Donepezil nanoemulsion induces a torpor-like state in non-hibernators tadpoles

DR. MARÍA PLAZA OLIVER

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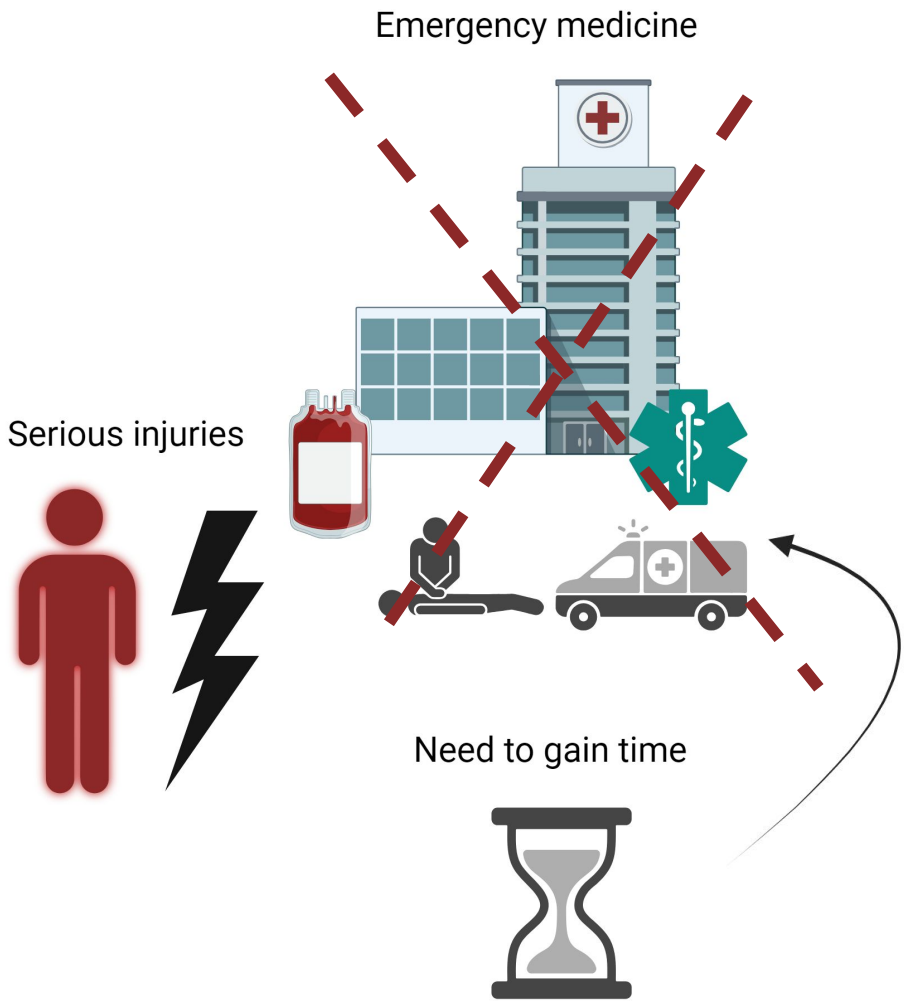
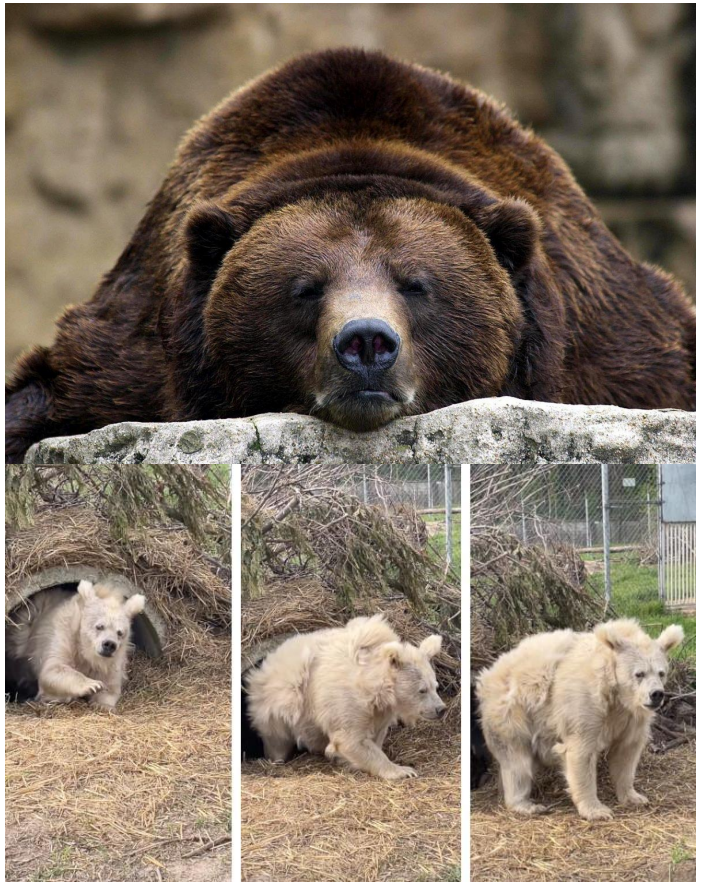
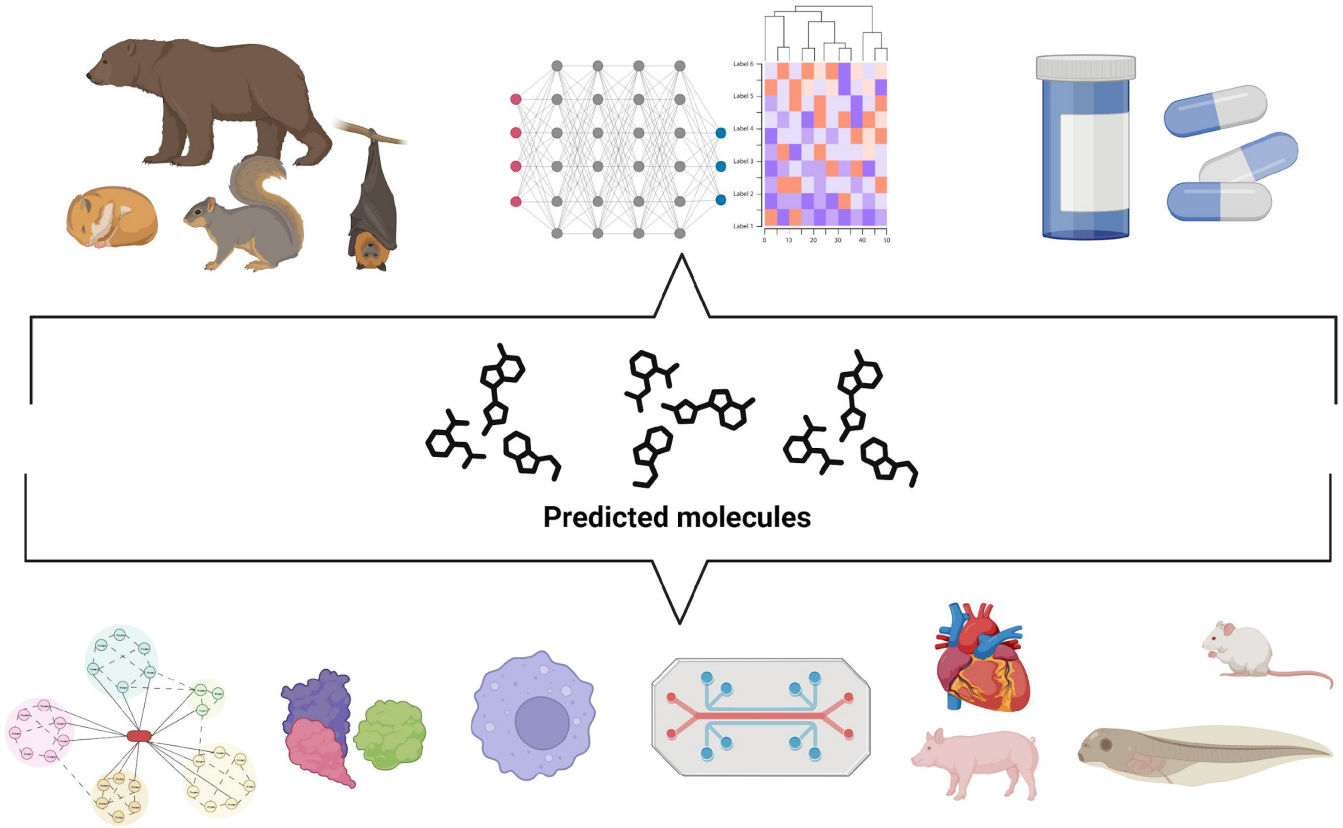




Example in nature: hibernating animals

DARPA Biostasis project

Development of therapeutics that slow biochemical activities while preserving molecule, cell, tissue, organ, and organism viability



DARPA Biostasis project

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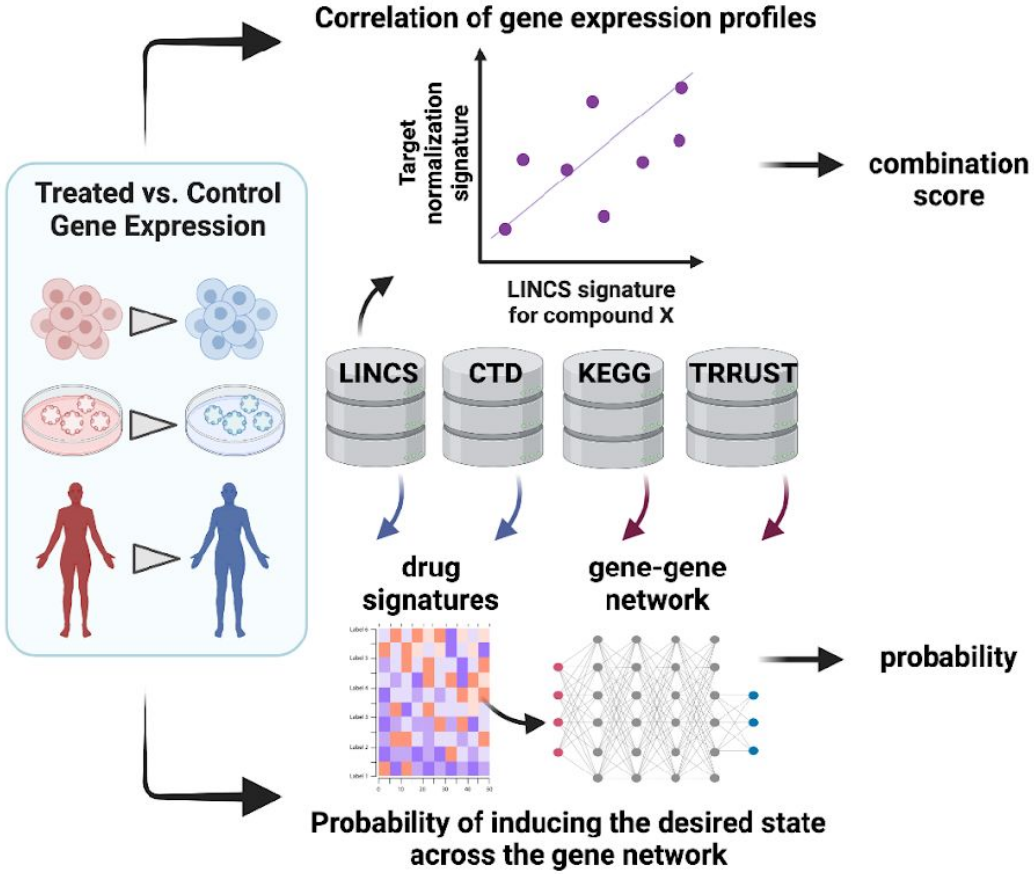
Medicine

Identification of pharmacological inducers of a reversible hypometabolic state for whole organ preservation

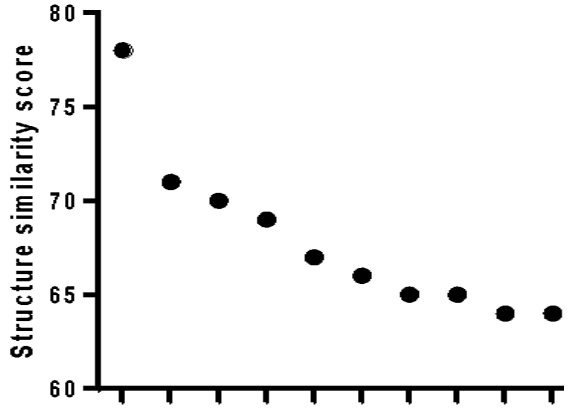
Megan M. Sperry, Berenice Charrez, Haleh Fotowat, Erica Gardner, Kanoelani Pilobello, Zohreh Izadifar, Tiffany Lin, Abigail Kuelker, Sahith Kaki, Michael Lewandowski, Shanda Lightbown, Ramses Martinez, Susan Marquez, Joel Moore, Maria Plaza-Oliver, Adama M. Sesay, Kostyantyn Shcherbina, Katherine Sheehan, Takako Takeda, Daniela Del Campo, Kristina Andrijauskaite, Exal Cisneros, Riley Lopez, Isabella Cano, Zachary Maxwell, Israel Jessop, Rafael J. Veraza, Leon Bunegin, Thomas J. Percival, Jaclyn Yracheta, Jorge Pena, Diandra Wood, Zachary Homas, Cody Hinshaw, Jennifer Cox-Hinshaw, Olivia G. Parry, Justin J. Sleeter, Erik K. Weitzel, Michael Levin, Michael Super, Richard Novak, Donald E. Ingber

Wyss Institute for Biologically Inspired Engineering at Harvard University • Department of Biology, Tufts University • DEVANA group, Faculty of Pharmacy, University of Castilla-La Mancha • Vascular Perfusion Solutions, Inc • RESTOR™, 59th Medical Wing, JBSA, Lackland AFB • Allen Center, Tufts University • Vascular Biology Program & Department of Surgery, Boston Children's Hospital and Harvard Medical School • Harvard John A. Paulson School of Engineering and Applied Sciences

Search of FDA approved drugs as new candidates



Drs. M.M.Sperry, T. Takeda & S. Kaushal



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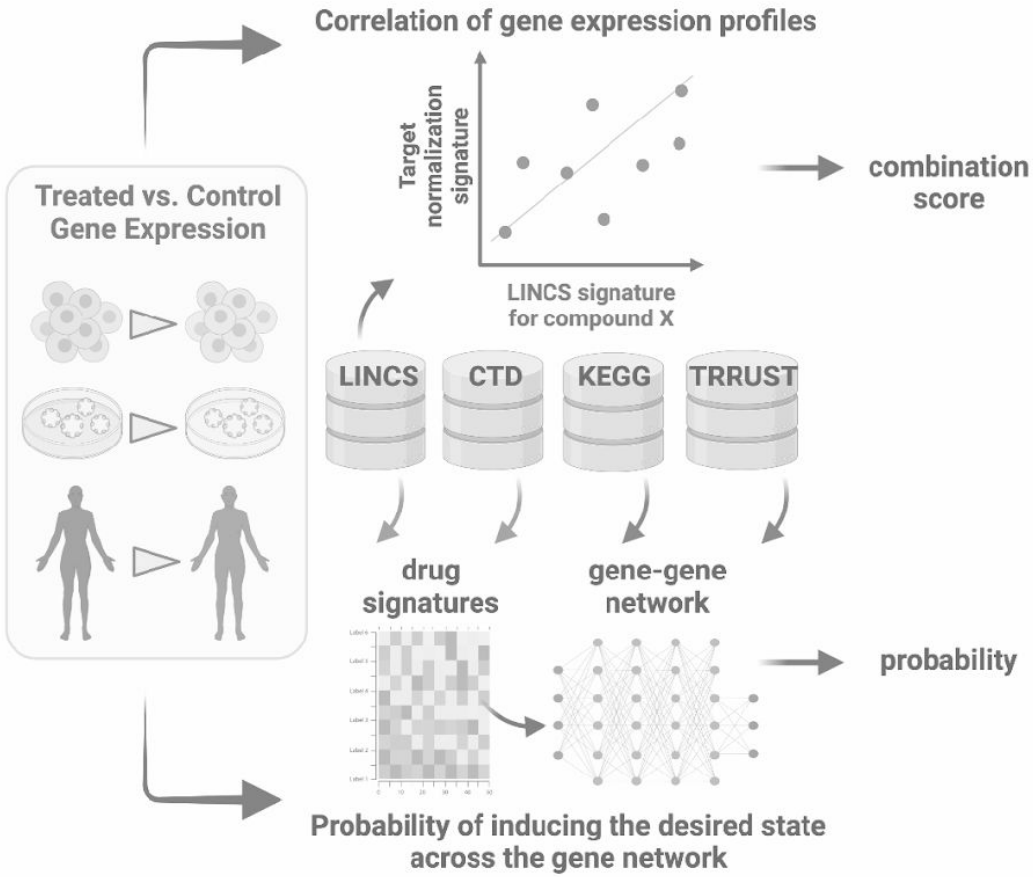
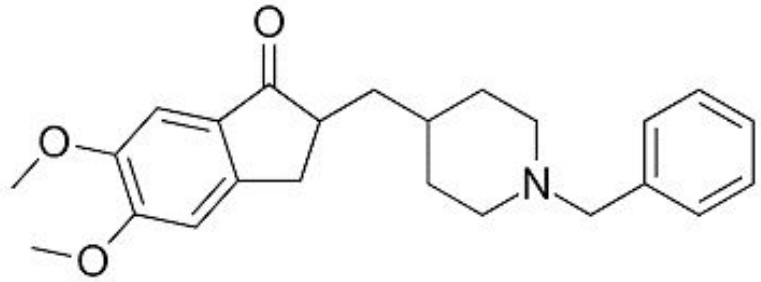
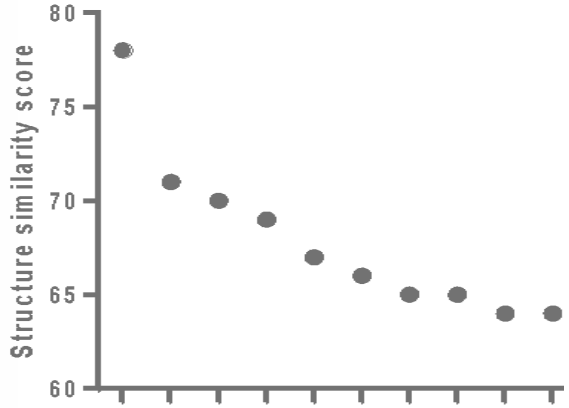


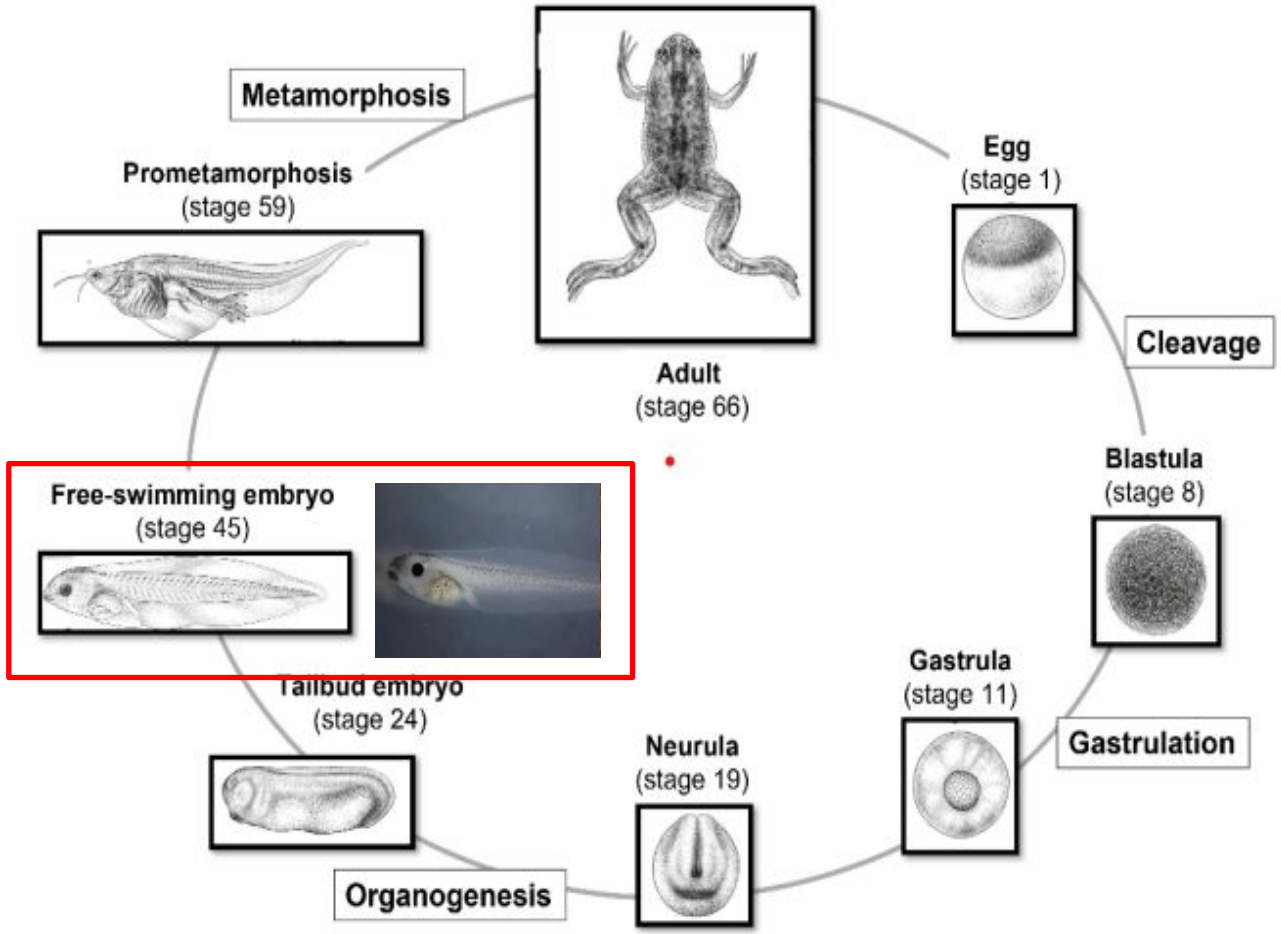


Donepezil



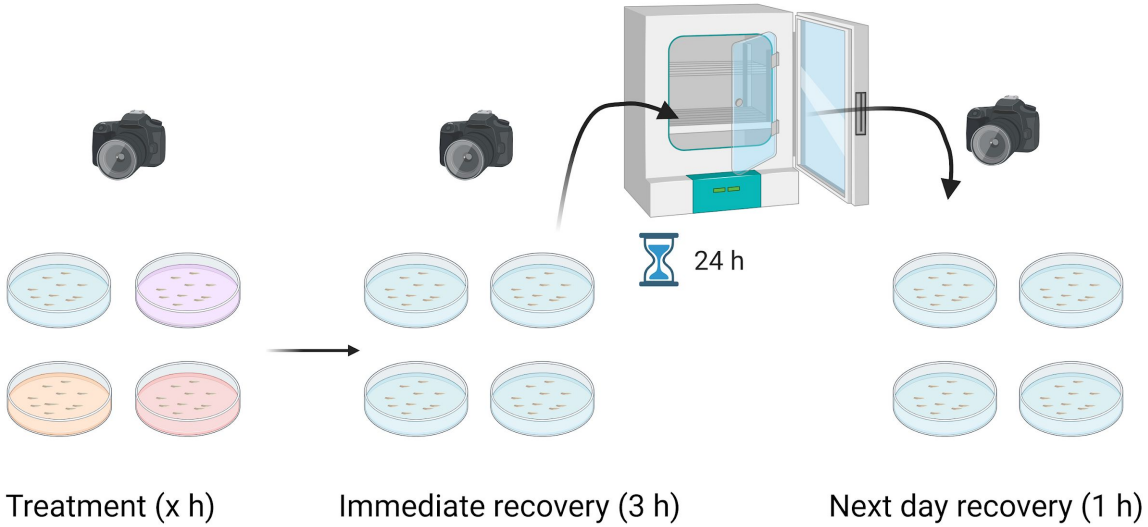
In vivo testing in *Xenopus laevis* tapdoles

- High-throughput
- *In vitro* fertilization
- Small size, lower cost
- Ease of handling
- Transparent model
- Less evolutionary distance compared to zebrafish
- Fully-sequence genome
- 90% of human disease homologs

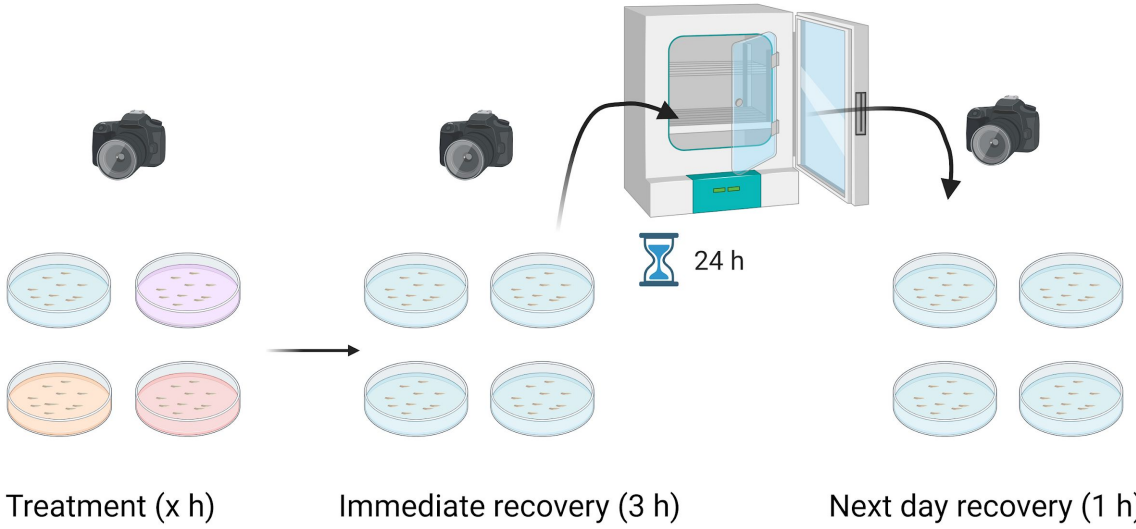


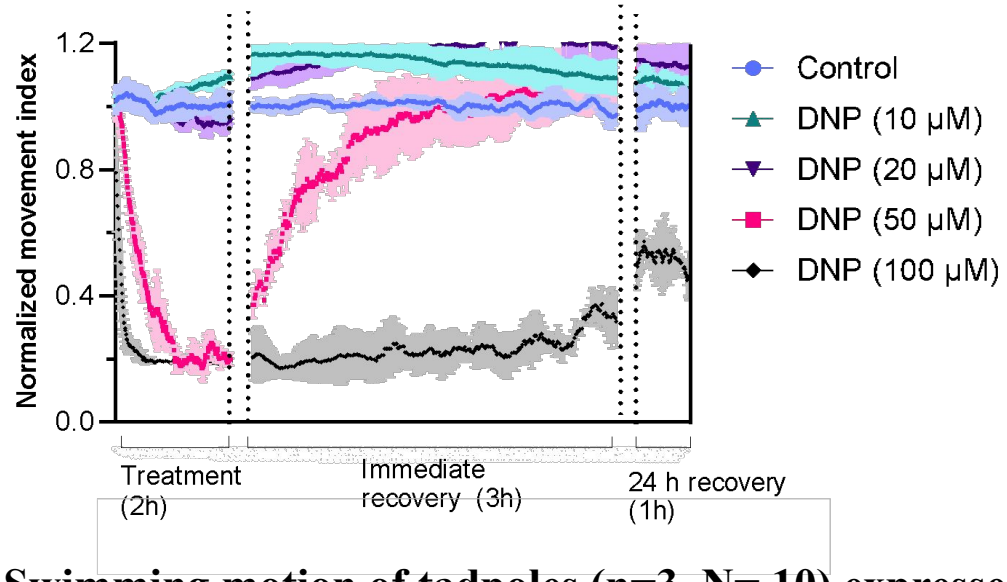
Adapted from R. Carotenuto et al. Biology 2023.

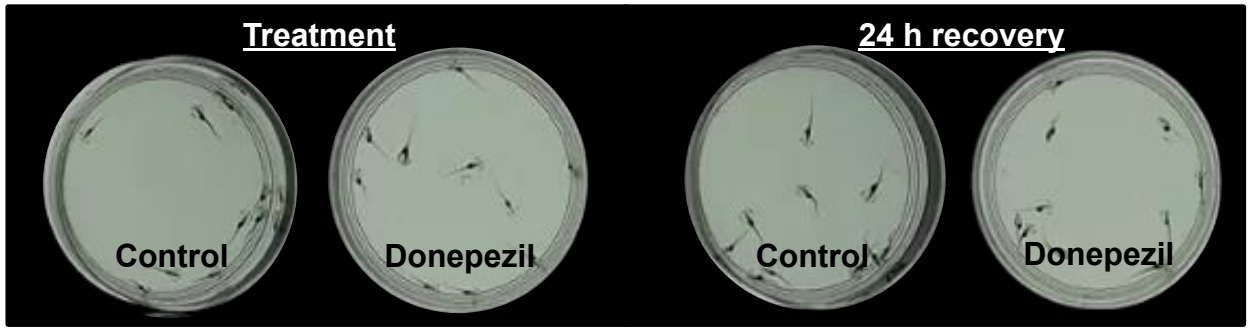
Donepezil decreases the swimming of tadpoles



Donepezil decreases the swimming of tadpoles

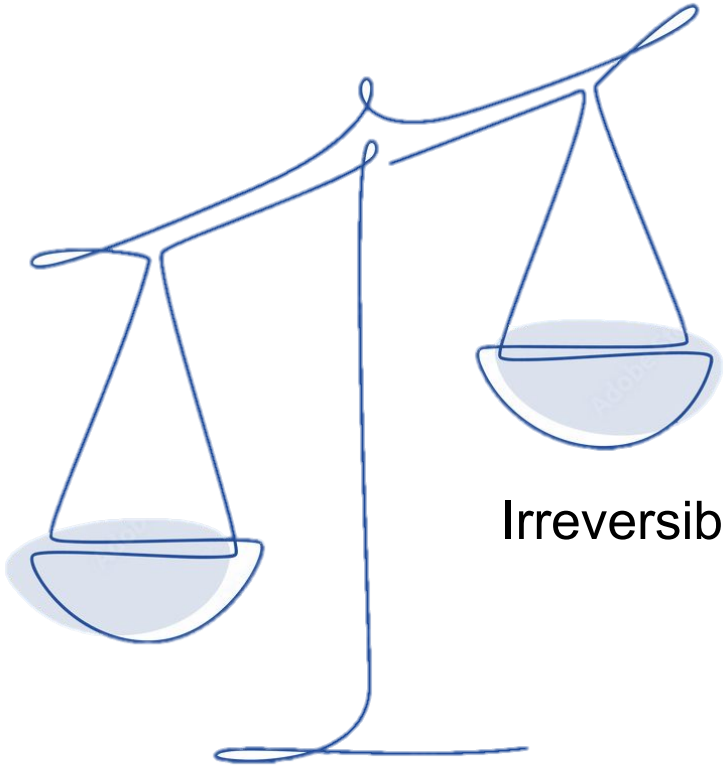




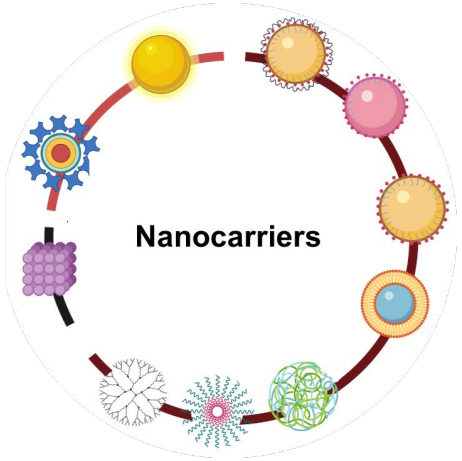


Swimming motion of tadpoles (n=3, N= 10) expressed by normalized movement index, being 0 indicative of lack of swimming and 1 of maximal swimming.

Balance between efficacy and toxicity needed



Prolonged biostasis

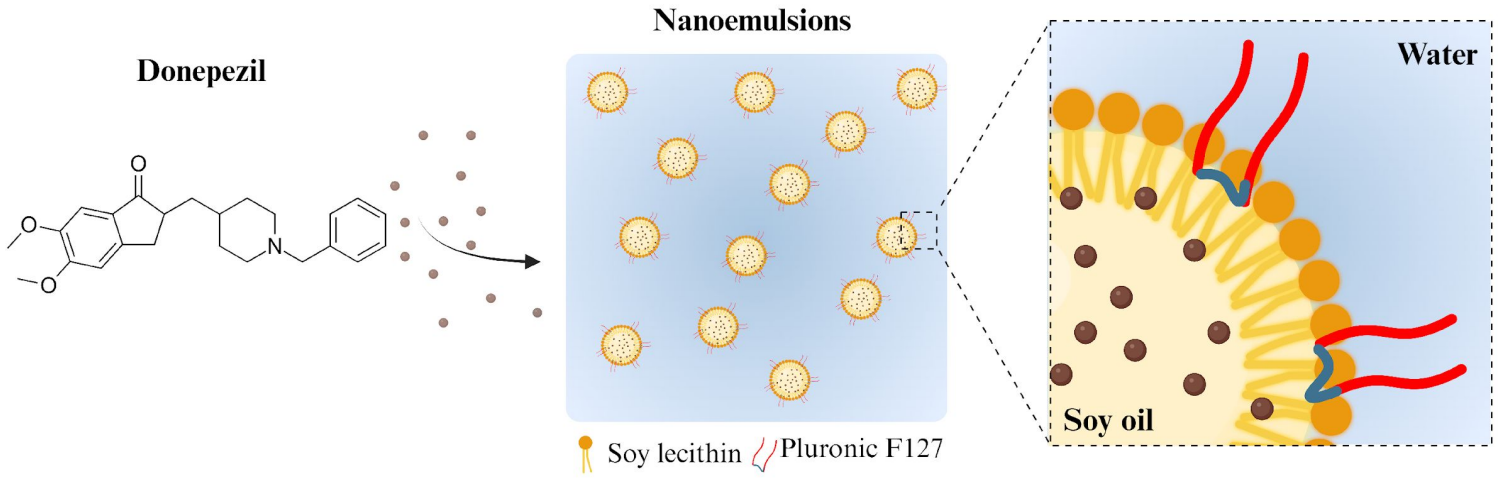


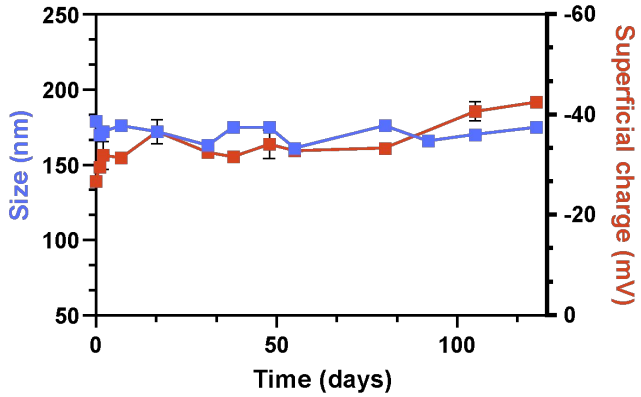
Irreversibility

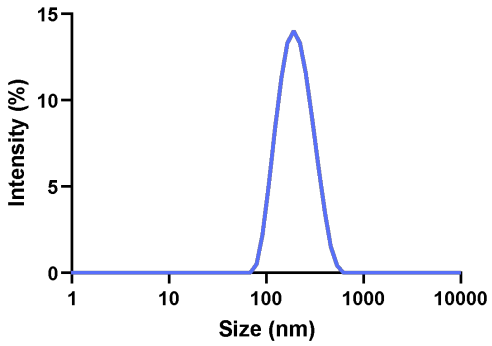
Prolonged biostasis

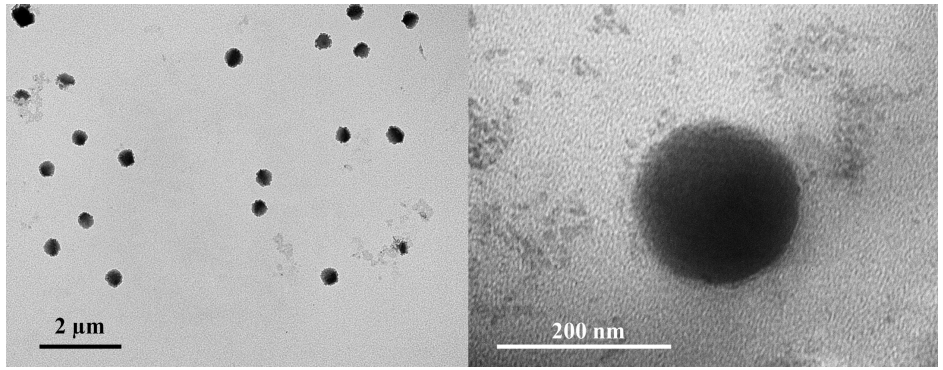
Reversibility

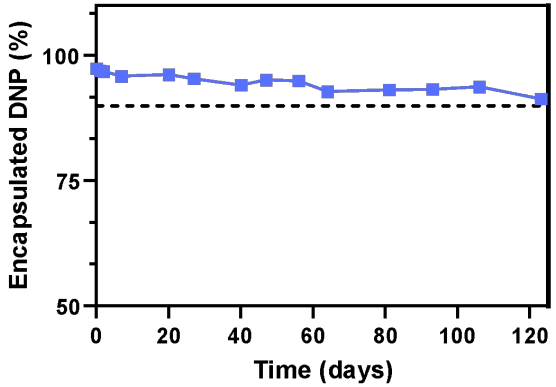
Donepezil nanoemulsion





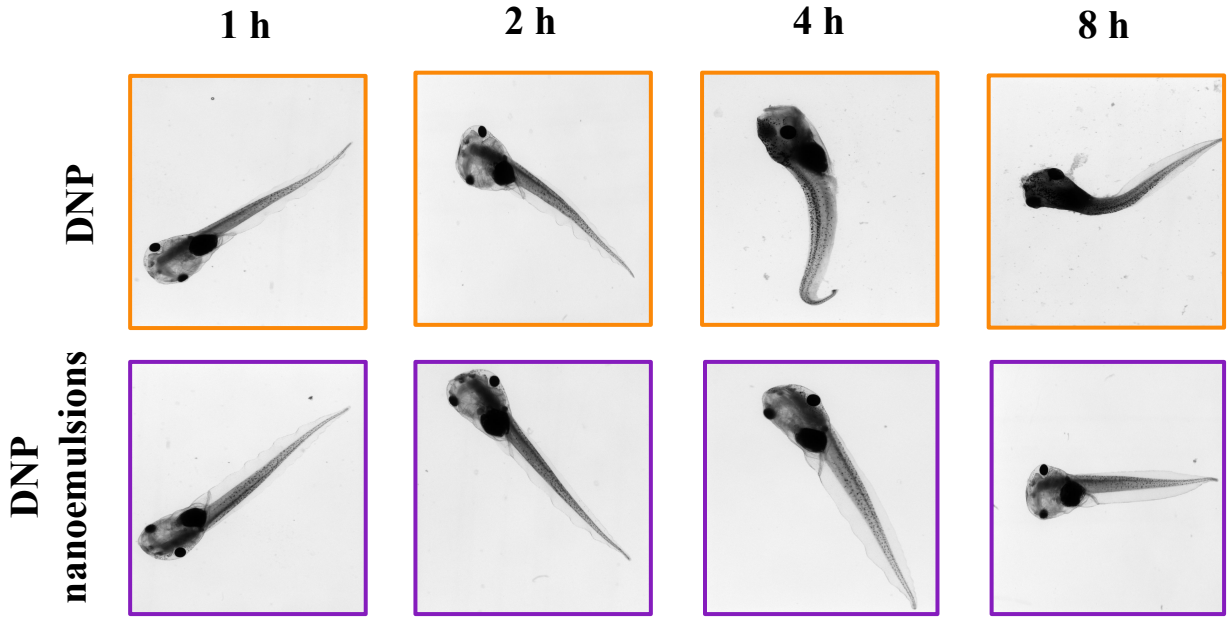


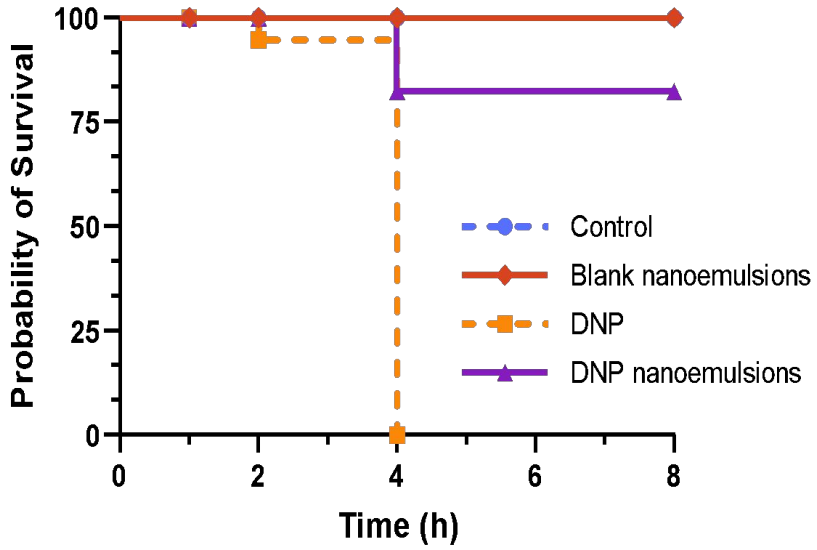




(n=3, N= 3)

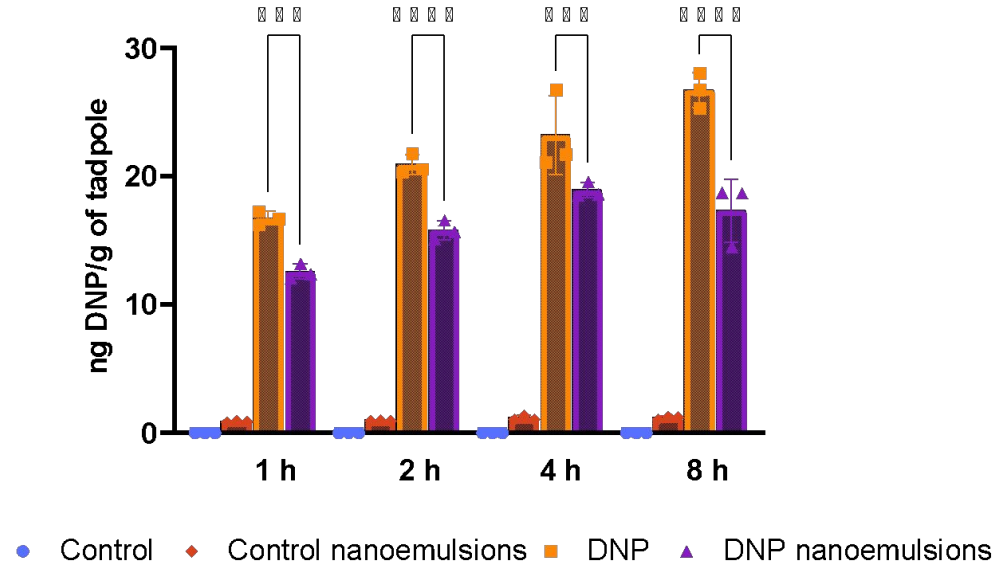
Nanoemulsion reduces toxicity of donepezil and enables longer treatments



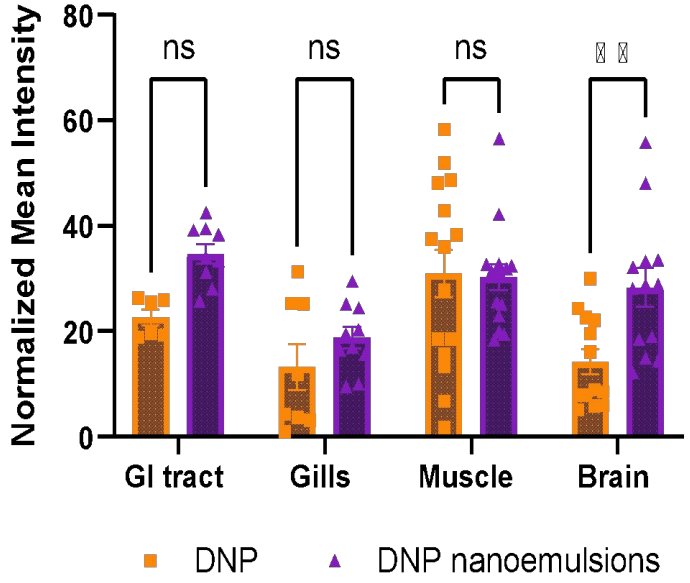


(n=10 per timepoint and group)

Nanoemulsion delivers donepezil in a more sustained way and increases brain concentration

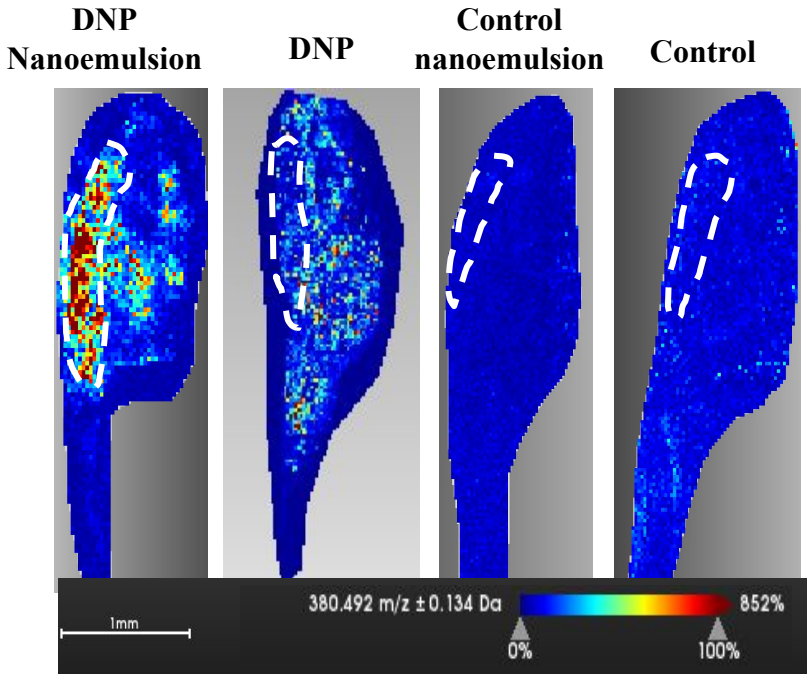


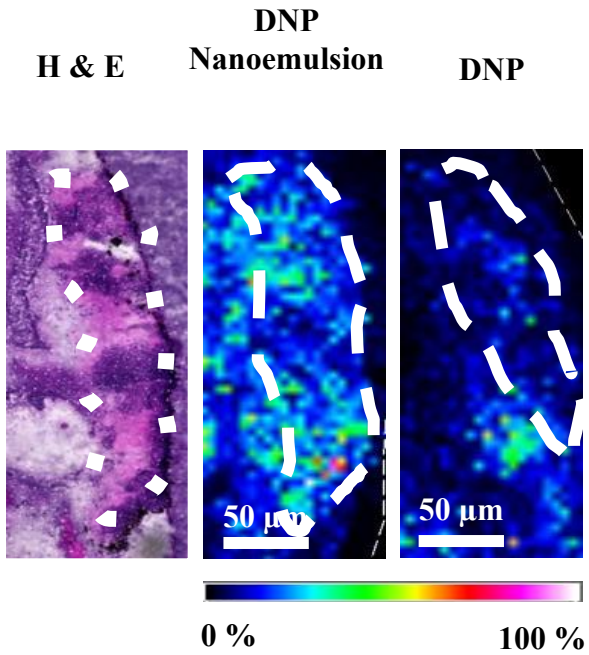
DNP concentration within whole tadpole lysates quantified by Mass spectrometry (n=3 , N= 3). Statistics 2-way ANOVA with Tukey's corrections.



Biodistribution of DNP after treatment of tadpoles with DNP or DNP nanoemulsion for 1 hour (n ≥ 6) assessed by MALDI-ToF. Statistics 2-way ANOVA with Sidak's corrections.

Nanoemulsion delivers donepezil in a more sustained way and increases brain concentration

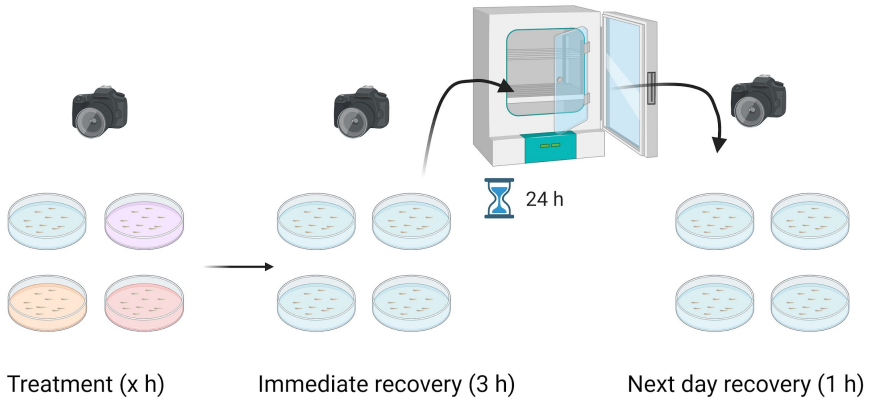




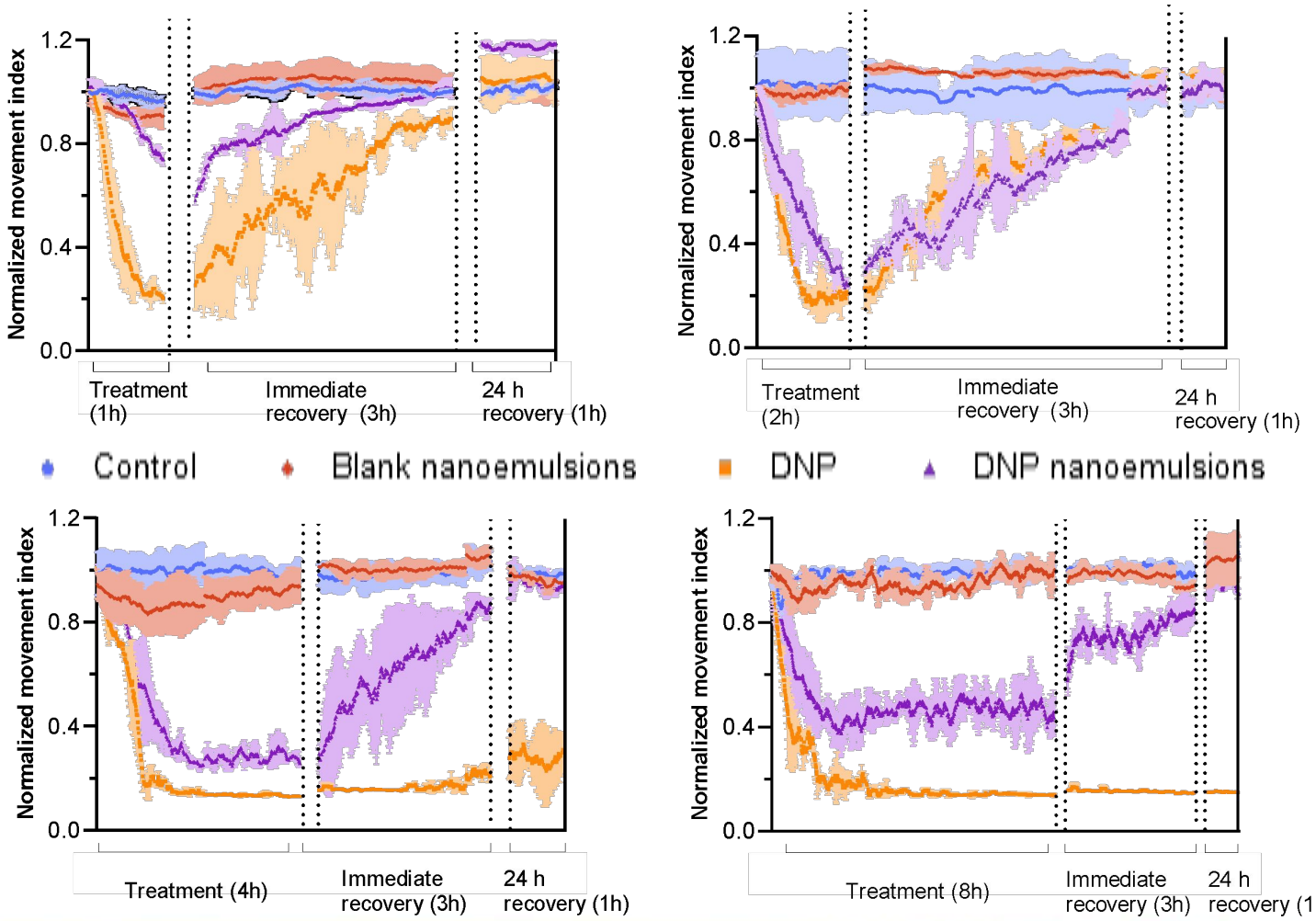
Dr. T. Lin

MALDI-ToF representative positive ion images (380.492 m/z [M+H]+) of DNP distribution in tadpoles treated for 1 h. Each pixel represents a mass spectrum. White dashes represent regions of the brain.

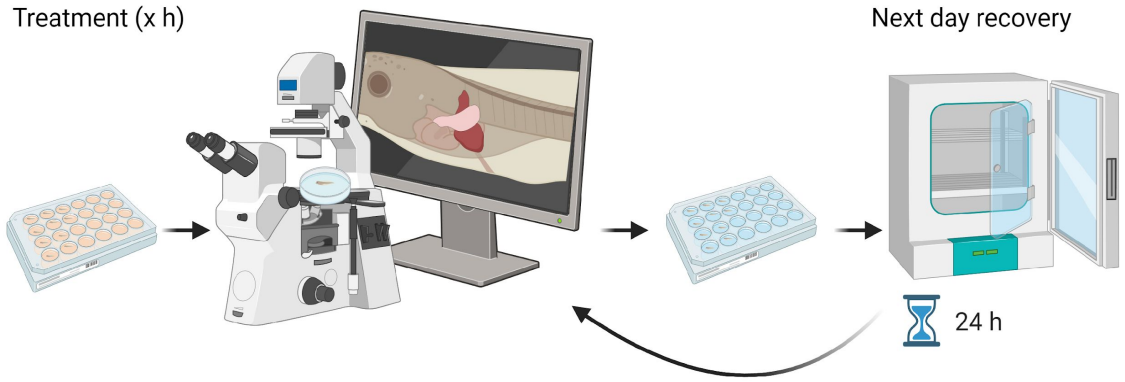
Donepezil nanoemulsion reduces swimming motion

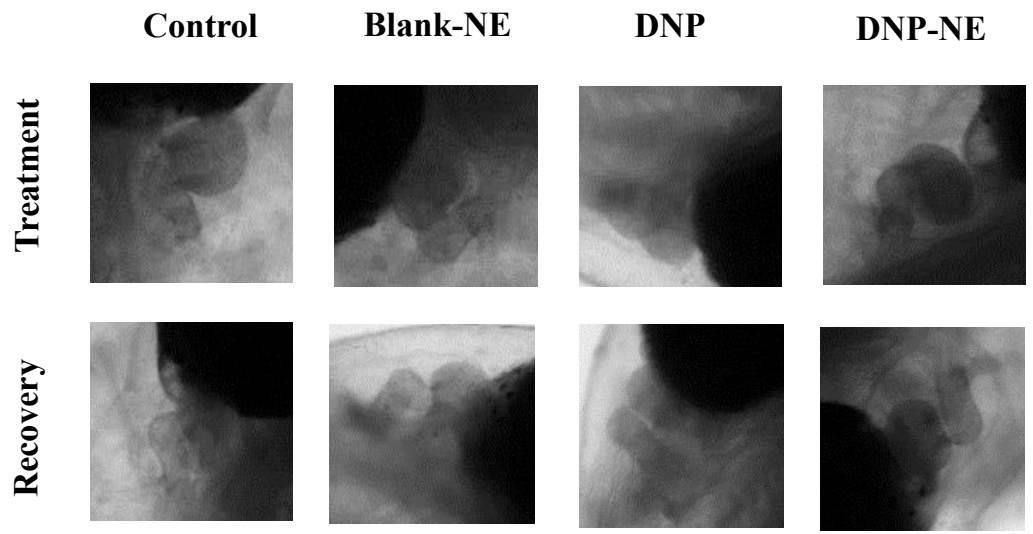


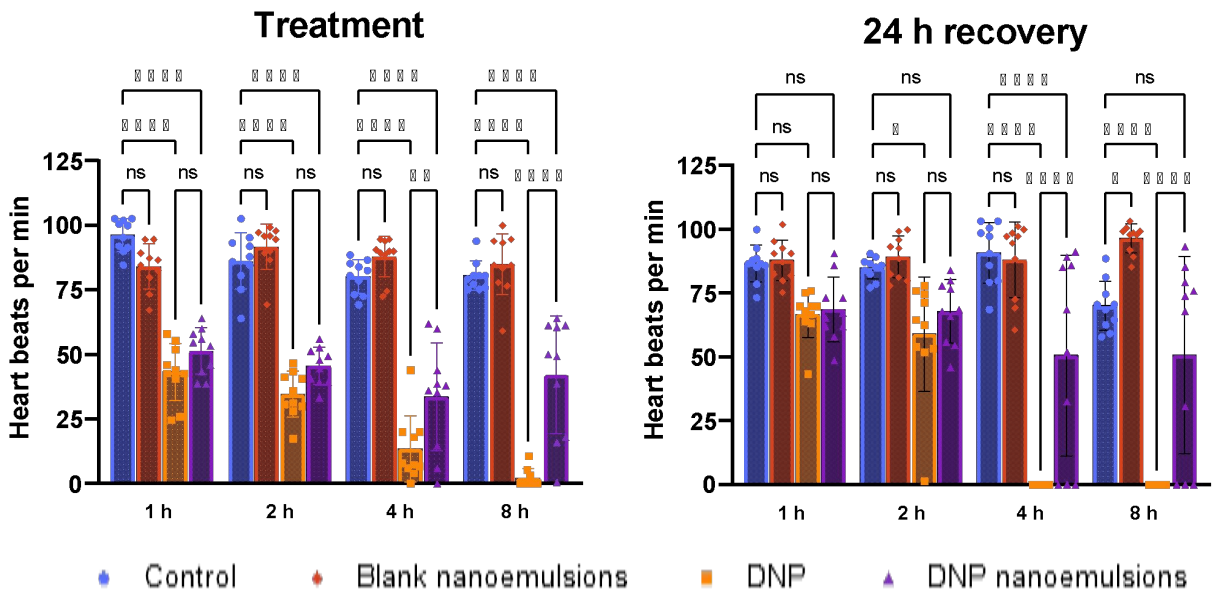
(n=2, N= 10)



Donepezil nanoemulsion reduces cardiac frequency

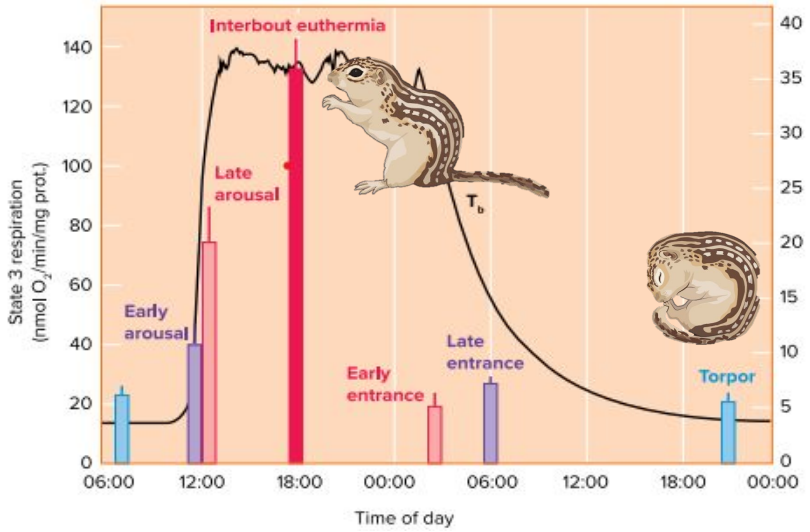






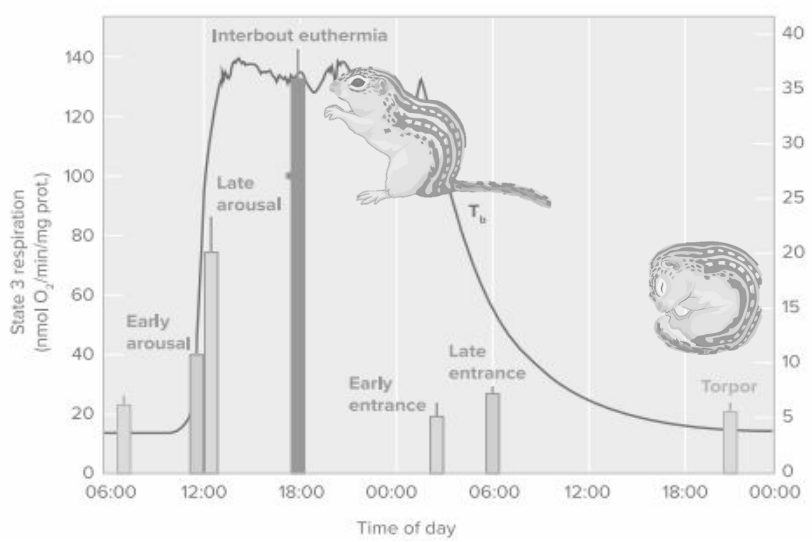
(n= 10 tadpoles per group and timepoint). Statistics performed using 2-way ANOVA with Tukey's corrections. Tadpoles not surviving are not included and not considered for the statistical analysis.

Donepezil nanoemulsion reduces oxygen consumption



Dynamics of mitochondrial metabolism throughout a torpor bout in ground squirrels. Adapted from J. F. Staples et al. Physiology 37. 2022.

Donepezil nanoemulsion reduces oxygen consumption



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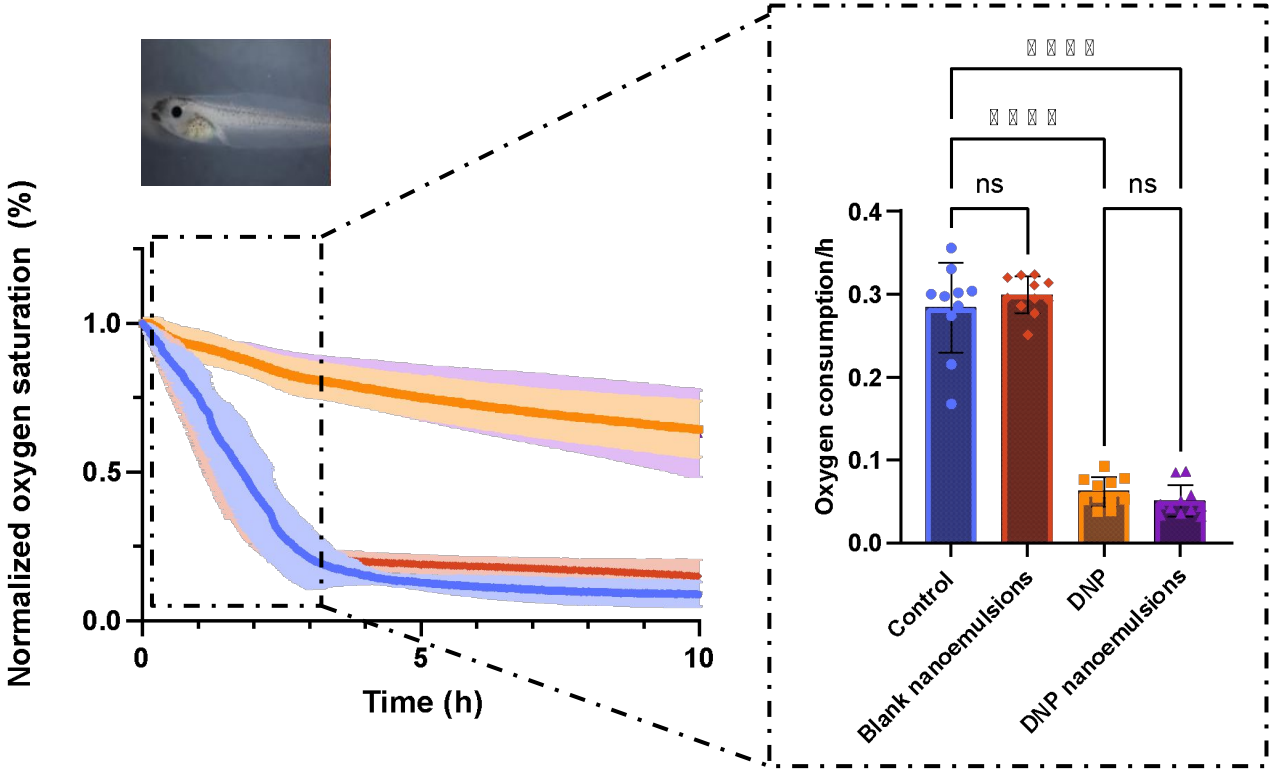


Effect of DNP and DNP nanoemulsion on oxygen consumption (n ≥ 10, N= 2) Statistical comparisons performed using 1-way ANOVA.

Conclusions

- Donepezil has potential to artificially induce a reversible torpor-like state
- Nanoemulsion reduces toxicity and increases brain concentration
- Further applications
- Tadpoles as a useful high-throughput model for in vivo testing of drug nanocarriers.

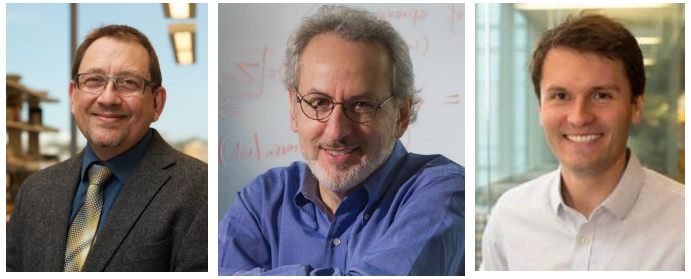
Acknowledgements

Boston, United States of America

DARPA DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

HARVARD UNIVERSITY

WYSS INSTITUTE



Dr. M. Super, D. E. Ingberg & R. Novak

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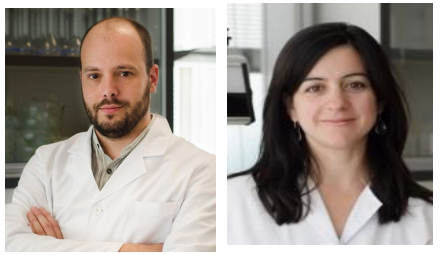

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