POSITIVE HUMAN-ANIMAL INTERACTIONS WITH RATS IN THE LABORATORY: INCREASING IMPLEMENTATION OF BEST PRACTICES TO IMPROVE ANIMAL WELFARE

by

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Dedicated to laboratory rats & laboratory animal personnel everywhere.

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ABSTRACT

Laboratory animal welfare is critically influenced by personnel working with animals through their decisions about housing, management, and enrichment of these animals. In particular, human-animal interactions can have major impact on both animals and research results. The first step in better understanding their effects is to define terminology, theories, and general applications (Chapter I). Rats are commonly used as model in laboratory research and have been shown to experience stress even during routine handling. A handling technique called heterospecific play or "rat tickling", which mimics aspects of rat rough-and-tumble play, has the potential to minimize stress, enrich a rat's life, and improve their welfare. Unfortunately, a survey of 794 laboratory personnel shows rat tickling implementation to be low (Chapter II). Commonly cited barriers to rat tickling includes a lack of time, difficulty with personnel (attitudes and training), and research factors. However, personnel were more likely to tickle their rats if they were more familiar with the practice, thought it was both good and under their control, and felt subject to social pressure to provide it. They also were more likely to tickle their rats if they wanted to provide more enrichment and generally had more positive behaviors towards laboratory animals.

Using those findings, an attempt was made to address those barriers to rat tickling implementation. Chapter III focuses on the barrier of time. This project compared the effectiveness of tickling rats for 15, 30 or 60 s for 1, 3, or 5 days. After the final day of tickling, rats were assessed for their in-cage behavior, human approach behavior, fecal corticosterone, and reaction to an intra-peritoneal injection. Results showed that the most time-efficient and effective rat tickling dosage is 15 s for 3 days before any aversive procedures, based on increased production of 50-kHz ultrasonic vocalizations (a measure of positive affect) and positive anticipatory behavior. Chapter IV focuses on the barrier of inadequate training. This project compared training laboratory personnel with online-only training or online + hands-on training as compared to a waitlist across 2.5 months. Results indicated that both training modalities increased personnels' reported correct implementation of tickling, self-efficacy, knowledge, and familiarity with rat tickling. Hands-on training also increased personnel's feelings of control related to rat tickling. Overall, this dissertation identified barriers to rat tickling and then attempted to address the barriers of time and beliefs/training to increase implementation of best practices of rat tickling to improve rat welfare.

CHAPTER 1. HUMAN-ANIMAL INTERACTIONS IN THE LABORATORY

Acknowledgement: This chapter will be published in the book Animal-Centric Care & Management.

1.1 Introduction

Human-animal interactions are a daily, significant, and often unavoidable component of laboratory animal science that can impact research animals, the people that work with them, and even research outcomes (1). After all, several regulatory bodies require that all animals be checked daily (2,3) and even this simple presence of a person in the housing room can influence the animals and research outcomes (1). Unfortunately, without deliberate effort, human-animal interactions can negatively affect both animals and humans alike. Animals may experience fear and stress from these interactions – which can negatively affect their behavior, physiology, and quality of life. Humans may also experience stress from negative interactions or from performing stressful procedures such as euthanasia. Finally, scientific quality may suffer from negative outcomes from human-animal interactions; research results from stressed animals may be less likely to translate to human research – thereby decreasing study validity.

It is not surprising that most human-animal interactions in the laboratory are initially negative. Think of a mouse. In the wild, mice undoubtedly experience fear when encountering a human; we are much larger and they likely view us as predators. Certainly, we are capable of causing them significant harm. In response, mice specifically try to avoid encountering humans by hiding or only entering human spaces in the dark, sticking to the periphery. If a mouse does meet a human, it may freeze and then immediately run away. But in the laboratory – even though the fear of humans still remains – the mouse cannot flee or even hide. In response, the mouse may freeze or even try to bite the handler as their only defense to get away, and hence experiences stress.

Many common laboratory species likely experience the same negative effects as mice. They also initially perceive humans as predators, therefore fearing even passive exposure or minor handling. In nature, these animals could cope with this fear by fleeing, hiding, or fighting. But in the laboratory, these coping mechanisms are hindered by the lack of space or hiding structures,

and necessary, forced, close interactions. In addition to simple handling, laboratory animals are often exposed to common laboratory procedures that are inherently stressful or painful. For example, procedures such as restraint, blood collection, injections, and oral dosing can increase blood corticosterone, glucose, heart rate, and blood pressure (4) – all indicators of heightened stress. The stress from these procedures affects gene expression, behavior, and immune function (5,6) which can harm experimental validity and cause unwanted variability between animals. The negative impacts of handling and procedures have been recognized for over 80 years(4–6) (7). However, these procedures often continue to be used without careful attempt to mitigate their negative impacts. Further, the consequences of stress from handling are often not accounted for in research studies.

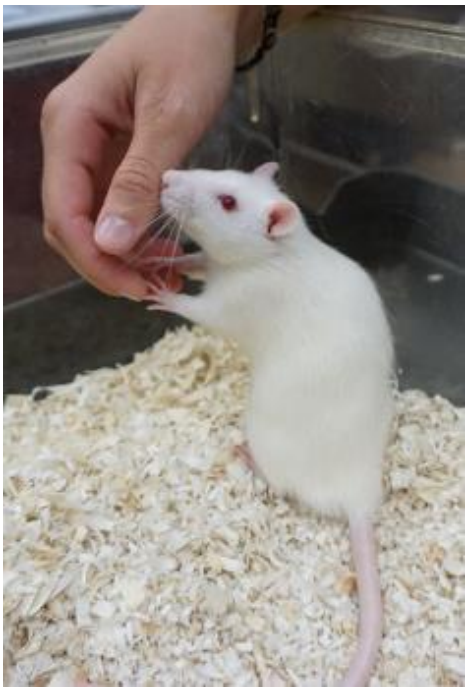

Figure 1.1 Positive human-animal interaction. A rat voluntarily seeks to interact with a human.

Fortunately, human-animal interactions in the laboratory do not have to be negative (**Figure 1.1)**. Instead they can be purposefully designed to benefit animals, humans, and research. When interactions are positive, they can lead to reduced stress in both animals and humans – making the experience enjoyable and enriching. Scientific research can be refined and scientific quality improved (8,9). However, regardless of the benefits to humans and research, optimizing

animal welfare should always be a priority. Providing laboratory animals with the best life possible is part of our ethical responsibility when engaging in animal research.

This introduction seeks to provide a broad-scale overview of human-animal interactions in the laboratory. What follows will address four main questions:

- What are human-animal interactions, and what types exist in the laboratory?
- How do these interactions affect laboratory animals and personnel?
- How should we interact with animals to improve human-animal interactions?
- What are the limitations to current research and potential avenues for future research?

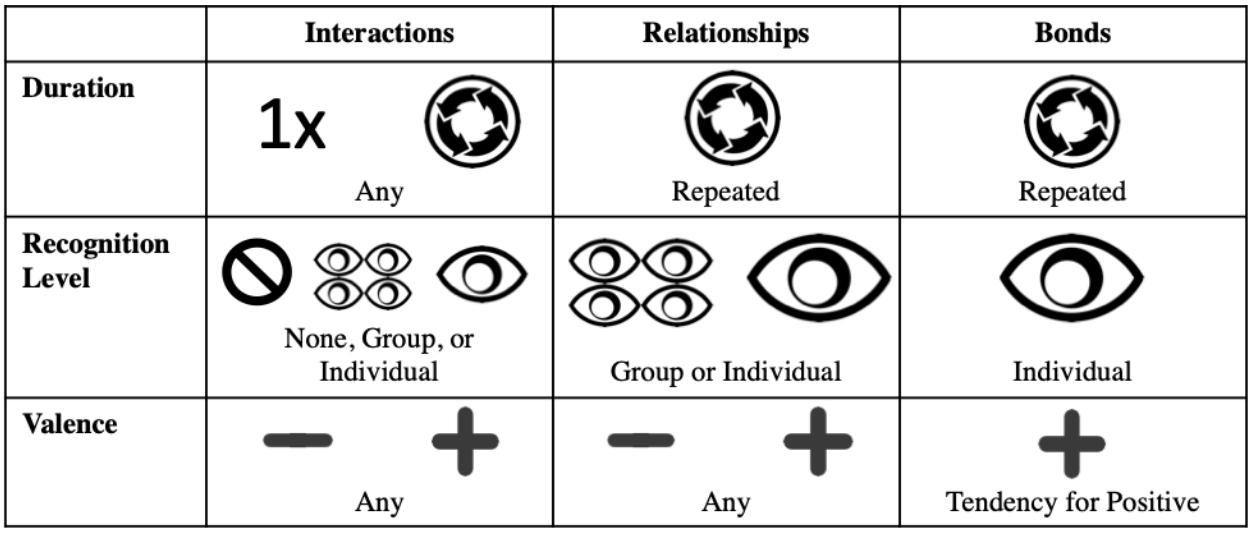

Figure 1.2 Human-Animal Interaction Terminology. A visual summary of the important distinctions between human-animal interaction terminology: human-animal interactions, human-animal relationships, and human-animal bonds. Duration indicates whether the interaction occurs a single time or is repeated. Recognition level indicates the level, if any, that both the human and animals recognize each other. Valence indicates whether the interaction(s) must be negative (-) or positive (+). Note that the depth and intensity of the interaction can vary for *all* definitions, although is typically higher for human-animal bonds.

1.2 Defining human-animal interactions

Because human-animal interactions can vary substantially between studies, animals, and even from day to day with an individual animal, clearly defining different human-animal interactions in the laboratory is necessary. Human-animal interactions can vary in duration (i.e., how long they last), depth (i.e., the intensity of the interaction), and valence (i.e., positive or negative quality of the interaction). Having clear, distinct, and consistent terminology for these interactions facilitates discussion and future research. A thorough review of terminology for

human-animal interactions across fields already exists (10), which will be briefly summarized here and supplemented with examples specific to the laboratory animal field (**Figure 1.2**).

The broadest and most widely applicable term, as demonstrated by its frequent usage in this chapter, is human-animal interaction. This term simply describes a sequence of behaviors that occur between a human and an animal (10). It applies whether the animals or humans recognize each other, interactions are repeated, or they are positive, negative, or neutral for either party. For example, this term is appropriate when a caretaker walks in a room to perform husbandry procedures, as well as when a researcher is habituating animals to specific research procedures. Overall, this term is the most commonly used and accepted across research publications and fields, in part because of its generality.

A slightly more restrictive term is human-animal relationship. This term generally refers to a series of interactions of any valence (i.e., positive, negative or neutral) between animals and humans known to each other (10). Humans may not recognize *individual* animals, but they must at least recognize the *group* of animals. Animals will typically recognize individual humans but may also recognize a group of humans (e.g., caretakers who wear a certain color of scrubs for performing certain procedures). A human-animal relationship could exist between a room of rats in a particular study and their regular husbandry or animal health technician. The caretaker recognizes this particular group of rats when she works with them over time; simultaneously, the rats also likely recognize this caretaker. Thus, human-animal relationships occur frequently in the laboratory.

The final and most restrictive term is human-animal bond. Although there is some disagreement over its exact definition, there are three relevant and agreed upon elements (11). First, a relationship must exist between a human and an *individual* animal. Second, the relationship must be reciprocal and persistent, meaning that *both* human and animal must recognize each other over a period of time. Third, interactions should tend to increase well-being for both parties. These bonds are most likely to form during long-term or small studies. They may also be more likely to form with animals with a closer evolutionary relationship to humans (e.g., non-human primates) or with common companion animal species such as dogs and cats.

All three terms can be used to describe different human and animal interactions in the laboratory environment. Human-animal bonds are likely the most beneficial human-animal interactions in the laboratory. However, human-animal interactions or relationships of a positive

valence are also highly valuable. Consistently using this terminology may increase understanding and communication of human-animal interactions within our own field and across different fields.

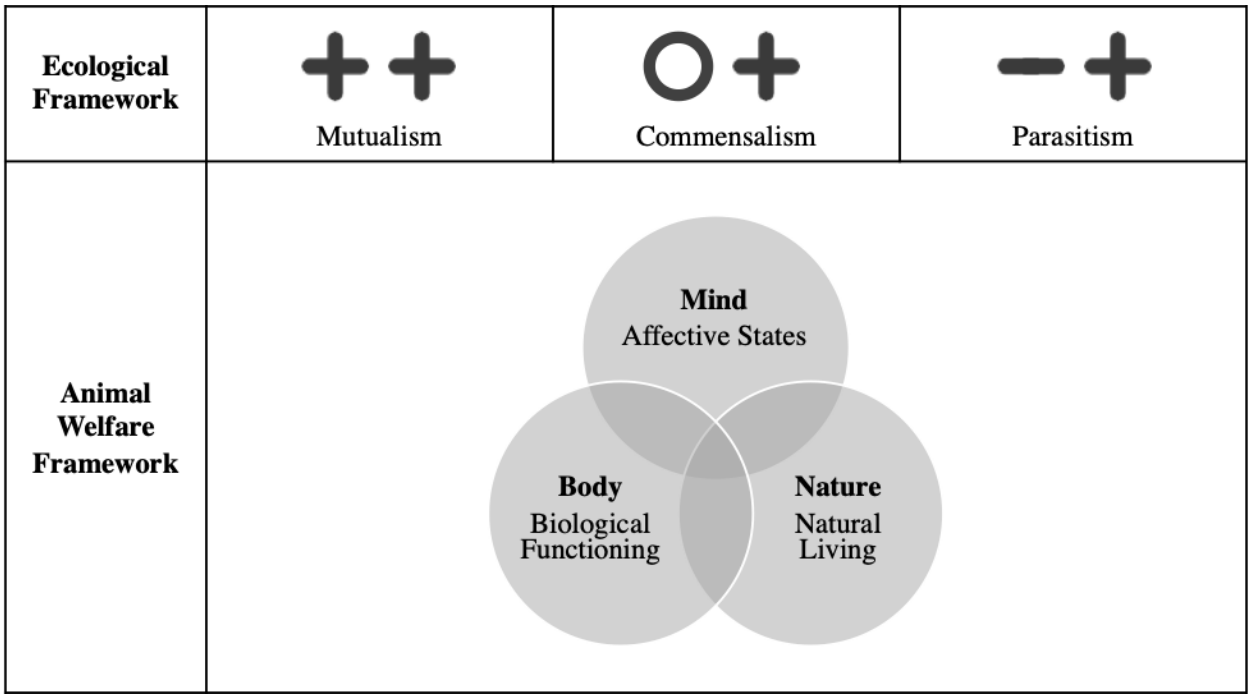

Figure 1.3 Frameworks for Human-Animal Interaction. A visual representation of key frameworks from ecology and animal welfare that can be applied to human-animal interactions. For ecological frameworks, the "plus" indicates a benefit for one species, an open circle indicates no effect, and a "minus" indicates a harm for one species. The animal welfare framework (12) indicates three key areas of welfare and that the area in the middle (where all three elements overlap) results in superior animal well-being.

1.3 Impacts of human-animal interactions

In this section, three types of frameworks will be introduced and used to interpret the potential impacts of human-animal interactions for both animals and humans (**Figure 1.3**). First, an overall ecological framework will be discussed that can be used to describe interactions from either the animal or human perspective. Then an animal welfare framework will be introduced and used to discuss harms and benefits of laboratory animal research to animals. Finally, several theoretical frameworks will be introduced to discuss human-animal interactions from the human perspective. Finally both those frameworks and other research will be used to discuss potential harms and benefits of laboratory animal research to humans.

1.3.1 Ecological Framework

In ecology, interactions between two different species can be categorized purely on the harms or benefits to each (**Figure 1.3)**. Using this framework can help us evaluate laboratory human-animal interactions with a broad lens. In *mutualism* both species benefit (13). In *commensalism* one species benefits while the other experiences neutral impacts (neither benefits nor harms) (13). In *parasitism* one species benefits (often using the other as a resource) while the other is harmed (13). In the laboratory, as well as in nature, interactions between species may not necessarily fit neatly into a single category and may change over time (14).

Using this ecological framework, human-animal interactions in the laboratory can be classified into mutualism, commensalism, or parasitism – depending on the specific research topic and laboratory environment. As a whole, animal research has the potential to be mutualistic when humans benefit from increased scientific knowledge and animals benefit from animal-centric care, protecting them from predation, malnutrition, and disease. Mutualism may also occur if medical discoveries benefit both humans and animals, especially when the laboratory environment is well-managed to support good animal welfare. Mutualism can also apply to positive reinforcement training where animals are trained to cooperate with research procedures – this benefits the animal (as mental enrichment and welfare), the human's experience, and research data. On the opposite side of the scale, parasitism may apply when humans benefit from research while the utmost priority is not placed on animal welfare or animals receive harms from research procedures. For example, using animals in chronic variable stress models are, by design, stressful/harmful to animals but used for human benefit. Commensalism is likely rare, though it may occur in observational studies with moderate enrichment and little harm to animals.

1.3.2 Animals

Human-animal interactions can also be discussed focusing on their harms and benefits to animals, especially through using a specific animal welfare theoretical framework.

Animal Welfare Theoretical Framework

Fraser et al. 1997 outlined a framework that evaluates welfare based on an animal's biological functioning, natural living, and affective states (12) (**Figure 1.3)**. This framework can

be used to assess the effects of human-animal interactions on animal welfare in the laboratory environment. Biological functioning is assessed by examining how interactions impact animal health and physiology. For example, standard laboratory routines such as restraint, injections, or oral gavage can increase corticosterone levels and decrease the immune function (4–6) – thereby decreasing welfare from the biological functioning perspective. Natural living is assessed by examining how interactions impact an animal's ability to express highly motivated natural behaviors. For example, rat tickling (a human-animal interaction that mimics aspects of rat social play) increases natural play behavior between pair-housed rats (15) – thereby improving welfare from the natural living perspective. Finally, affective states are assessed by examining how interactions affect the animal's emotions. For example, rat tickling increases positive emotions in laboratory rats (16) – thereby improving welfare from the affective states perspective. The affective states conception is considered the most vital element in animal welfare assessment because it addresses suffering or thriving. However, it is also the most difficult to measure.

Harms for Animals

Overall potential harms of human-animal interactions for animals were outlined in the introduction of this chapter.

Benefits for Animals

Avoiding negative human-animal interactions and promoting positive interactions in the laboratory can improve welfare for many species in a variety of ways. For example, avoiding physical corrections (such as slapping, using a snout noose, or electric prod) in favor of scratching can increase pig growth rates, decrease corticosteroids, and improve reproduction (17–19). In rats, using a modified, less restricted restraint technique for intraperitoneal dosing can decrease struggling, vocalizations, fecal count, and corticosterone (20). In non-human primates, cooperative training programs can eliminate the need for chair restraint, increase animal welfare by allowing earlier detection of clinical symptoms, and improve the validity of scientific results by eliminating model confounds from stressful events (21). Finally, exotic zoo animals (which share similarities to laboratory animals in long-term studies) can experience improvement to welfare when handled by fewer keepers and when spending more time with the familiar keepers (22,23).

Human-animal interactions can also be used to intentionally induce positive emotions in laboratory animals through promoting play, conducting positive reinforcement training, and providing positive attention or touch. As explained above, play can be promoted in rats via rat tickling (**Figure 1.4)** (15,16). Its positive nature is particularly evident in studies showing its use as a reward in operant conditioning paradigms (24,25). Positive reinforcement training itself is likely to engage the "seeking" circuit in the brain, which is considered to be highly rewarding and is associated with positive affect (26). In dogs, positive human contact such as attention or stroking has been shown to decrease the stress response (27). In cats, human petting increases positive affect and the production of secretory immunoglobulin A, which is beneficial for a healthy adaptive immune system (28).

Figure 1.4. Rat Tickling. A rat receiving the positive human-animal interaction of rat tickling.

1.3.3 Humans

Human-animal interactions can also be described by focusing on their harms and benefits to humans – several theories can be used to provide a framework for this discussion.

Human Theoretical Frameworks

Several theoretical frameworks have been proposed to explain human's attraction to and benefits from animals. This section will present the three most common theories outlined by leaders in the field (29) and how they can apply to human-animal interactions in the laboratory.

The "biophilia hypothesis" asserts that humans are genetically coded to respond to animals -- which explains our innate attention and attraction to them (30,31). In evolutionary history, there may have been genetic selection to be attentive to animals. For example, humans who were more attentive to other animals may have had greater fitness (higher survivability and reproduction) since paying attention to animals provides important cues about the environment (32). In fact, research suggests that animals innately hold our attention and that we experience physiological changes in response to this attention. When comparing brain scans from people viewing landscapes, people, and animals, there is greater, category-specific neurological activation in the amygdala when viewing animals (33). Animals can also provide a positive external focus of attention that may reduce cardiovascular responses to stress (34). Viewing fish tanks can even effectively hold the attention of individuals with advanced Alzheimer's symptoms during meal times to help increase weight gain (35). The biophilia hypothesis may also help explain our initial attraction to careers with animals, the reward we gain from positive interactions with laboratory animals, and why many of us find ourselves continually drawn to animals in our lives.

A second theory explaining human-animal interactions is the attachment theory. Attachment is a deep, enduring emotional relationship that connects two individuals and promotes a balance between physical closeness of the two individuals as well as independent exploration (36,37). This theory was first proposed by Bowlby in 1958. The four characteristics of attachment (often seen in species that require care after birth) are proximity maintenance, safe haven, secure base, and separation distress. These features are adaptive for survival since they increase the likelihood that caretakers will provide resources to their young, or the individual under their care. Attachment is usually discussed in respect to parent-infant relationships, but could also apply to

the human-animal bond (38–41). We may see strong, secure attachments between humans and laboratory animals with close human-animal bonds. These laboratory animals may prefer to be close to their caretakers (especially in stressful situations), be more willing to explore in the presence of those caretakers, and may even experience minor distress when separated from preferred caretakers. Their caretakers may be particularly responsive to these animals needs therefore promoting good animal care.

A third theory behind human's motivation to interact with animals is the social support theory, which is the perception or reality that one is cared for, has access to supportive resources, and is part of a supportive social network – which includes animals (29). This theory is often supported by experts in the field when exploring interactions between companion animals and humans (42) although results from pet ownership studies alone can be inconsistent depending on control factors (42). Regardless, there is evidence that animals can buffer a stress response and promote positive physical and mental health outcomes (43,44). This theory is more likely to apply with true mutualistic human-animal bonds in the laboratory where a caretaker feels they get support from their laboratory animals.

Harms for Humans

Of course, caretakers can experience harm from human-animal interactions. Simply performing or viewing stressful procedures (such as euthanasia) can lead to occupational stress or perpetration-induced traumatic stress (45,46). It can be stressful to care for an animal that you will eventually euthanize; this is sometimes termed a "caring-killing paradox" (47,48). In one study, laboratory animal personnel who performed positive human-animal interactions (i.e., petting, naming, and talking to their laboratory animals) also reported higher secondary traumatic stress (49). Furthermore, personnel may be subject to emotional harm from performing the "dirty job" of animal research that is often perceived negatively by society (50). These factors could lead to compassion fatigue, comprised of burnout and secondary traumatic stress (51). Having adequate social support (feeling like there is someone you can count on and talk to about your work with laboratory animals) is essential to account for the challenges of working in laboratory animal research (49).

Human-animal bonds in particular may increase negative ethical and emotional implications of interactions in the laboratory. Once a bond is formed with a laboratory animal,

ethical calculations around human-animal interactions may change. For example, most people feel greater responsibility to help and prevent harm to close friends versus strangers (11,52). Therefore, laboratory animal caretakers may feel greater responsibility to the animals they are bonded to versus those they have a more distant relationship with. Severing this bond abruptly and without health-related reasoning (such as an end of study euthanasia of a healthy animal) may feel like a betrayal of trust (11) and lead to feelings of guilt. Therefore, establishing institutional support or discussion groups for personnel will likely be beneficial. When possible, another option is to establish an adoption policy for eligible, healthy animals to allow bonds to continue beyond the end of the animal's research career.

Negative scientific implications are also possible from human-animal bonds. These bonds could accentuate possible conflict of interest between a caretaker's allegiance to their animals versus the scientific research (52). Having a bond with a certain animal could even lead to special care for that individual animal (e.g., providing extra treats or attention). Special care for one animal could cause unwanted variability in the response of that animal to experimental treatments or cause bias when assessing the animal during outcome evaluation. Overall, though, the potential harms from human-animal bonds can be mitigated with appropriate management – thereby allowing human-animal bonds to remain positive for all.

Benefits for Humans

Promoting positive human-animal interactions in the laboratory environment could benefit human psychological well-being, job efficiency, and professional quality of life. Psychological benefits could include reduced stress, anxiety, and more positive emotions at work. Anecdotally, people report feeling happier and more relaxed after tickling their laboratory rats or providing daily play to their laboratory cats. These innate feelings, combined with the knowledge of improving animal lives, could help mitigate moral distress from working with research animals and even promote compassion satisfaction. Furthermore, animal training and habituation could reduce the time required to perform various procedures (such as injections or restraint) and help these procedures be less stressful for handlers (16,21). Higher quality of life, including reduced likelihood of burnout, among laboratory personnel is positively associated with providing more animal enrichment and engaging in more frequent positive human-animal interactions (49).

Overall, establishing a culture of care that includes positive human-animal interactions will likely be beneficial to caretakers and the research institution's culture.

Positive human-animal interactions are also likely to make laboratory animals better scientific research models. Specifically, animals that are positively handled may be less stressed which can reduce variability in their response due to extraneous variables and may even increase reproducibility. For example, research with diabetic non-human primates models has shown that the utilization of positive reinforcement training enhances the value of translational research (53). Better models are beneficial to researchers and to the general public, who both benefit from scientific research.

1.4 Recommendations

1.4.1 Principles

From the time of the animal's arrival at a facility, it is crucial to implement practices and procedures that promote positive (or at least neutral), minimally stressful human-animal interactions because these early contacts shape animals' expectations for future interactions with humans (54). For example, animals should be carefully and gently handled when removed from shipping containers or separated from their mother after weaning. If possible, marking animals for identification should be done after a positive human-animal relationship has been established since marking often involves at least moderate restraint that can sometimes lead to distress (15). Similarly, ensuring positive experiences between human and young animals is particularly important, especially during their critical period of development (55), although evidence for this suggestion is mainly supported by research with dogs and cats. Therefore, if laboratory animals are not bred or received at a particular facility before this period, it may be beneficial to communicate with the vendor or provider about their protocols to ensure sufficient positive human-animal interactions is provided. It is important to note that although neutral interactions do not elicit negative responses, they do not have as positive an impact as positive interactions (56). Thus, one should aim for using positive interactions as much as possible.

In general, human-animal interactions should ideally improve an animal's control and predictability (57). Studies in animal welfare show that the psychological aspects of predictability and control are highly important to both mental and physical health. For example, control and

predictability influence the development of ulcers in rats undergoing electric shocks (58). Predictability can be provided by either performing interactions at the same time each day or by providing a reliable and distinct cue before interactions – and this may be most important for aversive interactions. For example, cage changing can always be done in the same order by working from top left to bottom right. Additionally, training animals with positive reinforcement to cooperate with procedures, even cage changing, can greatly increase predictability and control. Training allows animals to learn which interactions will result in rewards, choose whether to participate in interactions, and predict the interaction's outcome. Control in human-animal interactions in the home environment can be provided by allowing the animal choice in their location. For example, providing hiding structures can allow the animals to control whether they are viewed and reduces their fear of human. Perhaps counter-intuitively, this can lead to more interaction. Zebrafish housed in enriched tanks with hiding structures are more likely to approach the front of the tank than those housed in barren ones (personal communication).

Familiarity with, and understanding of, species-specific behaviors can help promote positive human-animal interactions. Knowledge of species-specific behaviors can help caretakers determine what movements or actions of their own may appear threatening or aversive. For example, direct staring and eye contact can be threatening for primates, but support development of a human-animal bond with dogs. When in doubt, most species prefer movements that are slow, controlled, and predictable. Furthermore, knowing species-specific behaviors will ensure that caretakers know when they may need to intervene in self- or peer-directed behaviors. For example, rat social play appears very rough and could be misinterpreted as fighting by someone unfamiliar with the behavior. However, knowing that the focus of the contacts differs between aggression (rump) and play (nape) facilitates the recognition of the behaviors and helps determine if an intervention is necessary.

In addition to learning species-specific behaviors, caretakers should also become familiar with specific effective human-animal interactions with each species. For more detailed information, see each species-specific guides in Animal-Centric Care & Management. In brief, here are some positive interactions that are recommended. Mice can be tunnel handled to reduce anxiety and increase control (59,60). Rats can be tickled to decrease fear of humans, reduce stress, and increase positive affect (16). Rabbits can be regularly, gently stroked from before six weeks of age through adulthood (61) and can be trained to cooperate with procedures (such as voluntary oral dosing

rather than oral gavage (62)) to reduce stress. Dogs can be trained to cooperate with procedures, socialized, and pet or played with regularly (27,63). Pigs can also be trained and scratched on the back (64). Non-human primates can also be trained to cooperative during handling (21,65). Zebrafish in enriched housing units providing opportunities to hide may benefit from calm, predictable attention such as slowly running fingers along the glass to promote schooling (personal communication). Each species likely can benefit from specialized, positive, human-animal interactions, one just need to learn about species-specific behavior and use some creativity.

1.4.2 Changing human behavior to improve animal welfare

The laboratory animal science field has a responsibility to improve the lives of the animals under its care. Therefore, it is essential to select laboratory animal personnel that are committed to animal-centric care and provide them with sufficient training and support to allow them to give the best care to laboratory animals. Individual contributions to human-animal interaction flourish best when supported by all levels of stakeholders, policies, and standards. Each individual contributes different knowledge, expertise, and experience (e.g., program administrators, managers, clinical veterinarians, caretakers, scientists, students). Cohesive support for positive human-animal interactions will produce the best policies, ease implementation, and encourage compliance.

But what happens if some individuals are not convinced of the importance of human-animal interactions? Research with farm animal stock people shows that attempting to change a person's beliefs about human-animal interactions through education can help enact positive change (66) and there is preliminary evidence this could be the case for laboratory animals as well (67). In one study, personnel were more likely to report implementing a positive human-animal interaction if they also reported stronger beliefs that the interaction was good, that their social and professional peers wanted them to do it, and, most importantly, that they had the ability to do it (e.g., time and education) (67). Therefore, management can first ensure that personnel feel that they have the ability to provide positive human-animal interactions by providing training on specific techniques and ensuring they are given adequate time to complete these tasks. During training, personnel should be instructed on both *how* and *why* improve human-animal interactions. Training can instruct personnel on the benefits and importance of human-animal interactions, as

well as outline the current social, public, and professional pressure for improved human-animal interactions in the laboratory.

1.5 Limitations & future work

Of course, there are limits to our current knowledge and application of human-animal interaction in the laboratory. There is comparatively little research about the impact of human-animal interactions on research model health, affective states, and, ultimately, research validity or reproducibility. Furthermore, the specific details of human-animal interactions (i.e., habituation procedures, cage changing handling and frequency, handling for experimental procedures, daily and health checks) are often not reported in peer-reviewed publications; articles may simply indicate that the animals were "habituated" which does not provide enough detail for replication. This lack of research and reporting promotes skepticism in regard to the importance of positive human-animal interactions.

Some individuals are even concerned about potential harms to their research models. Until knowledge is increased through research and reporting, it will be difficult to mitigate such concerns. For example, using rat tickling could potentially change a depression model by encouraging positive affect and reducing typical signs of depression -- therefore making the model ineffective. However, if the interactions between human and animals for husbandry or experimental procedures have a notable impact on research outcomes that may call into question the robustness, validity and translatability of this model. Therefore, the impact of human-animal interactions on various research models should be systematically evaluated and reported.

Providing science-based evidence of the benefits of positive human-animal interactions will encourage their widespread integration into standards, guidelines and procedures. It may also contribute to the development of new, more robust models. For example, rat tickling has been used to selectively breed rats that show an autistic-like phenotype (68).

Another major and often cited limitation to implementing positive human-animal interactions is lack of time. Planning, learning proper techniques, and actually performing positive human-animal interactions can take a significant amount of time, resulting in extra personnel expenses. Very often, personnel working with, and caring for, animals already feel overworked and overwhelmed with their current tasks. Thus, it does not seem realistic to add to their already busy schedule. Although these are realistic barriers, every attempt should be made to make positive

human-animal interactions a priority in animal laboratories and facilities. Keep in mind that changes do not have to be big and drastic. Often, even simple and relatively quick interventions can substantially change the human-animal relationship. The time needed to perform these interventions can be reduced as personnel get more experience in providing positive human-animal interactions. In addition, an animal responding to a positive intervention is more likely to approach and cooperate than attempting to escape and struggling. This may also contribute to reducing the time taken to perform procedures. Furthermore, systematic investigations have shown that significant, positive human-animal interactions can be gained in relatively short amounts of time. For example, just 15 seconds of rat tickling for 3 days (15) and just 5 minutes of training dogs for oral gavage for 4 days (69) are sufficient to obtain the desired response. Although it is important that all staff contribute to these efforts, having a behavior & enrichment specialist can facilitate the coordination and implementation of positive human-animal interactions.

1.6 Conclusion

In conclusion, human-animal interactions are just one aspect of animal-centric management, but a crucial one due to the vast number of interactions that are an integral part of animal-based research. Promoting positive human-animal interactions refines animal research by reducing pain, fear, and suffering – ideally promoting positive welfare. These interactions have the potential to improve research quality and personnel quality of life. When possible, fostering true, mutually beneficial human-animal bonds may be most advantageous. Overall, valuing and taking concrete steps to promote these unique relationships between laboratory animal personnel and their laboratory animals can ultimately improve both human and animal welfare.

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CHAPTER 2. LABORATORY ANIMAL WELFARE AND HUMAN ATTITUDES: A CROSS-SECTIONAL SURVEY ON HETEROSPECIFIC PLAY OR "RAT TICKLING"

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2.1 Abstract

Introduction. Laboratory rat welfare is critically influenced by laboratory animal personnel through their implementation, or lack of implementation, of various enrichment techniques. One such promising technique is heterospecific play, or "rat tickling", which mimics aspects of rat rough-and-tumble play and can contribute to improving welfare, but may be infrequently implemented. The theory of planned behavior can be used to study implementation by measuring intentions and beliefs about rat tickling, including behavioral attitudes (whether it is good or bad), subjective norms (whether there is social/professional pressure to provide it), and control beliefs (whether they feel in control of providing it). Therefore, the objective of this study was to identify current rat tickling prevalence and predictors among laboratory animal personnel in the United States (USA) and Canada. Our hypothesis was that rat tickling prevalence would be low and associated with beliefs about the practice, enrichment, and laboratory animals in general.

Methods. Laboratory animal personnel were recruited from widespread online promotion. A total of 794 personnel (mean ± SD = 40±11 years, 80% white, 80% female) completed at least 50% of the mixed methods online survey and met inclusion criteria of currently working with laboratory rats in the USA or Canada. The survey included questions about demographics, enrichment practices and beliefs, attitudes towards rats, general positive behaviors (e.g. talking to laboratory animals), and both practices and beliefs about rat tickling. Qualitative data were coded using thematic analysis. Quantitative data were analyzed using general linear models.

Results. Laboratory personnel reported low levels of rat tickling implementation, with 55% of participants reporting never using it. Laboratory personnel reported 2 key benefits (handling: 61%, welfare: 55%) and 3 key barriers (time: 59%, personnel: 22%, and research: 22%) to rat tickling using qualitative analysis. Current and planned rat tickling were positively associated with

more positive beliefs (social/professional pressure $p<0.0001$, control of providing tickling $p<0.0001$) and familiarity with tickling ($p<0.0001$). Current rat tickling was also positively associated with more positive general behaviors towards laboratory animals, such as naming animals ($p<0.0001$). Future rat tickling was positively associated with more positive attitudes about it ($p<0.0001$) and a desire to implement more enrichment ($p<0.01$).

Conclusion. Our findings show that even though rat tickling implementation is currently low, it is positively associated with personnel beliefs, familiarity, general attitudes, and a desire for more enrichment. That is, laboratory animal personnel were more likely to provide rat tickling if they were more familiar with it, thought providing it was both good and under their control, and felt subject to social/professional pressure, as well as if they wanted to provide more enrichment and generally had more positive behaviors towards laboratory animals. There is potential to increase rat tickling by increasing personnel familiarity with the procedure through training, decreasing the time required, and changing personnel beliefs – thereby improving rat welfare.

2.2 Introduction

To facilitate various types of basic, applied, and regulatory research, a number of animals are housed in laboratories. In this captive setting, these laboratory animals may experience stress as a result of housing, husbandry, and research practices (1). To mitigate these stressors it is recommended that captivate animals receive biologically relevant enrichments and handling improvements (2). Laboratory animal personnel are often the individuals responsible for implementing or recommending these enrichments and thereby improving animal well-being through their direct or indirect actions. These actions may either be supported or hindered as a result of many factors, which may include personnel role (e.g. animal care technician, laboratory manager, clinical veterinarian, principal investigator), institution type (e.g. universities, contract research organizations, government research agencies), or specific research type.

Handling of laboratory animal during everyday care or research protocols is often an underestimated source or stress that can cause unintended variability in data within and between laboratories and affect animal welfare. Handling can result in increased heart rate, corticosterone levels, glucose, and more (3). Rats, one of the most common laboratory animals, experience stress from handling (4,5) which can make handling difficult and contribute to poor animal welfare. Fortunately, handling can be improved by implementing habituation techniques such as

heterospecific play or "rat tickling." Rat tickling is a human-animal interaction that mimics aspects of rat rough-and-tumble play (6). It is more effective than exposure to a passive hand or minimal handling (7) and more efficient than other habituation techniques (8). It increases rat positive affect, habituation, and positive approach behaviors thus reducing routine handling stress (7).

Despite the known benefits of rat tickling, its current level of implementation and barriers to more widespread implementation are unknown (7). However, it is suspected that the prevalence of rat tickling implementation is relatively low, which would indicate that many rats are not receiving an enrichment that could be beneficial to their welfare. Anecdotally, laboratory animal personnel state that several factors prevent its widespread use (including its perceived time intensive nature, disbelief or lack of knowledge in its beneficial effects, and even the name "rat tickling" itself), but there is no scientific evaluation of these statements (7). Regardless of their specific reasons, rat tickling provision is ultimately a behavioral decision made by laboratory animal personnel.

Scientifically evaluating, understanding, and predicting human behavior can be complex and challenging. Fortunately, the theory of planned behavior has been successfully used across a wide variety of target behaviors (9). This theory is the explicit basis for over 832 published studies, is highly predictive, and can be used to develop interventions for behavior change in humans (9). The basis of this theory is that humans are more likely to perform behaviors when they plan to do them. In turn, those plans (or intentions) to behave in a certain way can be predicted using three main factors: beliefs about the consequences of a behavior (behavioral attitudes); beliefs about social and professional pressures to perform the behavior (subjective norms); and beliefs about control over performing the behavior (perceived behavioral control) (10). By measuring these factors, researchers can determine which beliefs may be the best targets for interventions aiming at increasing the performance of a behavior.

In the field of animal welfare, the theory of planned behavior has been used to evaluate the impact of stockperson beliefs on farm animal welfare, develop an intervention to change these beliefs, and, in turn, improve farm animal welfare (11). Stockpeople with more negative beliefs about farm animals and animal handling were more likely to handle them roughly which led to decreased animal productivity and welfare (12). However, when stockpeople were re-trained using an intervention focusing on improving their beliefs about the animals and their handling – based

on areas identified using the theory of planned behavior – they actually changed their behavior which, in turn, improved both farm animal productivity and welfare (13).

Our objective in this study was to characterize the current use of and beliefs about rat tickling in the status quo. Our specific aims were to (a) quantify the current prevalence of rat tickling and how it is used and (b) identify predictors that influence both intentions and past frequency of providing rat tickling including using the theory of planned behavior. Based on previous research using the theory of planned behavior and personal experience, we hypothesized that rat tickling will be provided more frequently by laboratory personnel with more positive attitudes towards rats and general behaviors towards laboratory animals, more familiarity, and more positive beliefs about rat tickling. With this knowledge, we hope to identify promising areas for future research and interventions to increase rat tickling prevalence thereby improving rat welfare.

2.3 Materials and methods

All procedures and informed consent protocols were approved by Purdue University's Human Research Protection Program Institutional Review Board, protocol #1712020004. No interactions occurred between the research team and animals during the course of the study; therefore, we did not seek approval from Purdue University's Institutional Animal Care and use Committee (IACUC).

2.3.1 Participants & procedures

Participants were recruited between February 22nd and March 26th, 2018 via widespread online promotions designed to maximize sample size (14). Online contacts were through seven modalities: direct emails to known laboratory personnel, list serves (e.g., CompMed, Laboratory Animal Research Enrichment Forum (LAREF), etc.), email lists (e.g., Canadian Association for Laboratory Animal Science (CALAS), Massachusetts Society for Medical Research (MSMR)), Facebook groups/pages/personal accounts (e.g., Laboratory Animal Sciences, Dog Spies), LinkedIn groups/personal pages (e.g., American Association for Laboratory Animal Science (AALAS), Animal Behavioral Biology), website advertising (CALAS & AALAS,) and online webinars (e.g., AALAS). All modalities were contacted up to four times with the same study flyer following recommended survey procedures (15). Additionally, all materials were translated into

French by a native French Canadian. Following voluntary informed consent, participants completed a 30 min online survey. For compensation for their time, participants could be entered into a drawing for a choice between $40 Amazon gift card or cash (chosen by 62.5% and 37.5%, respectively). Participants were included if they were over the age of 18 and report current work with laboratory rats in the United States or Canada.

2.3.2 Measures

This survey was developed by reviewing literature and consulting with experts in survey methodology, behavior theory, and laboratory animal enrichment. When possible, validated instruments were used (i.e. theory of planned behavior survey). When validated instrumentation did not exist, previous work was modified or new items were created, reviewed by experts, piloted, and revised as necessary. The survey question text and scales are available in **Appendix A, Table A.1**.

Demographics & work factors

Participants were asked about their demographics, current work, and percentage of time spent working with rats. Demographics included age, gender, race, and highest level of education. Current work questions related to current country of work (i.e., United States or Canada), role (e.g., animal care technician, veterinarian), type of institution (e.g., academic, contract research organization), primary type of research (e.g., applied, basic, regulatory), and both years and hours per week working with laboratory animals. Participants were informed that work was defined broadly and may include hands-on work such as changing cages or running procedures or hands-off work such as running a laboratory or research studies.

Enrichment, attitudes, & general behaviors

Enrichment use was evaluated with questions about general enrichment factors and frequency of using rat tickling. For general enrichment factors, participants were asked their degree of control over enrichment and if they wished they could provide more enrichment than they currently provided. At the beginning of this survey section, to counter the possibility of participants having different definitions or misunderstandings of enrichment, participants were instructed that

"in this study, we consider animal enrichment to be any attempt to improve animal welfare by enhancing the quality of a captive animal's care by providing stimuli necessary for psychological and physical well-being" (16).

Participants also received a Rattitude survey to assess their general attitudes towards rats and a general behavior survey, both adapted from Hemsworth & Coleman (11). Participants were asked if they agreed or disagreed with statements about laboratory rats, five negative (e.g., rats are smelly) and five positive (e.g. rats are entertaining). For general behaviors, participants were asked if they agreed or disagreed that they often observe, pet, talk to, or name their laboratory animals.

Rat tickling information

Current knowledge and use of rat tickling were evaluated via questions about current frequency of provision and familiarity with rat tickling. If participants were at least a little familiar with the technique, they were asked to select a pictorial representation of the technique used in their lab (**Figure 2.1**). The final question was included because anecdotally some individuals say they use rat tickling, but describe techniques that do not mimic aspects of rat social play, which is the basis of effective rat tickling.

At the end of this survey section, to counter the possibility of participants having misunderstandings or no knowledge of rat tickling – and prepare participants for the theory of planned behavior section – participants were instructed that "in this survey, rat tickling is defined as an interaction between a human and rat that mimics aspects of rat social play."

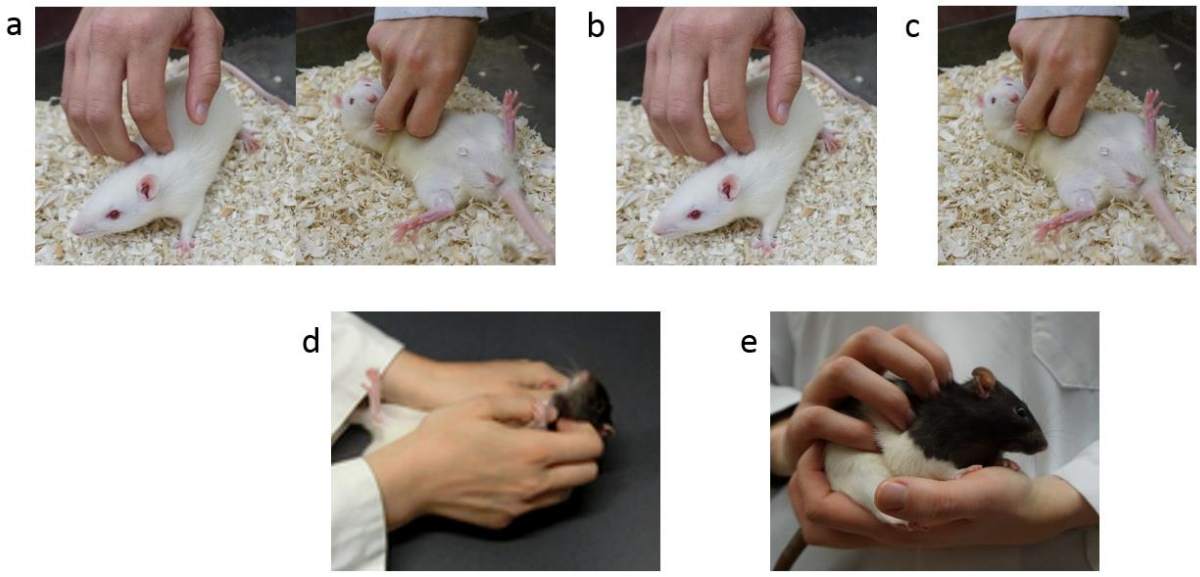

Figure 2.1. Pictorial Tickling Procedures. A: Dorsal contact and pin (standard, validated rat tickling procedure), B: Dorsal contact only or stroking in the cage, C: Pin only, D: Two-handed pin only, E: Stroking in the hand.

Beliefs: theory of planned behavior

The theory of planned behavior was used to assess rat tickling intentions, behavioral attitudes, subjective norms, and perceived behavioral control. Surveys constructed using this theory typically have excellent reliability and validity (9).

First, participants were asked open-ended, qualitative questions to allow participants to reply with their most salient answers without additional prompting. These questions were modeled after methods used in an elicitation study for the theory of planned behavior (9) and included asking participants what makes it difficult to tickle rats, easy to tickle rats, and what are the advantages to rat tickling, if any.

Then, participants were asked close-ended, quantitative questions in 10 sections that directly (and indirectly) assessed behavioral intentions, attitudes (behavioral beliefs x outcome evaluations), subjective norms (normative beliefs x motivation to comply), and perceived behavioral control (control belief power x control belief strength). Information on all items (including mean ± standard deviation, scale, summary scores, and Cronbach's alpha) is included in **Appendix A Tables A.5** and **A.6**. This survey was developed using a manual for constructing questionnaires based on the theory of planned behavior (9). At least 3 items were measured for

each construct. Summary variables were calculated using theory of planned behavior protocols (9). Overall, only one item was dropped from the survey based on its extremely low reliability, which was likely due to it being the only item that was negatively worded in the series (i.e., The decision to provide rat tickling to laboratory rats is… "*beyond my control*" versus "completely up to me" or "I am confident I can provide rat tickling".)

2.3.3 Data analysis

Variable coding

To ensure that all descriptive data reporting and summary scores indicate the same responses, only participants that answered 50% of questions in the survey were included for analysis. When comparing these participants to all respondents who started the survey, no obvious visual differences in demographics were seen.

Categorical data options that contained less than 20 responses were collapsed into larger categories to assist with analysis. Similarly, when fill-in responses for other text had more than 20 similar responses they were made their own category. Missing data for categorical variables (gender, race) were coded as "other." For gender, response options with too few frequencies were collapsed into other. Therefore, the other category for gender included the following: prefer not to answer, transgender man, transgender female, non-binary, or blank. For race, all individuals who selected multiple categories were coded as being of mixed race. For participant role, we also added the category of trainer based on the filled in responses of many participants.

Participants were asked to check all pictures to indicate how rats in their care were tickled. These responses were coded for clear and consistent interpretation. Responses that only included both dorsal contact and pin were coded as Dorsal Pin Only, which is the standard, validated technique for tickling. Responses that included dorsal contact and two-handed pin were coded as Dorsal Pin Double. Responses that included dorsal contact & pin with picking the rat up and stroking it in the hands were coded as Dorsal Pin Stroke. Responses that only included a pin without dorsal contact were coded as Pin, No Dorsal. Finally, responses that only included dorsal contact or stroking without a pin were recorded as Dorsal or Stroke Only.

Qualitative analysis

We used thematic content analysis to determine barriers, advantages, and improvements to rat tickling (17). Specifically, we used an inductive (bottom-up) and semantic analysis where codes were developed from the data, rather than a priori, from the explicit meanings. All coding and analyses were conducted with QSR International's NVivo 12 qualitative data analysis software.

An iterative process was used to code the entire qualitative data set. Within the dataset, each clause was treated as the unit of analysis and each clause given a code. Each clause was coded with as many codes as it contained. For example, the clause "time and buy in from management that it is a beneficial practice" would receive codes Time and Buy-In. Buy-in was defined as a belief that rat tickling is effective and worth the time and effort it requires. Within each response, if the same code was expressed twice, the second instance would be given *Redundant* so that frequencies would accurately represent the percentage of individuals expressing a particular sentiment.

The coding manual was refined via an iterative process in which responses were read multiple times. To assess the reliability of the coding scheme, once the manual was fully established, a second rater independently coded a random 20% of the data. Inter-rater reliability was then assessed using a two-way mixed, absolute-measures intra-class correlation coefficient (ICC) (18). The resulting ICC was in the excellent range (ICC = 0.97), which indicates that coders had a high degree of agreement and that a minimal amount of measurement error was introduced (18).

Quantitative analysis

Data analysis was conducted in Statistical Package for the Social Sciences (SPSS 24.0) using descriptive statistics and general linear models. Prior to testing, all assumptions of the general linear model were confirmed including independence of residuals, homogeneity of variance, normality of residuals, and multicollinearity in the data. For all summary scales, an average of individual items was calculated (excluding participants with >50% missing data in each measure).

The dependent variables for quantitative analysis were the current and planned level of rat tickling. The explanatory variables included theory of planned behavior beliefs (behavioral

attitudes, subjective norms, and perceived behavioral control), familiarity with rat tickling, attitudes & behavior (Rattitude and general behaviors), enrichment (control and desire), demographic, and work factors. Additionally, to confirm the validity of the indirect measures of the theory of planned behavior, linear regression models were used between the direct and indirect factors. Significance level was $p < 0.05$. Results are presented as mean ± standard deviation unless otherwise noted.

2.4 Results

2.4.1 Demographics & work factors

A total of 1449 individuals started the survey, but only 924 met the inclusion criteria for this study of currently working with laboratory rats in the United States or Canada. Of those, 794 completed at least 50% of the survey and therefore were included in the analysis. Detailed demographic and work information for all participants is displayed in **Table 2.1**. The laboratory animal personnel were primarily white (86%) females (80%) with an average age of 40. The majority had a bachelor's degree or higher (68%). They had worked with laboratory animals for an average of 14 years and currently worked an average of 35 hours per week with laboratory animals. In an average work week, almost half of participants (47%) spent less than 10% of their time working with rats. About two-thirds worked at a university while almost a quarter worked at a contract research organization. Finally, almost a quarter each were animal care technicians (24%), veterinary technicians (20%), or laboratory managers (20%).

Table 2.1. Demographic and Work Information for Laboratory Animal Personnel (N = 794).

Categorical Data	Category	N (%)
Country	United States	557 (70%)
	Canada	237 (30%)
Gender	Female	637 (80%)
	Male	110 (19%)
	Other	8 (1%)
Race	White	680 (86%)
	Other	42 (5%)
	Asian	30 (4%)
	Black	25 (3%)
	Mixed	17 (2%)
Education	High school diploma or equivalent	18 (2%)
	Some college, no degree	69 (9%)
	Associates or technical degree	173 (22%)
	Bachelor's degree	315 (40%)
	Graduate degree	219 (28%)
Institution	University	515 (66%)
	CRO	176 (22%)
	NonProfit	37 (5%)
	Government	35 (3%)
	Other	41 (5%)
Research Type	Applied	384 (48%)
	Basic	130 (16%)
	Product	70 (9%)
	Education	65 (8%)
	Regulatory	60 (8%)
	Other	78 (10%)
Role	Animal care or laboratory technician	190 (24%)
	Veterinary technician	162 (20%)
	Manager	157 (20%)
	Veterinarian	110 (14%)
	Other	68 (9%)
	Trainer	36 (5%)
	Other research staff	34 (4%)
	Other animal care staff	25 (3%)
	Principle investigator	12 (2%)

Table 2.1. continued

Time with Rats	<10%	373 (47%)
	11-20%	147 (19%)
	21-30%	84 (11%)
	31-60%	103 (13%)
	61-100%	81 (10%)
Continuous Data	**Mean ± SD**	**Range**
Age (M +- SD)	40 ± 11 years	20 - 78
Years working with lab animals	14 ± 10 years	0 - 50
Hours per week working with lab animals	35 ± 12 hours/week	0 - 66

2.4.2 Enrichment, attitudes, & general behaviors

Most laboratory animal personnel reported having at least a little control over enrichment (93%) and a desire to provide more enrichment (76%) (**Figure 2.2** and **Appendix A Table A.2**). However, only 45% of participants reported having a high degree of control of enrichment ("a lot" or "complete" control). The majority of participants (>90%) have positive Rattitudes and the minority (<10%) held negative Rattitudes. Finally, the majority of participants often engaged in positive behaviors towards their laboratory animals, with a notable 41% often naming their animals.

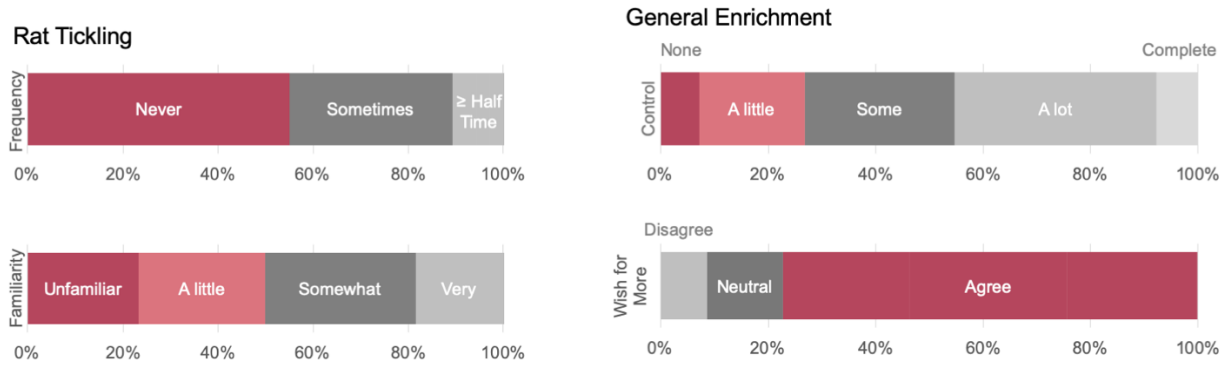

Figure 2.2. Rat Tickling & General Enrichment. Laboratory animal personnel's self-reported frequency and familiarity of rat tickling, as well as general control over enrichment provision and degree to which they agree that they wish they could provide more enrichment to their laboratory animals.

2.4.3 Rat tickling

Qualitative analysis of beliefs

Participant responses to open-ended questions about rat tickling were summarized into two central categories of *Benefits* (i.e., what are the advantages to tickling rats) and *Control Beliefs* (i.e., what are the factors that make it difficult or easier to tickle rats). These central categories were further split into themes and sub-themes, described below and summarized in **Figure 2.3**, and **Appendix A Tables A.3 & A.4.**

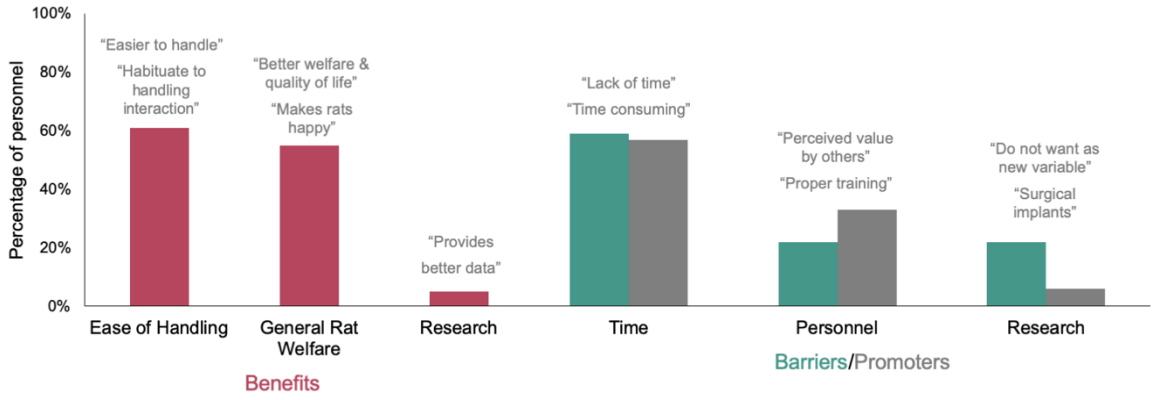

Figure 2.3. Benefits & Control Beliefs about Rat Tickling. The most common themes relating to benefits (advantages) and control beliefs (factors that are barriers that make it difficult or promoters that would make it easier) about rat tickling by 611 laboratory animal personnel currently working with rats. Graphic includes representative quotes. Sub themes and additional representative quotes are presented in **Tables A.3 & A.4.**

Participants indicated that rat tickling was beneficial primarily because it promoted *Ease of Handling* and *General Rat Welfare*, although a few participants indicated *Research* benefits or *No Benefits*. Over half of the participants indicated that rat tickling increases *Ease of Handling* and/or *General Rat Welfare.* More commonly participants specified these benefits came to both the handler and rat or, less commonly, just one species. About a tenth of these participants specifically mentioned that rat tickling promotes a bond. About a quarter of the overall participants specifically indicated that rat tickling reduces rat stress, anxiety, or fear. Additionally, 21% of participants indicated that rat tickling was a form of enrichment, with some specifying it was particularly good for socialization. Conversely, less than 10% of participants indicated that rat tickling was beneficial for *Research* or *No Benefits*. Some participants indicated rat tickling can

improve research data or outcomes. A few participants did not think it was beneficial at all for a particular study or compared to other enrichment techniques – alternatively some participants simply did not know enough to indicate benefits.

Almost 60% of participants indicated that *Time* was a key factor controlling rat tickling implementation. Most indicated that the time required made rat tickling difficult or that more time would make it easier. Additionally, some participants specifically mentioned time related factors such as limits imposed by *Staffing, Number of Rats,* or the *Consistency* needed for the technique. Despite the direct relationship between staff time and money to pay those staff, few participants mentioned *Money* as a limiting factor.

The second most common control beliefs were *Personnel* and *Research.* Within the theme of *Personnel* participants stated that a lack of *Buy-in* and *Education* of staff or (rarely) even *A Fear of Rats* may make rat tickling difficult, but that promoting *Buy-in* and *Education* may make it easier. Within the sub-theme of *Buy-In,* some personnel specifically stated that they thought implementing the technique was *Not My Problem* as it was not in their role or that *Official Approval* such as via IACUC (institutional animal care and use committee) or Principle Investigators would be beneficial to promote rat tickling. Within the sub-theme of *Education,* participants indicated general *Awareness* and lack of *Training* make rat tickling provision difficult and that increasing awareness and training opportunities would help. Within the theme of *Research* – which was more than three times more likely to be cited as a barrier than promoter – participants indicated concerns with introducing a *New Variable* to studies, that specific study-related *Rat Factors* or *Research Protocols,* or even just a *Short Study* may make implementation of rat tickling more difficult.

Participants less commonly mentioned control beliefs relating to *Rats, Safety, or Facility Factors,* and, of course, a few participants either indicated there being *No Barriers* or *Nothing Easier*. Within the theme of *Rats,* a variety of specific problems such as *Age* (older rats being less receptive), *Aggression* levels, *Individual Differences, Single-Housing, and Breeding Status.* Within the theme of *Safety,* participants mentioned concerns about maintaining *Biosecurity* or rat tickling resulting in *Harm to Personnel* either through bites or zoonotic diseases. Within the theme of *Facility Factors,* individuals indicated factors related to wanting more space in the rooms or housing rats in *Larger Cage Sizes.*

Quantitative analysis

Overall, participating laboratory animal personnel reported being fairly unfamiliar with rat tickling (50%) and the majority never tickle their rats (55%; **Figure 2.2** and **Appendix A Table A.2**). For those reporting using it, most do not report using standard, validated technique of a dorsal contact and single-handed pin (55%). Conversely, 21% of personnel indicated that rats were only given a dorsal contact in the cage or stroked in the hand, 17% indicated that rats were only pinned with one or two hands but did not receive dorsal contact, and 11% indicated that rats were tickled with a dorsal contact and either single or double-handed pin and also stroked. The rest of the participants indicated some combination of techniques, that they were unsure, or that the technique was not pictured.

For quantitative analysis, 656 and 591 participants were included for current and planned implementation of rat tickling, respectively. The former completed at least 50% of each scale in the quantitative theory of planned behavior section and the latter also reported their current level of rat tickling. These participants reported a very slightly positive *intention* to tickle rats in the next year (**Figure 2.4 and Appendix A Table A.5)**. From both direct and indirect measurements, participants had overall positive *attitudes* (e.g., they think rat tickling is good and are in favor of providing it), were relatively neutral to negative *subjective norms* (e.g., they do not feel social pressure to provide rat tickling), and neutral to negative *perceived behavioral control* (e.g., they are not confident they can provide rat tickling and there are barriers; **Figure 2.4**, and **Appendix A Tables A.5 & A.6**). In terms of measurement reliability, our direct measures had acceptable reliability within scales (**Appendix A Table A.5**; Cronbach's alpha >0.7 except for perceived behavioral control alpha = 0.66) and our indirect measurements were significantly associated with the direct measures (**Appendix A Table A.6;** significant standardized regression coefficients, $p < 0.01$).

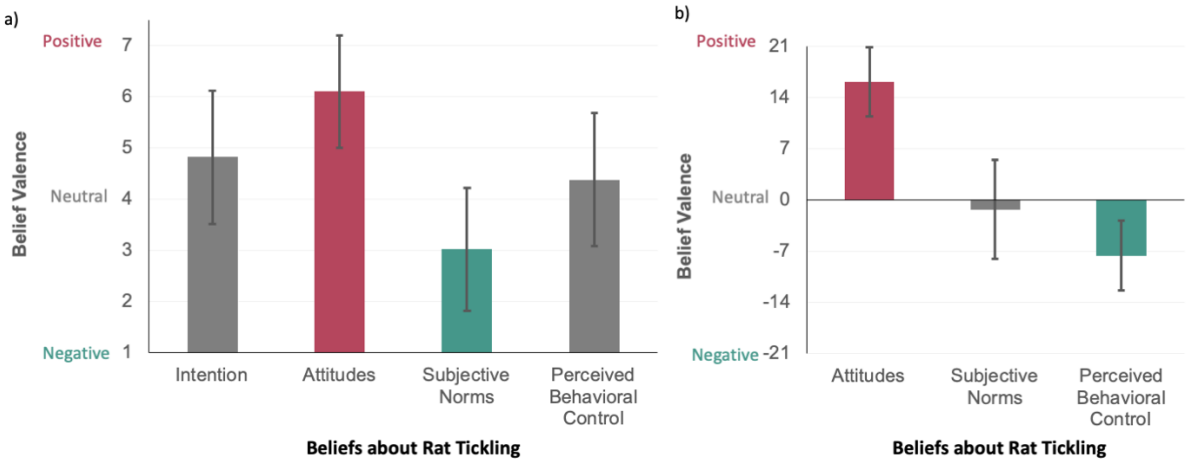

Figure 2.4. Beliefs about rat tickling. Laboratory animal personnel (N = 656) self-reported intention to provide and beliefs about rat tickling (M ± SD). All scales were developed from Theory of Planned Behavior protocols including beliefs about the consequences (behavioral attitudes), social and professional pressures (subjective norms), and control over (perceived behavioral control) providing rat tickling (a. direct, b. indirect).

In this study, current and planned rat tickling was associated with several factors (**Figure 2.5 and Appendix A Table A.7**). Both current and planned rat tickling were positively associated with subjective norms, control beliefs, and familiarity. Current and planned rat tickling were also positively associated with more positive attitudes towards laboratory animals in general or both more positive attitudes towards rat tickling and a higher desire to implement more enrichment in general, respectively. Additionally, working in Canada was positively associated with current rat tickling implementation ($\beta = 0.0249$, $p = 0.002$).

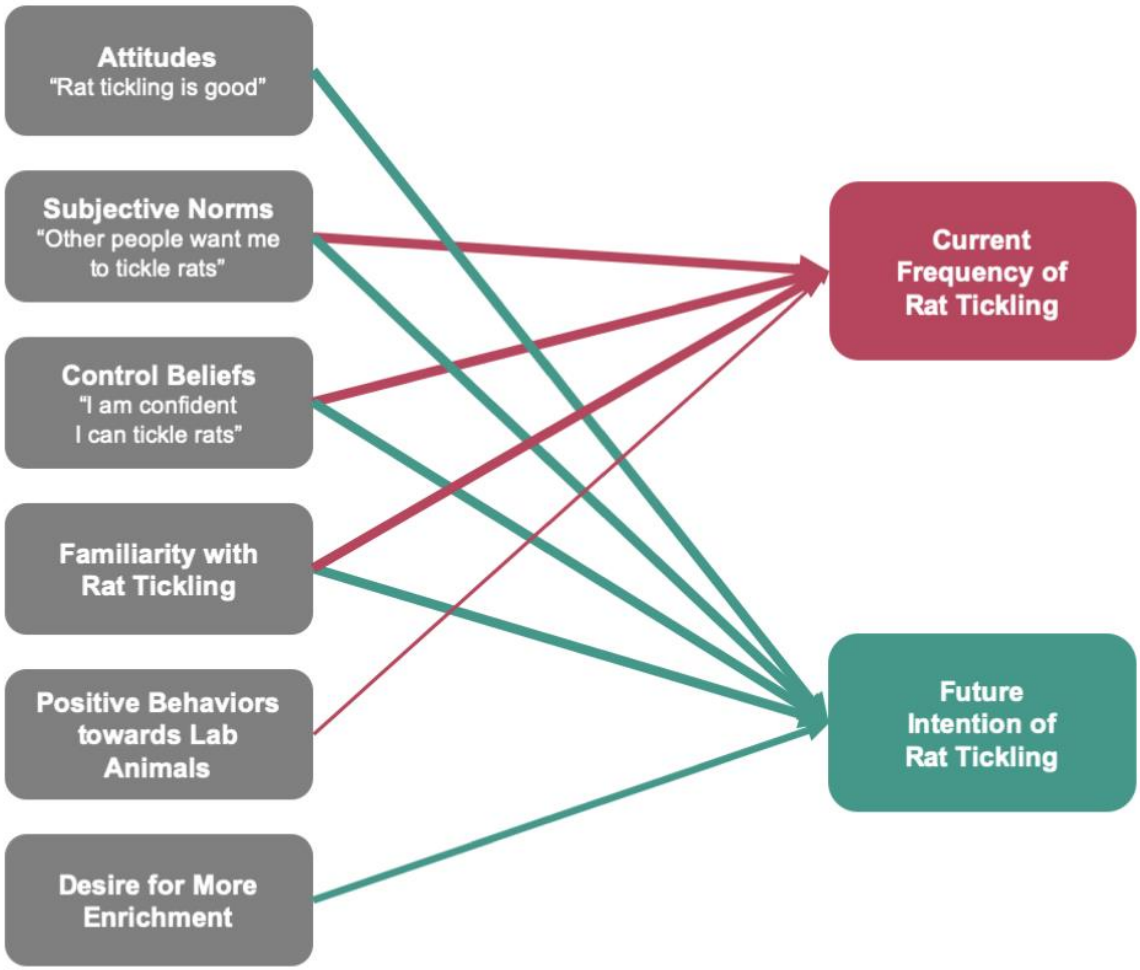

Figure 2.5. Significant associations between moderator factors and rat tickling implementation. This figure shows significant associations from self-report data from laboratory animal personnel about potential moderating factors and both current (top, N = 591) and future (bottom, N = 656) rat tickling. Lines only connect between significant associations. The thickness of the lines indicates the significance of association (thick = $p < 0.0001$, medium = $p < 0.001$, thin = $p < 0.05$). Non-significant moderators included positive and negative Rattitude and control over enrichment. Models were run controlling for age, years working, hours of work per week, % of time working with rats, gender, role, institution, research type, race, and highest education (none which were significant). Numerical data is reported in Appendix A Table A.7.

2.5 Discussion

To our knowledge, this study is the first to quantify current rat tickling prevalence and potential factors potentially influencing its implementation in the laboratory research environment. We successfully surveyed 794 laboratory animal personnel working in a variety of roles, institutions, and research types. Our results indicate that laboratory animal personnel report relative unfamiliarity with and infrequent implementation of rat tickling. When implementation of

rat tickling is reported, the technique used is often not the standard, validated one. Overall, our analyses indicate that although laboratory animal personnel generally believe rat tickling is beneficial, they do not feel confident in their ability to provide it due to lack of time and education. They do not feel social or professional pressure to provide this enrichment. On the contrary, there is indication that, they may even feel pressure not to provide it by other personnel or research staff (rather than neutral ambivalence). However, there is a positive association between rat tickling intention and both more positive beliefs about it and a greater familiarity with it. There are also positive associations between implementation of rat tickling and general positive behaviors towards lab animals and a general desire to provide more enrichment.

2.5.1 Current rat tickling implementation

In March of 2018, laboratory animal personnel in this population reported mostly no or low familiarity with and implementation of rat tickling. Although rat tickling originated in 1999 and has over 32 publications (7), the technique was not published as a habituation technique until 2008 (19), with its evidence base synthesized until 2017 (7), and with a peer-reviewed video standard operating procedure until May 2018 (after this survey was administered) (20). It is possible that peer reviewed journal articles are not the most effective form of communication for busy laboratory animal personnel.

Furthermore, even when personnel did report implementing rat tickling, many indicated using techniques that do not mimic aspects of rat rough-and-tumble play with a dorsal contact and pin (20). For example, 21% of participants indicated that their rats are "tickled" with only a dorsal contact, similar to stroking or petting. Unfortunately, stroking or light touch can be aversive to naïve rats, eliciting vocalizations indicative of negative affect (5) and fewer positive outcomes (7). Using non-validated techniques such as these may result in fewer positive results and even negative results (e.g., defensive posturing). In turn, this could contribute to less positive attitudes towards and implementation of rat tickling.

2.5.2 Beliefs & associations of rat tickling

Laboratory animal personnel overall had positive behavioral attitudes about rat tickling – that is, they generally thought that rat tickling is good and beneficial. Furthermore, participants

who had higher positive behavioral attitudes about the technique were also more likely to plan to provide rat tickling in the next year. In particular, rat tickling was cited to be beneficial for rat welfare and handling (but rarely research aims) in both qualitative and quantitative data. This may be a result of publications and video evidence demonstrating the handling and welfare benefits of rat tickling (7), while there are no publications showing improved research factors. Furthermore, handling and welfare benefits may be more salient to the laboratory animal personnel surveyed (only 2% of participants were Principle Investigators), while research factors may be more often a constraint. Regardless, it may be beneficial to develop research and case studies showing the feasibility and benefits of rat tickling for scientific research.

Overall, personnel had very low subjective norms about rat tickling – that is, they generally felt little to no social or professional pressure to provide this technique. However, participants who held higher subjective norms about rat tickling were more likely to indicate higher levels of current and planned rat tickling. Specifically, personnel may find the opinions – and social pressure – of accreditation staff, laboratory animal veterinarians, study leads, and principle investigators particularly important. In fact, some personnel indicated they felt study leads in particular should be responsible for initiating rat tickling implementation. Related to the current lack of professional pressure to provide rat tickling, some personnel cite its name "rat tickling" as a barrier to implementation. Our research team also promotes the term "heterospecific play" which is more commonly used in neuroscience publications (21).

Perceived behavioral control over providing rat tickling was overall reported to be neutral to negative – that is, that personnel do not feel in control of providing rat tickling. However, personnel who reported more positive control beliefs were also more likely to indicate higher levels of current and planned rat tickling. That is, individuals who felt confident that they *could* implement the technique were more likely to tickle rats. Specifically, personnel believe that having enough time, official approval, and sufficient training are very important. A lack of time was by far the most commonly cited barrier for rat tickling. This is unsurprising considering typical protocols recommend 2 min of tickling per rat for 5 days (10 min total per rat) (7). As our research team predicted this barrier, we completed a study (published 6 months *after* collecting this data) that reduces the time requirement down to 15 s for 3 days per rat (45 s total per rat) (8, Chapter 3). While this recommendation still requires additional time, it should be significantly more

manageable. Additionally, 3 days of tickling can easily fit within an institution's mandated, post-shipment, acclimation time, and prior to the start of the study.

In addition to beliefs about rat tickling, we also found positive associations between familiarity with rat tickling, behaviors towards laboratory animals (but not Rattitude), general enrichment desires, and rat tickling implementation. Unsurprisingly, a greater familiarity with the practice of rat tickling was a strong predictor of both current and future intention of rat tickling. More positive general behaviors (e.g. talking to or naming laboratory animals) predicted current rat tickling implementation. Laboratory animal personnel who perform these behaviors at higher levels may be highly motivated to seek out and implement enrichments such as tickling. These results mirror findings that farm animal stockpeople with more positive general behaviors towards their farm animals also have more positive human-animal interactions with them (11). Conversely, attitudes towards rats in general (e.g. beliefs that rats are smart, curious) was not associated with rat tickling implementation, which may be a result of a ceiling effect in that this sample had overwhelming strong positive and weak negative Rattitude (i.e. attitude towards rats). Finally, unsurprisingly, individuals with a stronger desire to provide more enrichment had a higher intention to implement of rat tickling in the future. Overall though, our study did not find any associations between work factors, demographic variables, and rat tickling implementation. This may indicate that it is feasible to implement rat tickling regardless of institution, research, or personnel.

Interestingly, in response to open-ended questions, some personnel indicated that using rat tickling to form a bond between handler and rat would be beneficial. However, creation of this bond was never cited as a barrier or disadvantage to rat tickling. Although research encourages bonds between laboratory animals and personnel (22), anecdotally we have heard that personnel may be hesitant to tickle rats because of fear that establishing a bond could make aversive procedures more difficult, and, in turn, increase compassion fatigue. In actuality, this does not seem to be a concern. Additionally, using further data collected during this survey, no association was found between frequency of rat tickling and compassion fatigue (burnout or secondary traumatic stress) (23).

2.5.3 More, better training is needed

Taken together, our results indicated there is a need for greater tickling education to promote implementation. Previous studies show that researchers can change personnel behaviors by holding trainings that directly target both behavior and beliefs (13). Educating laboratory animal personnel should not rely on peer-reviewed publication but include targeted training using appropriate educational theories to maximize behavior change. Based on this survey, training may include focus areas such as teaching participants proper technique while increasing their confidence, addressing common concerns about implementation, emphasizing its benefits to improve attitudes, and even emphasizing that rat tickling may become a social norm. Any efforts to increase perceived behavioral control (e.g. hands-on instruction in the technique or reducing time required) are likely to be particularly well received. Educating accreditation staff, veterinarians, study leads, and individuals particularly interested in enrichment may be particularly effective to increase social norms and professional pressure. Furthermore, it would be beneficial to show more evidence of rat tickling being used successfully in typical research studies, hopefully to the benefit of research data.

2.5.4 Limitations

There were several limitations to this project. First, since this study was cross-sectional it is impossible to determine the causation of any associations that we found. For example, perhaps the ability to currently implement rat tickling causes higher positive control beliefs, rather than more control beliefs causing higher current implementation of rat tickling. Future studies would benefit from randomly assigning laboratory animal personnel to educational workshops designed to change personnel beliefs to determine the causality of the association. However, this study provides insight into what those educational workshops could contain.

Second, since this survey only involved self-report data from laboratory animal personnel, there is the potential for subjective biases to occur. Our team did not directly measure the level of rat tickling, personnel behavior, or animal welfare. Therefore, it is possible that the personnel may have over or underestimated their current level or future ability to provide rat tickling. Future studies could implement a diary tracking method or allow for follow-up to see if rat tickling is indeed implemented with more positive attitudes and higher intentions. However, this study

provides valuable broad, exploratory insight into the perspectives of laboratory animal personnel and how those beliefs may impact enrichment implementation.

Finally, as this was a voluntary, survey-based convenience sample study, we are unsure if our sample is representative of the population or if participants were affected by sampling bias. One factor to consider is that we only translated the survey into French and not Spanish. It is unknown whether this low percentage of Hispanic and Latino participants is truly representative of the laboratory animal personnel field. If not, these participants could have characteristically different attitudes towards rat tickling. Regardless, the very large sample size obtained suggests that we have accurately characterized attitudes towards rat tickling.

2.6 Conclusions

In conclusion, as of May 2018, rat tickling appears to be relatively rarely implemented by laboratory animal personnel. Although laboratory animal personnel may believe rat tickling is beneficial for rat handling and welfare, they also believe there are key barriers to its implementation in the form of time, personnel, and research. Furthermore, there are statistical associations between higher current and planned rat tickling implementation and factors such as higher positive beliefs (attitudes, subjective norms, and control beliefs), familiarity with tickling, general positive behaviors to lab animals, and a desire to implement more enrichment. Laboratory animal personnel beliefs seem to be a key to promoting the widespread implementation of beneficial enrichment techniques. Overall, our results suggest that further research on reducing the time required to tickle rats, increasing personnel buy-in and education (therefore improving attitudes, subjective norms, control beliefs, and familiarity), and showing the benefit of rat tickling for research could help increase its prevalence and improve laboratory rat welfare.

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CHAPTER 3. PRACTICAL RAT TICKLING: DETERMINING AN EFFICIENT AND EFFECTIVE DOSAGE OF HETEROSPECIFIC PLAY

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3.1 Abstract

Laboratory rats may experience stress during handling which can reduce their welfare. Rat tickling, a handling technique that mimics aspects of rat rough-and-tumble play, has been found to induce positive affect based on production of 50-kHz ultrasonic vocalizations (USVs). However, current protocols for rat tickling are time-intensive, making implementation difficult. Our objective was to determine a time-efficient and effective dosage of rat tickling that could be practically implemented in the laboratory. We hypothesized that affect and handling can be improved by short, daily doses of tickling within a 5-day work week. Long-Evans rats (N=72) of both sexes, housed in pairs were sampled. Each pair was randomly assigned a tickling duration (15, 30 or 60 s per rat) and frequency (1, 3, or 5 days). After the final day of tickling, rats were tested for ease of, and reaction to, handling via an intraperitoneal injection of saline following a tickling session for their assigned duration. On test day, we measured production of USVs, home cage behavior (60 min before/after testing), approach behavior (30 s before/after testing), and fecal corticosterone. Periods before and after testing measured anticipatory and reactionary responses, respectively. In cage behaviors included social play, activity, and location. Approach behaviors included indicators of fear or anxiety such as rearing, location, and contact with the hand. Data were analyzed using general linear models. We found that 3-day rat tickling was most efficient and effective as it produced a higher rate of 50-kHz USVs *before* and *during* tickling ($p < 0.0001$), and rats played more and were less inactive in their cage for the hour *before* tickling and injection ($p < 0.003$) compared to 1-day of rat tickling, but there was no difference between 3- and 5-days of tickling. Only one outcome (play behavior after tickling) showed more positive results after 5- vs 3-days of tickling ($p = 0.002$). Tickling duration did not impact any outcome measures ($p > 0.05$). Neither tickling duration nor frequency impacted approach behavior, injection duration, or fecal corticosterone ($p > 0.05$). In conclusion, a time-efficient and effective rat tickling dosage was

identified to be 15 s for 3-days before any potentially aversive procedures are applied. This conclusion is based on increased 50-kHz USVs (a measure of positive affect) and positive anticipatory behavior, including play. Overall, our results suggest that minimal rat tickling can effectively habituate rats to handling and prepare them for research procedures within a work week.

3.2 Introduction

3.2.1 Importance

Rats naive to humans may find interactions frightening, which makes handling difficult and causes significant increases in behavioral and physiological indicators of stress (1,2). To minimize the effects of stress, it is common practice to habituate rats to human interaction. A variety of techniques are currently used. One experimentally evaluated procedure involves touching, stroking, lifting, talking, and offering food treats to rats for a total of 4.5 h per cage (3). This protocol resulted in decreased fear and improved ease of handling for up to six months (3). However, using this protocol to habituate 10 cages of rats would require an additional 40 h of dedicated employee time which is time-intensive and costly. Additionally, one of the procedures used – stroking rats naïve to handling – was found to elicit 22-kHz ultrasonic vocalizations (USVs), an indicator of negative affect in rats (1).

3.2.2 Current Knowledge

An alternative technique to habituate rats to human interaction and provide social enrichment is a type of heterospecific play called rat tickling (4). This technique appears to be an effective habituation protocol for use before common handling procedures such as injection (4–6). Rat tickling mimics aspects of rat rough-and-tumble play by alternating between touching the rat's nape and ventral surface with rapid and vigorous finger movements. This technique elicits the production of short, USVs in the 50-kHz range from rats. These vocalizations are thought to be indicative of positive welfare as shown by their production during rewarding social interactions, in anticipation of food, and in reaction to application of euphoragenic drugs (7–9). Tickling has also been found to improve both behavioral and physiological metrics of rat welfare (6).

3.2.3 Rationale

Previous applications of rat tickling methods have used a wide variety of session durations (30-600 s), frequencies (3-38 days), and total time investments ranging from 30 s to 100 min (6). The majority of these protocols result in a relatively large time investment per rat, especially when including daily trips to the animal room, between the animal and procedure rooms, and transitioning between cages. Anecdotally, when researchers from our laboratory (ML, SC, BG) have given presentations and workshops on rat tickling, laboratory personnel have expressed concerns about the time investment of rat tickling. They indicate that time is a barrier for implementation of this technique. Our rationale for this study was that laboratory personnel may be more likely to implement rat tickling in their facilities if an efficient and effective protocol is available.

3.2.4 Objectives

Our objective was to determine a time-efficient and effective rat tickling dosage. Our specific aim was to determine the minimum amount of time investment necessary for tickling to improve positive affect and ease of handling during a routine procedure. Based on previous research of various dosages of tickling rats, we hypothesized that affect and handling can be improved by short, daily doses of tickling within a 5-day work week. We predicted that rats tickled for less than 30 s for 3 days would show fewer positive responses during approach tests, fewer 50-kHz USVs during tickling, fewer positive behaviors in cages, and higher fecal corticosterone levels than rats that were tickled for at least 30 s for 3 days.

3.3 Materials and Methods

All procedures were reviewed and approved by Purdue University's Institutional Animal Care and Use Committee (IACUC), protocol #1605001415. All work was done in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

3.3.1 Animals, Housing, and Husbandry

This study was conducted at Purdue University in West Lafayette, Indiana, USA. Across two replicates, we sampled a total of 72 juvenile Long-Evans rats (36 rats per replicate (Rep); Crl:LE; Charles River, Kingston, NY, USA) equally split between male and females. The Long-Evans strain was chosen for its relatively frequent use in biomedical testing and history as the most frequent strain used in rat tickling research (6). We assessed both sexes based on the National Institute of Health (NIH) directive requiring both sexes be tested in animal trials (10). The rats arrived at our facility at the age of 35 days. Before data collection began, rats were allowed 3 days to habituate to the facility (without additional handling).

Rats were housed in same-sex pairs in static clear plastic cages with a wire lid (43 cm L x 22 cm W x 20 cm H). Each cage contained aspen bedding (Envigo Teklad, Madison, WI USA), and one red transparent plastic hut (10 cm H x 10 cm W x 15 cm L, BioServ, Flemington, NJ, USA). Food (rodent chow, Envigo Teklad, 2018, Madison, WI USA) and water were provided *ad libitum*. The room was maintained at a constant temperature (22 ± 0.2 ºC), humidity (37.5 ± 6.7 %) and 12 h light/dark cycle (lights on 0630-1830 in replicate 1, and 0600 to 1800 in replicate 2).

Laboratory personnel separate from the research team performed daily welfare checks and changed cages weekly. To reduce potential bias, cage cards were coded and identical in appearance so that caregivers were blinded to treatment during welfare checks. Researchers generally moved rats by cupping them in their hands, but would grasp rats by their tails to steady them if necessary. All researchers and laboratory personnel were female and wore disposable gowns and gloves when interacting with rats.

3.3.2 Experimental Treatments

A 3 x 3 factorial design was used to compare the effects of different durations (15, 30, or 60 s per rat) and frequencies (1, 3, or 5 days) of heterospecific play (**Fig 1)**. Our sample size was determined *a-priori* using Mead's Resource Equation with our smallest unit of measurement (cage) (11). Across both replicates, for each treatment combination of frequency and duration there were a total of 8 rats, housed in 4 cages split evenly between the sexes (4 male and 4 female rats). Depending on analysis (as detailed in section 2.6) this led to a total N of 72 rats and 36 cages. Heterospecific play for 60 s per rat for 5 days was selected as the "control condition" because this

is the most commonly used frequency and duration of heterospecific play that has previously been found to have positive outcomes (6). The median duration (30 s) was chosen as it is the shortest tickling duration previously used (6) and the shortest duration (15 s) was chosen as it is used for a single bout of tickling in the original description of rat tickling (12). The median frequency (3 day) was chosen as it was commonly used in previous studies whereas the shortest frequency (1 day) was selected as it is the shortest possible frequency of rat tickling. All animals were included during outcome assessment.

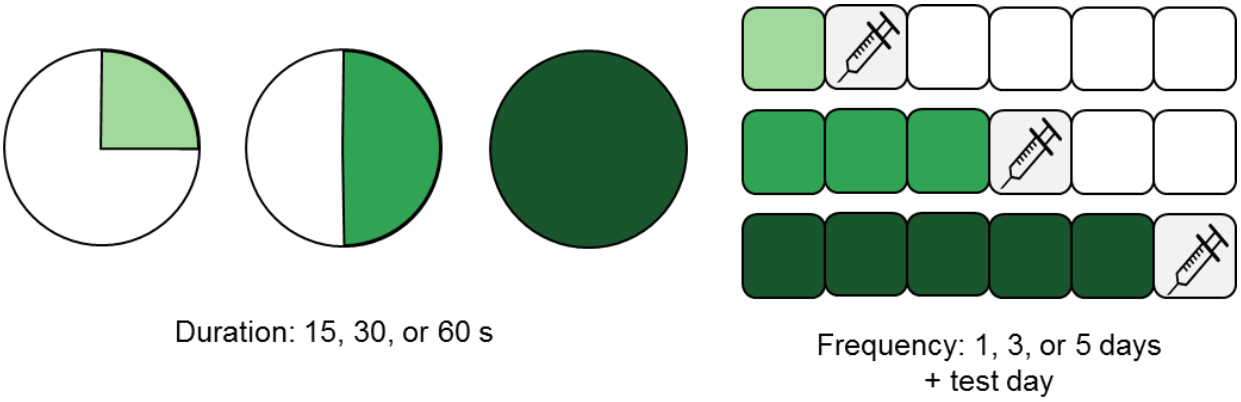

Figure 3.1. Tickling Dosages and Test Day. A schematic showing that rats were tickled in a 3 x 3 factorial design for 3 different durations (15, 30, or 60 seconds) and 3 different frequencies (1, 3, or 5 days). This treatment was followed by a test day (indicated by a square with a syringe) that included one tickling session followed by an intraperitoneal injection of saline (also referred to as tickling + injection). To assess the effectiveness of these treatments, all measurements (vocalizations & behavior) occurred on test day, except for collection of fecal corticosterone.

Efforts were made to reduce bias during randomization and housing **(Appendix B Table B.1).** Upon arrival at the housing facility, rats were randomly placed in cages using a randomly generated sequence list (random.org). To conceal the allocation sequence and ensure adequate randomization, treatments were assigned after rats were placed in them. Treatments were evenly balanced by the location in the room and tier level to control for potential confounds of light intensity, noise level, and locus of human activity in the room (13).

3.3.3 Procedures

On day 0, we collected fecal boli, changed cages, and marked rats for individual identification. First, fresh fecal boli (approximately 3-5 boli per cage) were collected. Then, all

rats received a cage change so that no cage changes would need to be conducted during the experiment. Finally, one randomly selected rat per cage was marked to allow for individual identification. In replicate 1, the tail was marked with a color band using a surgical grade marker (XL Prep Resistant Ink, Viscot Medical LLC, East Hanover, New Jersey, USA). In replicate 2, hair dye (Clairol NiceNEasy, Dark Caramel Brown) was applied to the haunches using a cotton swab using care not to contact the skin. Then, the rat was placed in its home cage with treats for distraction for 20 min to allow the hair dye to set. The hair dye was gently washed off with water and the fur dried with cotton swabs and paper towels. This change in marking technique was made to improve ease of distinguishing between rats for video analysis of approach tests and cage behaviour.

During the study, rats were tickled according to their assigned treatment (**Figure 3.1)** with a 30-s approach test before and after each tickling session. We used the Panksepp Method (dorsal contact and pin) modified only in duration and frequency (4,12). Rats' response to tickling treatment was assessed one day after the last tickling session for each condition (**test day, Figure 3.1**).

On test day the followed four procedures were performed. First, rats were assessed with a 30-s approach test. Second, rats were tickled as per their assigned treatment. Third, immediately after tickling, each rat was given an intraperitoneal injection of 1 mL/kg physiologic sterile saline (0.9% NaCl). To give the injection, the rat was restrained on the table on its back using one hand with the pointer and middle fingers applying pressure over the rat pelvic/inguinal area to prevent movement. The free hand was used to give the injection into the lower right quadrant of the abdomen using a 22 g x 1' needle inserted about 1 cm at a 45° angle toward the head. Fourth and finally, following injection, rats were again assessed with a 30-s approach test.

Our procedures were designed to closely mimic standard laboratory procedures, increase predictability, and minimize bias. To closely mimic standard laboratory procedures, tickling was performed in the home cage on standard bedding at normal light intensity for the light phase. One researcher (ML) always tickled the rats, while another researcher (Rep 1: TW, Rep 2: RS) ran the interval timer and sound equipment. During each tickling session, the cage was removed from the home rack, placed on a table within the main housing room, and had its wire top removed. To allow better predictability of the tickling procedure, within each cage, one randomly selected rat was always tickled first. Also, rats were tickled between 30 min to 1.5 h after lights-on during the

light phase of the photoperiod. To minimize bias, cages were tickled in a randomized order to prevent systematic transmission of olfactory cues between treatments. All cages receiving an injection were tested last in a randomized order, to prevent olfactory cues from affecting non-injection rats.

3.3.4 Measurements

All measurements (except body weight and fecal corticosterone levels) were taken on test day (i.e., the day that rats received tickling + injection; **Figure 3.1**). Vocalizations were considered the primary experimental outcome. Measurements taken directly *before* tickling (e.g., anticipatory) were considered to reflect anticipation of human interaction. Measurements taken directly *after* tickling + injection (e.g., reactionary) were considered to reflect reaction to human interaction.

Vocalizations

Ultrasonic and audible vocalizations were recorded at 4 time points on test day and classified using a standard coding scheme. The time points were before tickling (during a 30-s approach test), during tickling, during injection, and after injection (during a 30-s approach test). Vocalizations were classified as 22-kHz if peak frequency fell between 20-29 kHz and bandwidth was less than 4 kHz (7,14). These vocalizations were subdivided into short and long 22-kHz vocalizations when duration was less than 300 ms or more than 300 ms, respectively (Wright 2010). This classification was based upon the fact that long 22-kHz calls have been associated with negative state, whereas the role of short 22-kHz calls is yet to be determined (14). Vocalizations were classified as 50-kHz if peak frequency fell between 30 and 80 kHz, had a bandwidth between 2-7 kHz, and had a short duration between 10-150 ms (7,14). We did not sub-categorize the 50-kHz ultrasonic vocalizations and counted overlapping calls as one call (14).

Vocalization recordings were collected using two ultrasonic microphones capable of recording sounds in the 0-100 kHz range (Ultramic 200k; Dodotronic.com, CIBRA/University of Pavia, Castel Gandolfo, Italy). The first microphone was used for capturing tickling/approach vocalizations and was suspended 20 cm above the cage floor. The second microphone was used for collecting injection vocalizations and was suspended 20 cm above the injection site on the table. SEA Pro Ultra real-time high frequency ultrasonic vocalization recording software was used to

capture and visualize calls (Nauta, Milan, Italy). Vocalizations were sampled at a rate of 200 K with true 16 bit resolution during capture on a PC laptop.

Injection Duration

To evaluate ease of handling, we recorded the time it took to complete the injection using continuous focal sampling. The injection procedure was considered to begin and end as the glove holding the rat touches the invisible plane extending from the edge of the cage.

Approach Behavior

Rats were tested for their anticipation and reaction to human interaction using 30-s approach tests immediately before and after tickling + injection. We recorded behaviors to measure the rat's fear and anxiety (both generalized and specific to the handler;

Table 3.1). Continuous focal sampling was used to measure latency to contact the hand at the opposite side of the cage. Scan sampling every 2 s was used to quantify rears, contacts with the experimenters hand, burrowing, and location in the cage (near the hand, middle, or far away). Rats were considered to have lower fear and anxiety if they had a shorter latency to contact, more rearing, activity (line crossing), contacts with the hand, and time close to the hand.

Table 3.1 Cage and Approach Behavior Ethogram. Description of rat behaviors recorded during (1) 60 min in the home cage (Cage Behavior) before and after exposure to a tickling + injection procedure and (2) a 30-s approach test (Approach Behavior) used for assessing anticipation and reaction to human interactions. Behaviors were assessed in pair-housed male and female Long-Evans rats (N = 72) exposed to 15-60 s tickling session daily for 1, 3 or 5 days. Behaviors were assessed on test day (tickling + injection), one day after the last day of tickling treatment.

Cage Behavior	**Description**
Pin	One rat holds another rat down on its back in a supine position. The second rat has its belly up and at least 2 feet off the ground.
Inactive	Rat's body is still. This includes any behavior that does not involve major movement such as resting, sitting, or lying still.
Active	Rat's body is moving (other than rearing) which can include waling, eating, playing, or grooming. This does not include nose twitches or ear twitches.
Rearing	Rat stands up on hind legs with both forepaws raised off the floor. The forepaws can be in or out of contact with the walls.
Out of Sight	Rat cannot be seen well enough to determine its activity.
Locations: On the floor In the hut Top of hut Out of sight	A rat is in the hut if at least 50% of it's body is in the hut. A rat is on top of the hut if all 4 feet are on top of the hut. A rat is on the floor if it is not in hut/top of hut and its feet are on the bedding. Rat was considered out of sight if it could not be seen well enough to determine its location
Approach Behavior	**Description**
Rear	Rat stands up on hind legs with both forepaws raised off the floor, in or out of contact with the walls and its head peering up into the air. Hind paws may also be in or out of contact with the wall of cage. A rear starts when both forepaws come off the ground and ends when both forepaws are back on the ground.
Contact	Rat touches, or appears to touch (rat's nose overlaps the back of the handler's gloves, arm skin or shirt sleeve), the handler's hand with its nose or paws or body.
Burrowing	Obvious movement of bedding or the rat's face and front paws are no longer visible because it is beneath the bedding.
Locations: Hand Middle Away	A rat is considered in a certain location based on the location of its head and shoulders. The cage was divided into 3 equal sized quadrants: the quadrant with the experimenter's hand, a middle quadrant, and a quadrant on the opposite side of the cage where the rats were initially placed.

In detail, these are the procedures for the approach tests. The experimenter removed the cage from the rack, placed it on a nearby table in the housing room, removed the lid, picked both rats up at the same time, and placed them at the end of the cage farthest away from herself. The experimenter then rested her hand inside the cage, fingertips touching the bedding, against the wall closest to the experimenter. The approach test began at the moment both rats were placed at the far end of the cage. These methods were replicated from previous tickling literature where both

rats are placed on the opposite side of the cage from the experimenter (approximately 20 cm away) and allowed to move freely (6,15,16).

Cage Behavior

On test day, home cage behavior was recorded for 60 min directly before and after tickling + injection (

Table 3.1). Play behavior was assessed using continuous sampling by counting the number of pins per 5 min interval. Only pinning behavior was used because it has been shown to be highly correlated with overall social activity, has high inter-rater reliability, and high face validity (17). Behaviors and location were recorded using video cameras (Versiton Video Cameras, Model SV-GKN-A255, Sante Fe Springs, CA, USA), and later coded by observers blind to treatment, using scan sampling every 5 min. The sampling interval was calculated by comparing time budgets resulting from 2, 5, or 10 min scans and determining 5 min had an acceptable level of accuracy compared to 2 min.

Fecal Corticosterone

Fecal boli were collected at three time points, baseline, the final day of tickling treatment, and the test day. On tickling treatment day and test day boli were collected 7.5 to 9.5 hours post-tickling. Additionally, fecal boli were collected during the same period the day before rats were introduced to tickling to get a baseline fecal corticosterone value after habituation. The collection on the last day of tickling for each frequency treatment (day 1, 3, or 5) gave each cage a fecal corticosterone value in response to tickling only. The test day (day 2, 4, or 6) gave a value in response to tickling and injection. Only fresh boli (distinguished by their softness and wet appearance) were collected. These boli were immediately stored (to avoid steroid metabolite decomposition) in labelled Eppendorf tubes in a -20 ^{o}C freezer and moved to a -80^{o}C freezer within 24 h for long term storage. A researcher (AE) blind to treatment made the collections.

Fecal corticosterone was analyzed by Arbor Assays (Ann Arbor, MI, USA). In brief, samples were placed in a freeze dryer and lyophilized overnight to remove moisture. Samples were then individually ground and weighed. For every 100 mg of dried feces, 1 mL of Ethanol (190 proof) was added. Samples were agitated for 1 h, then centrifuged at 50000 x g for 10 min. The supernatant was drawn off. An aliquot of the samples was taken at 50 uL and dried in a rotary evaporator for 1 h at 30 ^{o}C, then reconstituted by adding 25 uL ethanol and vortexed. Then, the addition of 475 uL of Assay Buffer was used to bring the volume up to 500 uL, which represented 1:10 dilution. These samples were then evaluated using EIA in duplicate. The intra-assay coefficient of variation was 4.4%.

Body Weight

Rats were weighed to the nearest 0.1 g upon arrival, and on treatment days 1 and 7 using an electronic 2000g scale (SS Platform Digital Balance, OHAUS, Pinebrook, NJ, USA).

3.3.5 Data Quality & Bias Mitigation

Data quality for all vocalization and behavior data was maintained in several ways. First, each set of data was coded by one rater blinded to treatment. Second, to assess the reliability of the coding scheme another rater coded 20% of the data. Inter-rater reliability was then assessed using a two-way mixed, absolute-measures intra-class correlation coefficients (ICCs; (18). The resulting ICCs were all in the excellent range (ICC > 0.9), which indicates that coders had a high degree of agreement and that a minimal amount of measurement error was introduced (18).

Throughout the study, efforts were made to mitigate possible bias at every step by utilizing the Systematic Review Center for Laboratory Animal Experiments (SYRCLE) Bias Assessment tool as shown in **Appendix B. Table B.1**.

3.3.6 Statistical Analysis

All data was analyzed in JMP statistical software (JMP® 13.2.0; SAS Institute Inc, Cary, NC, USA) using general linear mixed models except for audible vocalizations during injection which were analyzed via generalized linear model with a Poisson distribution. The assumptions of general linear models were confirmed via Levene's test (homogeneity of variance) and visualization (normality of error and linearity). For all tests, the main level of statistical significance was set at $p < 0.05$ and subsequent custom contrast significance levels were set via Bonferroni corrections. Significant main effects and two-way interactions were analyzed using custom contrasts and Tukey tests. Three-way interactions were analyzed using Bonferroni corrected test slices and custom contrasts. Results are presented as least square mean ± standard error of the mean (LSM ± SEM), back-transformed where applicable.

All statistical analyses included only data from the test day, except for fecal corticosterone in which 3 time points were used in analysis (baseline, final tickling day, and test day). All models initially included the variables of tickling frequency, tickling duration, and sex to a third degree factorial. Models also, if possible, initially included blocking factors of replicate, rack number (1 or 2), and Tier (top, middle, bottom). However, in the final analyses, blocking factors or

interactions above p = 0.10 were excluded. Rack number nor tier was never included in final analyses as p > 0.10. Additionally, since test order was randomized and balanced by treatments, it was not included in statistical models.

To avoid pseudoreplication and accommodate repeated measures, analyses were blocked by either the experimental unit of rat nested in cage (approach behavior and injection duration: N = 72 rats, 8 rats per treatment combination) or cage (cage behavior, vocalizations, and corticosterone: N = 36 cages, 4 cages per treatment combination) with duration, frequency, and sex nested within them. Rat was treated as random and cage was treated as fixed. Duration and frequency were treated as categorical variables. Approach behavior and injection duration were blocked by the experimental unit of rat because we could distinguish between individuals. These analyses also included the blocking factor of rat marking (dyed, marker, none) as the methods used differed between rats of a pair and between replicates.

Overall, an exemplary initial analysis used was:

> Dependent variable = frequency + duration + sex + frequency*duration + frequency*sex + frequency*duration*sex + replicate + rack + tier + cage(frequency, duration, sex)

In select analyses, we modified or added variables. For anticipatory 50-kHz USVs, a log10 transformation was necessary to meet the assumption of homogeneity of variance. For tickling 50-kHz vocalizations, data was analyzed using the average number of 50-kHz USVs produced per 15 s as the dependent variable in order to compare rat responses without biasing longer durations. For vocalizations during injection, we also included injection duration as a covariate. For pins, data was agglomerated by taking the total number of pins per cage per day per time period (anticipatory or reactionary). For fecal corticosterone, we also included time (baseline, final day of tickling, test day).

For cage behavior and location, data was agglomerated by taking a summary score per cage per day per time period (anticipatory or reactionary) of the percent time budget the rats displayed each behavior or location. Any observations coded as out of sight were eliminated from the data set; thus, budgets do not equal 100% and variables are not co-linear. That is, changes in one behavior or location category do not necessarily influence another category.

Overall, an initial time budget analysis used was:

> % time budget = behavior + frequency + duration + sex + behavior*frequency + behavior*duration + behavior*sex + behavior*frequency*duration +

behavior*frequency*sex + behavior*duration*sex + frequency*duration*sex + replicate + rack + tier + cage(frequency, duration, sex)

For fecal corticosterone, data was analyzed in absolute numbers. We used a WLS-GLM procedure where each data point was weighted using the inverse of the estimated variance (calculated from the CV for each sample). Therefore, corticosterone values with very high variation (high CV) received low weights and corticosterone values measured more precisely (low CV) received high weights (19).

All data was included for each outcome except for injection vocalizations. For injection vocalizations, the microphone did not record for the very first injection, so data from that first injection is missing and therefore that data set is unbalanced.

3.4 Results

All results presented (except fecal corticosterone) were taken on test day (i.e. the day that rats received tickling + injection). Below we report only main effects and interactions with significant post-hoc analysis. When both main and interaction effects were significant, only the highest level interaction effects are reported. Results are presented below in the chronological order that they were measured during the procedure.

Tickling duration never significantly impacted any outcome measure. Main statistical tests for the impact of tickling duration and frequency on outcome measures are reported in

Table 3.2 with corresponding post-hoc comparison contrasts reported in **Table 3.3**.

Table 3.2. Statistical tests on the Impact of Tickling Dosage & Outcome Measures. The statistical tests resulting from general linear models of the effect of tickling frequency (1, 3 or 5 days) and duration (15, 30 or 60 s) on various outcome measures in male and female Long-Evans rats (N = 72 rats in 36 cages) assessed on test day (tickling + injection), one day after the last day of tickling treatment. Bold indicates a significant effect.

Period	Measure	Frequency	Duration
Anticipatory	Pins	**$F_{2,30}$ = 8.0, p = 0.002**	$F_{2,30}$ = 0.08, p = 0.8
	Cage Behavior	**$F_{4,60}$ = 12.1, p < 0.0001**	$F_{4,60}$ = 0.2, p = 0.9
	Cage Location	**$F_{4,60}$ = 7.8, p < 0.0001**	$F_{4,60}$ = 1.4, p = 0.3
	50-kHz Vocalizations	**$F_{2,30}$ = 6.0, p = 0.007**	$F_{2,30}$ = 0.2, p = 0.8
Tickling & Injection	Tickling 50-kHz Vocalizations	**$F_{2,29}$ = 14.8, p < 0.0001**	$F_{2,29}$ = 0.2, p = 0.8
	Injection 50-kHz Vocalizations	$F_{2,24}$ = 0.4, p = 0.7	$F_{2,24}$ = 0.06, p = 0.9
	Injection Duration	$F_{2,60}$ = 0.3, p = 0.7	$F_{2,60}$ = 0.7, p = 0.5
Reactionary	Pins	**$F_{2,30}$ = 7.0, p = 0.003**	$F_{2,30}$ = 2.3, p = 0.1
	Cage Behavior	$F_{4,60}$ = 1.2, p = 0.3	$F_{4,60}$ = 0.8, p = 0.5
	Cage Location	$F_{4,60}$ = 1.7, p = 0.2	$F_{4,60}$ = 0.3, p = 0.8
	50-kHz Vocalizations	**$F_{2,30}$ = 4.5, p = 0.02**	$F_{2,30}$ = 0.5, p = 0.6
Overall	Fecal Corticosterone	$F_{2,41}$ = 1.2, p = 0.3	$F_{2,41}$ = 0.9, p = 0.4

Table 3.3. Post-hoc Tests on the Impact of Tickling Frequency Outcome Measures. The post-hoc custom contrast statistical tests results from significant effects of tickling frequency (1, 3 or 5 days) on various outcome measures in male and female Long-Evans rats (N = 72 rats in 36 cages) assessed on test day (tickling + injection), one day after the last day of tickling treatment. Bold indicates a significant effect.

Period & Measure	1 vs 3 days of tickling	1 vs 5 days of tickling	3 vs 5 days of tickling
Anticipatory			
Pins	**$F_{1,30}$ = 10.9, p = 0.003**	**$F_{1,30}$ = 13.1, p = 0.001**	$F_{1,30}$ = 0.1, p = 0.8
In Cage Inactivity	**$F_{1,60}$ = 15.2, p = 0.0002**	**$F_{1,60}$ = 28.2, p < 0.0001**	$F_{1,60}$ = 2.0, p = 0.2
In Cage Activity	$F_{1,60}$ = 6.0, p = 0.02	**$F_{1,60}$ = 14.2, p = 0.0004**	$F_{1,60}$ = 1.8, p = 0.2
50-kHz USVs	**$F_{1,30}$ = 7.1, p = 0.01**	**$F_{1,30}$ = 10.5, p = 0.003**	$F_{1,30}$ = 0.3, p = 0.6
Tickling			
50-kHz USVs	**$F_{1,29}$ = 25.1, p < 0.0001**	**$F_{1,29}$ = 18.8, p = 0.0002**	$F_{1,29}$ = 0.4, p = 0.5
Reactionary			
Pins	$F_{1,30}$ = 0.2, p = 0.6	**$F_{1,30}$ = 12.0, p = 0.002**	**$F_{1,30}$ = 8.8, p = 0.006**
50-kHz USVs	$F_{1,30}$ = 4.7, p = 0.04	**$F_{1,30}$ = 8.2, p = 0.008**	$F_{1,30}$ = 0.5, p = 0.5

3.4.1 Anticipatory Period

Anticipatory Cage Behavior

On test day during the anticipatory period, tickling frequency significantly impacted rat in cage play behavior (measured via number of pins), general behavior, and location (**Figure 3.2;**

Table 3.2). Rats tickled for at least 3 days showed more pins, less inactivity, and less time in huts than rats tickled for only 1 day, with no differences between 3 and 5 days (**Figure 3.2; Table 3.3)**. Rats tickled for 5 days also showed more activity than rats tickled for 1 day, with rats tickled for 3 days showing intermediate response to 1 or 5 days (**Figure 3.2; Table 3.3)**. For blocking factors, sex also impacted rat behavior ($F_{2,60} = 13.3$, $p < 0.0001$) and location ($F_{2,60} = 11.6$, $p < 0.0001$). Specifically, female rats spent less time inactive (21 vs 48 ± 4%; $F_{1,60} = 17.9$, $p < 0.0001$) and less time in their huts (27 vs 55 ± 5%; $F_{1,60} = 15.5$, $p = 0.0002$) than male rats.

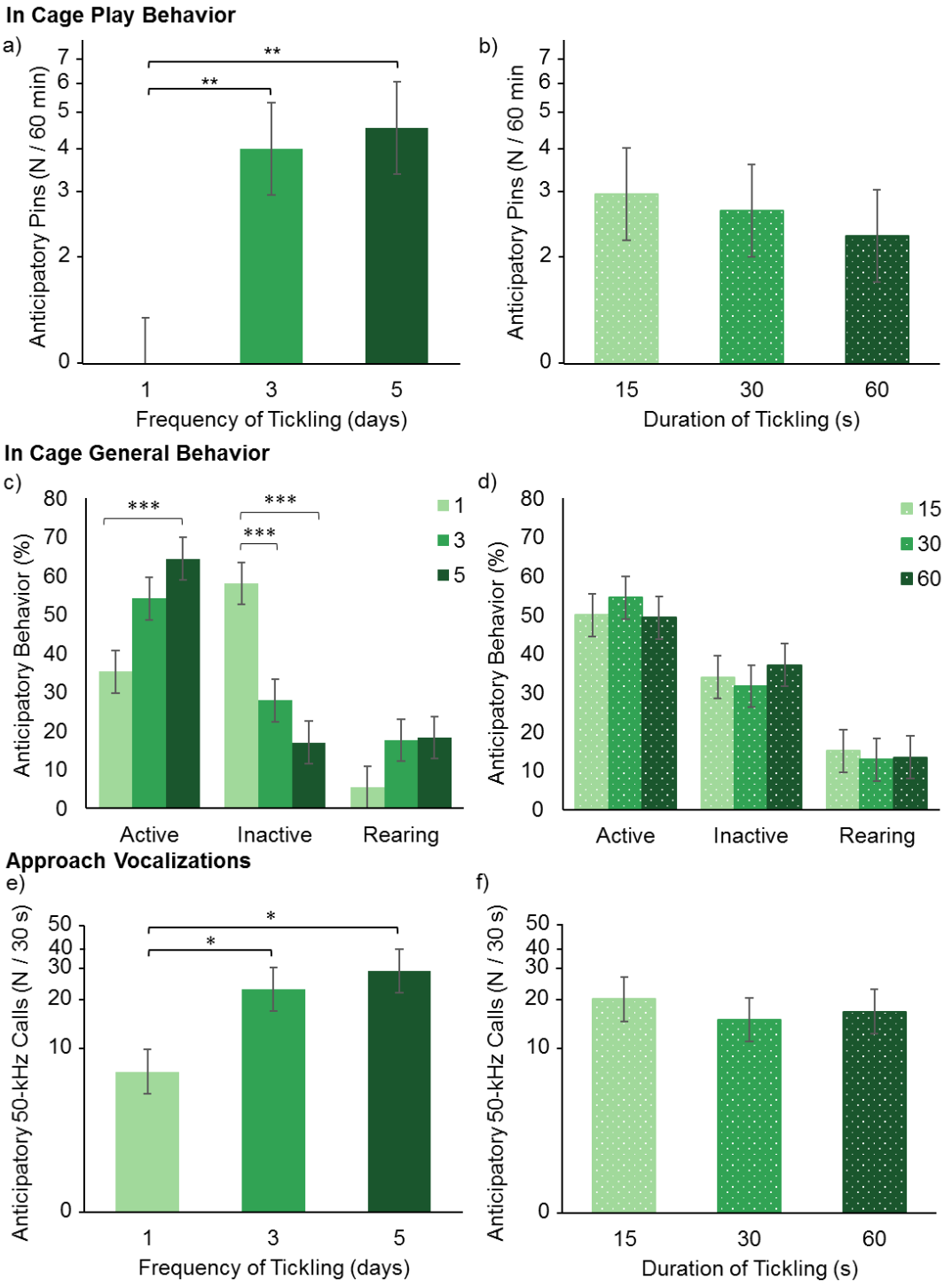

Figure 3.2. The Impact of Tickling Frequency and Duration on Anticipatory Measures. The impact of tickling frequency (left column; 1, 3, or 5 days, n = 12 cages per frequency) and duration (right column, 15, 30, or 60 seconds, n = 12 cages per duration) on in cage play behavior (a, b) and general behavior (c, d) for 60 min prior tickling on test day and total 50-kHz vocalizations (LSM ± SE) during a 30 s anticipatory approach test on test day (e, f). Total N = 36 cages with 4 cages per treatment combination. The scales of a, b, e, and f are back-transformed from log10. *p<0.05 **p<0.01 *** p<0.001 between groups via a custom contrast.

Anticipatory Approach Vocalizations

During a 30-s approach test before tickling + injection, tickling frequency significantly impacted rat 50-kHz USVs (**Figure 3.2;**

Table 3.2). Rats tickled for at least 3 days produced at least 59% more vocalizations than rats tickled for only 1 day, but did not differ from each other (**Figure 3.2; Table 3.3**). We note that the majority of rats did not produce any 22-kHz calls (94%) and no cages of rats produced audible vocalizations. For blocking factors, female rats produced more 50-kHz USVs before tickling than males (33.8 vs 7.2 ± 1.2 50-kHz USVs/cage/30 s; $F_{1,30} = 19.9$, $p = 0.0001$).

Anticipatory Approach Behavior & Location

During a 30-s approach test before tickling + injection, latency to contact the hand was significantly impacted by an interaction of frequency and sex ($F_{2,64} = 4.3$, $p = 0.02$**)**. Male rats tickled for 5 days had a longer latency to contact than male rats tickled for 1 day (Tukey, $p < 0.05$). For blocking factors, male rats reared less than female rats ($F_{1,52} = 6.9$, $p = 0.01$) and rats in replicate 1 reared more than rats in replicate 2 ($F_{1,52} = 9.5$, $p = 0.003$). We also note that burrowing behavior was too rare for statistical analysis as 85% of rats never burrowed during approach (0.67 ± 0.24 burrowing attempts).

3.4.2 Tickling and Injection

Vocalizations during Tickling

On test day, tickling frequency significantly impacted the rate of 50-kHz USVs produced during tickling (**Figure 3.3;**

Table 3.2). Rats tickled for at least 3 days produced at least a 58% higher rate of 50-kHz calls than rats tickled for only 1 day, but did not differ from each other (**Figure 3.3; Table 3.3)**. For blocking factors, female rats produced a higher rate of 50-kHz calls than males (46.4 vs 34.7 ± 2.2 USVs/15 s; $F_{1,29} = 20.1$, $p = 0.0009$). Also, replicate 1 rats produced a higher rate of 50-kHz calls than replicate 2 rats (46.1 vs 34.9 ± 2.2 USVs/15 s; $F_{1,29} = 12.7$, $p = 0.001$). We note that the majority of rats did not produce any long 22-kHz USVs (97%, 0.12 ± 0.1), short 22-kHz USVs (92%, 0.16 ± 0.08), or audible vocalizations (92%; 0.24 ± 0.2).

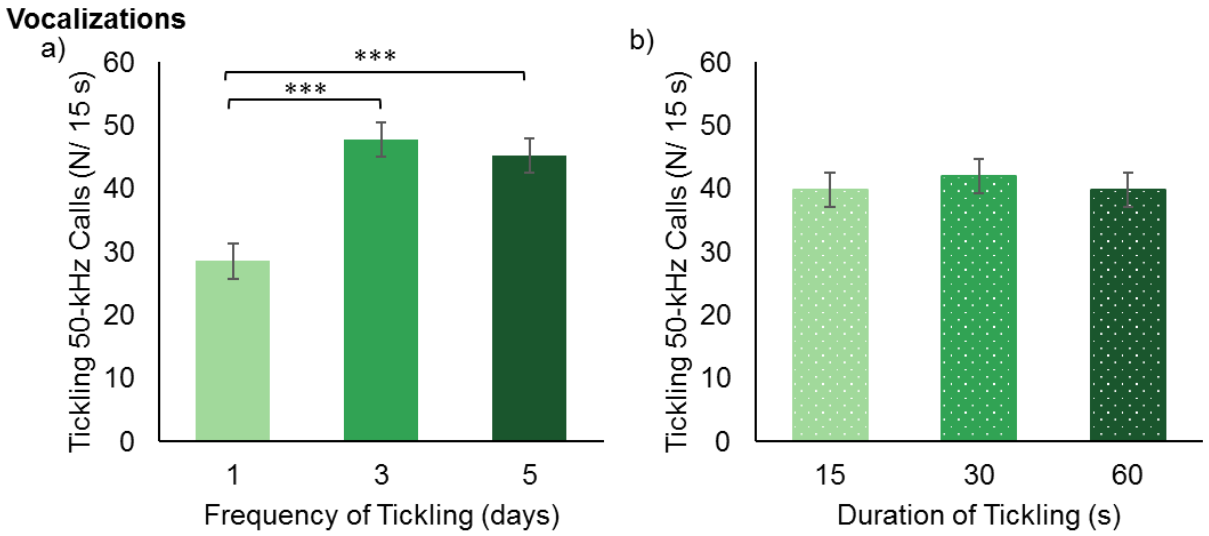

Figure 3.3. The Impact of Tickling Frequency and Duration on Vocalizations during Tickling. The impact of tickling frequency (left column; 1, 3, or 5 days, n = 12 cages per frequency) and duration (right column, 15, 30, or 60 seconds, n = 12 cages per duration) on average 50-kHz vocalizations per 15 seconds (LSM ± SE) during tickling on test day. Total N = 36 cages with 4 cages per treatment combination. *** $p<0.001$ between groups via a custom contrast.

Vocalizations during Injection

During injection, rats in replicate 1 produced more 50-kHz USVs than replicate 2 (4.6 vs 2.6 ± 1.2 USVs; $F_{1,24} = 6.3$, $p = 0.02$). We note that during injection, all cages of rats produced at least some 50-kHz USVs (4.3 ± 0.4 USVs), no cages of rats produced any long 22-kHz USVs and only one rat uttered one short 22-kHz USVs. We also note that rats did produce a few audible vocalizations during injection (0.58 ± 0.15 total), but these were not impacted by either tickling frequency or duration.

Injection Duration

Injection duration was impacted by marking ($F_{2,60} = 5.3$, $p = 0.008$) and tier ($F_{2,60} = 5.4$, $p = 0.007$). Rats marked with a marker had a longer injection duration than unmarked rats (11.9 ± 1.1 vs 9.6 ± 1.0 s; $F_{1,60} = 10.3$, $p = 0.002$), with dyed rats (10.6 ± 1.1 s) showing an intermediate response. Rats housed on the top tier had a longer injection duration than lower tier rats, with middle tier rats taking an intermediate duration (T: 12.0 ± 1.1 vs L: 9.5 ± 1.1 vs M: 10.7 ± 1.0 s; Tukey, $p < 0.05$).

3.4.3 Reactionary Period

Reactionary Approach Vocalizations

On test day during a 30-s approach test after receiving an injection, rat 50-kHz USVs production was significantly impacted by tickling frequency (**Figure 3.4;**

Table 3.2). Rats tickled for 5 days produced 157% more vocalizations than rats tickled for only 1 day, but there was no difference between vocalization production for rats in the ticking frequency conditions 3 and 5 or 1 and 3 days (**Figure 3.4; Table 3.3**). For blocking factors, female rats produced more 50-kHz USVs than males (42.7 vs 17.9 ± 5.0 50-kHz USVs/30 s; $F_{1,30}$ = 12.4, p = 0.001). We note that no cages of rats produced audible vocalizations and very few 22-kHz calls (0.17 ± 0.1 22-kHz USVs, 94%) were produced.

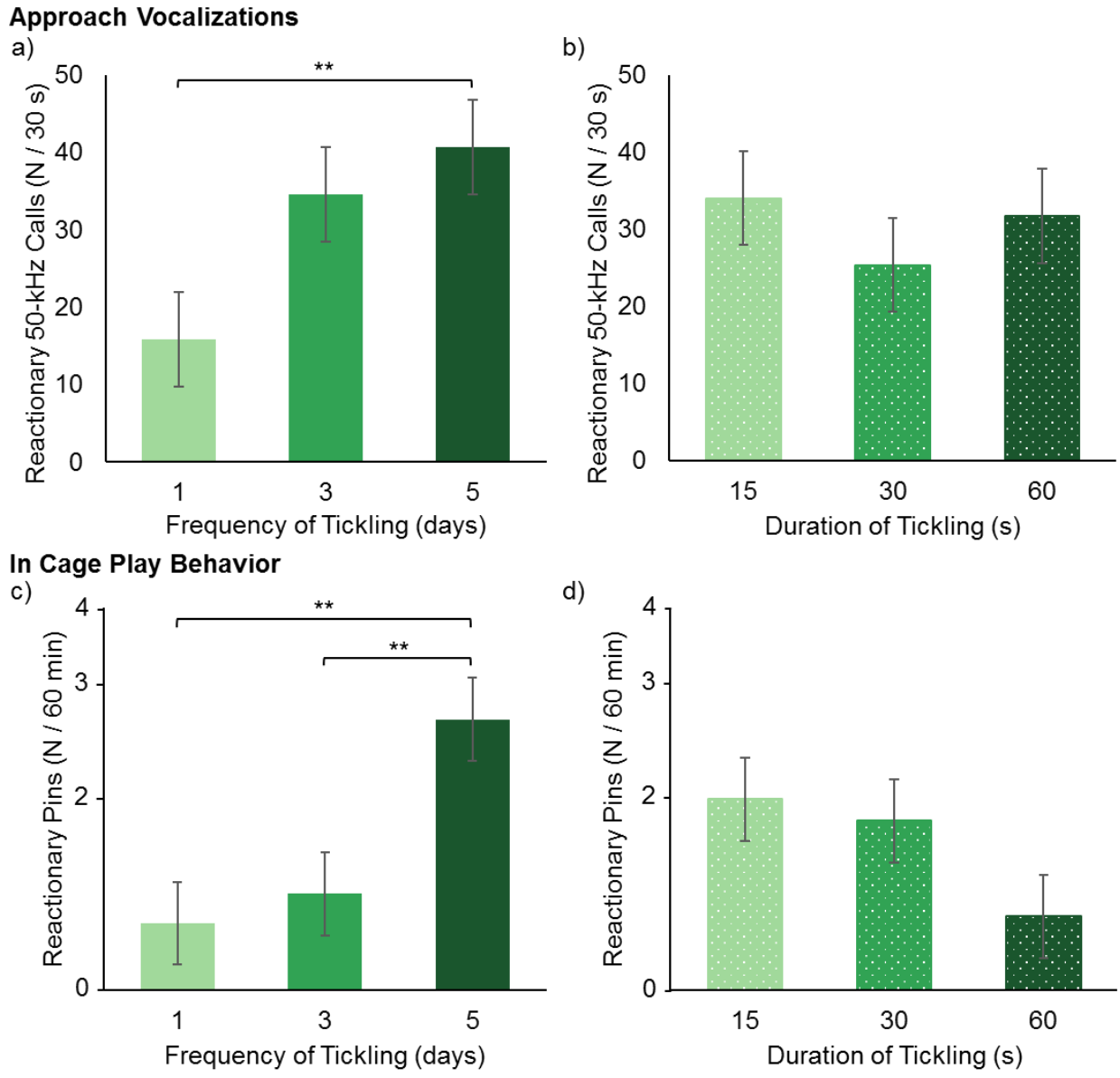

Figure 3.4.The Impact of Tickling Frequency and Duration on Reactionary Measures. The impact of tickling frequency (left column; 1, 3, or 5 days, n = 12 cages per frequency) and duration (right column, 15,

30, or 60 seconds, n = 12 cages per duration) on total 50-kHz vocalizations (LSM ± SE) during a 30 s approach test after an injection on test day (a, b) and in-cage rat play behavior 60 min after that. Total N = 36 cages with 4 cages per treatment combination. The scales of c & d are back-transformed from log10. **p<0.01 between groups via a custom contrast.

Reactionary Approach Behavior & Location

During a 30-s approach test after receiving an injection, male rats had a longer latency to contact (13.3 vs 9.6 ± 1.1; $F_{1,66} = 5.0$, $p = 0.03$) and fewer rears (2.7 vs 5.2 ± 0.5; $F_{1,66} = 12.9$, $p = 0.0006$) than female rats.

Reactionary Cage Behavior

During the reactionary period, tickling frequency significantly impacted rat in cage play behavior (**Figure 3.4;**

Table 3.2). Rats tickled for 5 days pinned more than rats tickled for 3 or 1 day (**Figure 3.4; Table 3.3**). For blocking factors, sex impacted reactionary cage behaviors ($F_{2,60} = 19.7$, $p < 0.0001$) and location ($F_{2,60} = 9.3$, $p = 0.0003$). Female rats spent more time active (65 vs 52 ± 3%; $F_{1,60} = 8.9$, $p = 0.0004$), less time inactive (14 vs 36 ± 3%; $F_{1,60} = 26.6$, $p < 0.0001$), and more time on the floor (56 vs 43 ± 4%; $F_{1,60} = 12.1$, $p = 0.0009$) than male rats.

3.4.4 Fecal Corticosterone

Rat fecal corticosterone metabolites were not significantly affected by tickling frequency or duration or any other blocking factors.

3.5 Discussion

3.5.1 General Discussion

This study is the first investigation to compare different dosages of heterospecific play, commonly known as rat tickling. Results show that a tickling frequency of 3 days and duration of 15 s (45 s total) was the most efficient and effective combination of our treatments at improving positive affect and handling. This conclusion is based on findings that first, across all measures, duration of tickling did not impact results. Second, rats tickled for at least 3 days showed (1) more 50-kHz USVs before and during tickling and (2) more play, less inactivity, and less time in their huts in anticipation of tickling. Only one outcome (play behavior after tickling) indicated that 5 days of tickling was more beneficial than 3 days of tickling. Although we did not see any significant effects in fecal corticosterone and relatively few positive effects in approach tests, we believe this study supports the efficacy of a shorter dosage of tickling than typically previously used (6).

3.5.2 Tickling Dosage

Vocalizations

In this study, results from our primary outcome of vocalizations indicate that tickling for 15 s for 3 days is most efficient and effective. As stated above, more 50-kHz vocalizations were produced before and during tickling after 3 days of tickling (versus 1 day, with no differences

between 3 and 5 day), while duration never impacted results. In the present study, we tickled rats during the course of 1 week for various practical dosages most commonly used in previous literature (6). In standard laboratory settings, tickling for 3 days for 15 s may be a reasonable length of time – especially since it could fit within a work week and the recommended habituation period after rat transportation.

If time allows, it may be beneficial to tickle rats for a longer duration or frequency. Rats tickled for a longer duration produce more *total* 50-kHz USVs (since they produce a similar rate of vocalizations for longer) which indicates a longer positive experience. And some previous work by (20) suggest that rat vocalization rate increases even further across 2 weeks. However, when time is limited, our results indicate tickling for only 15 s per rat per day for 3 days is beneficial.

Our results may suggest that rats experience a rapid increase in reward value of tickling through 3 days of tickling. Previously, assessment of animal behavior in the anticipatory period has been used to evaluate affective states and make inferences about animal welfare. It is suggested that anticipatory behavior is influenced by the perceived reward value of the forthcoming stimulus (21). In dolphins, greater anticipatory behavior to a human-animal interaction is correlated to greater participation during the interaction (22). For rats, higher production of 50-kHz USVs during the anticipatory would indicate a higher reward value of tickling (indicated by higher production of 50-kHz USVs during tickling). Of course, an alternative and complementary explanation could be that rats tickled for only one day simply had not yet learned to anticipate human contact leading to tickling. Regardless of its ultimate explanation, these findings supports a rat tickling dosage of at least 3 days.

Approach Behavior

During the approach tests, it was surprising that a reduction in approach latency was not found after 3 days of tickling. Previously, 7 experiments have found shorter approach latencies in tickled rats compared to non-tickled control rats (6). Therefore, we expected that a greater frequency of tickling would lead to a faster approach and more positive behaviors.

There are two likely explanations for these null results. First, any amount of tickling may be effective to increase approach, so that the difference between rats tickled for 1 day vs 3 days is undetectable. Previously, rats tickled only once for 15 s had a decreased approach latency compared to controls (15). A second explanation could be that, as rats were exposed to repeated

approach tests, they explored more (showing increased rearing), became more comfortable with the handler, and learned tickling would be delayed by 30 s. Approach tests have been criticized for their lack of specificity between fear and curiosity (23). An increase in familiarization with the human and, therefore, general exploration may have caused no difference in approach latency across days. In this study, rats were given an approach test before and after every tickling session (although only results from the final approach test were analyzed to allow for accurate interpretation). This experimental design choice was made to enhance predictability and take advantage of the standard 15 s rest period taken before tickling to collecting data. However, most previous experiments only conducted a single approach test (6) and one conducted a series of quick 15 s approach tests during tickling (15). Despite these null results, since our primary outcome and other outcomes did show significant differences, a rat tickling dosage of 15 s for 3 days is supported.

Cage Behavior

During the hour before and after tickling, rat home cage behavior generally supported our main conclusions. Before tickling + injection, rats tickled for at least 3 days played more (as measured by number of pins). Play behavior is considered a good indicator of positive welfare because it promotes positive affect, generally only occurs in absence of poor conditions, and spreads to other individuals (24). In this study, it was especially positive to see an increase in play because tickling mimics aspects of rat rough-and-tumble play. Since rats would be getting some outlet for play via tickling, conspecific play could have decreased, rather than increased. Instead, tickling rats may have actually promoted positive emotional contagion, prompting play behavior between individuals in the cage. If time allows, a longer frequency of tickling may be more beneficial for spreading play behavior since, after tickling + injection, rats tickled for at least 5 days played more than rats tickled for 1 or 3 days,

Rats tickled for at least 3 days also spent less time inactive and less time in their huts in the hour prior to tickling. Overall, rats housed in standard caging under standard protocols are sedentary which causes a variety of negative health consequences that could impact their utility as models for humans (25). Thus, increasing their activity should be beneficial for their welfare and utility as research models. Additionally, an increase in rat general activity has been linked to anticipation of rewarding stimuli such as sexual contact or access to an enriched cage (26,27). It

is possible that an increase in general activity after 3 days of tickling could indicate that the reward value of tickling increased across the days and/or that the rats had learned to pair the presence of the experimenter with tickling.

3.5.3 Sex Differences

In this study, we found that juvenile female Long-Evans rats produced more 50-kHz vocalizations, before and during tickling than male rats. These findings are contrary previous studies findings that during tickling juvenile males produce more 50-kHz vocalizations (28) or no difference between sexes (20,29). We speculate that our different results could be an interaction between rat sex and handler sex, since previously, tickling research in the laboratories of Panksepp, Mällo, and Wöhr has primarily been conducted by male handlers, whereas in this study the handler was a single female. This speculation is based on findings showing that rats, especially female rats, suppress pain responses in the presence of male handlers (30).

3.5.4 Replicate and Marking

Unexpectedly, rats had more positive responses to tickling in replicate 1 than replicate 2. This was unexpected since as procedures grow throughout time, more positive responses are expected in replicate 2. Instead, replicate 2 rats produced a lower rate of 50-kHz USVs during tickling, fewer 50-kHz USVs during injection, and fewer rears during an anticipatory approach test compared to rats from replicate 1.

We speculate these findings could have resulted from a significant change in marking procedure between replicates. Procedures changed from using permanent marker on the tails to a semi-permanent hair dye to facilitate individual identification (although individual identification in home cage video was ultimately unsuccessful). This alternative marking method has previously been successfully used for individual identification in Wistar rats (31) and in mice (32). It may be more aversive than sharpie marker odor which has previously been found to be negative for rats (33), though surgical markers were used in this study since they are less pungent. However, this procedure may be particularly aversive as it includes intense smells, restraint, and wetting the fur. Additionally, it may have been aversive for the dyed rats' cage mates, which was present in the

cage when the dyeing procedure was done. Thus, if hair dye is the only viable marking option, it may be beneficial to tickle rats before application.

3.5.5 Limitations

There are a few limitations to this project. First, we decided to not include a non-tickled group of rats. This decision was made because the purpose of the project was not to show the benefits of tickling (as they have already been established in previous publications, see LaFollette et al. 2017), but rather to compare and determine a practical dosage of tickling as an efficient habituation procedure. Therefore, our conclusions are only in relationship to a standard amount of tickling rather than an un-tickled, control group.

Second, we were unable to analyze our results by individual calling rates which have previously been shown to impact outcomes (6). This is because we tickled pair-housed rats within their home cage rather than separating them to decreases overall time commitment and increases practicality. This was done since our main goal was to demonstrate that rat tickling is a procedure that can be practically integrated into laboratory practice.

3.6 Conclusions

In conclusion, our results show that the most time-efficient and effective rat tickling dosage is 15 s per day for 3 days, a total time investment of 45 s per animal. This recommendation is based on the increased production of 50-kHz USVs (an indicator of positive affect), increased anticipatory activity in the cage, and increased play behavior. This knowledge can be used to apply tickling in a more widespread manner to improve rat husbandry and welfare. Overall, our results suggest that relatively minimal rat tickling applied in an efficient manner is an effective habituation technique for laboratory rats.

3.7 Funding

This work was supported by Grants for Laboratory Animal Science (GLAS) and the Animal Welfare Institute (AWI). Neither GLAS nor AWI had any involvement in study design; collection, analysis and interpretation of data; writing of the report; and the decision to submit the article for publication.

3.8 Acknowledgments

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CHAPTER 4. CHANGING HUMAN BEHAVIOR TO IMPROVE ANIMAL WELFARE: A LONGITUDINAL INVESTIGATION OF TRAINING LABORATORY ANIMAL PERSONNEL ABOUT HETEROSPECIFIC PLAY OR "RAT TICKLING"

4.1 Abstract (350 words max, currently 337)

Despite evidence for the animal welfare benefits of rat tickling, the technique is rarely implemented. One barrier to its implementation is a lack of targeted training, especially since in-person workshops are resource intensive. The objective of this study was to determine the efficacy of online-only or online + hands-on training programs in comparison to a waitlist control condition. Our hypothesis was that training would increase important outcomes such as implementation, knowledge, self-efficacy, familiarity, and beliefs in rat tickling and that hands-on training would have an additive effect.

Laboratory animal personnel currently working with rats in the USA were recruited via widespread online promotion. After completing a baseline survey, participants were semi-randomized to either an online-only training (n=30), online + hands-on training (n=34), or waitlist control group (n=32). At baseline, treatment groups were not significantly different in demographics or outcome measures (ANOVAs, p's>0.05). Both training groups received an interactive, visual training course in rat tickling. The hands-on training group also received a 30-minute training session specifically reviewing the hands-on components of rat tickling. Participants received a survey directly after their assigned training and a final survey 2-months later. In each survey, participants answered questions related to their beliefs, self-efficacy, knowledge, and implementation of rat tickling. Data were analyzed using general linear mixed models.

Compared to baseline, both training groups reported increased implementation, self-efficacy, knowledge, and familiarity of rat tickling at 2-months follow up (Tukey's, p's<0.05), while the waitlist group stayed the same. Compared to baseline, online + hands-on training participants also increased in their perceived control beliefs at 2-months (Tukey, p's<0.05). At the follow-up survey hands-on training participants also had higher self-efficacy and familiarity as compared to the waitlist (Tukey, p's<0.05).

Our findings show that both online-only and online + hands-on training improves laboratory animal personnel's implementation, knowledge, self-efficacy, and familiarity with rat tickling. Hands-on training also improved perceived control beliefs and had greater benefits for self-efficacy and familiarity. Overall, this study shows there is potential to improve animal welfare through the creation of targeted, interactive training courses.

4.2 Introduction

Although scientific research has provided a variety of well-supported strategies to improve animal welfare across species and settings, these findings are often not widely implemented. Examples of issues where a lack of implementation has been reported despite known strategies include pig aggression (1), lameness in dairy cows (2), and laboratory rodent handling (3). This lack of implementation should be a serious concern to researchers and funding agencies who aim to use science to ultimately improve animal welfare. If research findings are not translated to practice, then their applied benefits are unrealized.

One example of these circumstances can be found in laboratory rats. These animals can be negatively affected by handling, especially when naive to human interactions, which can result in fear, stress, anxiety, and even more difficult handling (4–6). During initial handling and, if intentional effort is not made, negative effects can be further increased by common laboratory procedures such as marking the animals for identification, restraint, injection, and blood draws (7). Beyond negatively impacting rat welfare, stress is also a potential confounding factor for scientific experiments (8). Therefore, the negative effects of stress during handling both reduces the possible benefits that can be gained from scientific research (i.e., by reducing study validity and reliability) and increase the costs of scientific research (i.e., by harming rat welfare).

A refinement to rat research and improvement to rat welfare can be made through the use of the positive handling technique heterospecific play, or "rat tickling". This technique mimics aspects of rat rough-and-tumble play (9). A systematic review of 53 experiments in rat tickling shows that this technique increases rat positive affect, habituation to handling, and positive approach behaviors, thus reducing stress associated with routine handling (10). Rat tickling can even reduce or eliminate negative responses to repeated intraperitoneal injections when administered just prior to the injection (11).

Despite the strong scientific evidence, our 2018 survey of almost 800 laboratory animal

personnel indicated that 89% of participants never or only rarely use rat tickling (3). Their use of rat tickling was strongly associated with their beliefs about and familiarity with the technique. For example, participants were more likely to provide rat tickling if they more strongly believed that rat tickling was beneficial, expected by their peers, and that they were confident in their ability to implement rat tickling. When participants were asked in a free response question to identify what made it difficult to provide rat tickling, the most common identified barrier was lack of time (stated by 60% of participants). However, recent research shows that only 15 seconds of tickling per day for 3 days is sufficient to elicit positive responses from the rats (12). Lack of proper training and buy-in by personnel was also commonly listed as a barrier to use.

Despite the demonstrated benefits of heterospecific play and associations between personnel beliefs and application, there is a lack of knowledge of a causal link between education and implementation. However, in the field of farm animal welfare, research shows that targeted in-person training can effectively improve stockperson beliefs and increase implementation of positive handling techniques (13). Furthermore, experts recommend that welfare findings should be communicated outside of the primary research community, especially with key initial stakeholders, and under close supervision to ensure success (14). Despite this, to our knowledge, similar study has not been conducted on training laboratory personnel in the complex research environment.

Moreover, it remains unclear what training modality is necessary to implement change. Laboratory personnel self-report that hands-on courses are influential to learning handling techniques (15). However, it may not be feasible to rapidly disseminate new information about welfare enhancing techniques through hands-on workshops alone, due to time and financial limitations. Furthermore, even if hands-on workshops are available, providing background material online before participants attend the training would save instructors significant amounts of time. Online education is advantageous since it can efficiently reach a larger number of participants with minimal costs. However, its efficacy in teaching hands-on procedures is unknown. Based on current stress of rats in response to handling, but lack of implementation of the effective technique of rat tickling, there is a critical need to assess the impact of rat tickling training materials, in both online-only and online/hands-on formats, on laboratory animal personnel attitudes, knowledge, and implementation.

This study's objective was to conduct a methodologically rigorous longitudinal trial to quantify the efficacy of training programs on laboratory animal personnel with the end goal of improving animal welfare. Our specific aims were to quantify the efficacy of different training programs of laboratory personnel beliefs, self-efficacy, knowledge, and implementation of rat tickling. Based on previous research using the theory of planned behavior and personal experience, we hypothesized that, relative to a waitlist control, laboratory personnel who undergo training programs would report improved attitudes, self-efficacy/knowledge, and implementation of rat tickling. We further hypothesized that there would be an additive benefit to hands-on training relative to online-only training. With this knowledge, we hoped to experimentally identify effective training programs to not only increase rat tickling prevalence, but other animal welfare enhancing techniques.

4.3 Materials and Methods

All procedures and informed consent protocols were approved by Purdue University's Human Research Protection Program Institutional Review Board, protocol #1712020004. All interactions between researchers, study participants, and rats during the study were approved by each individual university's Institutional Animal Care and use Committee (IACUC): Harvard University protocol #14-02-189-1, University of California San Francisco protocol # AN180239-01B, Indiana University Purdue University Indianapolis protocol #11426, and Purdue University protocol #1201000547.

4.3.1 Participants

Participants were recruited between August 21nd and September 4th, 2019 via widespread online promotions designed to maximize sample size (16). Online contacts were through seven modalities: direct emails to known laboratory personnel, emails to individuals at each host institution, list serves (e.g., CompMed, Laboratory Animal Research Enrichment Forum (LAREF), etc.), email lists (e.g., MSMR), Facebook groups/pages/personal accounts (e.g. Laboratory Animal Sciences), LinkedIn groups/personal pages (e.g., American Association for Laboratory Animal Science (AALAS), Animal Behavioral Biology). All modalities were contacted up to three times with the same study flyer following recommended survey procedures (17).

Participants were included if they were over the age of 18 and reported that they had worked with laboratory rats in the last 12 months or planned to work with laboratory rats between August to December 2019 in the United States. We informed participants that work was defined broadly and included both hands-on (e.g., changing cages, performing procedures) and hands-off work (e.g., supervision as a clinical veterinarian, principle investigator, or manager). To compensate participants for their time, they received one entry into a drawing for a choice between a $40 Amazon e-gift card or cash (chosen by 76% and 22%, respectively with one participant donating their prize back to the research team to use for future research) each time they completed a survey (50 prizes available).

4.3.2 Experimental Treatments

Figure 4.1 details treatment group assignment and a timeline of procedures. Three treatment groups were evaluated in this study. The **online-only training group** received an online training course about rat tickling. The **online + hands-on training group** received the same online training course as well as a small group, in-person, hands-on training session. The **waitlist control group** received no interventions during the study period but were told that they would receive the online training course after completing the final survey.

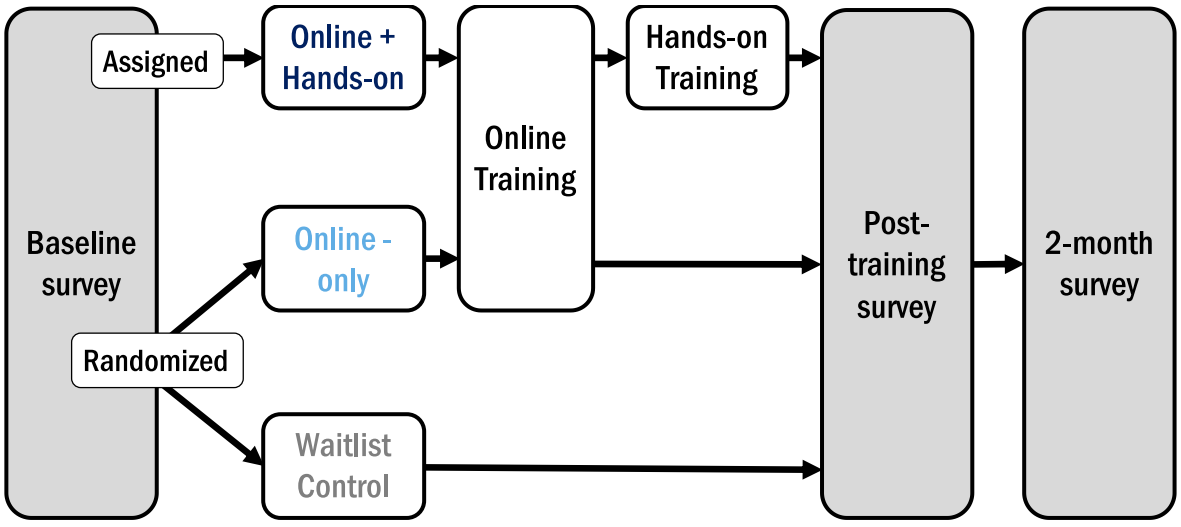

Figure 4.1. Timeline of procedures & assessment for laboratory animal personnel. The timeline of procedures and surveys for laboratory animal personnel in this study. All participants were given a baseline survey before group assignment. Then they were either assigned or randomized to groups and then received training. Immediately after training (or a similar time point for waitlist) participants received a post-training survey. Two-months later they received a final follow-up survey.

All treatment groups were evaluated at three time points. First, participants completed a baseline survey before being assigned to treatment group (**baseline)**. Second, participants completed a survey directly after completing their assigned training (i.e., the online training course for the online-only group or the hands-on training for the online + hands-on group; **post-training)**. The waitlist received a second survey at a similar time point. Third, participants completed a final survey approximately two months after the second survey (**2-months)**.

A partially randomized controlled trial design was used in this project in order to maximize sample sizes in each group. Although a completely randomization design was intended, not enough participants were recruited that were available on the available hands-on training session days. Therefore, after completing the baseline survey (in which they indicated their availability for such a hands-on session), participants were assigned to one of three treatment groups. If they were available to participate in the hands-on training, they were assigned to that group. Otherwise, they were randomized using a random number generator (random.org) to either the online-only or waitlist control treatment groups. Additionally, 4 individuals were originally assigned to the online + hands-on training group and took the online course in preparation, but then were unable to attend the workshop – these individuals were re-assigned to the online training group.

Participants in the online-only training group were given a link to the post-training survey upon course completion to ensure they had truly completed the course. Participants in the online + hands-on group were required to send a screenshot of their course completion certificate before they were allowed to participate in the hands-on training. They then received a paper hands-on post-training survey after completing the hands-on training.

Online Training Course

The online training course was carefully and intentionally developed from our work conducting 7 hands-on workshops in the USA and Canada across 3 years. Participants in these prior hands-on workshops included diverse perspectives from a variety of roles (students, animal caretakers, veterinarians, researchers, managers), research types (neuroscience, behavioral, training, basic), and institutions (industry, academic). These prior workshops included both informational lecture and practical hands-on sections. During these prior workshops, we identified and addressed common difficulties with the hands-on procedures, misconceptions, and frequently

asked questions. During three years of teaching we refined our methods and explanations of teaching individuals the tickling technique.

Therefore, an online training course was developed for this study based upon our prior work. This course was designed using Articulate Storyline software to create a seamless course complete with multiple interactive elements as well as extensive video and pictorial examples of rat tickling. It was designed to take an average of 30 minutes to complete. Course topics included the rationale behind rat tickling, detailed pictorial/video instruction on the hands-on technique (including videos of both what to do and what not to do), guidance on implementation, how to assess rat response to the technique (including videos of positive and negative rat reactions to rat tickling), and a series of frequently asked questions. Multiple videos and pictures from a variety of angles were used to communicate the hands-on technique as clearly as possible. In order to advance to the next section within the course, participants had to complete a quiz about that section's content. Slides were carefully designed to engage participants and communicate in a clear manner. The most updated version of this course can be found at: bit.ly/RatTicklingCertificate.

Hands-on Training Session

The hands-on training course was focused on teaching hands-on skills, rather than the theory behind rat tickling. Each session had a maximum of 5 participants and lasted approximately 45 minutes. Two instructors (MRL & BNG) led each session. Participants first observed the session leaders prior to attempting the technique. Participants were given immediate, individualized feedback on their hands-on technique throughout the workshop. Session leaders also noted key rat behaviors during the workshop indicative of either positive or negative responses to tickling. Refinement techniques were used to first train participants with stuffed rats, then pre-tickled rats, and then naïve rats (although to minimize rat numbers, not all participants were able to work with complete naïve rats). This order of interaction also allowed participants to work with rats of increasing difficulty. Finally, bat detectors were used during the hands-on sessions to allow participants to hear positive ultrasonic vocalizations of the rats they were tickling. Many participants noted that they felt that having a bat detector was extremely beneficial for them to get immediate feedback from the rats.

4.3.3 Measures

This survey was developed by reviewing literature and consulting with experts in survey methodology, behavior theory, and laboratory animal enrichment. When possible, validated instruments were used (i.e., theory of planned behavior survey). When validated instrumentation did not exist, previous work was modified or new items were created, reviewed by experts, piloted, and revised as necessary. Several measures were based off of work done in LaFollette et al. 2019 which was a cross-sectional survey of rat tickling in the laboratory. The survey question text and scales are available in **Appendix C Table C.1**. Unless otherwise noted, each questionnaire was given during every survey.

Demographics & work factors

Participants were asked about their demographics and current work in the baseline survey only. Demographics included age, gender, race/ethnicity, and highest level of education. Current work questions included role (e.g., animal care technician, veterinarian), if they supervised others, type of institution (e.g., academic, contract research organization), primary type of research (e.g., applied, basic, regulatory), highest level of certification, and years working with laboratory animals.

Baseline Factors

Baseline factors that could influence the uptake of the rat tickling procedure were measured only in the baseline survey. Participants were asked how many hours they work with rats in a typical work week, the degree of stress or pain most of their rats' experience, and how confident they were in their general rat handling skills. Participants were also asked how much control they have over the provision of enrichment to their rats, if they wished they could provide more enrichment than they do currently, and how, if at all, they had previously heard about rat tickling (e.g., journal article, popular press article).

Current & future implementation of rat tickling

Rat tickling current and planned use were assessed by a few key questions. Participants were asked how often they provided rat tickling to laboratory rats in the past 2 weeks (baseline

and post-training surveys) or 2 months (2-month survey). Answer options for this question included never (0% of instances working with rats), rarely, sometime (50% of instances working with rats), often, or always (100% of instances working with rats). Participants were also asked about their intentions (e.g., want, expect, and intend) to provide rat tickling in the next year using protocols from the Theory of Planned Behavior. Individuals who supervised others were also asked these questions about those that they supervised.

In the final survey, to determine if individuals could identify the correct scientifically support tickling technique, participants were asked to identify which picture most accurately represents the correct method for how laboratory rats should be tickled (**Figure 4.2)**.

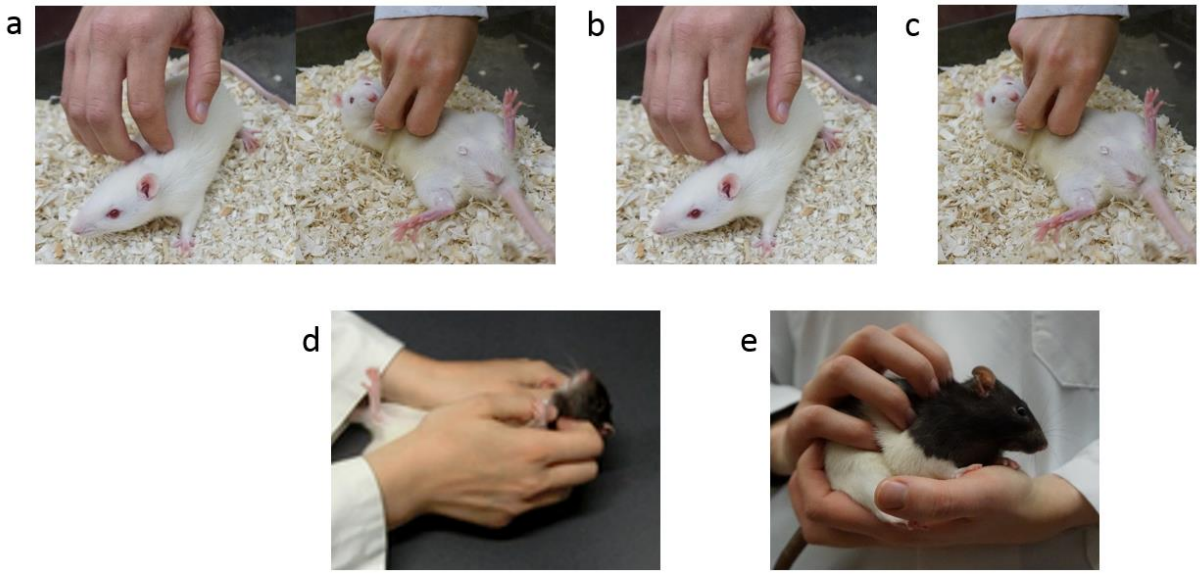

Figure 4.2. Pictorial Tickling Procedures. A: Dorsal contact and pin (standard, validated rat tickling procedure), B: Dorsal contact only or stroking in the cage, C: Pin only, D: Two-handed pin only, E: Stroking in the hand.

Knowledge, self-efficacy, & familiarity with rat tickling

Participants were asked several questions to determine their knowledge, self-efficacy, and familiarity with rat tickling. Self-efficacy to tickle rats was assessed via 5 questions modified from a general self-efficacy scale (19). This scale asked participants about their confidence about their ability to tickle rats in general, naïve rats, and complete the three components of rat tickling: dorsal contact ("nape"), flip, and pin ("on belly"). Specific self-efficacy questions were included as

during previous training sessions, the flip and pin are considered the most challenging – but arguably the most important – components of rat tickling. Knowledge about rat tickling was assessed via 7 questions about knowledge pertaining to the technique. Participants were also asked about when tickling should be implemented in relation to procedures and study timeline, duration, frequency, rationale, whether tickling or stroking is better, and whether adult rats should ever be tickled. Finally, familiarity was assessed via a single question asking participants about their rat tickling familiarity (both general knowledge and hands-on technique).

Beliefs about rat tickling

Beliefs about rat tickling were assessed using a brief Theory of Planned Behavior questionnaire based off our previous research (3). Surveys constructed using this theory typically have excellent reliability and validity (18). Participants answered 9 close-ended, quantitative questions about their behavioral attitudes (consequences of rat tickling), subjective norms (social and professional pressures to provide rat tickling), and perceived behavioral control (general confidence/control over the ability to apply rat tickling). The perceived behavioral control variable is characteristically different from self-efficacy as it asks participants about external control factors such as whether providing rat tickling is actually under the control of the participant (e.g., an animal caretaker may be confident in their ability to provide rat tickling, but not allowed to provide it to their rats because of managerial decisions).

General human-rat interactions

Participants also received a general behavior survey, both adapted from Hemsworth & Coleman (20). Participants were asked if they agreed or disagreed that they often observe, pet, talk to, or name their laboratory animals.

Qualitative questions

At several points throughout the survey, participants were asked to answer open-ended, qualitative questions. These questions allowed participants to reply with their most salient answers without additional prompting. During each survey, after asking about implementation, participants were asked if they had any further comments about their previous experiences with rats or rat

tickling. Then, participants answered questions about rat tickling benefits (i.e., what are the advantages to rat tickling) and barriers (i.e., what makes it difficult for you to tickle rats). At the end of each survey participants were asked if they had any final comments.

4.3.4 Data analysis

Participant inclusion & variable coding

A total of 182 laboratory animal personnel began the baseline/screening survey and answered all 3 screening questions. Of those, 16% (n = 29) were excluded for not being located in the United States or not currently working with laboratory rats. Of those remaining, only individuals that actually completed the baseline survey (80%, n = 122) were invited to participate in the second survey, although only 80% (n = 97) actually completed the second survey. Those that completed the second survey were then invited to complete the third survey, of which 90% (n = 87) actually completed the third survey. Of those individuals who completed the first two surveys, to ensure that all descriptive data reporting and summary scores indicate the same responses, only participants that answered at least 50% of questions for each scale in the survey were included for analysis (ultimately this only excluded 1 additional participant from the analysis). Also, to assist with analysis, categorical data options that contained less than 4 responses per treatment group were collapsed into larger categories when possible.

Quantitative analysis

Data analysis was conducted in JMP Pro 14.0.0 using descriptive statistics, chi-squared tests, and general linear mixed models. Prior to testing, all assumptions of the general linear model were visually confirmed including independence of residuals, homogeneity of variance, and normality of residuals. For all summary scales, an average of individual items was calculated (excluding participants with >50% missing data in each measure). Significant main effects and two-way interactions were analyzed using Tukey tests. A chi-squared test was used to analyze the dependent variable of correct identification of rat tickling technique compared to treatment group.

The dependent variables for quantitative analysis via general linear mixed models were rat tickling implementation (i.e., implementation, intent), knowledge, self-efficacy, familiarity and, beliefs (i.e., behavioral attitudes, subjective norms, and perceived behavioral control). The

independent variables of interest were treatment (i.e., online-only training, online + hands-on training, waitlist control), time point (i.e., baseline, post-training, 2-months), and an interaction of treatment and time point. To control for potential confounding effects, we also included baseline variables of rat stress/pain level, enrichment factors (control and desire), and confidence in rat handling skills. These potential confounding effects were removed from the model if $p > 0.10$. To avoid pseudoreplication and accommodate repeated measures, analyses were blocked by the random experimental unit of participant with treatment nested within. Significance level was $p < 0.05$. Results are presented as mean ± standard deviation unless otherwise noted.

Overall, an exemplary initial analysis used was:

Dependent variable = treatment + time point + treatment*time point + rat stress/pain + enrichment control + enrichment desire + rat handling experience + participant(treatment).

Qualitative analysis

We used thematic content analysis to analyze responses to open ended questions. We were interested in determining participant identified benefits and barriers to tickling as well as specific comments about the online or hands-on trainings. An iterative process was used to code the entire qualitative data set. Within the dataset, each clause was treated as the unit of analysis and each clause given a code. Each clause was coded with as many codes as it contained. For example, the clause "time and buy in from management that it is a beneficial practice" would receive codes Time and Buy-In. Buy-in was defined as a belief that rat tickling is effective and worth the time and effort it requires. The coding manual was developed from our previous research coding 794 responses to of laboratory animal personnel to similar questions (3). New codes were added as needed to accurately describe this new dataset. The coding manual refined via an interactive process in which responses were read multiple times.

4.4 Results

4.4.1 Demographics

A total of 96 participants completed at least the first two data collection points in the study and therefore were included in the study (30 = online-only, 34 = hands-on, 32 = waitlist). Additionally, 86 participants completed all 3 timepoints (28 = online-only, 28 = hands-on, 30 =

waitlist). Detailed demographic information is displayed for all participants in Table 1. Overall participants were primarily white (79%) females (82%) with a bachelor's degree or higher (79%). On average, they were 37 years old, worked with rats 7 hours a week, and had been working in the laboratory animal field for 11 years. Participants worked mostly in universities (75%), but in a variety of roles (e.g., 16% managers, 22% laboratory technicians, 16% veterinarians) and research types (e.g., 48% applied). The majority had some sort of laboratory animal certification (86%). Less than half of participants (42%) currently supervised others working with rats. The only demographic variable that differed by treatment group was highest certification level (**Table 4.1**)

Table 4.1. Demographic and work characteristics of laboratory animal personnel across treatment groups (N = 97). As treatment groups were semi-randomized, they were tested at baseline for the possibility of significant differences in demographic distribution via chi-squared tests (X^2) for categorical data or analysis of variance (F-tests). M = mean, S.D. = standard deviation.

	Group			
Categorical Data N (% of group)	**Online + Hands-on**	**Online-only**	**Waitlist**	**Group difference**
Gender				
Female	28 (83%)	24 (80%)	27 (84%)	
Male	6 (17%)	6 (20%)	5 (16%)	$X^2 = 0.2$, $p = 0.9$
Race				
White	24 (71%)	6 (20%)	28 (88%)	
Person of color	10 (29%)	6 (20%)	4 (13%)	$X^2 = 2.9$, $p = 0.2$
Highest Education				
< Bachelor's degree	7 (23%)	5 (17%)	7 (22%)	
Bachelor's degree	15 (43%)	13 (43%)	13 (41%)	
Graduate or Veterinary degree	12 (34%)	12 (40%)	12 (38%)	$X^2 = 0.4$, $p = 0.9$
Role				
Laboratory technician	9 (26%)	6 (20%)	6 (19%)	
Veterinary technician	3 (9%)	5 (17%)	5 (16%)	
Manager	4 (11%)	7 (23%)	5 (16%)	
Veterinarian	7 (20%)	5 (17%)	4 (13%)	
Other	11 (32%)	7 (23%)	12 (38%)	$X^2 = 4.4$, $p = 0.8$
Institution				
University	26 (77%)	23 (77%)	23 (77%)	
Contract research organization	5 (14%)	3 (10%)	3 (9%)	
Other	3 (8%)	4 (13%)	6 (19%)	$X^2 = 1.7$, $p = 0.8$
Research				
Applied	15 (43%)	17 (57%)	15 (47%)	
Basic	9 (26%)	5 (17%)	6 (19%)	
Education	4 (12%)	4 (13%)	3 (9%)	
Other	6 (18%)	4 (13%)	8 (25%)	$X^2 = 2.7$, $p = 0.8$
Certifications				
None	7 (20%)	4 (13%)	3 (9.4%)	
Lab animal certification	15 (44%)	9 (30%)	15 (47%)	
Registered veterinary technician	16 (49%)	16 (53%)	22 (69%)	
Graduate or veterinary degree	6 (18%)	7 (23%)	7 (22%)	
Board-certified veterinarian	5 (15%)	3 (10%)	0 (0%)	$X^2 = 16.8$, $p = 0.03$
Continuous Data M ± S.D.	Online + Hands-on	Online-only	Waitlist	Difference?
Age	38 ± 10	37 ± 9	38 ± 10	$F_{2,93} = 0.1$, $p = 0.9$
Years working	12 ± 8	12 ± 9	12 ± 8	$F_{2,93} = 0.2$, $p = 0.8$
Hours per week working with rats	9 ± 12	5 ± 8	7 ± 9	$F_{2,93} = 1.1$, $p = 0.3$

4.4.2 Baseline Characteristics

Most participants self-reported that most of their rats generally experience minor stress or pain of a short duration based on the USDA pain scale (55%). Although 93% of participants indicated they had at least a little control or influence over their rats' enrichment, only 20% indicated that they had complete control. The majority of participants (76%) wished they could provide their rats more enrichment than they currently do. The majority of participants (90%) were also confident in their general rat handling skills. At baseline, over half of participants (62%) indicated that they were at least moderately familiar with rat tickling. In fact, over half of participants (62%) had seen an educational talk about rat tickling previously. Participants had also heard of rat tickling through technical articles (49%), peer-reviewed journal articles (32%), and YouTube videos (33%).

At baseline, there were almost no differences in outcome measures or covariates between treatment groups (p's > 0.05 for implementation, intent, familiarity, self-efficacy, knowledge, attitudes, norms, control beliefs, enrichment control, enrichment desire, rat handling experience; **Table 4.2**). The only difference was the self-reported rat stress/pain based on USDA pain categories. Higher rat stress/pain was reported by the hands-on group compared to the waitlist.

Table 4.2 Baseline characteristics of laboratory animal personnel across treatment groups (N = 97). As treatment groups were semi-randomized, they were tested at baseline for the possibility of significant differences in baseline characteristics via analysis of variance (F-tests). M = mean, S.D. = standard deviation.

	Group			
Continuous Data (M ± S.D.)	Online + Hands-on	Online-only	Waitlist	Difference?
Rat Stress/Pain	2.4 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	$F_{2,88} = 5.9$, $p = 0.004$
Enrichment Control	3.2 ± 0.2	3.2 ± 0.2	3.1 ± 0.2	$F_{2,93} = 0.2$, $p = 0.8$
Enrichment Desire	5.4 ± 0.2	5.7 ± 0.3	4.9 ± 0.3	$F_{2,93} = 2.5$, $p = 0.09$
Rat Handling Experience	5.7 ± 0.2	5.9 ± 0.2	6.0 ± 0.2	$F_{2,93} = 0.8$, $p = 0.4$

4.4.3 Impacts of Treatment, Time, & Other Factors

Implementation, Intention, & Technique

Self-reported implementation of rat tickling was significantly impacted by the interaction of treatment and time (**Figure 4.3, Table 4.3**). Compared to baseline, the online + hands-on training groups had higher implementation immediately and 2-months post-training (Tukey, p's < 0.05),

while the online training group only had higher implementation at 2-months post-training (Tukey, p's < 0.05). Within each time point, no group was significantly different from the others (Tukey, p's > 0.05). Waitlist participants experienced no change in knowledge over the study period (Tukey, p's < 0.05). Implementation was also positively associated with control over enrichment implementation (**Table 4.3**).

Intent to provide rat tickling in the next year was significantly impacted by time (**Figure 4.3, Table 4.3**). Compared to baseline, all treatment groups had higher intentions to tickle rats post-training and 2-months later (main effect of time, Tukey, p's < 0.05). Intent to provide rat tickling in the next year was also positively associated with control over enrichment implementation and desire to provide more enrichment in general (**Table 4.3**).

At the third time point, all participants were asked to identify the picture which showed the most correct rat tickling technique. In this case, the picture of dorsal contact + pin would be correct and all other answers would be incorrect. At this time point, significantly more participants in the trained groups correctly identified the scientifically supported technique (96%), as compared to waitlist participants (73% correct; X^2= 0.008; **Figure 4.3)**.

Implementation by individuals that participants currently supervised was significantly impacted by time ($F_{2,71}$ = 30.6, p < 0.0001) by not treatment or their interaction ($F_{2,80}$ = 1.6, p = 0.2; $F_{4,71}$ = 1.5, p = 0.2). Supervisee implementation was higher at the final 2-month follow-up survey compared to baseline or directly post-training (Tukey, p's < 0.05).

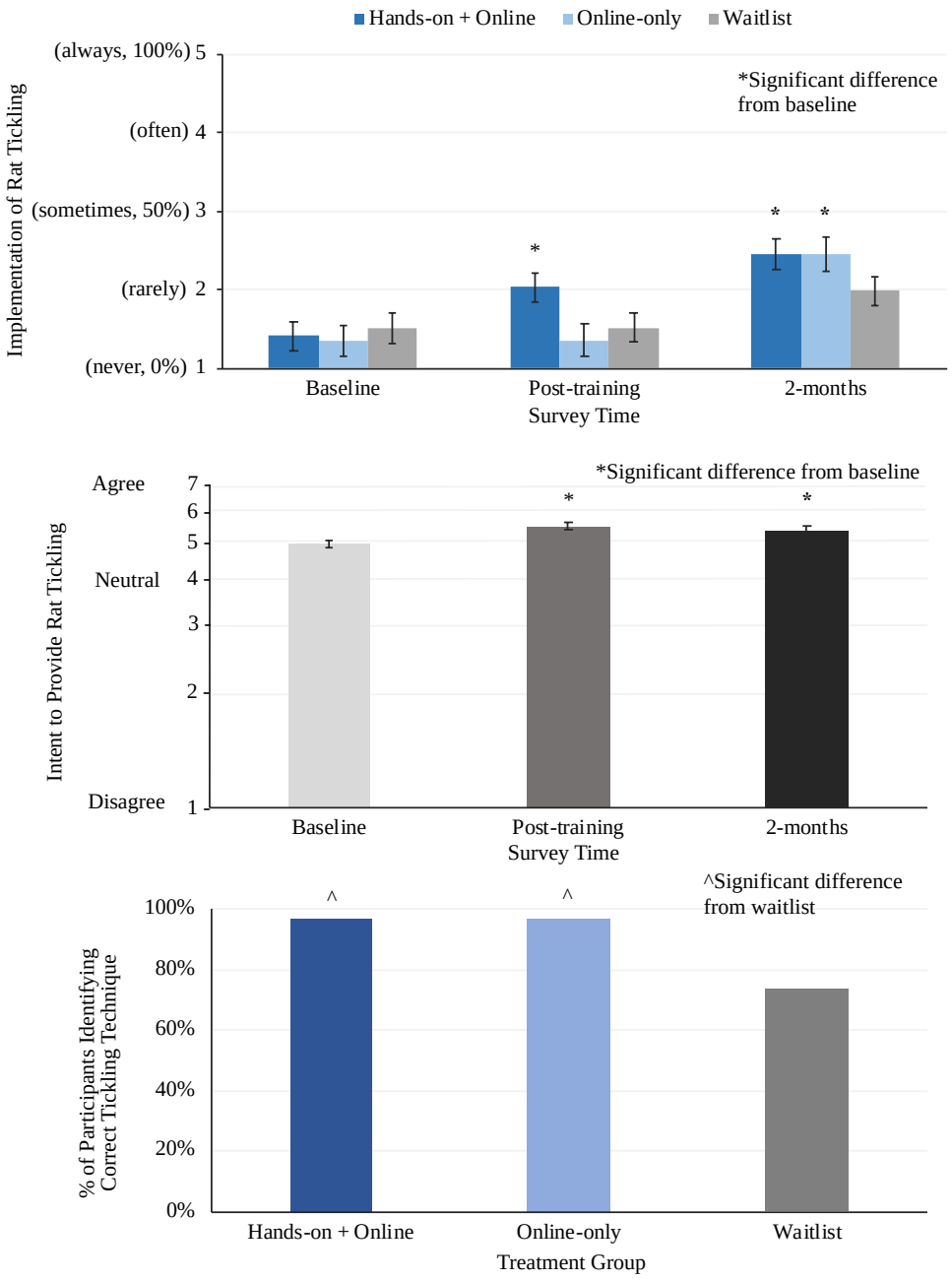

Figure 4.3. Implementation & intent to provide rat tickling / Correct identification of technique. *Top/middle:* This figure shows the highest order significant associations from general linear mixed models that impacted participants current implementation & intent to provide rat tickling. Implementation and intent were measured via a self-report survey. Models were run controlling for potential confounding variables. Both scales display only the range of possible responses. The scale of intent is back transformed from log10. Top: *Indicates a significant difference from baseline within treatment group. Middle: *Indicates a significant different from baseline. *Bottom:* The bottom graph shows the percentage of participants that selected a dorsal contact + pin picture when asked to identify which picture indicates correct rat tickling technique. ^Indicates a significant different from waitlist.

Table 4.3. Implementation & intent to provide rat tickling. The associations from general linear mixed models of self-reported data from laboratory animal personnel. Participants were asked about their current implementation and intent to provide rat tickling to their laboratory rats. Blank cells indicate that the covariate was not included in the final model. Bold indicates a significant effect with $p < 0.05$. (+) indicates a positive association.

	Implementation	Intent
Timepoint	**$F_{2,154} = 31.0$, $p < 0.0001$**	**$F_{2,178} = 13.8$, $p < 0.0001$**
Treatment	$F_{2,163} = 0.16$, $p = 0.9$	$F_{2,165} = 1.3$, $p = 0.26$
Timepoint*Treatment	**$F_{4,154} = 3.5$, $p = 0.009$**	$F_{4,178} = 1.2$, $p = 0.3$
Control over Enrichment	$F_{1,90} = 5.5$, $p = 0.02$	**(+) $F_{1,92} = 6.4$, $p = 0.01$**
Enrichment Desire		**(+) $F_{1,92} = 6.9$, $p = 0.01$**

Knowledge, self-efficacy, & familiarity

Factual knowledge of rat tickling was significantly associated by an interaction of treatment and time (**Figure 4.4, Table 4.4**). Compared to baseline or the waitlist, both training groups had higher knowledge of rat tickling directly post-training and the 2-months follow-up (Tukey, p's < 0.05). Online-only training participants experienced a significant decrease in knowledge from directly post-training to the 2-month follow-up (although this remained higher than at baseline or waitlist participants; Tukey, p's < 0.05). Conversely, waitlist participants experienced no change in knowledge over the study period (Tukey, p's < 0.05).

Rat tickling self-efficacy was significantly affected by the interaction of treatment and time (**Figure 4.4, Table 4.4**). Compared to baseline, participants in both training groups increased in self-efficacy at post-training and the 2-month follow-up (Tukey, p's < 0.05). Conversely, waitlist participants, experienced no change in rat tickling self-efficacy over the study period (Tukey, p's < 0.05). The only time point with significant differences between groups was directly post-training. At this time point, the online + hands-on training group had higher self-efficacy than the waitlist (Tukey, $p < 0.05$). Additionally, rat tickling self-efficacy was positively associated with baseline rat handling experience (**Table 4.4**).

Familiarity with rat tickling was significantly associated by an interaction of treatment and time (**Figure 4.4, Table 4.4**). Compared to baseline and the waitlist, both training groups reported an increase in their familiarity with rat tickling at the 2-month follow-up (Tukey, p's < 0.05). Also compared to baseline, hands-on + online training participants reported an increase in familiarity at directly post-training (Tukey, p's < 0.05). At baseline, groups were not significantly different in familiarity from each other. However, compared to the waitlist, only the online + hands-on group

had higher familiarity immediately post-training and at 2-month follow-up (Tukey, p's < 0.05). Waitlist participants experienced no change in familiarity over the study period (Tukey, p's < 0.05). Additionally, rat tickling familiarity was positively associated with baseline rat handling experience (**Table 4.4**).

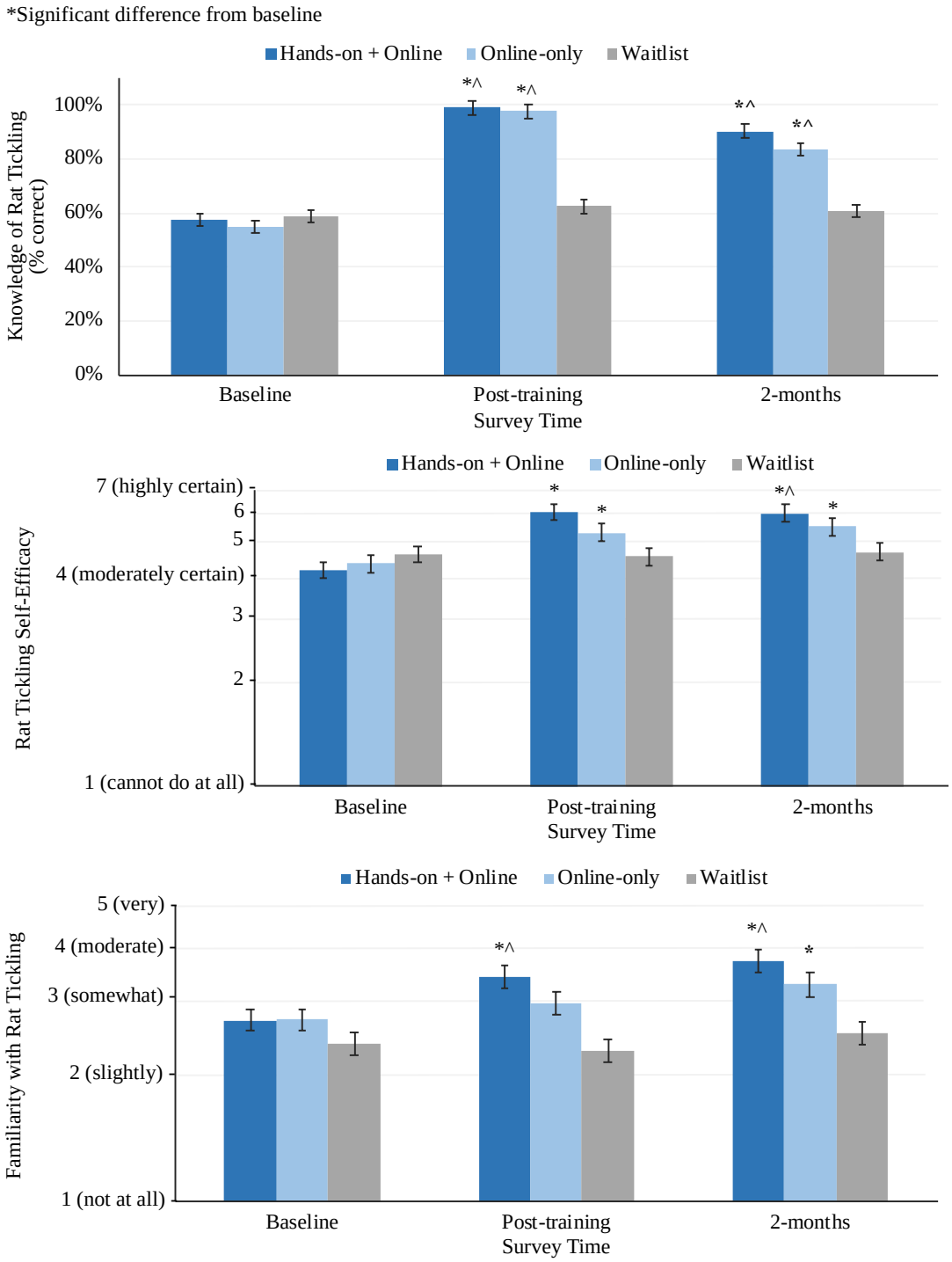

Figure 4.4. Knowledge, self-efficacy, and familiarity with rat tickling. This figure shows the highest order significant associations from general linear mixed models of knowledge, self-efficacy, and familiarity with rat tickling. Variables were measured via a self-report survey. Models were run controlling for demographic, work, and potential confounding variables. The scales of self-efficacy and familiarity are back transformed from log10. Scales display only the range of possible responses. *Indicates a significant difference from baseline within treatment group. ^Indicates a significant difference from the waitlist within time point.

Table 4.4. Knowledge, self-efficacy, and familiarity with rat tickling. The associations from general linear mixed models of self-reported data from laboratory animal personnel. Participants were asked about their factual knowledge, self-efficacy, and familiarity with rat tickling. Blank cells indicate that the covariate was not included in the final model as $p > 0.05$. Bold indicates a significant effect with $p < 0.05$. (+) indicates a positive association.

	Knowledge	Self-efficacy	Familiarity
Timepoint	**$F_{2,179} = 161.7, p < 0.0001$**	**$F_{2,177} = 24.5, p < 0.0001$**	**$F_{2,178} = 18.8, p < 0.0001$**
Treatment	$F_{2,234} = 0.7, p = 0.5$	$F_{2,187} = 0.8, p = 0.5$	$F_{2,186} = 0.6, p = 0.5$
Timepoint* Treatment	**$F_{4,179} = 32.0, p < 0.0001$**	**$F_{4,177} = 7.4, p < 0.0001$**	**$F_{4,178} = 4.5, p = 0.002$**
Rat Handling Experience		**$F_{1,90} = 16.0, p = 0.0001$**	**(+) $F_{1,91} = 9.8, p = 0.002$**

Beliefs

Attitudes towards rat tickling were significantly associated with time point (**Figure 4.5,**

Table 4.5). Compared to baseline, all treatment groups had more positive attitudes directly post-training and at the 2-month follow-up (Tukey, p's < 0.05). Additionally, more positive attitudes were seen in participants who more strongly agreed that they had a desire to provide more enrichment to their rats (

Table 4.5).

Subjective norms towards rat tickling were significantly associated with time point (**Figure 4.5,**

Table 4.5). Compared to baseline, all treatment groups had more positive attitudes directly post-training and at the 2-month follow-up (Tukey, p's < 0.05).

Perceived behavioral control to provide rat tickling was significantly associated with an interaction of treatment and time (**Figure 4.5,**

Table 4.5). Compared to baseline, hands-on + online training participants increased in perceived behavioral control post-training and the 2-month follow-up (Tukey, p's < 0.05). Conversely, both online-only training and waitlist experienced no change in perceived behavioral control over the study period (Tukey, p's < 0.05). Within each time point, no group was significantly different from the others (Tukey, p's > 0.05). Additionally, perceived behavioral control was positively associated with desire to provide more enrichment, control over enrichment, and baseline rat handling experience (

Table 4.5).

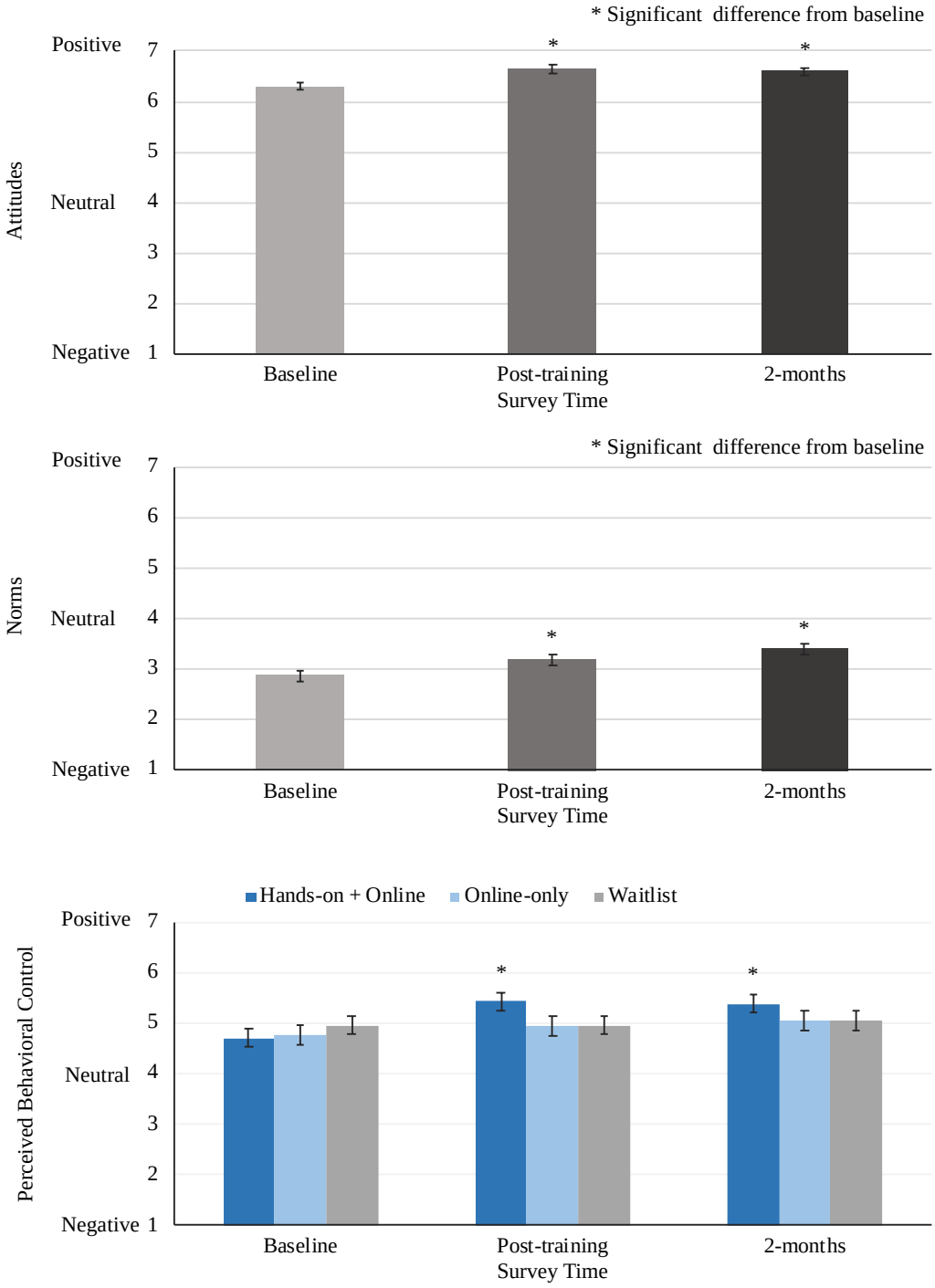

Figure 4.5. Beliefs about rat tickling. This figure shows the highest order significant associations from general linear mixed models of attitudes, subjective norms, and perceived behavioral control with rat tickling. Variables were measured via a self-report survey. Models were run controlling for potential confounding variables. Scales display only the range of possible responses. Top/Middle: *Indicates a significant difference from baseline. Bottom: *Indicates a significant difference from baseline within treatment group.

Table 4.5. Beliefs about rat tickling. The associations from general linear mixed models of self-reported data from laboratory animal personnel. Participants were asked about their attitudes, subjective norms, and perceived behavioral control with rat tickling. Blank cells indicate that the covariate was not included in the final model. Bold indicates a significant effect with $p < 0.05$. (+) indicates a positive association.

	Attitudes	Subjective Norms	Perceive Behavioral Control
Timepoint	**$F_{2,178} = 13.5$, $p < 0.0001$**	**$F_{2,179} = 14.0$, $p < 0.0001$**	**$F_{2,178} = 8.3$, $p = 0.0003$**
Treatment	$F_{2,176} = 1.2$, $p = 0.3$	$F_{2,177} = 1.0$, $p = 0.2$	$F_{2,144} = 0.5$, $p = 0.6$
Timepoint* Treatment	$F_{4,178} = 2.4$, $p = 0.05$	$F_{4,179} = 2.1$, $p = 0.07$	**$F_{4,178} = 3.5$, $p = 0.009$**
Enrichment Desire	**(+) $F_{1,92} = 15.3$, $p = 0.0002$**		**(+) $F_{1,91} = 4.5$, $p = 0.04$**
Control over Enrichment			**(+) $F_{1,91} = 7.5$, $p = 0.008$**
Rat Handling Experience			**(+) $F_{1,90} = 5.1$, $p = 0.03$**

General human-rat interactions

General human-rat interactions were not significantly associated with treatment, timepoint, or their interaction (p's > 0.05). However, human-rat interactions were positively associated with control over enrichment and a desire to provide more enrichment ($F_{1,92} = 6.8$, $p = 0.01$; $F_{1,92} = 5.9$, $p = 0.02$).

4.4.4 Qualitative Data

Participant responses to open-ended questions about rat tickling were summarized into two central categories of *Benefits* and *Barriers* to rat tickling. These central categories were further split into themes and sub-themes, described below and summarized for trained individuals in **Figure 4.6** and by group in **Appendix C Tables C.2.**

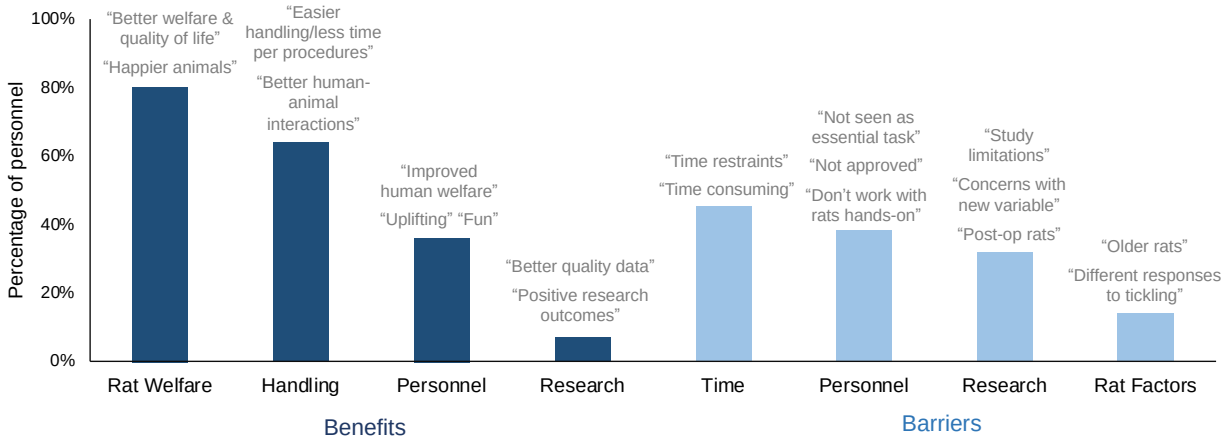

Figure 4.6. Trained personnel - Benefits and Barriers to Rat Tickling. The most common themes related to benefits (advantages) and barriers (factors making it difficult) to tickle rat by 86 laboratory animal personnel in a 2-month follow-up survey. Graphic includes representative quotes. Sub themes and additional representative quotes are presented in **Table C.2**.

The majority of participants, regardless of training group, indicated that rat tickling was beneficial primarily for *Rat Welfare* (79% of all participants) and *Handling* (63%). Benefits for *Personnel* were also commonly listed (38%). Less commonly, participants also indicated benefits of rat tickling for *Research* (9%) such as better recovery time, more reliable physiologic reactions, and better quality data. Within the category of *Rat Welfare* benefits, participants often specifically noted its benefits for reducing stress or anxiety (29%), providing enrichment (22%), and socialization (12%). Within the category of *Handling* some participants also mentioned the benefit of rat tickling to help develop a bond (7%). Several subcategories under *Personnel* were mentioned. Most commonly participants indicated benefits to personnel well-being describing rat tickling as being fun, uplifting, and even reducing human stress (15%). Other potential benefits for personnel included increasing empathy for the animals and increasing monitoring. One participant indicated "…we've also noticed a measurable positive difference in the research personnel's demeaner in the lab, their approaches to rat research, and deeper understanding of rat welfare needs."

Participants identified several barriers that still remained preventing rat tickling including *Time* (44% of participants), *Personnel* (43%), *Research* (26%), and *Rat Problems* (14%). Within the category of *Time*, some participants mentioned difficulties related to the quantity of rats, staffing limitation, time needed to train personnel in the technique, and consistency. Within the category of *Personnel*, access was commonly an issue (16%) as some participants simply were not directly involved with hands-on rat work and therefore personally may not be able to implement

rat tickling. Additionally, participants mentioned a need to train individuals to do it properly (15%). Many individuals had concerns or difficulties getting approval or buy-in for rat tickling. Only one participant each mentioned that they were not responsible for this issue or a fear of rats by students may be an issue. Within the category of *Research*, most commonly there was either concern with adding a new variable or research-related rat factors (e.g., head implants) that may prevent tickling rats for a certain period of time. Finally, in terms of *Rat Problems*, participants mentioned only having older rats or concern with individual differences or aggression of rats. Barriers that were infrequently cited (2%) include having *Too Few Rats* at an institution, *Small Caging*, or simply *No Barriers.*

4.5 Discussion

To our knowledge, this is the first study to experimentally evaluate the efficacy of training laboratory animal personnel to improve important outcomes related to enrichment implementation. We compared training laboratory animal personnel about rat tickling via an interactive, highly visual, online-only training module versus the same module supplemented with hands-on training, as compared to a waitlist control. We successfully sampled 97 participants at baseline and after training, with 86 of those completing a final survey two months later.

Results indicated that training laboratory animal personnel with either online-only or online + hands-on modules was beneficial to important outcomes related to rat tickling implementation. At the end of the study as compared to baseline, trained personnel reported higher frequency of implementation and significantly more could correctly identify the scientifically supported method for rat tickling. Furthermore, at the end of the study compared to baseline, trained personnel had higher knowledge, self-efficacy, and familiarity with rat tickling. These results are in agreement with previous farm animal welfare research and recommendations from experts that targeted training can improve beliefs and implementation of positive handling techniques (1,2). Furthermore, in previous research, more familiarity with rat tickling was shown to be strongly associated with implementation of rat tickling (3). In this study, that training groups were more able to identify correct technique and had higher factual knowledge should help ensure that rat tickling is applied in a scientifically supported manner and reduce perpetuation of misconceptions about rat tickling. Furthermore, correct knowledge and implementation should help improve rat

welfare and create a positive feedback loop when personnel see these positive effects. Overall, training personnel in rat tickling has multiple positive benefits.

There were a few advantages to online + hands-on training over online-only training. First, only the online + hands-on training group increased in perceived behavioral control compared to baseline. That is, personnel who went through both the online and hands-on training modules felt that tickling was easier to implement, more up to them, and overall felt more confident that they could provide rat tickling (as compared to baseline). Second, at the end of the study, only the online + hands-on training group compared to the waitlist had significantly higher self-efficacy (i.e., confidence in tickling naïve/experience rats and doing all components of rat tickling) and familiarity with rat tickling. These results may be seen as hands-on participants received immediate positive verbal feedback on their rat tickling technique during the workshop and had an opportunity to tickle pre-trained rats. Therefore, hands-on participants can feel confident that their technique is correct and may also be more able to accurately assess themselves compared to the online-only training group. Our previous research in a sample of over 700 laboratory animal personnel indicates that perceived behavioral control shows an extremely strong correlation with current implementation and intent to tickle rats (3). Therefore, when feasible, we recommend also providing hands-on training in addition to the online training module because it improved perceived behavioral control and had greater benefits to self-efficacy and familiarity. However, if time and cost are prohibitive factors then the online training module is still a very good option for training laboratory animal personnel.

Although we did improve implementation in all groups and correct implementation in trained groups, at the end of the study trained personnel were implementing rat tickling less than 50% of the instances they work with rats. Although this could seem concerning, it may still be enough to be effective, considering that rat tickling can be effective even after only 3 days of tickling for 15 seconds (12). Future research on rat tickling implementation may ask personnel to consider what percentage of rats they work with have been tickled for at least 3 days to capture the spread of rat tickling within the laboratory. However, it is also important to consider that rat tickling is not appropriate for all rats or models, particularly adult rats that have never been tickled before.

Qualitative data also supported both training and perhaps changes in the overall perspective of rat tickling since our survey in 2018. In terms of benefits, in this study approximately 20% more

participants indicated benefits of rat tickling for rat welfare. Additionally, a new them of responses – benefits for personnel – was seen indicating that participants believe in the benefits of rat tickling for the personnel implementing this enrichment. In terms of barriers, approximately 15% less participants indicated time as a barrier. However, about 20% more participants indicated that personnel were a barrier and in trained participants about 15% more participants indicated that research was a barrier. These results indicate that even if new tickling protocols may require less time than previously, that they still do require additional time that must be supported by other personnel and carefully considered how to implement in current research paradigms.

Participants made several comments at the end of the study that support the use of training modules to increase rat tickling implementation. For example, several participants mentioned that they were sharing the training module widely with colleagues and implementing it in their internal animal handling courses. They also reported success in using rat tickling with a variety of different research paradigms such as pharmacokinetic, diabetic, and tumor lesion rat models. They also indicated seeing observable differences in both the rats and the staff. They noted that staff morale improved, and they even believed that the staff's approach to rat research and rat welfare was improved. These qualitative comments further indicate the positive reactions participants had about receiving training in rat tickling.

At the end of this study, all participants regardless of treatment, reported more positive attitudes, a higher intent to provide, and higher subjective norms of rat tickling. This may be a result of the Hawthorne effect, in which participants alter their behavior simply due to their awareness of being observed. In this study, simply by being asked about rat tickling, waitlist participants may have sought out additional information about rat tickling during the study period that may have changed their opinions or encouraged them to start attempting implementation. For example, our team has published an online downloadable handout, a video protocol, and a general information video that are all freely available online (4–6). Furthermore, as participants were asked about their intent to tickle rats over the next year, we may not have seen significant differences since most waitlist participants appeared eager to take the training module once the study period was complete. Regardless, the positive results seen in all treatment groups seem to be a positive indicator for establishing rat tickling as a more common intervention in the laboratory.

One limitation of this study is that it only involved self-report data and therefore there is the potential for subjective biases or misconceptions to occur. For example, at the end of the study 25%

of waitlist participants were unable to identify the scientifically supported rat tickling technique out of a series of pictures. Furthermore, in open-ended comments a few participants indicated that their version of tickling involved only dorsal contact, sometimes mentioning that the flip or pin was difficult for them so they just did not do it. Therefore, there may be many misconceptions about what rat tickling really is. Although we told participants that rat tickling was defined as an interaction between a human and rat, which mimics aspects of rat social play, we refrained from showing them pictures of the technique to keep any "training" to a minimum for waitlist participants. Also, as participants were trained, their definition of tickling may have become more conservative or accurate over time therefore suppressing results. Therefore, our results may be even stronger than indicated because many waitlist participants may not truly understand what rat tickling is and therefore over-report their current implementation and beliefs towards the practice. Furthermore, if participants stroke rats instead of tickle them, the rats will not receive the same beneficial outcomes from tickling (5,7). Despite this limitation, our study still provides support for training being beneficial to importance outcomes related to rat tickling.

4.6 Conclusions

In conclusion, targeted training of laboratory animal personnel in rat tickling appears to be effective in improving implementation, knowledge, self-efficacy, and familiarity with rat tickling. Hands-on training also improved perceived behavioral control and was more beneficial for self-efficacy and familiarity. These results indicate that providing hands-on training is best, but that online-only training is still quite beneficial. However, it is also important to note that our online training module included multiple interactive elements, extensive video and pictorial examples, and was designed from extensive hands-on training workshops to extensive details and counteract common misconceptions and difficulties implementing the technique. A less interactive or detailed online training module may not have the same effects. Overall though, this study demonstrates that curated online training modules are beneficial and can optionally be supplemented with hands-on training. These trainings are an effective means of promoting implementation of animal welfare-enhancing techniques.

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CHAPTER 5. CONCLUSION

Humans are a key factor in the lives of laboratory animals and as such they can have huge impacts on animal welfare. Unfortunately, without deliberate effort, human-animal interactions in the laboratory can be negative. After all, most laboratory animals are prey species, which are not provided with the ability to flee during close interactions with humans, a potential predator. Fortunately, increasing efforts are being made to improve human-animal interactions and promote animal welfare.

One key area where human-animal interactions can be improved is in laboratory rat handling. When rats initially interact with humans, they often experience fear and stress (1,2) – which are further compounded by common laboratory procedures that involve restraint or injections. This fear and stress can reduce rat welfare and even make handling more difficult for personnel. Furthermore, stress in rat handling even has the potential to harm experimental validity and reliability. These effects increase the costs and decrease the benefits of laboratory rat research.

Fortunately, the positive handling technique of heterospecific play or "rat tickling" can help. This procedure mimics aspects of rat rough-and-tumble play with several benefits. Our systematic review on rat tickling in 2017 found over 50 published experiments with several consistent benefits of rat tickling (3). For example, in comparison to a control condition, rat tickling was found to increase 50-kHz ultrasonic vocalizations (indicative of positive emotional states), increase approach behavior, decrease generalized anxiety and fear, and even improve handling (3).

Unfortunately, simply developing and researching this species-specific, relevant, and beneficial technique is not enough to improve rat welfare. Laboratory animal personnel working with rats must actually implement rat tickling in their laboratories. Anecdotally, implementation appeared to be low. Therefore in 2018, we conducted a widespread survey of laboratory personnel across the United States and Canada (4). Results showed that, in fact, rat tickling implementation was quite low with 55% of personnel never using the technique. In response to open ended questions, personnel frequently cited barriers to rat tickling to include a lack of time, lack of buy-in, lack of training, and difficult research factors. Furthermore, using quantitative methods, results showed that personnel with more familiarity and more positive beliefs about rat tickling, especially confidence/control in the ability to implement it, were strongly associated with higher levels of implementation.

To address the barrier of time, we conducted a project to determine the most efficient and effective dosage for rat tickling (5). We compared 3 different durations (15, 30, and 60 seconds) and 3 different frequencies (1, 3, or 5 days) on outcomes before, during, and after rat tickling – including rat response to an injection. Results showed that duration did not affect outcomes, but that frequency did. Overall, 3 or 5 days of tickling led to increased 50-kHz ultrasonic vocalizations and in-cage play behavior (indicative of positive emotions) as compared to just 1 day of tickling. Therefore, the most efficient and effective dosage of rat tickling is just 15 seconds per day for 3 days, which is a 1000% time reduction from traditional protocols of 2 minutes per rat per day for 5 days.

Finally, we attempted to address the barrier of a lack of training as well as directly trying to increase the implementation of rat tickling in laboratories across the United States. In this project, we created a highly visual, interactive online training course in rat tickling that was based off of experiences from our extensive in-person workshops. The efficacy personnel being training with only the online training course was then compared to an online + hands-on training group versus a waitlist control across a 2.5-month period. Results showed that, compared to baseline, participants in both training groups increased in their correct implementation, knowledge, self-efficacy, and familiarity with rat tickling while the waitlist experienced no change in these outcomes. Furthermore, compared to baseline, only the online + hands-on training participants increased in their perceived behavioral control of rat tickling – which was the strongest association in our initial survey. Therefore, it appears that targeted training in rat tickling was effective in increasing important outcomes related to rat tickling implementation.

Of course, there is still more research and efforts needed to increase rat tickling in the laboratory. Some researchers are still concerned about the effects of rat tickling on various models and therefore both targeted training and research for these individuals could be beneficial. For example, targeted research in rat tickling could establish null or even positive effects on research models such as reduced experimental variation as is seen in tunnel-handling mice (6). There is even the potential to use rat tickling to explain experimental variation by measuring the ultrasonic vocalizations that occur during rat tickling as a measure of individual differences (3). Additionally, many laboratories receive rats past the juvenile age – which is their prime receptive period for tickling – we recommend conducting research on the effects of tickling rats at the vendor. These

initial interactions have the potential to reduce shipping stress and improve future interactions with humans.

In conclusion, these series of projects have attempted to evaluate the status quo of rat tickling implementation, identify barriers, and then address these barriers – all with the end goal of increasing rat tickling and improving rat welfare. At this time, our laboratory recommends tickling rats using scientifically supported technique (dorsal contact and pin) for 15 seconds per rat for at least 3 days before starting any procedures, then again during initial procedures, and then once a week at cage change after that. During each tickling session, individual rat response should be monitored closely as individual rats may react differently to this interaction. Rat tickling should be reported in publications to help increase awareness and implementation. Overall, through these projects and the creation of an open-access course in rat tickling, we hope we have begun to achieve our aim in increasing implementation of the positive handling technique of rat tickling to improve rat welfare.

5.1 References

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APPENDIX A. CHAPTER 2 SUPPLEMENTAL FILES

Table A.1

Scale	Question	Responses & [Coded Value]
Demographics	What is your age?	Any value
	What is your gender?	Male; Female; Transgender Man; Transgender Female; Nonbinary; Other; Prefer Not to Answer
	How would you describe yourself? (Choose one or more from the following groups)	Native American; Asian; Black; Pacific Islander; White; Other
	What is the highest degree or level of school you have completed? (If you're currently enrolled in school, please indicate the highest degree you have received.)	Less than a High School Diploma; High School Degree or equivalent; Some College, No Degree; Associate Or Technical Degree Bachelor's Degree; Graduate or Professional Degree
Current Work	Where do you currently work with laboratory animals?	US; Canada; Other
	What is your current role in working with laboratory animals?	Undergraduate; Animal Care or Laboratory Technician; Veterinary Technician; Graduate Student; Animal Facility or Laboratory Manager; Post-doctoral Researcher; Principle Investigator; Laboratory Animal Veterinarian; Other Animal Care Staff; Other Research Staff; Other
	What type of institution do you currently work with?	University, College or Medical School; Contract Research Organization; Non-profit Organization; Other
	What is the primary, broad type of research that you conduct?	Basic; Applied; Regulatory; Product; Education; Other

	How many years have you worked with laboratory animals? Slide the light gray bar to the appropriate number of years. Note: Work is defined broadly, this may include hands-on work such as changing cages or running procedures or hands-off work such as running a lab or research studies.	Any value
	Approximately how many hours per week do you work with laboratory animals? Slide the light gray bar to the appropriate number of hours. Note: Work is defined broadly, this may include hands-on work such as changing cages or running procedures or hands-off work such as running a lab or research studies.	Any value
	During an average work week, for each type of laboratory animal, please indicate the approximate percentage of time you spend working with laboratory rats. Percentage should include all possible work hours. Note: Work is defined broadly, this may include hands-on work such as changing cages or running procedures or hands-off work such as running a lab or research studies	Any value
Enrichment	How much influence or control do you have over the type or amount of enrichment that is used with the laboratory animals you work with?	None [1] A little [2] Some [3] A lot [4] Complete [5]
	I wish I could provide more enrichment to my animals than I currently do.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]
Rat Tickling Use	In the past year, how often were the following enrichments provided to the laboratory rats you work with? - Rat tickling	Never [1] Sometimes [2} About half the time [3] Most of the time [4] Always [5] Unknown [missing]
Rat Tickling Familiarity	How familiar are you with rat tickling?	Unfamiliar [1] A little familiar [2] Somewhat familiar [3] Very familiar [4]
Rat Tickling Method	Which picture most accurately represents how the laboratory rats in your care are tickled?	DorsalPin; Dorsal; Pin; DoubleHands; Stroking; NotPictured; Unsure

Theory of Planned Behavior (TBP) Qualitative	What factors or circumstances, if any, make it difficult or impossible for you to provide rat tickling to laboratory rats?	Free response
	What do you believe are the advantages, if any, to providing rat tickling to laboratory rats?	Free response
	What factors or circumstances, if any, would make it easier or enable you to provide rat tickling to laboratory rats?	Free response
TBP - Behavioral Beliefs	**If I provide rat tickling to laboratory rats:**	
	I will feel like I am doing something positive for the rats	Extremely Unlikely [1] Unlikley [2] Somewhat Unlikley [3] Neither Likely nor Unlikely [4] Somewhat Likely [5] Likely [6] Extremely Likely [7]
	I will enjoy my job more	
	My mood will improve	
	Rat health will be improved	
	Rat welfare will be improved	
	Rats will be easier to handle	
	Scientific results will be more valid	
TBP - Control Belief Strength	Providing rat tickling to laboratory rats will take a lot of time.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]
	A lot of training is required to provide rat tickling to laboratory rats.	
	Providing rat tickling to laboratory rats requires a lot of money.	
	Providing rat tickling to laboratory rats requires official approval from others.	
TBP - Intention	I intend to provide rat tickling for my laboratory rats in the next year.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]
	I expect to provide rat tickling for my laboratory rats in the next year.	
	I want to provide rat ticking for my laboratory rats in the next year.	
TBP - Outcome Evaluation	**Complete each statement based on whether it is desirable or undesirable:**	
	Doing something positive for the rats is...	Extremely Undesirable [-3] Undesirable [-2] Somewhat Undesirable [-1] Neither Desirable nor Undesirable [0] Somewhat Desirable [1] Desirable [2] Extremely Desirable [3]
	Improvement in my mood is...	
	Enjoying my job is...	
	Improving rat health is...	
	Improving rat welfare is...	
	Improving ease of rat handling is...	
	Improving scientific results is...	
	Other laboratory animal colleagues provide rat tickling to their laboratory rats.	Strongly Disagree [-3] Disagree [-2]

TBP - Normative Beliefs	Accreditation staff encourage people like me to provide rat tickling to laboratory rats.	Somewhat Disagree [-1] Neither Agree nor Disagree [0] Somewhat Agree [1] Agree [2] Strongly Agree [3]
	Laboratory veterinarians think I should provide rat tickling to laboratory rats.	
TBP - Attitudes	**Overall, I think that providing rat tickling for my laboratory rats is:**	
	Bad -- Good	Bad [1] to Good [7]
	The wrong thing to do -- The right thing to do	The Wrong Thing [1] to The Right Thing [7]
	Worthless -- Useful	Worthless [1] to Useful [7]
	Harmful -- Beneficial	Harmful [1] to Beneficial [7]
TBP - Motivation to Comply	**In general, how important to you are doing the following things?**	
	Doing what other laboratory animal colleagues do is important to me.	Not at All [1] to Very much [7]
	Doing what accreditation staff encourage me to do is important to me.	
	Doing what laboratory veterinarians think I should do is important to me.	
TBP - Control Belief Power	I am _____ to provide rat tickling if I have more time.	Less Likely [-3] to More Likely [3]
	I am _____ to provide rat tickling if I have enough money.	
	I am _____ to provide rat tickling if I receive sufficient training.	
	I am _____ to provide rat tickling if I get official approval from others.	
TBP - Subjective Norms	Most people who are important to me think I should provide rat tickling to laboratory rats.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]
	I feel under professional pressure to provide rat tickling to laboratory rats.	
	It is expected of me that I provide rat tickling to laboratory rats.	
TBP - Perceived Behavioral Control	I am confident that I could provide rat tickling for my rats.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]
	The decision to provide rat tickling to laboratory rats is beyond my control.	
	Whether or not I provide rat tickling to laboratory rats is completely up to me.	
	Overall, for me to provide rat tickling for laboratory rats is:	Extremely Difficult [1] to Extremely Easy [7]

Rattitude	**Please indicate how strongly you agree or disagree with the following statements.**	
	Rats are often nervous.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]
	Rats are smelly.	
	Rats are ugly.	
	Rats do not feel pain.	
	Rats are aggressive.	
	Rats are curious.	
	Rats are calm.	
	Rats are entertaining.	
	Rats are friendly.	
	Rats are intelligent.	
General Behaviors	**Please indicate how strongly you agree or disagree with the following statements.**	
	I often observe the laboratory animals I work with.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]

Table A.2

	M	SD	Range	% Agree
Enrichment control (N=794)	**3.19**	**1.06**	**1 to 5**	**-**
Desire for more enrichment (N=793)	**5.38**	**1.438**	**1 to 7**	**-**
Positive Rattitude (N = 703)	**6.14**	**0.64**	**2.8 to 7**	-
Curious	6.60	0.60	3 to 7	99.1
Intelligent	6.55	0.70	3 to 7	97.9
Entertaining	6.28	0.87	1 to 7	96.0
Friendly	6.08	0.93	1 to 7	93.7
Calm	5.20	1.11	1 to 7	76.4
Negative Rattitude (N = 703)	**2.37**	**0.79**	**1 to 5**	-
Nervous	3.89	1.51	1 to 7	45.2
Smelly	3.10	1.59	1 to 7	26.5
Aggressive	2.13	1.21	1 to 7	6.0
Don't feel pain	1.20	0.78	1 to 7	1.6
Ugly	1.50	0.88	1 to 7	0.9
General Behaviors (N = 694)	**5.41**	**1.08**	**1.75 to 7**	-
Observe	6.27	0.96	1 to 7	95.8
Talk to	6.02	1.34	1 to 7	89.8
Pet	5.63	1.49	1 to 7	82.7
Name	3.74	2.06	1 to 7	41.0
Familiarity with Tickling (N = 794)	**2.45**	**1.04**	**1 to 4**	**-**
Current Use of Tickling (N = 706)	**1.67**	**0.93**	**1 to 5**	**-**

Table A.3

Advantages	N (%)	Representative quotes
Ease of Handling	**371 (61%)**	**"easier to handle;"**
		"habituate to handling interaction"
Benefit Both	182 (30%)	"positive experience for both rat and handler"
Bonding	*74 (12%)*	*"connection, a bond"*
		"[the rats] would likely develop a positive relationship with the technician."
Benefit Handler	49 (8%)	"improves technician well-being," "stress relief for the handler," or "animals become more friendly to humans"
Benefit Rat	27 (4%)	"I believe… it makes them [the rats] calmer when being held"
General Rat Welfare	**338 (55%)**	**"better welfare and quality of life"**
		"makes the rats happy and calm"
Less Stress	156 (26%)	"a positive interaction to help relieve the stress of negative interaction."
Enrichment	128 (21%)	"rat enrichment"
		"mimics natural behaviors."
Socialization	*51 (8%)*	*"if they are singly housed it provides them with social interaction"*
Research	**28 (5%)**	**"provide better data,"**
		"it lowers their stress levels which could possibly improve experimental result"
No Benefits	**34 (6%)**	**"none"**
Unaware of Advantages	26 (4%)	"not familiar with the benefits of rat tickling"

Table A.4

	Barriers	**Promotors**	**Representative Quotes**
Time	**363 (59%)**	**347 (57%)**	**"time," "lack of time," /** **"more time within the day"**
Staffing	54 (9%)	95 (16%)	"limited staff" / "additional staff"
Number of rats	54 (9%)	16 (3%)	"the number of rats housed in our facility" / "less rats"
Consistency	10 (2%)	18 (3%)	"time to perf orm tickling on a regular basis"
Money	4 (1%)	9 (1%)	"funds for staffing for this cause" "The only 'disadvantage'… is the impact to labor… costs"
Personnel	**134 (22%)**	**204 (33%)**	
Buy-in	74 (12%)	105 (17%)	"perceived value [of rat tickling] by others"
Not my problem	*35 (6%)*	*11 (2%)*	*"a better PI initiated enrichment"*
Official approval	*3 (0%)*	*25 (4%)*	*"study/PI staff approval", "adoption by IACUCs [institutional animal care and use committees]"*
Education	61 (10%)	110 (18%)	
Training	*44 (7%)*	*96 (16%)*	*"proper training is needed but unavailable"*
Awareness	*17 (3%)*	*16 (3%)*	*"not all of the staff is aware of rat tickling"*
Fear of rats	4 (1%)	2 (0%)	"personnel scared of rats"
Research	**135 (22%)**	**35 (6%)**	
New variable	45 (7%)	2 (0%)	"researchers do not want [rat tickling] as a variable" "[rat tickling] would alter experiment outcome." / "understanding if [rat tickling] will affect a researcher's model outcomes"
Rat factors	43 (7%)	1 (0%)	"rats have surgical implants that do not make this method of handling feasible."
Research protocol	11 (2%)	21 (3%)	"having it mandated in an SOP [Standard Operating Procedure] would help."
Short study	11 (2%)	1 (0%)	"acute studies"
Rats	**54 (9%)**	**6 (1%)**	**"some rats don't approach us" "many rats are aversive to any human touch."**
Age	13 (2%)	5 (1%)	"older rats ordered in and are unused to handling so are defensive"
Aggression	13 (2%)	1 (0%)	"some of my rats are more aggressive and do not tolerate hands in their cage,"
Individual differences	5 (1%)	0 (0%)	"some rats like it, but not all do"
Single-housed	3 (0%)	0 (0%)	"single housed rats"

Breeding status	2 (0%)	1 (0%)	"breeders may not be as willing unless exposed to tickling before they are bred which would take more time."
Safety	**30 (5%)**	**8 (1%)**	
Biosecurity	16 (3%)	6 (1%)	"maintaining bioprotection can be a challenge as you have to keep the rat in the cage."
Harm to Personnel	13 (2%)	1 (0%)	"rats may bite" "most have zoonotic diseases (e.g., hsv, adenovirus)."
Facility Factors	**4 (1%)**	**15 (2%)**	**"more space in the rooms"**
Cage size	1 (0%)	4 (1%)	"larger caging or enrichment areas for laboratory personnel and animal care technicians to play with the rats"
No Barriers	**29 (5%)**	**0 (0%)**	**"none"**
Nothing Easier	**0 (0%)**	**31 (5%)**	**"none"**

Table A.5

Direct Measurement	Score	*a*
Intention: I ____ to provide rat tickling in the next year	**4.82 ± 1.3**	
Want	5.28 ± 1.4	0.86
Intend	4.66 ± 1.4	
Expect	4.52 ± 1.5	
Behavioral Attitudes: I think providing rat tickling is ___ to __	**6.11 ± 1.1**	
Bad to Good	6.32 ± 1.1	0.92
Harmful to Beneficial	6.11 ± 1.3	
Worthless to Useful	6.02 ± 1.3	
The wrong to the right thing to do	5.97 ± 1.4	
Subjective Norms	**3.02 ± 1.2**	
People who are important to me think I should provide rat tickling	3.94 ± 1.4	0.76
I feel under professional pressure to provide rat tickling	2.6 ± 1.4	
It is expected of me to provide rat tickling	2.51 ± 1.4	
Perceived Behavioral Control	**4.38 ± 1.3**	
I am confident that I could provide rat tickling	5.24 ± 1.5	0.66
Overall, it is *(easy to difficult)* for me to provide rat tickling	4.39 ± 1.7	
Providing rat tickling is completely up to me	3.54 ± 1.9	
The decision to provide rat tickling is beyond my control.^*	-	

Table A.6

Indirect Measurement	Score	β to Direct
Behavioral Attitudes = behavioral beliefs x outcome evaluations	**16.18 ± 4.7**	**.501*** **
Easier handling	17.52 ± 4.99	
Improved rat welfare	17.48 ± 4.68	
Something positive for the rats	17.37 ± 5.2	
Improved rat health	16.39 ± 5.11	
Increased job enjoyment	15.52 ± 6.03	
Improved mood	14.46 ± 6.58	
Improved scientific results	14.39 ± 5.76	
Subjective Norms = normative beliefs x motivation to comply	**-1.31 ± 6.8**	**0.451*** **
Accreditation staff	-2.55 ± 8.74	
Laboratory veterinarians	-1.04 ± 9.01	
Other laboratory animal colleagues	-0.3 ± 6.83	
Perceived Behavioral Control = control beliefs power x control belief strength	**-7.59 ± 4.8**	**.118** **
Time	-10.44 ± 7.06	
Official Approval	-10.33 ± 7.71	
Training	-6.94 ± 5.96	
Money	-2.62 ± 5.16	

Table A.7

	Scale	M (N=656)	SD (N=656)	Current Rat Tickling β (N=591)	Future Rat Tickling β (N=656)
Theory of Planned Behavior					
Attitudes	1 to 7	6.11	1.13	0.061	**0.320*** **
Subjective Norms	1 to 7	3.02	1.16	**0.118*** **	**0.177*** **
Control Beliefs	1 to 7	4.22	1.29	**0.203*** **	**0.433*** **
Tickling Familiarity					
Familiarity with Tickling	1 to 4	2.49	1.03	**0.300*** **	**0.163*** **
Animal Attitudes/Behaviors					
General Attitudes	1 to 7	5.41	1.08	**0.108***	0.063
Positive Rattitude	1 to 7	6.15	0.62	-0.108	0.064
Negative Rattitude	1 to 7	2.36	0.78	0.065	-0.043
Enrichment					
Desire for More Enrichment	1 to 7	5.35	1.45	-0.023	**0.089** **
Control of Enrichment	1 to 5	3.19	1.05	0.012	0
Demographic & Work Factors					
Age	Any	39.8	11.28	-0.004	-0.005
Years Working	Any	34.19	12.09	0.009	-0.002
Hours of Work per Week	Any	13.57	9.84	0.003	0
% Time Work with Rats	0 to 100	26.2	23.3	-0.005	-0.014

APPENDIX B. CHAPTER 3 SUPPLEMENTAL FILES

Table B.1. Bias Assessment. Detailed reported of steps to mitigate bias using the Systematic Review Center for Laboratory Experiments Bias Assessment Tool.

Item	Type of bias	Domain	Description of domain
1	Selection bias	Sequence generation	A random number generator (random.org) was used to generate the sequence of assigning rats to cages, treatments to cages, marking to rats, and which rat would be tickled first in each tickling session.
2	Selection bias	Baseline characteristics	All rats were the same age at the start of the experiment and sex was equally distributed among treatment groups. All rats were weighed on day 2. Vocalization rate to tickling was assessed systematically during the experiment. Both weight and calling rate were included as covariates in all statistical analysis to adjust for any unequal distribution of this highly relevant baseline characteristic.
3	Selection bias	Allocation concealment	The investigator could not foresee assignment to the intervention or control group as the cages were assigned after the animals were assigned and this sheet was contained on a separate page.
4	Performance bias	Random housing	During the randomization process, we used a balanced process to house the animals randomly throughout the experiment. Each rack and row contained each treatment and the specific location of each treatment was rotated between replicates.
5	Performance bias	Blinding	ID cards of each cage were identical in appearance and a code was used to indicate treatment. Additionally, room caregivers who performed welfare checks and refilled food and water were not involved in the experiment. Finally, the individual collecting fecal pellets was not informed of the code and was also blind to treatment allocation.
6	Detection bias	Random outcome assessment	In-cage behavior and fecal corticosterone were assessed at the same time. Rats were tickled in a random order each day. All animals were selected for outcome assessment.
7	Detection bias	Blinding	• **Vocalizations** – Coders were blind to frequency treatment. However, coders could not be blind to duration treatment since the files were either 1.5 min, 2 min, or 3 min long. • **Approach Tests –** Approach test coders were blind to treatment • **Injection Test** – Coders were blind to treatment • **Cage Behavior –** Coders were blind to treatment • **Fecal Corticosterone** – Fecal samples were processed by an independent laboratory blind to the project and treatments. Tubes were only marked with the date and cage number.
8	Attrition bias	Incomplete outcome data	All data was included for each outcome except for injection vocalizations. For injection vocalizations, the microphone did not record for the very first injection, so data from that first injection is missing and therefore that data set is unbalanced.

APPENDIX C. CHAPTER 4 SUPPLEMENTAL FILES

Table C.1. Survey Questionnaire.

Scale	Question	Responses & [Coded Value]
Demographics	What is your age?	Any value
	What is your gender?	Male; Female; Transgender Man; Transgender Female; Nonbinary; Other; Prefer Not to Answer
	Please select one category for your Race / Ethnicity	White (Not Hispanic or Latino); Black or African American (Not Hispanic or Latino); Hispanic or Latino; Asian (Not Hispanic or Latino) Native American, Metis, Inuit, or Alaska Native (Not Hispanic or Latino); Native Hawaiian or Other Pacific Islander (Not Hispanic or Latino); Two or More Races (Not Hispanic or Latino); Prefer Not to Answer
	What is the highest degree or level of school you have completed? (If you're currently enrolled in school, please indicate the highest degree you have received.)	Less than a High School Diploma; High School Degree or equivalent; Some College, No Degree; Associate or Technical Degree Bachelor's Degree; Graduate or Professional Degree
Current Work	What is your current role in working with laboratory animals?	Undergraduate Student; Animal Caretaker or Laboratory Technician; Veterinary Technician/Nurse; Graduate Student; Animal Facility or Laboratory Manager; Post-doctoral Researcher; Principle Investigator; Trainer in Laboratory; Laboratory Animal Veterinarian; Other Animal Care Staff; Other Research Staff; Other
	What type of institution do you currently work with?	University, College or Medical School; Contract Research

		Organization; Non-profit Organization; Other
	What is the primary, broad type of research that you conduct?	Basic; Applied; Regulatory; Product; Education; Other
	What is your highest level of certification?	Board certified veterinarian (ACLAM, DACAW); Graduate or Veterinary Degree (MS, PhD, DVM); Certified, licensed, or registered veterinary technician; Laboratory animal certification (ALAT, LAT, LATG, CMAR, ARLAT, ARLATA, RLAT, RMLAT); None of the above
	How many years have you worked with laboratory animals? Slide the light gray bar to the appropriate number of years. Note: Work is defined broadly, this may include hands-on work such as changing cages or running procedures or hands-off work such as running a lab or research studies.	Any value
	During an average work week, how many hours do you work with laboratory rats? Note: Work is defined broadly. It include hands-on work such as changing cages or running procedures. It also includes hands-off work such as overseeing research as a clinical veterinarian, principle investigator, or manager.	Any value
	Overall, how much stress or pain do most of the rats you work with experience	Little or no discomfort or stress [1]; Minor stress or pain of a short duration [2]; Moderate stress or pain of a short duration [3]; Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals [4]; I don't know [Exclude]
	How much influence or control do you have over the type or amount of enrichment that is used with the laboratory rats you work with? Note: In this study, we consider animal enrichment to be any attempt to improve animal welfare by enhancing the quality of a captive animal's care by providing stimuli necessary for psychological and physical well-being.	None [1] A little [2] Some [3] A lot [4] Complete [5]

Rat Baseline	I wish I could provide more enrichment to my animals than I currently do.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]
	I am confident in my general rat handling skills.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]
	Which, if any, are ways that you have previously heard about rat tickling?	Educational talk Hands-on workshop Science article or blog post Popular press story Peer-reviewed journal article YouTube video Other (fill in) I have never heard of rat tickling before
	How familiar are you with rat tickling? Please think about both your general knowledge and hands-on technique	Not at all familiar [1] Slightly familiar [2] Somewhat familiar [3] Moderately familiar [4] Very familiar [5]
Tickling Core	In the past 2 weeks/2 months, how often did you provide rat tickling to laboratory rats?	Never (0% of the instances I worked with rats) [1] Rarely [2] Sometimes (about 50% of the instances I worked with rats) [3] Often [4] Always (Every instance I worked with rats) [5] I did not work with rats in the past two weeks [Exclude]
	Do you have any further comments about your previous experience with rats or rat tickling?	
Rat Tickling Method	Which picture most accurately represents how the laboratory rats in your care are tickled?	DorsalPin; Dorsal; Pin; DoubleHands; Stroking; NotPictured; Unsure

TBP - Attitudes	**Overall, I think that providing rat tickling for my laboratory rats is**	
	Bad -- Good	Bad [1] to Good [7]
	Worthless -- Useful	Worthless [1] to Useful [7]
	Harmful -- Beneficial	Harmful [1] to Beneficial [7]
TBP – Subjective Norms	Most people who are important to me think I should provide rat tickling to laboratory rats.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]
	I feel under professional pressure to provide rat tickling to laboratory rats.	
	It is expected of me that I provide rat tickling to laboratory rats.	
TBP - Perceived Behavioral Control	I am confident that I could provide rat tickling for my rats.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]
	Whether or not I provide rat tickling to laboratory rats is completely up to me.	
	Overall, for me to provide rat tickling for laboratory rats is:	Extremely Difficult [1] to Extremely Easy [7]
TBP - Intention	I expect to provide rat tickling for my laboratory rats in the next year.	Strongly Disagree [1] Disagree [2] Somewhat Disagree [3] Neither Agree nor Disagree [4] Somewhat Agree [5] Agree [6] Strongly Agree [7]
	I want to provide rat ticking for my laboratory rats in the next year.	
	I intend to provide rat ticking for my laboratory rats in the next year.	
Self-efficacy	**At this moment, from 0 to 10, please rate how certain you are that you can tickle a rat generally, with certain characteristics, or certain components**	[1] Cannot do at all [2] [3] [4] Moderately certain I can [5] [6] [7] Highly certain I can
	Tickle a rat in general	
	Tickle a rat that has never been tickled before	
	Tickle a rat on the nape of its neck	
	Flip a rat over to tickle it on the belly	
	Tickle a rat on its belly in the cage	
Knowledge of Rat Tickling	At minimum, how long should each tickling session last per rat (duration)	15 s [1]; 30 s [0]; 60 s [0]; 120 s [0]
	At minimum, how many days should you tickle rats before their first procedure?	1 [0]; 3 [1]; 5 [0]; 7 [0]
	You are working with rats that you need to give injections to. You have already tickled them for several days. Now it's the day of the first injection, when should you tickle them?	Several hours before the injection [0] Just before the injection [1] Just after the injection [0] You should not tickle them on an injection day [0]

	When you first handle rats, is it better to tickle or stroke them?	Tickle [1] Stroke [0]
	True or false. Adult rats should never be tickled	True [0]; False [1]
	What is the scientific basis for rat tickling?	Mimics aspects of rat social play [1] A standard way to stroke or pet rats [0] Teaches the rat to submit to you [0] Mimics human tickling [0]
	Ideally, when in a project should rats FIRST be tickled?	A day or two after arrival or weaning AND before any procedures or marking [1] Right after marking the animals [0] After acclimation, right after the project starts [0] After data collection [0]
	Any final comments you would like to share with us about rat tickling, this study, or otherwise?	Free response

Table C.2. Qualitative Responses

	Hands-on + Online	Online-only	Waitlist	
Benefits				
Rat Welfare	**23 (82%)**	**22 (79%)**	**23 (77%)**	**"Overall, it is a great refinement technique that promotes animal welfare" "happier animals"**
Less Stress	5 (18%)	10 (36%)	8 (27%)	"Less stress for the animal"
Enrichment	3 (11%)	8 (29%)	8 (27%)	"Enrichment for the animals"
Socialization	1 (4%)	5 (18%)	4 (13%)	"Better socialization"
Ease of Handling	**17 (61%)**	**19 (68%)**	**18 (60%)**	**"Easier handling/less time per procedures" "Better human/animal interactions"**
Bonding	2 (7%)	1 (4%)	3 (10%)	"Can improve the human-animal bond" "Bond with animcal care technician and researcher"
Personnel	**10 (36%)**	**9 (32%)**	**5 (17%)**	**"Effect on staff is positive" "Possible advantages to people handling the animals"**
Affect	8 (29%)	4 (14%)	1 (3%)	"Enrichment for researchers and staff" "It's uplifting to provide something enjoyable" "Good enrichment for humans"
Attention/Empathy	1 (4%)	2 (7%)	2 (7%)	"Increases monitoring of animals for health issues by staff" "empathetic researchers" "Building this bond can be a tremendous incentive for the technicians to spend a few extra seconds or minutes on observations"
Injury/Handling	0 (0%)	1 (4%)	1 (3%)	"Safer handling for the caretakers and investigators
Research	**2 (7%)**	**4 (14%)**	**2 (7%)**	**"…positive for… research outcomes" "More reliable physiologic reactions" "If they are less stressed they provide better quality data"**
Barriers				
Time	**11 (39%)**	**14 (50%)**	**13 (43%)**	**"Time", "Time consuming"**
Quantity of Rats	3 (11%)	2 (7%)	1 (3%)	"The number of rats in a room," "So many rats, so little time"
Staffing	0 (0%)	3 (11%)	0 (0%)	"staff availability"
Training	0 (0%)	1 (4%)	1 (3%)	"Time it takes to train tickling"
Consistency	0 (0%)	1 (4%)	0 (0%)	"If it is done on one experiment, it has to be done on every other study"
Personnel	**11 (39%)**	**10 (36%)**	**16 (53%)**	**"Personnel"**
Access	5 (18%)	4 (14%)	5 (17%)	"I don't work directly hands-on with rats"
Training	2 (7%)	4 (14%)	7 (23%)	"Previously did not have the knowledge" "I know there is a proper method to tickling rats and I have not been trained"

Approval	4 (14%)	3 (11%)	4 (13%)	"Not currently approved at my institution" "Some protocols forbid extraneous handling"
Buy-in	4 (14%)	1 (4%)	3 (10%)	"Convincing others will take time" "It's not seen as an essential task"
Fear/Injury	1 (4%)	1 (4%)	0 (0%)	"Some of my direct reports are a bit scared of handling rats and are worried about getting bitten"
Research	**6 (21%)**	**12 (43%)**	**4 (13%)**	**"Study limitations" "Behavior studies" "I don't want to interfere with scientific work going on"**
Rat Factors	2 (7%)	4 (14%)	2 (7%)	"Animals that are immediately post operative cannot handle rough play but were asier to handle and less stress by handling" "implanted animals"
New Variable	0 (0%)	5 (18%)	0 (0%)	"In some tox and behavioral studies could be considered a variable that may affect data" "Concerns that different responses to tickling may manifest as additional experimental variables"
Rat Factors	**6 (21%)**	**2 (7%)**	**4 (13%)**	**"Animals that are not used to being handled"**
Age	4 (14%)	2 (7%)	2 (7%)	"Older rats" "It is much easier to do this with younger rats. Older, larger rats who have never ben tickled do not readily accept tickling"
Individual differences	1 (4%)	0 (0%)	1 (3%)	"Some are frightened by human touch"
Aggression	1 (4%)	0 (0%)	1 (3%)	"Animals that… are aggressive"
Too few rats	**2 (7%)**	**1 (4%)**	**2 (7%)**	**"We rarely have rats in our facilities so its hard to try tickling when we don't have any available"**
Facility	**1 (4%)**	**0 (0%)**	**0 (0%)**	**"Small caging"**
None	**0 (0%)**	**0 (0%)**	**1 (3%)**	**"None"**

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8. LaFollette, M.R., O'Haire, M.E., Cloutier, S., Gaskill, B.N. 2018. A happier rat pack: The impacts of tickling pet store rats on human-animal interaction and rat welfare. *Applied Animal Behaviour Science, 203,* 92-102. https://doi.org/10.1016/j.applanim.2018.02.006

9. LaFollette, M.R., O'Haire, M.E., Cloutier, S., Blankenberger, W.B., Gaskill, B.N. 2017. Rat tickling: a systematic review of applications, outcomes, and moderators. *PLOS One.* 12(4), e0175320. https://doi.org/10.1371/journal.pone.0175320