Q&A

Do we actually understand what we mean by “anxiety” in rodent models of anxiety?

In the webinar on September 7th, 2023, Dr. Mu Yang and Steffen van Heijningen showed you the major differences between contemporary, highly automated methods and “old school”, ethologically based methods of measuring behaviors.

CONTENT

REVIEW THE WEBINAR

Can the animal's evolutionary context and their environment (last two points under 'old school') be applied to the automated system?

I believe automated systems can greatly facilitate studying naturalistic behaviors of rodents species in a semi-natural environment. Advanced tracking systems can identify animals and their movements without taking them out of the home environment. I think the fundamental differences between "old school" and contemporary approaches are at the conceptual level, rather than on the technical level.

Would you consider both reactions (instinctive) and actions (goal-directed behaviours) defensive behaviours?

"Defensive behaviors" are usually understood in the context of anti-predator behaviors. But aversive stimuli such as odors and foot shocks can also elicit defensive behaviors. "Reactions" can be defensive behaviors depending on what the animal is reacting to.

I think that some smells are engraved as threatening. Novelty is not generally considered threatening. It could constitute an interesting differentiation in the threat/fear distiction presented here?

Novelty and/or novel environment are not threatening, but they usually elicit some risk assessment behaviors, at least at the beginning.

Can animal develop resilience for anxiety/fear? And how it's correlating with human resilience mechanism?

This might be related to coping strategies. Please see the studies on SAL (short attack latency) and LAL (long attack latency) mice by Jaap Koolhaas and van Oortmerssen. https://pubmed.ncbi.nlm.nih.gov/7598668/.

For elevated plus-maze, would you consider rats or mice spending more time in the open arm as part of that risk assessment behavior even without the stretch posturing?

While I would agree the animals are gathering sensory information when they are on the open arm, I would not equate spending time on the open arm (location) and risk assessment (a behavior with distinct forms).

Is the disctinction between fear and anxiety in rodents comparable in humans? (and do they serve the same purpose)?

Great questions! The causes and functions of fear and anxiety are surprisingly similar in humans and rodents. Caroline Blanchard has several papers on this:

https://pubmed.ncbi.nlm.nih.gov/36343691/

https://pubmed.ncbi.nlm.nih.gov/28716675/

https://pubmed.ncbi.nlm.nih.gov/21056591/.

I had a question about the behaviors elicited by fox urine with mice. Some of my collegues use this to assess anxiety-like freezing responses? Do you think this may not be true?

Fox urine is a natural predatory stimulus. In the right context, mice should exhibit some kind of defensive behaviors.

What do you think about the Light-Dark test version that uses a tunnel to connect the dark and lit space. In my opinion this works fairly well to assess risk assessment of mice.

That is a great idea. A tunnel that connects a safe area and a riskier area seems be a good way to elicit risk assessment in mice.

In rodents, the convention to measure anxiety with first exposure to the EPM or open field. Does it only measure anxiety or combination of anxiety and novel exploration ability which can be variable from mouse to mouse. Do our measurements of anxiety confound with novelty exploration abilities. Which brings to a question - should mice be exposed to EPM more than once to measure anxiety?

Novelty exploration is a behavior. Anxiety is a state, not a particular behavior. Anxiety is *functional* during novelty exploration or any other potentially risky behaviors, to minimize risks. Sandra File had some early studies on effects of running EPM more than once. https://www.sciencedirect.com/science/article/pii/009130579502171X.

CONTENT

REVIEW THE WEBINAR

Would it be possible to automate measures of stretch-attend postures automatically with head and tail fixed laser points?

That's a good point. There are a few tracking systems that supposedly able to measure stretch attend posture. Worth looking into how they work.

I have cats and mice as pets in my house. The cats watch the mice run around. Sometimes the mice seem curious about the cats or they ignore the cats.

I don't doubt that at all. Lee Silver has a fascinating book that describe the origin of modern lab mice and pet mice. https://www.informatics.jax.org/silver/contents.shtml. Bred for docility and coat color, contemporary lab mice and pet mice are quite different from *Mus Musculus*.

What would be your opinion on the best pharmacological ways to test if the anxiety could be exacerbated or reduced?

I would use mice whose baseline anxiety level has been tested and replicated. Then I would probably have a positive control group that receives a drug (e.g, diazepam) known to change the anxiety-like behaviors in this mouse line. These are essential for testing any drug with unknown effects on anxiety.

So when considering mice anxiety. What are you thoughts of OFT vs EPM in terms of risk assesment?

Risk-assessment can be easily observed and quantified in OFT and EPM. I would recommend quantifying risk-assessment with location as a modifier.

Is there a recommended battery of behavior tests for Autism in mice?

After working in the field of mouse models of Autism, I actually doubt that there is a valid test battery for autism in mice. This can be an entire panel discussion.

Are you also saying that the elevated-plus maze is fine. But we should also pay attention to what the mouse is doing in each arm in the maze?

Yes I think EPM is a perfectly fine test. I do not agree with the practice of equating %time spent in arms as the sole/key measure of anxiety-like behaviors. Location measures and posture measures (with location measure as a modifier) should be combined to have a more comprehensive view of the data.

How frequently do others find mice fall from the open arm of the EPM. How should one deal with such data?

Is there a rim around the open arms of your maze? I usually do not use data from mice that "fell". But if certain group of mice tend to jump off (as opposed to fall off), you may need some other tests to pick up the phenotype.

Is anxiety measurable in mice home cage? If yes how?

I am not sure it is something that can be easily done in the conventional shoe-box cages. In modern automated homecage systems, one can imagine building out tunnels that connect the home cage to some kind of risky area. It might be feasible to measure avoidance and risk assessment in this kind of setup.

What do you think of Marble burying assay?

I would score time spent in burying, rather than number of marbles buried. And I refrain from calling the burying behavior by any other names (anxiety, stereotypies, OCD, etc. etc.).

When considering infectious diseases as a potential agent causing behavioral changes. Is risk assessment appropiate?

Martin Kavaliers has some fascinating studies on behavioral changes in mice with parasites. E.g. https://pubmed.ncbi.nlm.nih.gov/7567094/.

CONTENT

REVIEW THE WEBINAR

Please compare the EPM and the zero maze.

Conceptually, they are highly similar. Zero maze doesn't have the junction area which makes scoring easier. But tracking/recording zero maze can pose some technical challenges due to its shape.

How do you define simple versus complex animal behaviors? I agree we need to be careful labeling behaviors but even simple behaviors can say a lot. Is nail biting in humans considered simple of complex? That is often associated with anxiety or boredom. Anxiety and the like are composed of simple behaviors?

I don't view behaviors based on their complexity. Rather, I focus on the functions of the behaviors. I am not saying "time spent on the open arm" (on the plus maze) is necessarily a simple behavior, and I am not saying we can't rely on simple behaviors. I am saying though, that it is way too common that we resort to use tests and measures that are simple for *human* to understand and perform, and prefer to call those anxiety-like, autism-like, etc. etc..

In human, anxiety is a behaviour that does not necessarily involve an immidiate threat (unlike fear response). Would you reccommed to test anxiety in a homecage/semi-natural housing conditions to monitor mice's regular behaviours to assess anxiety?

I am a big fan of observing behaviors in the animals' natural environment. I actually think a safe homebase is necessary for mice to exhibit robust risk assessment behaviors to assess the risk, you need to know where is safe first. I think the current automated cage systems can be outfitted with detachable "novel chambers" through tunnel. I imagine we will see avoidance and risk assessment behaviors.

Animals respond to handling. Their initial avoidance response quickly disappears which could be construed as resilience?

I would probably call it habituation in a safe environment. I think resilience is not quite the right description here.

How can you interpret (evolutionary/philosophycally) the innate anxiety/fear differences between inbred and outbred mice? I had struggled to replicate the urine avoidance tests in C57 mice?

Good question. First, see this book by Lee Silver https://www.informatics.jax.org/silver/contents.shtml. The origin of contemporary lab mice were "pet mice" bred for docility and coat color. Strong defensive behaviors, such as fight and flight, were "annoyances" for early biologists who needed mice for non-behavioral research. Currently used inbred strains have greatly diminished defensive behaviors compared to Mus Musculus. Second, you may need to create a "homebase" (a safe spot that the mice can seek shelter in if it needs to hide). Exposing B6 to predator stimuli in a bare arena is not likely to elicit "structured" behavioral response.

Can you elabrate more about the diference between inbred and outbred mice internms of anxiety and depression behavior? Which is better (stronger phenotypes) to evaluate anxiety or depresion?

Inbred mice are preferred for genetic studies. Outbred mice are closer to Mus Musculus and have stronger defensive behaviors (which are used to study fear and anxiety). Depression is the reaction to stressors over a prolonged period of time. I don't think "depression tests" that are based on the animal's reaction to transient stress (forced swim, tail suspension) are actually tapping into depression-like behaviors.

Do you believe there is value in analyzing behaviors in the distal and proximal portions of the open arms in EPM separately?

I think there is value in reporting any measures ----- we don't always know or need to know what we are measuring when we are measuring it. What I do caution against is the tendency to make a story out of everything that is significant. By dividing up the behaviors (and increasing the number of parameters), one can always get significant results on something. This can be a panel discussion on its own. Good topic!

CONTENT

REVIEW THE WEBINAR

I use BALB/c work for assessing anxiety-like beavior and was wondering if it is okay to manipulate the duration of the assay depending on the strain?

That might be against the trend of standardization. Personally, I do think test conditions need to be adjusted based on the strain.

Can we use radial arm maze test for assessment for Anxiety like behavior in animals along with memory or cognitive function?

Yes. I think you can quantify risk assessment (stretch attend posture and head-dip) as you can do in elevated plus maze.

Do you recommend measuring head dips in the EPM as a risk-assessment behavior?

Yes. It is a classic measure that we used to measure. It can be very tricky to distinguish head-dip and stretch attend in the EPM though.

Thank you!

We would like to thank al participants of this webinar. We value your attendance and questions! We'll continue to host virtual meet-ups and Noldus user groups where customers will be able to connect with Noldus employees, industry experts, and most importantly, one another. We hope to welcome you again at one of these virtual meetings.

- Check out our upcoming meetings
- Missed a webinar, you can find an overview of all webinars on our website and replay the webinar of your choice

CONTENT

REVIEW THE WEBINAR