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Change Management in Animal Research

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Slide 1: Change Management in Animal Research

>> *Nicolette Petervary:* Good afternoon. I'm Dr. Nicolette Petervary, part of the NIH Office of Laboratory of Animal Welfare. Today is Thursday, June 27th, 2024, and I'm pleased to welcome you and our speakers to our webinar today on Change Management in Animal Research.

Slide 2: Housekeeping

There are just a few housekeeping details before we get started. The slides, transcript and webinar recording will be available after the webinar on our website. They need to be processed for 508 compliance compatibility before posting, and this can take a few weeks, so please bear with us. Please be aware that OLAW is unable to offer RACE or CPIA credit at this time, so please check with your licensing boards or accrediting organizations with questions about continuing education credit. We will have both the Q and A box and chat enabled for the webinar. Please use the Q and A box for questions to be addressed by the presenters and the chat for comments and discussion. The Q and A box does allow questions to be submitted anonymously. We'll be taking questions at the end of the webinar, but if we run out of time or if the question is a little more nuanced or context-specific, we'll forward the questions to the presenters after the webinar, and then we'll append the question and answer to the

end of the transcript. We'll monitor the chat as best we can, and we encourage you to use it to interact with us and with other participants. Finally, please note that we ask you to abide by the OLAW Code of Conduct, which is available on the OLAW Webinar and podcasts page. We'll also post a link in the chat.

Slide 3: Speaker Introduction

And now I'd like to introduce our speakers.

Dr. Natasha Karp is a Director of Statistics within AstraZeneca leading a team of statisticians supporting preclinical research. In addition, Natasha is an active researcher publishing papers with a focus on the challenges within preclinical research with a particular interest in improving replicability, reproducibility, and generalizability of the studies. In recent years, the research has focused on meta-research exploring how to enable and nudge scientists into better research practice. Natasha started her scientific career with a degree in biochemistry from the University of Warwick and a PhD in Chemistry from Imperial College London. Natasha has also worked in academia as a postdoctoral researcher at the University of Cambridge, and at the Wellcome Trust Sanger Institute. For over a decade, Natasha has been an active contributor to working group activities to drive improvement in research practice. For example, contributing to the development of the NC3R's Experimental Design Assistant. More recently, she has supported the UK Medical Research Council Experimental Design Working Group, which culminated in recommendations that supported the new 'Sex in Experimental Design' mandate for inclusion of both sexes in preclinical research as the default position.

Dr. Brianna Gaskill received her PhD in Animal Behavior and Wellbeing from Purdue University. She completed a 3-year postdoctoral position at Charles River Laboratories and then returned to Purdue as a faculty member in 2014 and was awarded tenure in 2020. Her research is focused on developing new animal welfare assessment methodologies, rodent well-being, and elucidating the scientific impact of welfare problems in animal-based research. In 2020, Dr. Gaskill was hired by Novartis as their 3Rs scientist, where her primary focus is applying 3Rs strategies in concert with disease area scientists to improve both animal welfare and drug discovery. She has published over 80 peer-reviewed manuscripts, and her work has been acknowledged with awards from the NC3Rs, the Swiss Laboratory Animal Science Association, and the International Society for Applied Ethology. Natasha will be presenting first. Natasha, welcome. The floor is yours.

Slide 4: Driving change: sex inclusive research as a case study

>> *Technical Support*: I think you're muted, Natasha.

>> *Natasha Karp*: You'd think I handle that by now but thank you very much for the invitation and thank you very much for everyone joining us today to talk about this topic. I am going to be focusing around the topic of driving change and then presenting a case study around sex-inclusive research.

Slide 5: Disclosure

I have my disclosures that I work with the pharmaceutical industry, but I also have a number of academic collaborations both here in the UK with the Medical Research Council and the NC3Rs.

Slide 6: Outline

So as an outline, I'm first of all going to focus on the processes and ideas around driving change and focus in on the case study giving a bit of background, exploring the barriers, and then presenting a nudge strategy. There's some general conclusions and some ideas that I hope can help you with your own change story that you're trying to drive. So, driving change.

Slide 7: 1. Driving change

What is my personal driver for this direction that I've taken?

Slide 8: My personal driver

A number of years ago, I gave a talk at an ethical review board meeting, and I met someone I hadn't seen for a number of years from my first postdoc. And they said, "It's lovely to see you. I haven't seen you in so long. You're doing so well." Then they said, "You're still banging on about experimental design," and I felt a little bit insulted. Then I reflected on this, and I thought, "Well, do I want to spend another 20 years of my career still banging on about experimental design?" I felt like I was having a drop in the ocean impact, and I wasn't really driving change or making anything happen. I thought "I need a new strategy. I need a new approach. What can I do?" I decided to go and look at how business drives change.

Slide 9: Organisational Culture: 'the way we do things around here

I started reading around the topic and studying a number of different methods, and I'm going to share some of those today that I have found helpful in understanding the challenges that we face that then can be used to try and have your change journey be more effective and more impactful.

The first thing to consider is that we all operate within what's called an organizational culture, which is "the way we do things around here," and our organizational culture leads to our behavior intention, how we are going to conduct ourselves, which then gives a signal to other people of what's the norm, the society expectations. And then, in effect, this establishes pathways of how we are going to conduct ourselves and how we're going to conduct our research in these behavioral paths, which then reinforces the culture which sets the intention. So you can see how culture is really driving the activity on the ground and how we operate within our environments.

And there's this onion model that really tries to capture what drives culture. What it says is that at the core of the culture is our underlying assumptions. These are our core beliefs, that are our unquestioned truths of the matter. We wouldn't even need to think about articulating them. But they do lead to some espoused values. The summaries of our position that we take. Then from those values, we have the visible artifacts of our beliefs, and we will see those in a business setting as the formal guidelines and the processes. Too often, when we're thinking about change, we start at the point of "let's change the guidelines." But the guidelines are the outer layer of the onion and if you peel off the outer layer of an onion that has very little impact. If you want to make a change, you actually have to reach in to tackle those underlying assumptions, the unspoken truths that are leading to the behavior that you are conducting.

Slide 10: Step 1- recognising that this is CHANGE

The first step of any situation is to realize is that what you're trying to do is drive change. Typically, that means you will be raising the expectations of people who are often very stressed and busy. You will need a plan, and you will need a community of support because the reality is, change is hard, and change is a journey that you are going to go on, and it will take time. You need realistic expectations that it's going to be an incremental journey and the time it's going to take to actually, really, drive that change and embed it within your communities.

Slide 11: Personal reaction to change

The next strategy I found helpful was thinking about people's personal reaction to change, and what we're seeing here in this diagram is a method that was originally proposed for the concept of grief, how people react to grief, and has now been applied to business. What we can see on the x-axis is the integration of change; on the y-axis is engagement. It says at the beginning of that journey people react with denial and disbelief and resistance, and that's because they're looking backwards. They're looking back to the state before and they want to return to it. Generally, this is the first reaction people greet you with when you're talking about a change event.

The first thing is to note not to take it personally and to ride this emotional reaction that you're going to get and to look out for movement into the step where people are starting to look forward and starting to explore and experiment with the change idea that you're proposing. As they start that exploration, they can then move into the commitment where they actually implement that change into their practices. There is a risk in this curve, which is there's a danger zone that when you implement your activities, if you do not help people travel beyond this inflection point, they'll just return back to the old ways of working. The other thing to appreciate about this, is that you yourself have already gone through this change curve and have become committed to this change event. But every time you meet someone new, you are starting with them at the beginning of the curve, and that is a natural process and part of the journey of change. The real thing we need to look at is what can we do that can help people move through this change curve more effectively and more quickly so that we can get on and hit that commitment state.

Slide 12: What strategies can we use to drive engagement?

Gary Yukl explored the topic of strategies that you can use and he said that these strategies could be grouped into three types depending on their outcomes. There are some strategies that lead to resistance, which is people actively digging in their heels and undermining your change journey. There are some strategies that lead to reluctant compliance, which is when you're watching them people will do that changed behavior, and as soon as you're not paying attention they'll revert back to the old behavior. What we need are the strategies that lead to a commitment, a commitment such that people then become advocates for the change journey that you are trying to drive.

He grouped the tactics that you could use into two types, those that are positive that lead to commitment and those that are negative that can tend towards resistance. The negative strategies are all about trying to force people, putting something upon people. If you apply pressure, if you say "Well, that person over there is doing it so you should do it" — legitimizing. That's why coalition building can be

in that negative category because it can give that sense of pressure that this is being forced upon you. The positive strategies are around rational persuasion, the giving of the talk, the winning of the heart and mind, trying to reach into that inner onion to change those beliefs. It can be an exchange strategy. You give something in return such as the NIH maybe with a funding approach to a new way of working. But consultation is where you talk to people and negotiate and discuss and explore the new behaviors, and so this collaborative approach will lead to a more commitment outcome, and therefore more positive engagement. Then inspirational appeals where the value that you're going to get those that align with being a cutting-edge, better scientist; it's going to help women's health, et cetera, because that, again, reaches into the core of the onion.

Slide 13: Lewin's Force field analysis

>> *Nicolette Petervary*: Natasha, I'm just going to interrupt briefly because the audio seems to be going in and out for some people. Perhaps if you turned off your camera, that sometimes helps with this. If everyone's okay with that, we'll have Natasha turn off her camera and proceed. Thank you.

>> *Natasha Karp*: Okay, so let's try this now. One of the methods that I found really helpful was to use a Lewin's Force Field Analysis. A Lewin's Force Field Analysis is a really good visual way to explain why we're in a status quo of no change, because what you do is you quantify the forces that are driving change on a scale of one to four, and you quantify the force that's resisting change on a scale of one to four. If the forces driving change are weaker than the forces resisting the change, nothing is going to change. What you need to do is to strengthen the driving forces and weaken the resisting forces, and then the status quo will unfreeze, and you will refreeze into a new situation. It's a very visual way and quick summary to get people on board of what the step should be to move forward and why the status quo is as what it is.

Slide 14: Institute level plan

The last management strategy I'm going to present, because I find it helpful, was an institute-level plan, and this came from a book written by John P. Kotter, called "Leading Change." He argued that there is an eight-step change process that you need to embrace for change to successfully occur and truly embed in a culture. He says that you need to go through all of eight steps. Initially, you start off sequentially, though later they might all be running at once. But if you don't keep going to the end, it won't be embedded in the culture.

If you look at the first four steps, they're all around the leadership and this is all around changing the hearts and minds, the core beliefs, and the later stages are all about management. Too often, he argues, we jump to the management issues rather than focus on the leadership issues for a change event. But the first step was creating urgency, a burning platform, a reason to bother change because otherwise (because it's hard) nothing's going to happen. Then he argues to form a powerful coalition across the organizations, so everyone has a voice from right down to the junior labs up to the senior leaders and senior management to engage, to give value to the change that you are driving forward. From that, you need to create a vision for the change, where a vision is a sound bite, and it has to be a sound bite rather than a long document because you're going to have to communicate this with a high repetition to enable people to really embed this into a new way of working when you consider how much information [they] typically receive in a working week. Then you can start to start thinking about the barriers to remove those obstacles, those blocking forces for the change. As you remove those obstacles, you can

create short-term wins which can give the energy and the impetus to keep going on the vision, and you can build on that change, and then it will become anchored in the culture. You can see now this is why I said at the beginning you're going to need a plan because it is a multistep process to really drive that change and for it to become embedded in culture.

Slide 15: 2. Case study: sex inclusive research

Let's have a look at a case study, and this one is sex inclusive research.

Slide 16: Gender/Sex- does terminology matter?

The first thing I'm going to cover is the terminology of gender and sex. We often use these terms interchangeably, but sex refers to a set of biological attributes that define us as male or female whilst gender refers to socially constructed roles, behaviors, expressions, and identities of men and women. Both sex and gender impact health outcomes, but when we are conducting research on animals, we cannot explore gender. We are only capable of explaining variation in our outcomes as a function of sex, and therefore it is not appropriate for us to use the word gender to represent these health outcomes.

Slide 17: Sex and gender matters

I'm going to argue that both sex and gender matter to health outcomes. And we know that from personal experience when we see that in differences in the prevalence, the symptoms, the progressions and the side effects we experience to a disease depending on whether we're male or female. If we look at the more topical COVID-19 case, in the height of the pandemic, it became very obvious that the prevalence was higher in women, but there was a higher morbidity and mortality in men. This was due both to sex and gender differences. At the sex level, there's a higher expression of the ACE 2 receptor for the coronavirus in males, and there are immunological differences driving the outcome difference. At the gender level, we can see differences such as women having more contacts and work in care roles and males having a lower uptake of preventative measures demonstrating how both sex and gender impact health outcomes.

Slide 18: Simplification underpins experimental research

What drives the issue with sex in research is that when we conduct experimental research, we rely on a process of simplification. We take a homogeneous pool of test subjects, and we randomly allocate them to a treatment group, apply an intervention, measure the effect as an outcome of interest, analyze the data, and then generalize to the broader population. We typically discard sex along with other sources of variation.

Slide 19: Embedded neglect of sex within preclinical research

This approach has led to an embedded neglect of sex within preclinical research. We see this at all stages of the research pipeline, and here I'm highlighting just some of the papers that have been raising this over a 26-year period. For example, in experimental design, a meta-analysis conducted first in 2009 and then again in 2019 looked across nine fields of biology, and they did see an increase in the number of papers reporting studies on both sexes. In that time period, it increased from 26 to 48 percent of papers. But that is still under half the papers that were included both sexes somewhere in that manuscript. In the analysis, what we find is that if both sexes are collected, such as when you breed the animals in-house, only 42 percent of the scientists conduct a sex-based analysis, and those that are

reporting sex differences aren't using the appropriate statistical tools to back up those conclusions. So, we have two problems. We have a problem with inclusion of both sexes and also the appropriate data analysis when we have collected the data.

Slide 20: Sex matters but it isn't perceived as a doable problem

This is happening because scientists believe sex matters but don't believe inclusion is a doable problem. And we know this from research such as a sociological exploration. This was a piece of work conducted by Annika Gompers, and she did an analysis where she interviewed some scientists and identified themes. She found that scientists believed that it was important to embrace variation, to understand biological differences and sex really mattered. However, there was a need to avoid complexity to make science doable to be able to make progress and draw conclusions. In practice there was a tension between the generalizability and avoiding complexity, and it wasn't a doable issue. Likewise in the UK, the MRC conducted a survey and 95 percent of researchers saw the benefit of including both sexes, but there were barriers or concerns around the cost of the experiments, the complexity of the research design, and the compliance with the 3R principals.

Slide 21: 3. Case study: understanding the barriers

I'm now going to present some case studies where the first step is to really understand the barriers. There are lots of different strategies you can take to understand the barriers.

Slide 22: The 2hr workshop intervention

I'm going to share with you a piece of work because I felt it was quite cool, where we ran an experiment to see whether a 2-hour workshop intervention would have the potential to change people's behavior around sex inclusive research. This experiment was based on a parallel group design where we were testing people attending a conference (so we had a baseline group), which were those who were looking at posters, just generally attending the conference. We then also tested people who attended a related symposium. Finally, we tested a community who attended the workshop intervention where the workshop was trying to raise awareness of the changing expectation. It was looking to challenge perceived misconceptions, it was to link to new resources, and to upskill the scientists in both how to design experiments that are inclusive and in the data analysis.

Slide 23: Survey delivers?

We used a survey to collect data from the scientists. From this survey, we can quantify the scale of the issues, the misconceptions, so we really know what the barriers are. We can see if the workshop had any impact on the knowledge and whether it could address the cultural and knowledge barriers that are preventing inclusive research, and we can also understand what is driving behavior. And you might ask "how can I achieve that with a survey?"

Well, what we did, is we used the Theory of Planned Behavior. In this construct, we can't actually measure behavior directly because it takes a long time to be publishing papers of whether you included both sexes or not. Instead we can quantify is people's intention to include both sexes. We can also quantify three other aspects which relate to people's intention. The first, their attitude (whether they believe sex matters and [that] you should conduct inclusive designs), their perceived subjective norm (the society pressure and where other people are in their attitude to inclusive research), and also the

perceived behavioral control (whether they feel that they are able to include both sexes in their research process).

Slide 24: Survey construct- total 39 questions

There were 39 questions in the survey and you can see we had questions around consent, inclusion criteria, demographics, what they thought the advantages, the barriers were, the knowledge, and some questions for each of those Theory of Planned Behavior aspects.

Slide 25: Example TPB questions construct

For example, for the perceived behavioral control questions, you would have three questions. The first question would make a statement, and you would give a reply, and that reply would get a numeric number. So: "I feel confident in my ability to include both sexes." The second question is "Whether or not I include both sexes is completely up to me." The third one is "*Overall, using both sexes is easy or difficult.*" Through this we get a quantitative score from those three questions and then we can calculate an average score that represents their perceived behavioral control to conduct inclusive research.

Slide 26: Data study 1

This led to a data set from 194 people, where 102 met the inclusion criteria. This is predominantly from academic institutes and most the participants have been involved with animal research for over 13 years.

Slide 27: The workshop rescued embedded cultural and knowledge barriers

What we showed with the data from this intervention was that the workshop could rescue embedded cultural and knowledge barriers. What we can see here on the y-axis is we have the cumulative knowledge score, and there is no difference between the baseline and the interested group, but the intervention group has a statistically significant increase in the score. Intention score likewise also significantly increased after the intervention.

Slide 28: Intention positive correlates with attitude and subjective norm

But more interestingly is [that] we can use the dataset to understand what is driving intention. Here we have three graphs where the y-axis is intention, and the x-axis are the different aspects of the Theory of Planned Behavior attributes. We can see attitude (whether they believe sex matters) and we can see a positive correlation between the attitude and the intention. So as people values it more they have a higher intention to include. But the thing to note is that the average score of the population is quite high, so most people think inclusive designs matter, and therefore trying to do activities that raise people's awareness of sex inclusion and the importance of it won't make much difference to their actual intention because most people already value it. We didn't see any correlation with behavioral control, but we did see, again, a positive correlation with subjective norm. Meaning that when there was a higher social pressure, there was a higher intention score. Here, the average score there is nearer the middle; there is more scatter. Now that means that if we conduct activities, such as raising policies, giving talks, highlighting case studies, getting it on the table of meetings, we will shift people's intention along this score as we raise the perceived subjective norm in that community.

Slide 29: Barriers

We can also look at some of the questions particularly the knowledge questions and how many they answered correctly. We can see that the intervention for most of the questions related in improvement in the questions and they were answered correctly. But looking at this data, what we find is that no one selected data analysis concerns as a barrier, but 70 percent of researchers thought that data could be pooled, and 71 percent of researchers thought data should be disaggregated. This really highlights that there is a problem, that they don't know how to analyze the data correctly when they have collected both sexes. It also highlights, the scale of the misconceptions, which are leading to barriers to inclusion, were really high in this community. Seventy percent of the baseline and the symposium group thought inclusion would double the n , which has been shown to not be true. But that misconception becomes a barrier that hinders scientists from studying both sexes. 80 percent thought inclusion increased variability, which therefore meant they would increase the number of animals used, and they thought it wasn't ethical to include both sexes and actually again, there is evidence to show that that is not a true statement.

Slide 30: Take home message for your change project?

The take-home message here from this point for your change project is that you really need to actually truly understand the barriers and this comes through that consultation process that is really critical. To work with people, the Gary Yukl idea [is] that you have to consult with people to truly understand why they're not doing the behavior that you expect. Scientists want to do the right things, so if they want to do the right things and they're not doing it, then what is getting in the way of that? Then you have to address those barriers and you also then need to raise the cultural expectation to normalize it so it becomes the normal thing to do within a culture. Listen carefully, conduct a root cause analysis to understand the resistance, look for themes. I really recommend pilots before implementation because in the process of pilots you generate those success stories and you can start to take people on that journey from looking backwards to looking forward at the pace that really is going to work with them.

Slide 31: 4. Case study: a nudge strategy

So the final thing I'm going to present on this is that we've now moved into a sort of a nudge strategy that has been rolled out more widely.

Slide 32: SIRF: Sex Inclusive Research Framework

This is the Sex Inclusive Research Framework, which you can access on the website through this [URL](#). Now this was put together because there are numerous funding or regulatory bodies that now need to assess whether a research proposal is appropriate from a sex-inclusive position because they are moving from a justification for inclusion to a justification for exclusion. But we know that frequently [there are] the barriers of misconceptions, so what we need to do is to nudge people out of giving them misconceptions as the reason for why they're only studying one single sex. We need to transparency in the decision-making process so that people really start to reflect in a constructive way of whether they truly can or can't include both sexes. So, what is it? It's a decision tree of 12 questions and associated supporting information, that gives information to evaluate the answers to those questions that's been put in the grant proposal or the research proposal for the ethical review board, and then there's information that explains why that question exists. It delivers an evaluation outcome that's either green (the proposal is appropriate); amber (there's some caution required; there's some risks either in the design or the analysis); or red (the justification is not sufficient).

Slide 33: Sex Inclusive Research Framework diagram

This is what the diagram looks like, and I won't be able to talk through it in this way because it's far too complex in this situation, but I can give you some highlights from it.

Slide 34: Examples "Caution is required"

For example, when would you get a caution? You would get a caution statement if you were going to do an unbalanced inclusive design, because there are generalizability and analysis risk. You would also get caution if you do not express how you're going to include sex in the analysis because it introduces analysis risks, for example. You also get a generalizability risk if you're working within a model where the disease occurs in both sexes, but the model can only be induced in one. Now, this doesn't say that you can't conduct these experiments. It's just saying there is a risk and that it is up to your funding body and your ethical review board to decide what to do in that situation. For example, we have a published justification here on the right, and it's stating that in all experiments, male and female littermates will be pooled together and analyzed as one group. It is inclusive. There are both males and females, and the groups will be mathematically compared, but they are not including sex as a variable in the analysis. It is a sex-inclusive design but has some risk.

Slide 35: Examples "Single sex not appropriately justified"

A situation where you could get single-sex as not appropriately justified could be because you are putting forward a common misconception, a generic statement such as "Females are more variable", "Including both sexes will double the sample size needed." Or, you could be making a statement that is about avoiding change or fear: "My previous data has all been in one sex," or, "Sex hasn't been shown to date to matter." If sex hasn't been shown to date to matter, that's great news. You can embrace both sexes and make a generalizable estimate.

Let's have a look at the example. "We plan to use male mice, as female mice tend to have twice the level of circulating cortisol as males, and these levels may shift in response to the stage of the estrogen cycle." There's a baseline difference in the cortisol level. That doesn't matter. We can account for that by including sex as a variable in our analysis with the right statistical tools. Then they're saying, "And these levels may shift." They don't have evidence for that. There's no power calculation to say that would be a problem. They are making a generic statement around variability to say female animals are more variable, and so single-sex is not appropriately justified.

Slide 36: Examples "proposal is appropriate outcomes"

What about in green, a proposal is appropriate? It could be a single-sex study. For example, it's an exception such as female mice have been implanted with patient-derived ovarian cancer tumors. In this situation, of course, it would be appropriate to only work with female animals. It's an acceptable exception. Or it could be a harm and/or cost evaluation versus benefit. The last question is where you evaluate whether you consider costs that could be ethical, logistical, or welfare issues to explore the benefit of studying both sexes versus those costs to make a decision. If you find those costs are too high, the single-sex study would be justifiable.

Slide 37: What is the SIRF trying to do?

What the SIRF is trying to do, it is trying to alter the forces that are at play in the Lewin's Force Field. It's trying to give resources to the ethical review boards and the funders to strengthen the driving force by giving real clarity of whether it's a good justification or a poor justification. It's trying to weaken the forces resisting change and trying to tackle those misconceptions so that it's a true reflection of whether it can be included or not. Please reach out to the framework, and if you want to reach out to myself directly, you're welcome to.

Slide 38: 5. Conclusions (no dialogue)

39: Conclusions

In conclusion, I have been talking about sex-inclusive research for many years, and suddenly the tide is turning. I would argue, don't be disheartened. Incrementally, you can drive change, and this situation can significantly improve. But you do need a strategy, and I would recommend looking at the business change management strategy to develop your plan but also communicate the approach to management and others to get resources to help you with your change journey. The first step is to truly understand the barriers and the drivers for change so that you can generate that burning platform and weaken the barriers that are hindering the progress. Find ways on the way to win, and celebrate the progress you are making. Then we can be solution focused and truly deliver real impact in how we're conducting our research, whatever your change journey is. Thank you.

Slide 40: Acknowledgement

I need to acknowledge numerous people who have been helping me on the way including Brianna, who is my Theory of Planned Behavior expert, and the people in the working group who've helped me develop the SIRF framework.

Slide 41: References

And [for] any of the references I've mentioned today, the details are available in this slide if you wish to read further.

Slide 42: Confidentiality Notice

Thank you.

>> *Nicolette Petervary*: Thank you, Natasha, and now Brianna will begin her presentation.

>> *Brianna Gaskill*: All right. Great. So just for a little bit of background, when I was asked to give this particular presentation, OLAW was really interested in providing

Slide 43: Changing human behavior to improve animal welfare: rat tickling as a case study

some actual welfare changes that might be able to provide some application of some of these ideas that Natasha has just pointed out and how we can potentially utilize them in real-world practices. So, this is just kind of a case example of how my lab at Purdue University has utilized the Theory of Planned Behavior in order to try to move forward with implementation of animal welfare techniques.

Slide 44: Disclosure

And so just as usual— the usual disclaimer, this is based on work that I completed at Purdue University and does not necessarily reflect the opinions of my current employer.

And we're going to use rat tickling as an example.

Slide 45: Acknowledgements

And before I get started, I want to make sure that I acknowledge the person behind the majority of this work, Megan LaFollette, and some other really fantastic women that helped as part of her Ph.D. thesis on really understanding how we make behavioral change in the welfare space.

Slide 46: How does change happen in laboratory animal science?

So before we get started, I want to just take a second to tell you a little bit about rat tickling and why we decided to focus on this and then provide some kind of structure around how we approach this challenge, this animal-welfare-related technique. So before we get started, I just want to take a couple seconds for you to think about how does change actually happens in the field of laboratory animal science? Is it data-driven? Is it based on scientific evidence? How do we facilitate these changes if we actually have these two pieces? And does it happen naturally?

Well, for the longest time, I felt like I had a perception of “If you build it they will come,” and if I put out the best-quality data people will take it up and start to implement it. But towards the end of my time at Purdue, I was really starting to realize that that really wasn't necessarily the case and that we probably needed to take a little bit different approach to how we were presenting, communicating, and providing information to people in order to try to get these new ideas implemented so that it would improve animal welfare. And so for years, I've been drawn to this handling technique that a good friend of mine, colleague Dr. Sylvie Cloutier, had been utilizing and applying in the laboratory animal field. And she had gone to conferences. She talked a lot about it. I just didn't see a lot of people doing it. I really wanted to see where we could maybe try to apply it in real-world circumstances. So what we're going to do is we're going to use rat tickling as this example of how we can use social science techniques in order to make change and improve animal welfare.

Slide 47: Affective Neuroscience

So just a little bit of background on rat tickling. This was developed by Dr. Jaak Panksepp, and he is considered the father of affective neuroscience. He was really interested in developing a technique to systematically study the neurological basis of positive affective states such as play. And I think we can all believe that play, across humans and other species, is considered a very positive experience. And Dr. Panksepp really wanted to study play in rats so he could better understand the brain and how it was working.

Slide 48: Rat play video

And so this is what rat play looks like. Hopefully the video is coming through well. You can see these two juvenile rats running and jumping on top of each other, chasing each other, flipping over on their backs. You can even see it in adult males, as well, so even once they've gone through sexual maturity, we can

still see them occasionally playing. Granted, we've seen the majority of this in the juvenile stage, but rats will play as they get older, just not to the same degree.

Slide 49: Rat play has 2 key components

Now, there's two main concepts of rat play, and the first of which is the dorsal contact, which hopefully you saw in that first video of those two little rats. One jumps on top of the back of the other. And the other aspect is the pin. Usually, the rat will jump on or will roll over on its back, and the other one will jump on top of it. And if any of you, which I think quite a few, are very familiar with dog play, rat play has a lot of similar aspects. And so you might be able to see those when you see your rats playing.

Slide 50: Rat tickling mimics 2 key aspects of rat rough-and-tumble play

Now, what Dr. Panksepp wanted to do was he wanted to be able to study play when he wanted to study play instead of just catching it naturally. And so what he decided to do was to start mimicking social play with his hand using ... and mimicking these two aspects. So he would utilize the dorsal contact by doing kind of a tickling motion on the nape of the neck of the rats. Then he would flip the rats over and do kind of a jiggle, tickling motion on their backs. And so he utilized this in order to facilitate play when he was wanting to do data collection.

And what he found is that rats

Slide 51: 22-kHz vocalizations reflect negative affect

have different kinds of vocalizations, and they give them off in different situations. He's done a lot of really amazing work, neuroscience work, to really understand these vocalizations. And this is where rats are really cool. So rats give off a negative vocalization. It's considered a 22-kHz vocalization, and this is actually a spectrograph to kind of visually illustrate what this vocalization looks like. And it may just look like a solid line that's just going straight across in this image, and this is actually what it sounds like: *[plays recording]*. And so you don't hear a lot of intonation, it's a very flat line. And we see that these vocalizations in particular are produced in situations that we know are negative such as electric shock, the presence of predators or predator odor, social defeat, and the application of noxious drugs. And on top of this, these are produced in anticipation of these negative events, and not only that but they correlate with typical behavioral outcomes that we would use to assess negative emotions such as avoidance behavior or perhaps freezing behavior.

Slide 52: 22-kHz= cholinergic activity & are correlated with magnitude of anxiety

But to go a step further, he found that they were reliable predictors of cholinergic activity in the brain that correlated to a magnitude of anxiety. Areas of the brain light up that we would associate with a negative experience in perhaps a human brain. So, this gives some really good context that when the rats give off these vocalizations it means that it's something they don't like.

Slide 53: 50-kHz vocalizations reflect a positive affect

On the flip side, the more fun side, there are positive vocalizations. Now these are called 50-kHz vocalizations, and they indicate a positive affect or a positive emotion, something that they like. And these look quite a bit differently. We call these frequency-modulated vocalizations, or trills, and they sound a little something like this *[plays recording]*. These sound very differently than the 22-kHz calls.

And again, we see these produced in situations that are positive such as the anticipation of tasty treats, euphorogenic drugs, during play, and even during copulation. And similarly, again, we see these in anticipation of these positive experiences, and correlating with levels of satiation.

Slide 54: 50-kHz= dopaminergic activity & are correlated with magnitude of reward

Further, we see that these are indicative of dopaminergic activity and are correlated, again, with the magnitude of reward. So, providing some really strong neuroscience evidence to say that when the rats give off these vocalizations, this is something that we would consider to be positive.

Slide 55: Rat tickling video

This is what rat tickling looks like. This is Franny. We got to tickle her in San Francisco, and she was one of the best rats we've ever tickled [*plays recording*]. So those vocalizations you heard were from a bat detector that was above the cage so that we could hear those vocalizations. Now, if you've worked with rats for years, you're probably like, "*I have never heard rats ever give off these vocalizations before,*" and that's because we can't hear it with the naked ear. We need specific equipment in order to actually hear these vocalizations. And so obviously this is a really good example of a rat that was very much enjoying the tickling. Usually, the response we get when people watch this video is, "*Wow, that was really rough. Are you sure they actually like it?*" And based on the vocalizations, the fact that they will come back and seek interaction with your hand, we believe that this is something that they enjoy. It is exceedingly rare to see the 22-kHz or negative calls occur during a tickling session.

Slide 56: That's cool but... why should I tickle rats?

So, okay, that's cool. But why should we actually tickle rats?

Slide 57: ...the vocalizations tell us how a rat is feeling!

So when we approach questions about animal welfare or how we think about animal welfare, most welfare scientists have embraced the Three Conceptions model where we look at various aspects of the animal to understand kind of the whole picture. Now, the first of which is biological functioning. This has to do with the basic biology of the animal. Is it diseased? Is its physiology functioning in the way that it was intended? Are they healthy? And that, obviously, health is going to have an effect on welfare. The next has to do with natural living, so as an animal in an environment that naturally provokes aspects of their innate, natural behaviors. And so this is something that we tend to lean more towards natural behaviors that we're seeing in the environment where the animals are living. And then the last has to do with affective states or emotions. Is the rat feeling pleasure? Is the rat feeling pain? Things along those lines, and this ultimately is one of the most important conceptions of the animals' experience but is probably the most difficult to actually measure. We do have aspects of actually being able to measure this, but it's very challenging, and that's where rats and their vocalizations come in that's so amazing. Based on the work that Dr. Panksepp did, this gives us some indication of how the rats are actually feeling. So it gives us some indication of this aspect of affective state and how they're feeling right now.

Slide 58: Rats experience stress during handling

But of course, in the laboratory, rats experience a fair amount of stress just simply from being handled. A lot of this comes down to the fear of not knowing what's going to happen to them. And of course, when we have to handle the animals, restrain them for various procedures, and they're not acclimated

or habituated to this, this causes fear and anxiety. And this fear and anxiety leads to behavior change, hormone changes, and even affects brain structure. And ultimately this harms animal welfare, experimental validity, as well as reliability. So it's an animal welfare issue, and it's also a scientific issue.

Slide 59: Rat tickling is an effective intervention

So in terms of rat tickling as an effective intervention, this is a systematic review that Megan did as part of her master's project to really see, *“Okay, well, we think this is a good idea. We think that the field of laboratory animal science should be doing this. But is this really what the literature is saying?”* And so what she did is she looked at all of the literature that had utilized tickling or tickling-related procedures in the literature and just kind of looked at, what kind of outcomes were we seeing? And she found that if they measured the 50-kHz vocalizations, those positive vocalizations, overwhelmingly we saw that when the rats were tickled, we see them giving off these positive calls. We also see that they're more likely to approach a human handler, a human hand. We also see a reduction in fear or anxiety behaviors, and lastly, we find that when those animals do have to be handled, they're a lot easier to handle. And ultimately found no negative effects in any of these publications. Either it was neutral, or it was a positive effect. So it really gave us an indication that based on all of this literature that had been done that this was something that was positive and that the literature in whole was indicating that this is something that could be beneficial for their welfare.

Slide 60: Does anyone use it?

So our next question was, *“Well, this is great, there's good evidence for it, but does anybody actually use it?”* And so this is where we start to really kind of get into that change management and Theory of Planned Behavior, really trying to understand the perceptions of the people in the field to this particular technique.

Slide 61: Laboratory animal personnel cross-sectional survey

And so what Megan did for one of her first Ph.D. thesis projects was to do a survey. We needed to know how many people were utilizing this in the United States and Canada. We felt like those were probably a little bit more similar environments than how it was being utilized perhaps over in Europe. And so we focused on these two populations to see whether people were actually applying this. So if anyone took this survey, thank you so much. We know it was a little bit longer than expected, but we've got an overwhelming wealth of information from this.

Slide 62: Measures: 3 main sections

So in this particular survey, we had three main sections. The first, of course, is basics, demographics. We looked at rat-tickling frequency, and then we utilized the Theory of Planned Behavior that Natasha talked about in her last talk to look at intentions and beliefs around rat tickling. We got a fantastic number of people who wanted to participate. Almost 1,500 people started the survey. Nine hundred people met the inclusion criteria. And once we kind of filtered through and made sure that people had answered 50 percent of our questions, we had close to about 800 participants that we included in this data set, which our social scientists (who assisted with Megan's thesis) were quite overwhelmed with the response that we got back. She was quite shocked, and she said it's clear that this group of people really have something that they want to say. So it's really exciting to see that.

Slide 63: Theory of planned behavior

So to kind of just remind you, this is... we're going to look at the Theory of Planned Behavior, and if we work backwards, really what we're interested in is that changed behavior. Did somebody change their behavior based on whatever implementation or intervention we've done? So the Theory of Planned Behavior can be used to study human behavior but really look at their intentions. And because, as Natasha said, quite often it's hard to get at the true behavior, a lot of times we have to look at intention. But it's very predictive of behavior later on. So we look at attitudes, so... *"is rat tickling something that's good?"* We also looked at behavioral norms, *"people I respect want me to tickle rats."* Those are some example questions. And then last, we looked at behavioral control or perceived behavioral control. *"I am confident that I can tickle rats."* So this has to do with whether you understand the technique and feel like you can control and do it in an effective manner? So this is kind of the general model that we utilized in part of the survey.

Slide 64: Lab personnel tickle rats infrequently

It was not surprising, but we ultimately found that lab personnel tickle rats really infrequently. So 89 percent never or rarely tickled their rats. And remember, this was out of nearly 800 diverse respondents, so almost 90 percent have never or rarely tickled their rats.

Slide 65: Theory of planned behavior

Now, when we looked at the Theory of Planned Behavior, and in this particular study, we were only able to focus on future intention.

Slide 66: Beliefs are associated with rat tickling intention

What we found was that attitude significantly affected future intention. So did subjective norms. And finally, behavioral control or perceived behavioral control also affected those future norms or those future intention to tickle rats.

Slide 67: Handling & welfare was a benefit of tickling

And when we ask people about, *"Well, what do you think the benefits of tickling rats were?"* we found that over 55 percent of people felt like this increased rat handling as well as rat welfare in terms of the advantages.

Slide 68: Time was the biggest barrier to rat tickling

Now, that's great and all, but we're really curious about, what was blocking people from doing it? And ultimately time was the number one response that we got back from folks who took the survey, that it took too long (the phrase *"lack of timing"*, *"It's time consuming,"* various phrases like that).

Slide 69: Personnel & Research were other barriers

And then we also found that people had comments about personnel and research. Granted, this was substantially less, but these were still barriers that we wanted to think about and attempt to address.

Slide 70: Forcefield analysis

Now, we can take this information that we collected from the surveys and apply it in this Lewin's Force Field analysis as well. And so here we specifically pulled out time, training, and research as some of these main barriers that are pushing against ... are forces against change.

Slide 71: Can we address these forces against change and improve implementation?

So we decided, can we address these forces and hopefully improve implementation?

Slide 72: Current tickling practices are time-intensive

So time was one of the biggest aspects that people mentioned, and so we felt like this is probably the most important one we needed to address. This is actually something that, at least in the published literature, it does not appear that Dr. Panksepp had actually looked into. But based on his general protocol for tickling rats, it took 2 minutes, and you needed to tickle rats over 5 days. And if you had a study of 50 rats, that would potentially take 5 hours per study in order to get rats through the protocol so that they would be more likely easier to handle, and less fear, that sort of thing. So it's not surprising that people thought that this was very time-consuming.

Slide 73: What dose of tickling is sufficient?

So we decided to look into, what is an effective dosage of tickling to get these positive behavioral outcomes for the rats but with the smallest time investment needed? So what we did is we looked at different durations of tickling, so 15, 30, or 60 seconds. And one of the things that's a little confusing based on Panksepp's protocol, even though it said 2 minutes, you're oscillating between 15 seconds of tickling and 15 seconds of rest. So within that 2 minutes, you're only tickling rats for a maximum of 60 seconds. And so we decided to look at that as our maximum duration of tickling. Then we looked at three different frequencies. We thought ideally this is something that could fit within an acclimation period, at least within a work week. So we looked at one, three, or five days of tickling in a factorial design.

Slide 74: Tickling for 15 s was most efficient & effective

And what we found was that tickling for 15 seconds was actually the most efficient and effective. We saw no differences between any of the durations of tickling as a main effect variable.

Slide 75: Tickling for 3 days was most efficient & effective

However, we did find that tickling for 3 days was probably more efficient... we got a higher response, measured by those 50-kHz positive vocalizations, when we tickled rats for 3 days instead of 1. But we didn't find much of any significant difference if we tickled them for 3 or 5 days. So we felt that 3 days was the most efficient as well as effective.

Slide 76: Tickling for 15 s over 3 days was most efficient & effective tickling dose

So based on this, we recommend tickling your rats for 15 seconds over 3 days, and ultimately if we look at how this changes the investment of time, again, the original protocol, it would take approximately 5 hours. But with this new recommended protocol it would take 38 minutes for 50 rats. So we felt like this was something that was much more likely to be fit into an acclimation period before animals actually

went on study. And actually was over 1,000% reduction in time investment. We're hoping that this might be a little bit more likely, weakening some of those barriers associated with time.

Slide 77: Can we address personnel barriers through training?

Next, can we address some of these personnel-related barriers through training? And so this is something that was actually quite fun. Megan and I went on a tickling tour across the United States in order to do some various trainings.

Slide 78: Tickling tour study

Now, in this particular study, we had three groups of people. We had a group of people that received an online and hands-on training session, (live, hands-on training session). And then we had a group that only received an online training session. And then last, we had a group of people who were interested in learning about tickling but were put on a waitlist. We were actually able to recruit 96 people to participate in our survey, and everyone received a baseline survey. But then these two groups received the online training, which I would highly recommend.

Slide 79: Online tickling training

It's a beautiful online training platform. It's colorful. It's interactive. There are videos. It's really, really fantastic, and I'll provide [a link to that](#) in just a second.

Slide 80: Practical explanations

But we provide aspects of practical explanations, so we realized through some of these trainings that to really understanding how to grip the rat in order to do the flip was very challenging for some people based on how people were trained to do different restraint techniques.

Slide 81: How to fit tickling into your study

How to fit tickling into your study, some recommendations around that.

Slide 82: FAQs and tips!

Then finally, some FAQs and tips. So tickle first and manipulate rats later. So the first thing that you should be doing before you do any kind of manipulations is starting that tickling protocol and then trying to do those manipulations after that's been completed.

Slide 83: Example rat tickling certificate

And you, too, can receive your very own certificate of completion as a certified rat tickler if you take this course. So this is actually what we used to make sure that our participants had actually gone through the training and appropriate training before we moved on to their hands-on training.

Slide 84: Tickle tour diagram

And so we wanted to make sure that everyone had gotten through and finished all the materials for that. But this one group went on and received a 30 to 45-minute hands-on training session.

Slide 85: Hands on training map

And Megan and I went all over the US. We started in the Boston-Cambridge area doing various tickle training sessions. And then we went out to San Francisco and did some trainings there and then back at Purdue at our home institution as well as IU School of Medicine. We got a nice breadth across the United States with those different locations.

Slide 86: Tickle tour diagram

So after everyone had received their treatments, everyone received a post survey. And then 2 months later, we did a follow-up to really kind of get at that general behavior. Did they implement the behavior after this particular intervention?

Slide 87: Theory of planned behavior

We're looking at our Theory of Planned Behavior

Slide 88: Beliefs about tickling

here and kind of focusing on these beliefs.

Slide 89: Beliefs about tickling: Attitudes

We found that attitude was affected. However, it was only a timepoint-related aspect. So it wasn't due to our intervention strategies. But basically, at that post survey time and 2 months later, we saw that there was an increase in their attitudes towards rat tickling, but nothing to do with our interventions.

Slides 90/91: Beliefs about tickling: Norms

If we look at those subjective norms, we find again a time-related aspect where we see an increase from baseline from their social norms.

Slides 92/93: Beliefs about tickling: Control

And then last, in terms of perceived behavioral control, we did finally see an effect of our treatments in that in the hands-on, online group, they saw a significant increase from baseline in their perceived behavioral control of rat tickling.

Slide 94: Theory of planned behavior

Now really what we want to know is, did this affect behavior? Can we change beliefs are really interesting, but we really want to know, did it influence the behavior? And so this is what we were able to look at, at those 2-month-later time points.

Slide 95: Training improved implementation

And we were very excited to see that training did improve implementation. So if we look at the post-training time point for our hands-on group, we see a significant increase in implementation from the baseline. And we also see it at the 2-month-later time period in that people in both groups, either the online or the hands-on group, both increased their implementation from those baseline values. So that was really exciting to see, that we had effectively changed behavior.

Slide 96: Other applications for welfare change

Megan has gone on to bigger and better things, she is the director at The 3Rs Collaborative, and she's utilizing this technique in other ways. For instance, hopefully you've all heard about the refined mouse handling push from both the NC3Rs and The 3Rs Collaborative. And this is where she's utilized the Theory of Planned Behavior in order to focus on that particular rodent-related refinement. But she's also utilizing it in different ways too, utilizing it for environmental health monitoring in order to move away from sentinel testing. So these are some examples of where this technique is being applied in other places.

Slide 97: Conclusions

And then finally to just conclude, one of the things that I think we need to be more conscious about is that people are big barriers to animal welfare improvements, and that a social science framework can be really helpful in improving implementation. But as Natasha talked about, we need to listen to our stakeholders and really identify what the challenges are that they're facing with these new procedures, so that we can adequately address those barriers and provide solutions to those challenges. And ultimately, providing data and experience back to the end user is really, really helpful for them to understand that you have listened, provide some benefits to them, show them what you've collected, so that you can provide that information back.

Slide 98: Acknowledgements

And then lastly, just again huge thanks and acknowledgments to Megan for all of her hard work on this project, our collaborators, and this work was supported by GLAS as well as the Animal Welfare Institute. Now if you're interested, you can get to the rat tickling certification there. And I think we're ready for questions. So thank you.

End of slides

>> *Nicolette Petervary*: Thank you, Brianna. And I encourage everyone to become a certified rat tickler because that is an awesome thing to put on your CV.

>> *Brianna Gaskill*: And it's fun, too.

>> *Nicolette Petervary*: And it's fun.

>> *Brianna Gaskill*: I have to get in there. It's fun, too.

>> *Nicolette Petervary*: So we have ... first I'll go, Brianna, to a question about a slide because I think we want to do that while it's still fresh. Based on the dosage slide for tickling, the recommendation was 15 seconds of tickling over the 3-day period. Does the tickling stop once the research trial starts?

>> *Brianna Gaskill*: So in that particular study, what we did is we were just looking at that timeframe. Could we get the reactions and behaviors out of the rat that were positive within this intervention? Now, we didn't go on beyond that in that particular study, but there have been studies to indicate that if you periodically continue to tickle the rats, over time... and we generally have a recommendation maybe during cage change once a week, every 2 weeks. As long as they continue with some of that tickling on a periodic basis, you can still see the benefits. One of the things to recommend is that, remember, rat play kind of decreases over time. So you may find that once the rats get too big or they're just too old, they may not really want to do it that much anymore, but it's a really individual preference. And so Megan's

got all kind of FAQs, tips, and tricks about how to read your rats, how to be aware and be looking for some of these things because it's a natural progression, and we understand that. And so [it] really kind of depends on the situation, but she's got tons of information and recommendations, suggestions of how to apply it in a normal basis, as part of a normal research study.

>>*Nicolette Petervary*: And we can access all of those when we go to the certificate?

>>*Brianna Gaskill*: Yes.

>>*Nicolette Petervary*: Perfect. We have another question, and this is for both speakers. It's a little more nuanced. So, if you need some time to think about it, that's fine. But maybe we can explore some initial thoughts. Are there any thoughts on how to encourage change in a contract research organization? The funders provide their plan, and we often have to accept their plan or decline the funds. It can put us in a very tough spot. Biases toward male animals in research is a perfect example that we are struggling to address.

>>*Brianna Gaskill*: You want to take that one first, Natasha?

>>*Natasha Karp*: That is a difficult situation. And I think it depends how your relationship is with your clients. In some areas of the business, AstraZeneca has a long-term partnership with a contract research organization, and actually they are considered trusted partners with a lot of experience. If you can demonstrate that experience, you can get buy-in. But basically, you're in a similar situation which I guess I find myself in, where you're trying to read the room of the openness to the idea. You can't take a hard stance. You have to assess whether they're interested, whether there's room to try something and whether they perceive the social pressure and then whether they're open to try something. But I do think you're in a challenging situation, but I think it's the same. Then is it that different within an organization if there's a standard way of working, and people have always done it that way, and then you've got a new, young scientist who goes, "*Well, I've heard this, and I want to try it.*" They're in, again, that similar situation, that it can be quite hard. I think there are parallels. I don't know if that helps.

>>*Nicolette Petervary*: Thank you.

>>*Brianna Gaskill*: I would say the same thing. I think we have challenges even internally, where some of our scientists say, "*Well, why are you challenging this? The CRO has already approved it.*" And so I think it goes both ways, right? It's something that, as Natasha said, you kind of have to test out the openness. I think providing suggestions and well-supported suggestions is not necessarily a bad thing. But obviously if it's a situation where there's not a lot of openness, it's going to be very hard to make that change. But I feel like there's a definite culture push in the last few years that people are starting to think about 3Rs-related aspects more commonly. And so I'm hoping that people are more open to these ideas and to be creative and try new things. And maybe they work sometimes and sometimes they don't, and I think that's okay to fail, as long as it's an honest attempt. It's not just like, "*Oh, we went through the motions, and, no, it didn't work., So we're not doing it anymore.*" But I think it's important to definitely try, but it's a challenging situation for sure.

>>*Nicolette Petervary*: And while, Brianna, you're answering all of these questions, we've gotten a lot come in. So I'm going to try and go back through the rat tickling and then go back to Natasha, just for the sake of efficiency. So there's one comment that, anecdotally, some people posit that just as some humans don't like to be tickled, there may be some rats that don't like to be tickled. When doing

ultrasound monitoring of rats during tickling, is there a 100-percent incidence that 50 kilohertz vocalization across all animals tested?

>>*Brianna Gaskill*: No, there is not 100 percent, and just to say, just because we call this tickling, it's not the same thing as human tickling. It's just a fun way to label this social interaction, this positive social interaction with the rats, a positive... we're playing. We're playing with them. We're not actually tickling them like you would a small child. It's just that sometimes the finger motions look a little bit similar. So just making sure that that's clear. We're mimicking social play with the rats, not tickling them. But I think it's a really good point. We have found a few rats... so throughout Megan's thesis work, she tickled a lot of rats, and we did find a couple (not very many, maybe a handful at the very most) who ultimately, we decided, "*You know what? We're not going to tickle these guys.*"

And she's got an entire section in the training that's "Read Your Rat." This is what it looks like if a rat doesn't want to be tickled. I think the vocalizations helps you understand that. So if you can see the 22-kilohertz calls being given off. But they will give some audible calls as well that you can physically hear while you're doing it. But honestly just the behavioral postures, the behavioral cues that you can get from the rats... you can figure [it] out without those vocalizations. One of the things that Megan and I talked about quite often is whether you had a "floppy" rat or not. So when they are giving off a lot of vocalizations as you're tickling them, they are loose, and they were relaxed, and literally they feel floppy like a rag doll. But if you have a rat who is not enjoying the tickling and not giving off the 50-kilohertz calls or potentially [is giving off] some 22s, they are stiff. They are rigid, and so they feel very differently. And so you can tell just by ... as you're doing the technique probably whether the rats like it or not. But like I said, Megan has got an entire section on there on making sure that you're not doing this to an animal who doesn't want it done to them, so that you are reading the animals they go through.

And obviously if you've got animals on a research study, where maybe they don't feel well, this is probably not necessarily a good situation for you to be doing that to them. Because if they don't feel well, if they have some kind of incision, you don't want to necessarily do this rough and tumble play which is what it's ... how it's described, for health-related reasons. And it's possible that animals may not like it, especially if they don't feel well. So... and she's got some really good scenarios on there to help you think through, should I be actually applying tickling or not? And that's why we generally say it a good thing to do during acclimation, before anything happens to the animals. So that way, they have a positive experience with the humans first off.

>>*Nicolette Petervary*: And we have another interesting question for you, Brianna. Is it important who is doing the tickling? Is this a personal connection between the tickler and the rat?

>>*Brianna Gaskill*: So yeah, I would... Sylvia Cloutier did some really interesting work with this as well, on whether it was really important for the person doing the tickling to be doing the procedures as well. And ultimately, yeah, it kind of is. So you can't just necessarily... I think there is a little bit of association that does happen, but if it's something new, a lot of rodents are novelty averse. And so this is something... Thinking about you, you smell differently. They can tell individuals because we all smell differently. They can tell the difference between a carnivore and a vegetarian. They understand that we are different, and they have such an amazing sense of smell. And so it's generally... we recommend that whoever will be doing the manipulations, doing an injection, whatever with the animals on the study, that those people be doing the tickling beforehand.

>>*Nicolette Petervary*: Thank you. We have a lot of questions. And this is sort of a question and a comment. All of our animal care staff are rat ticklers. However, it seems like the research personnel need to be the crowd to buy into this welfare technique. And so I'm guessing that this attendee is asking maybe how to bring them into the fold.

>>*Brianna Gaskill*: Yeah, how to bring them into the fold, and that's where a lot of this comes in. So we focused primarily during our studies on laboratory animal professionals, not necessarily the scientists. And so usually what we recommend is, if you're going to be trying to pitch this ... This is very similar to one of the earlier questions. How do I get people to buy in? Again, it really depends on the openness of that particular researcher. They may not want anything to change, and that may be a very hard situation for you to try to implement these techniques. I think Megan has put together a fantastic overview of how the stress can affect physiology, and if we eliminate that stress, you're probably going to get a clearer signal when you do your study to provide some good support of why this should be important. And usually if you can put it in those contexts, most of the time the scientists will be open to maybe trying the idea. But again, it just really depends on the openness of that particular person. But it's... it can be a challenge, but if you approach it in the right way, with some good support, it's not just like, "*This is fun.*" Yes, it is, but if you can provide them with the data to support why this would be important for not only the animals but for their science, that's probably one of the best approaches for a PI, anyways.

>>*Nicolette Petervary*: And I'm guessing if you find one early adopter who champions this, it'll sort of take on and become more popular with the rest of the researchers as well, hopefully.

>>*Brianna Gaskill*: Exactly, and if we're thinking about that planned behavior or Theory of Planned Behavior and some of the outlines that Natasha put forward, having those early adopters quick... making sure you get good quick wins. Yeah absolutely, that all kind of falls into it. Displaying your certificate and showing, "*Look what I have. I have this cool certificate,*" like the social, "*Oh, well, I want that, too.*" Kind of like the Ice Bucket Challenge fever that happened, I think there's a real possibility of that, too. If it's something cool and interesting that everybody wants to be a part of, there's a good possibility that social pressure can help influence the uptake as well.

>>*Nicolette Petervary*: Awesome. We have some more questions. I'm glad we made this an hour and a half webinar. Can social housing replace the need for tickling?

>>*Brianna Gaskill*: No. So that's another beautiful study that was done by Sylvia Cloutier, where she looked at group-housed and singly-housed rats. And basically, the socially-housed rats are not as interested as ... in being tickled as the isolated rats. And so her idea was not don't tickle the group-housed rate, but that if you have to singly-house a rat, tickling may be a proxy to social interaction for that individual animal and might be more beneficial for them than it is for the group-housed rats. But not saying that it's not beneficial for the group-housed rats. They're basically socially satiated because they have those interactions all the time in their cage, and so they're not as desperate for it as perhaps that individual animal. So I wouldn't say that it's something that can replace it, but it's just kind of a nice additive.

>>*Nicolette Petervary*: Right. And it also can assist with handling, when humans are handling those animals. So there's always that positive to consider as well. I love this next question. This may be outside

the scope of this talk, but do you know if there have been any other similar studies to see if play helps with other animals in research?

>>*Brianna Gaskill*: So that is a that is usually the first question, and I usually have a slide already cued up for that. Do not tickle your mice. Absolutely don't even attempt this. You will be bitten. Do not do it. And it has to... it comes down the fact that play in mice is functionally very different than the play in rats. Rats are very similar in terms of play as dog play is, and so it really comes down to what type of play. So rats do a lot of social play. Mice do a lot of physical play. And so just very different in how those animals play, and that's where interactions like this might be able to work. So for instance, I get down on the floor and I play with my dog. I do a play bow. And so we can interact in very similar ways. I don't flip him over, but you can mimic some of those aspects of that social play a lot similarly. So it really comes down to what kind of play does that animal do, and is that conducive to social interactions and social play?

>>*Nicolette Petervary*: Right. And now, before we go on to more questions for Natasha, I just love this comment: "I would bring up all of these ideas about rat tickling." I'm presuming that's what they're referring to, to your IACUC and present the data to them as something to implement for the welfare of the animals. That is my plan after hearing this webinar.

>>*Brianna Gaskill*: Great, that's exciting.

>>*Nicolette Petervary*: So we have ... Oh, I'm sorry. We have one more question for Brianna. This is ... They just keep coming in. What kind of play behaviors do mice exhibit? And I'm guessing the follow-up would be, can we exploit that for their welfare?

>>*Brianna Gaskill*: That's challenging with mice because they do a lot of ... It's called popcorning, where they kind of jump up and then they kind of dart around. But there is some aspect of social contagion that can happen with this as well. So you see one little pup kind of do a popcorn and race around, and then all of a sudden you see the other pups just jumping up and down. And so that is going to be very difficult to mimic, and so especially when it's not an interaction, direct interaction. Granted, mice will do some social play, but it's a very small percentage of the play that they do. So it's just not something anybody's necessarily tried. We don't see play in adults like we do [see] play in adult rats to the same degree, and so ... and I would say there is a very short window where you see play in mice compared to rats. So if somebody wants to give it a try, go for it, but really making sure that you understand how they play and making sure that you can mimic that is going to be the challenge. But if somebody can come up with a way to do that, that'd be great.

>>*Nicolette Petervary*: Yes, carefully, please, because species differences matter.

>>*Brianna Gaskill*: Yes, and there is a high likelihood you will be bitten. So be careful.

>>*Nicolette Petervary*: Okay. So now I will go back to Natasha because we have another question for you. Do your references include guides on recommended ways to analyze sex effects and calculate power? Do you recommend testing for interactions between sex and other independent variables? If you want power for that, I suspect it could increase the required sample size.

>>*Natasha Karp*: Okay, so that's a good question. We have two types of experiments we're conducting when we're including both sexes, one when we're actively looking for sex differences. So, they should be powered for that. But the other type, which is what we're trying to encourage, is when you're including

both sexes to make a generalizable estimate. And in those situations, what you're recommended to do is to calculate the n you need and then share it between the two sexes. Now, this might sound counterintuitive because sharing the end, do you not decrease the power? The answer is, no, you don't if you embrace the right statistical test. Because you're not going to desegregate the data and separate it, and you're not going to pool the data where you've got a baseline difference. You're going to embrace factorial analysis. And at this point, when you embrace factorial analysis, the power is maintained because you estimate it from both the males and the females simultaneously.

Now, if the effect is very different between the sexes, by very different, I mean they're in opposite directions, or it only occurs in one sex. You will lose statistical power for the main effect, but it passes to the interaction term. And so therefore, it's okay because you found a major difference. Now, if this feels like, "*Really? I don't believe you,*" well to answer this, we conducted loads of simulations where we constructed the data, going, "Here's a baseline difference. Here's a different size effect, or when it goes in opposite directions." And that work was published last year in "PLOS Biology." that paper is reference in the material. We have shown that power is maintained.

The whole concept around sex inclusion research is that you're estimating a generalizable effect, the average effect that represents both. When it is very different between the two sexes, it passes to the interaction. That is talked about in the material accessible on the website. Now, does it include guides on how to analyze sex effects. The tool is designed to represent lots of different biological questions, different data types, different experimental designs. We cannot give a checklist "if you've done this, this is the statistical test" because we can't cover all the situations. We can talk about best practice, but we've also had to write it in a way to say, this is the recommendation. But basically, it says, "have you come up with a plan to include sex as a biological variable?" The research out there says that not only do we need tools like this, but we do need more training and more resources to help us with how to analyze data. I'm just doing the corrections to the manuscript which have a little bit more about how to analyze the data. There will be a bit more information, but I actually think there will be more coming into the space to help with this. Because as we said, there's two gaps: inclusion and appropriate analysis of the data. But it is a little bit more complex because it depends exactly what you're asking of your experiment. I can't give a really clear answer.

>>*Nicolette Petervary*: Thank you, Natasha. We have another question. I think you might have answered this, but due to potential sex variability, do you need twice as many controls, or is there a better way to have the baseline?

>>*Natasha Karp*: No. You just have to embrace the right statistical test. This might seem counterintuitive, but if you go to the website there's a seminar that you can watch. And then there's a little bit in there where I talk about the statistics and how it works which I didn't include here because there was not enough time. No, with factorial analysis, you can account for the variation that's due to a baseline sex difference which is really common and separate that so it's not unexplained variation, and you will maintain your sensitivity to detect a treatment effect. So that's one of the misconceptions because we're imagining T tests, and we're imagining pooling the data and then going, "*It's adding the variation.*" The gap is, is understanding how factorial analysis works. So have a watch of the seminar. The resources are linked in and hopefully that will help you.

>>*Nicolette Petervary*: Thank you. We are coming to the end of our webinar. I thank you, all for staying to the end. We will have another webinar in the fall on a topic to be determined. I'd like to thank our

speakers for this incredible information. I was so excited to have them both here, and I'm excited to have all of you listening in. We will see you the next time. Thank you.

Note: There were no additional questions after the event