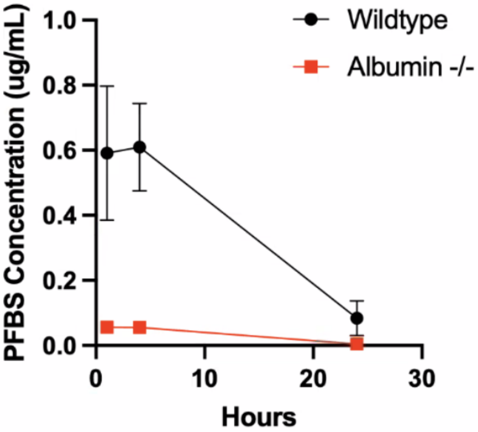
Experimental design and statistical analysis

Marie-Abele Bind

STEEP Trainees seminar

March 2025

STEEP Project 3



Florian : "You don't need statistics in neuroscience"

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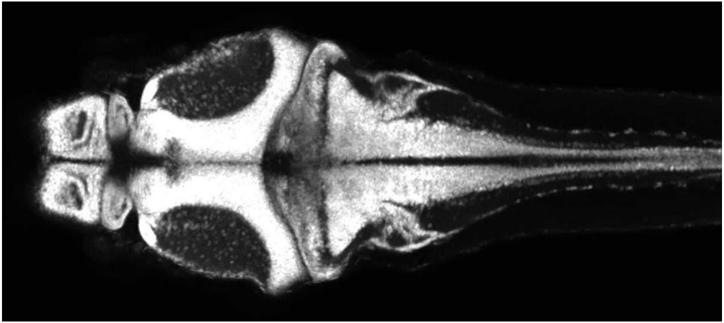
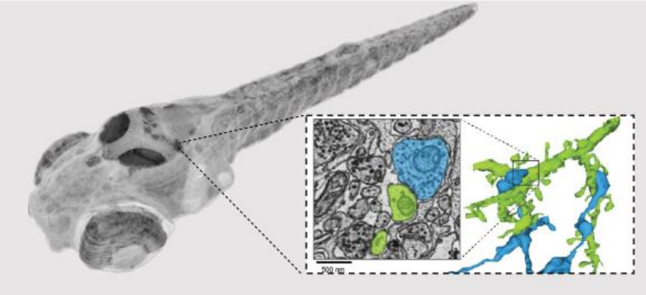
Florian's lab



Florian's lab



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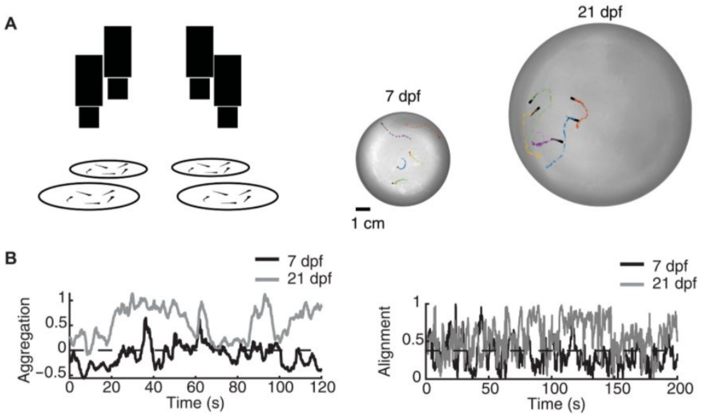
Neuroscience application



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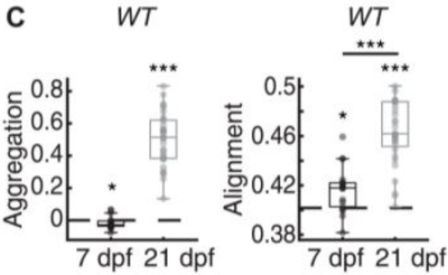

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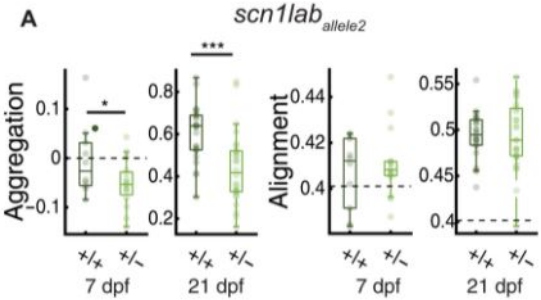

Paper submitted to Science Advances (April 2021)

Collective behavior emerges from genetically controlled simple behavioral motifs in zebrafish

Ariel C. Aspiras*[1], Roy Harpaz*[2,3], Sydney Chambule[1], Sierra Tseng[1], Florian Engert[2,3], Mark C. Fishman[1#§] & Armin Bahl[2,3,4§]

1 Harvard Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge 02138, USA.

2 Department of Molecular and Cellular Biology, Harvard University, Cambridge 02138, USA.

3 Center for Brain Science, Harvard University, Cambridge, Massachusetts 02138, USA.

4 Centre for the Advanced Study of Collective Behaviour, University of Konstanz, Konstanz 78464, Germany.

Reviewer's feedback

- "The authors repeatedly and systematically misinterpret the p-values calculated for their statistical tests and consequently make a large number of false and misleading claims about the meaning of their results.

Reviewer's feedback

- The most charitable explanation for this is that the authors are simply ignorant of how to correctly interpret p-values despite their extensive shared expertise of quantitative data analysis."

Reviewer's feedback

- "The authors do not discuss effect sizes in any meaningful way. If the authors do not discuss effect sizes and what they mean biologically (some of which are very small and probably not meaningful) then they are abdicating their central role as scientists."

Reviewer's feedback

- "The reasoning for the choice of tests as well as the assumptions of each test and how these assumptions were validated for the data to which the tests were applied are not explained."

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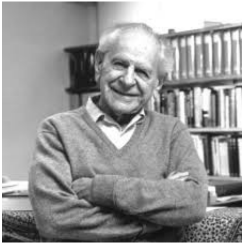

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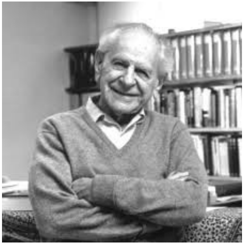
Popper, 1962



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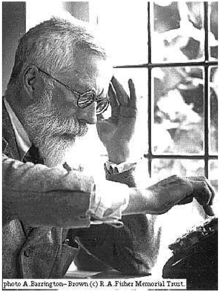

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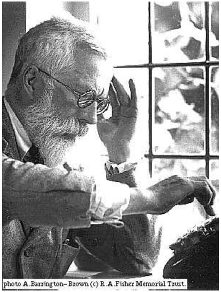

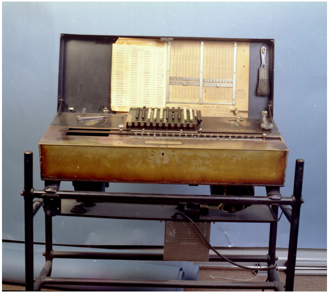

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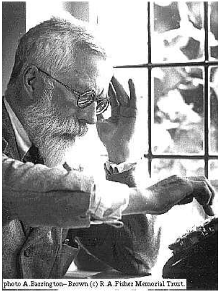

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  Example of myth : $\forall i \in \{1, ..., N\} \quad Y_i(G_i{=}1) = Y_i(G_i{=}0)$

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$$\underline{\text{Ex. of T}} : \quad T(G, Y^{obs}) = \frac{\frac{1}{N_t}\sum_{i:G_i=1} Y_i^{obs} - \frac{1}{N_c}\sum_{i:G_i=0} Y_i^{obs}}{\sqrt{\frac{s_t^2}{N_t} + \frac{s_c^2}{N_c}}}$$

  - Statistic : function of the observed data.
  - Sensitive to expected departures from the null hypothesis.
  - Example of more extreme : $T(W, Y^{obs}) < T^{obs}$

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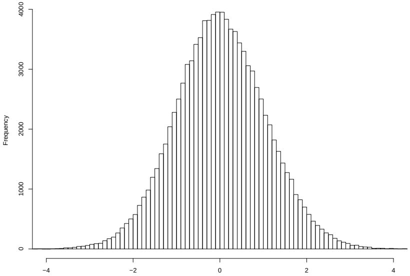

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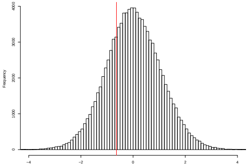

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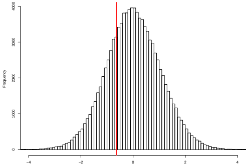

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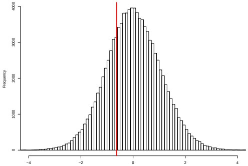

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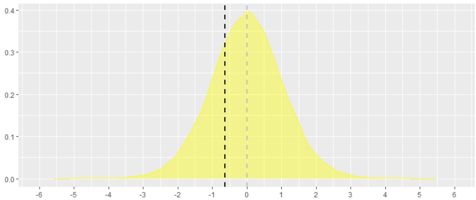
The Fisher-exact p-value corresponds to the proportion of values of the test statistic that are as extreme or more extreme than the observed value of that test statistic.

Beyond hypothesis testing

- For the alignment experiment, we could have reported a counternull value, which was introduced by Rosenthal and Rubin (1994) as a nonnull value of an effect (e.g., difference in alignment between *scn1lab*$^{+/+}$ and *scn1lab*$^{+/-}$) that is supported by exactly the same amount of evidence as the null value of the effect.

Counternull sets in randomized experiment

- Here, we can equate the randomization-based null and counternull p-values.



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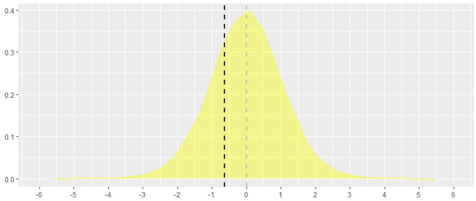

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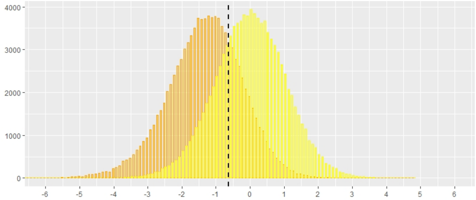

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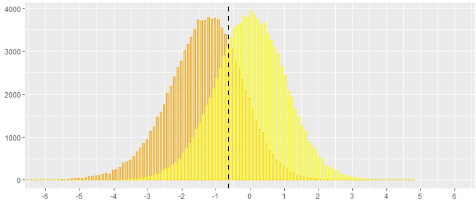

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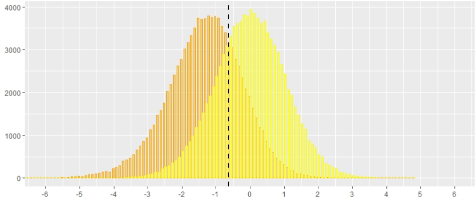

Advantage of reporting counternull values

- The counternull approach helps avoid misinterpretations when testing a null hypothesis.

Advantage of reporting counternull values

- First, the counternull set is associated with a p-value ≈ 0.27. My colleague did not reject the null and subsequently was tempted to accept the null hypothesis. In this situation, reporting the counternull set forced a discussion on accepting the null value, but also the counternull set, which did not contain substantial values!

Advantage of reporting counternull values

- Now consider a counternull value of an effect that is associated with a low p-value, but with low magnitude. In this situation, the counternull value is also worth reporting, because, if small, it would indicate that my colleague's intervention is not necessarily biologically relevant.

Happy ending


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RESEARCH ARTICLE | NEUROSCIENCE

Collective behavior emerges from genetically controlled simple behavioral motifs in zebrafish

ROY HARPAZ, ARIEL C. ASPIRAS, SYDNEY CHAMBULE, SIERRA TSENG, MARIE-ABÈLE BIND, FLORIAN ENGERT, MARK C. FISHMAN, AND ARMIN BAHL Authors Info & Affiliations

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Discussion

- The counternull hypothesis does not have to be about constant and additive effects.

  An alternative counternull hypothesis ($H_{Counternull}$) is :

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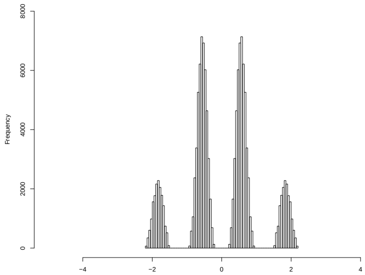

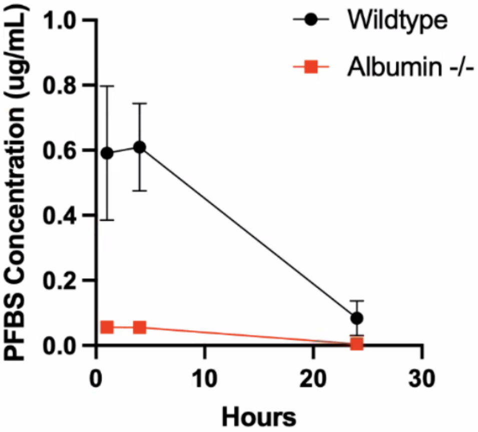
PFBS Concentration (ug/mL)
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
Hours
Wildtype
Albumin -/-


thank you