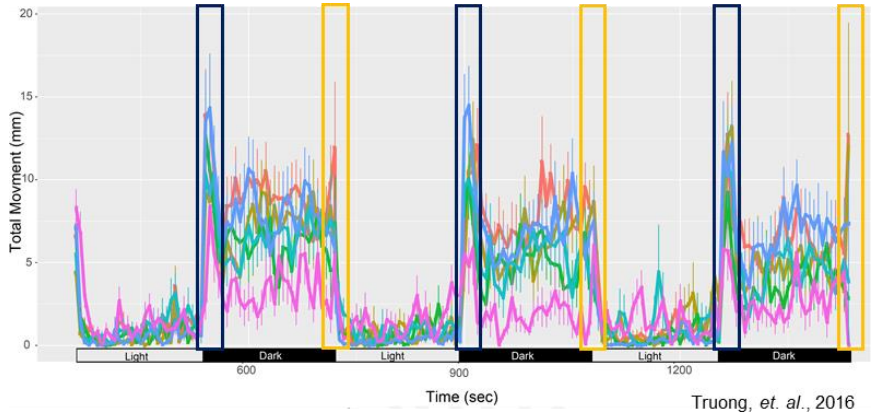


At 2:00 min the lights were turned off

Larval zebrafish respond to alternating light and dark photoperiods as measured by electrical firing activity using the MEA



n = 1 larvae



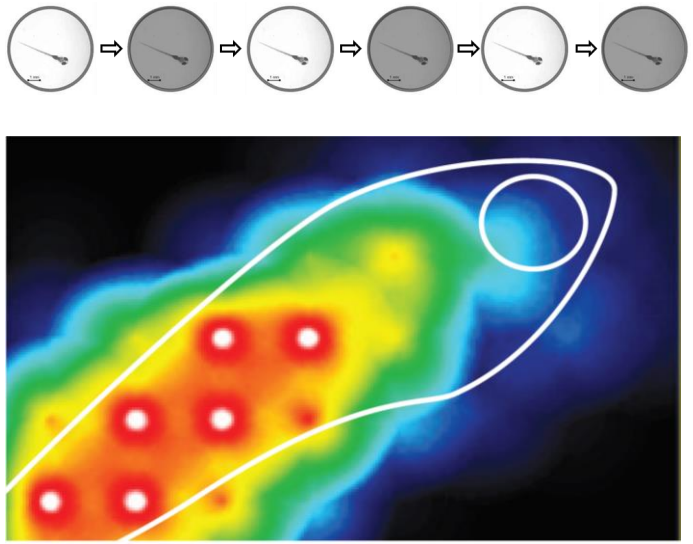
Truong, *et. al.*, 2016

The zebrafish MEA data potentially corresponds to what we typically see in the light-dark behavioral assay

“Fish on a Chip”: Linking network function and behavior

Phase 3: Demonstrate zebrafish MEA light-dark assay for DNT testing proof-of-concept by exposing larval zebrafish to chemicals known to alter neuronal activity.

Novel zebrafish light-dark MEA assay

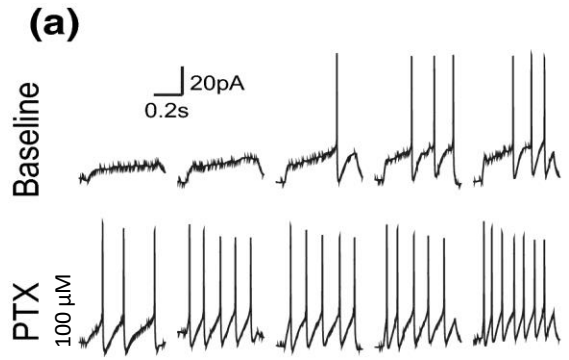


Chemical exposure: picrotoxin and tetrodotoxin are known to produce changes in brain activity and help provide proof of concept.

1.Picrotoxin
- a $GABA_A$ receptor antagonist;
- Known to increase activity
- 100µM

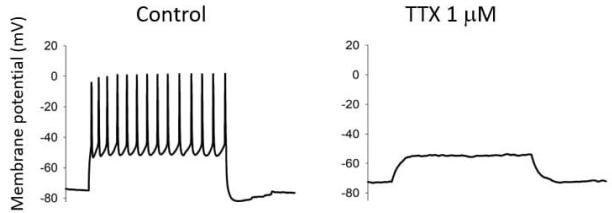
2.Tetrodotoxin
- a Na^+ channel blocker
- known to decreases activity
- 100 µM

An example of picrotoxin’s effects in mouse brain ventral tegmental area (*ex vivo*)



Modified from Tossell, et al., 2021 (PMID: 33522050)

An example of tetrodotoxin’s effects in rat hippocampus (*ex vivo*)



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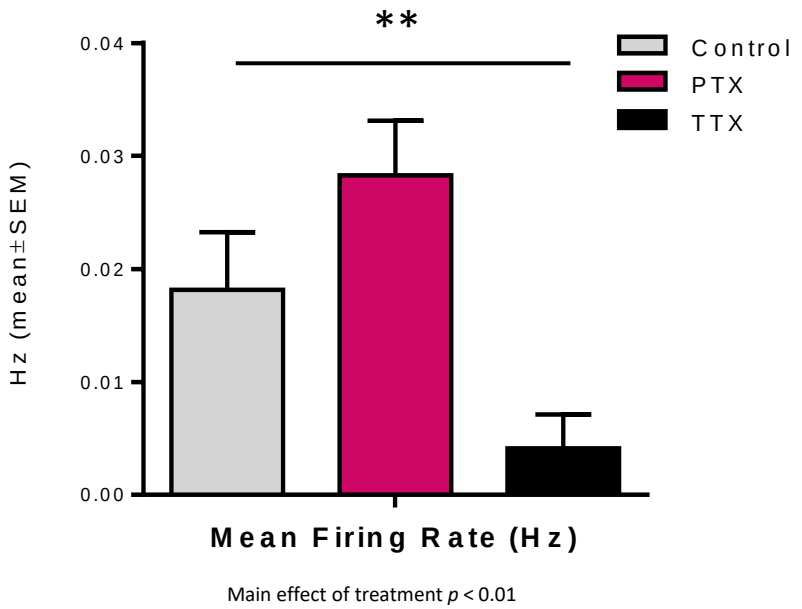
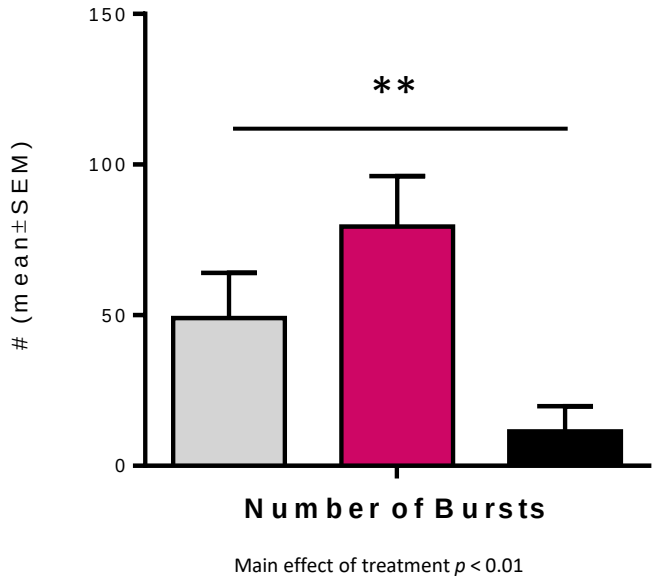
Picrotoxin and tetrodotoxin alter neuronal activity in larval zebrafish

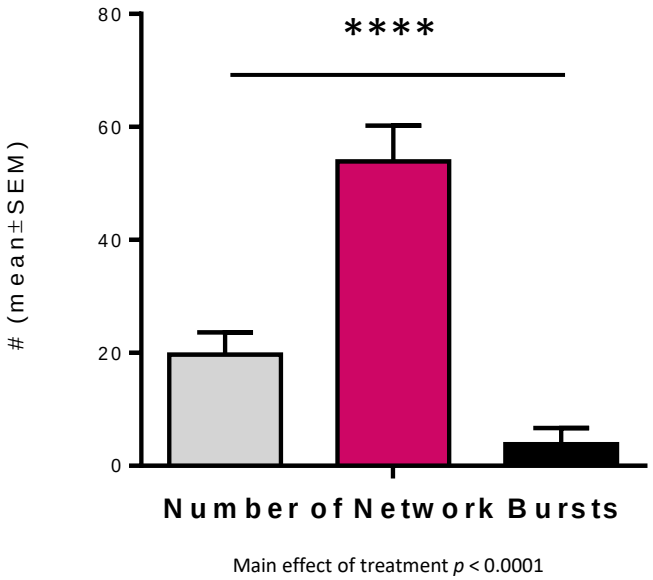
Picrotoxin(PTX): 100µM
Tetrodotoxin (TTX): 100 µM
n = 20-40/treatment group



Main effect of treatment $p < 0.01$

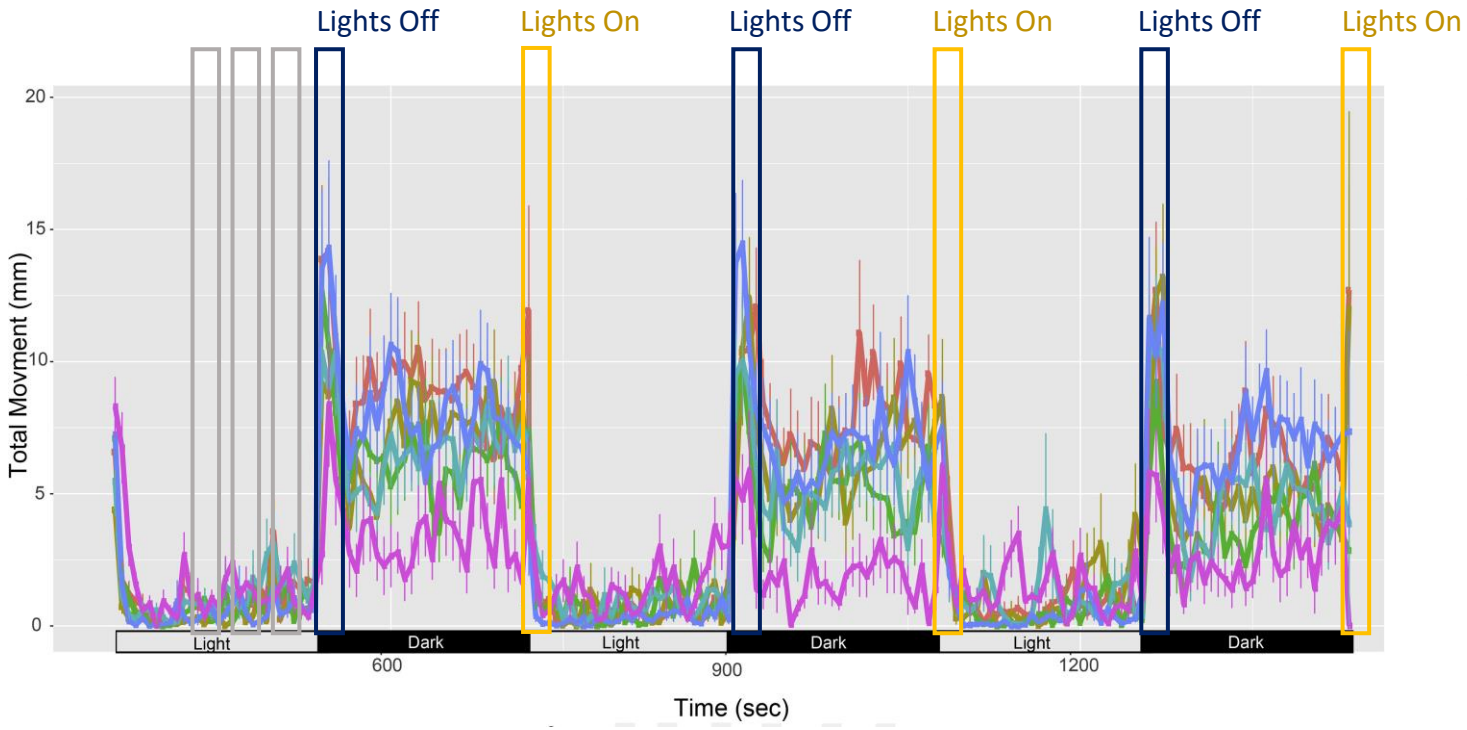


Main effect of treatment $p < 0.01$

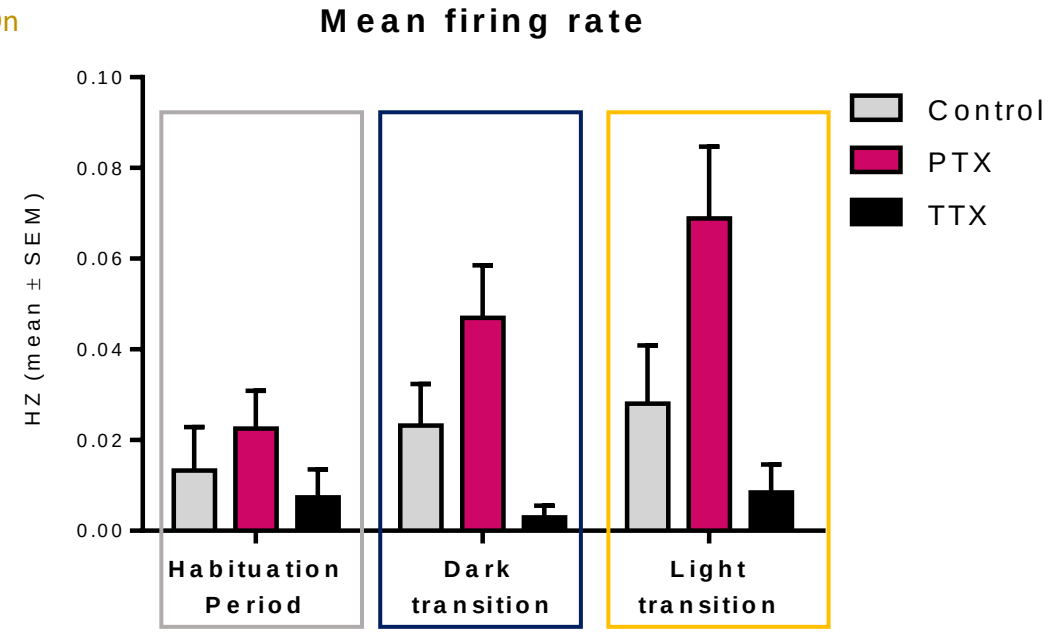


Main effect of treatment $p < 0.0001$

Changes in the transition period can be measured



Modified from Truong, et al., (2016) (PMID: 27593350)

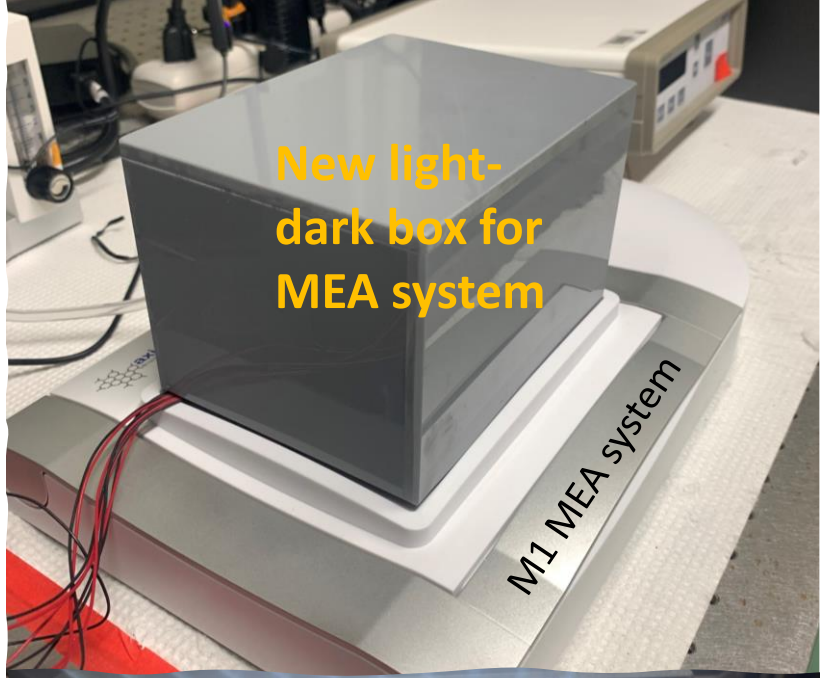


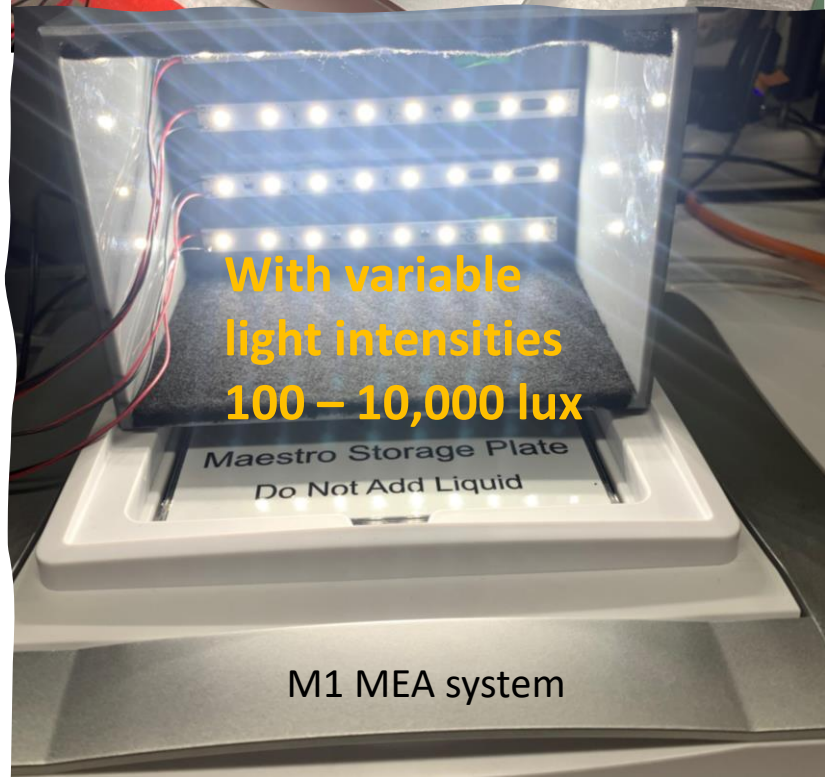
Picrotoxin(PTX): 100µM
Tetrodotoxin (TTX): 100 µM

Main effect of period $p < 0.05$
Main effect of treatment $p < 0.01$
Main effect of subjects (matching) $p < 0.0001$
n = 20-40/treatment group

Conclusions & future objectives:

- Here we show that MEA technologies can be used to record the electrical brain activity from larval zebrafish and that a novel zebrafish light-dark assay can be used to measure changes in electrical activity following chemical exposure.
- Currently conducting dose-response curve experiments for PTX and TTX (0 µM – 100 µM).
- Constructing an apparatus that will give us better control over the amount of light the during the dark (0.5 lux) and light (300 -3500 lux) photoperiods.
- Increase the length of time of the photoperiods.
- Use assay-specific controls (chemicals active in a light-dark assay) to demonstrate link between behavioral and electrophysiological responses.





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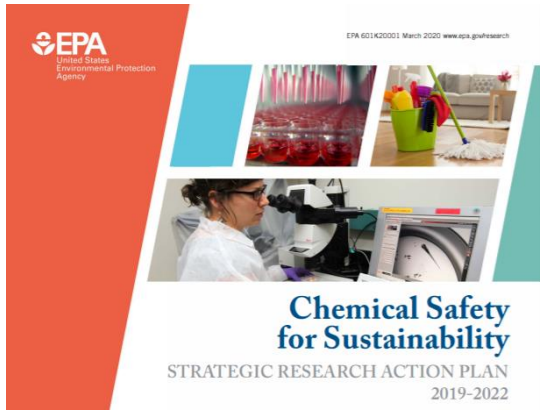

Thank you!