Video Article

Tickling, a Technique for Inducing Positive Affect When Handling Rats

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Abstract

Handling small animals such as rats can lead to several adverse effects. These include the fear of humans, resistance to handling, increased injury risk for both the animals and the hands of their handlers, decreased animal welfare, and less valid research data. To minimize negative effects on experimental results and human-animal relationships, research animals are often habituated to being handled. However, the methods of habituation are highly variable and often of limited effectiveness. More potently, it is possible for humans to mimic aspects of the animals' playful rough-and-tumble behavior during handling. When applied to laboratory rats in a systematic manner, this playful handling, referred to as tickling, consistently gives rise to positive behavioral responses. This article provides a detailed description of a standardized rat tickling technique. This method can contribute to future investigations into positive affective states in animals, make it easier to handle rats for common husbandry activities such as cage changing or medical/research procedures such as injection, and be implemented as a source of social enrichment. It is concluded that this method can be used to efficiently and practicably reduce rats' fearfulness of humans and improve their welfare, as well as reliably model positive affective states.

Video Link

The video component of this article can be found at https://www.jove.com/video/57190/

Introduction

Handling of small animals, such as domestic rats (*Rattus norvegicus*) kept in laboratories and as pets, can cause significant increases in behavioral and physiological indicators of stress[1], as well as aversive responses suggestive of emotional distress[2]. Handling rats can trigger fear-based responses towards humans[3,4], which may make further handling more difficult, increase the risk of injury to both animal and handler, and limit the validity of experimental data[5].

To avoid negative effects of handling on experimental results and human-animal relationships, it is common practice to allow some time for newly acquired young animals to habituate to human interaction prior to assigning them to experimental treatments. During this acclimation period, caretakers may expose rats to a series of events that can be highly variable in both form and duration. These may include passive exposure to people, touching, lifting, petting, holding, talking, and offering food treats. Although combinations of such procedures have been reported to reduce fear of humans[6], it is unclear which specific practices are most effective for use by busy animal caretakers. There are reports that rats can have either adverse responses, or at least no favorable response, to some of these individual techniques[2,7,8,9].

In contrast, abundant evidence supports the conclusion that tickling, a handling technique that mimics aspects of the playful rough-and-tumble behavior of rats, is a robustly beneficial form of social contact, especially for juvenile rats[10,11,12,13]. Rats are highly social mammals, which is clear from observing the time they spend playing together as juveniles[14] and their strong motivation to re-establish and maintain social contact with conspecifics[15,16,17]. Rat tickling draws upon the playful social nature of rats to convert handling into a positive experience. This concept was originally developed as a model for investigating the neurobiological basis of positive affective states evoked by social play behavior[7]. Since then, rat tickling has been used in over 56 different experiments, 22 of which compared tickling to other handling techniques[13].

The rat's rough-and-tumble play is characterized by two key behaviors: "dorsal contact" and "pin" (**Figure 1**)[18,19]. During rough-and-tumble play, a rat initiates play by making dorsal contact with the partner's nape. The recipient responds by rolling supine and is pinned down by the initiator standing above[19]. The play continues with the rats taking turns being in each position, on top and below[18]. Human handlers are able to mimic the two key components of rough-and-tumble play by alternating between contact with a rat's nape (dorsal contact) and ventral surface (pin) using vigorous, quick movements of the fingers similar to those used when tickling a child. Of course, tickling is not an exact copy of rat rough-and-tumble play because rats cannot pin the human hand during tickling. Indeed, the level of engagement of individuals in rough-and-tumble play and tickling can differ[20,21]. Despite this, it appears that tickling invokes the same reward mechanisms as rough-and-tumble play among

conspecifics. Tickling is actively solicited by the animals and can serve as a reward for operant learning, contributing evidence that being tickled is substantially rewarding for young rats[7,11,12,13,22]. Even though rats have also been reported to learn an operant task in exchange for gentle stroking as the reward, they were faster to approach the hand of a person offering tickling compared to stroking[7].

The pins used in tickling may seem similar to common restraint procedures but there is evidence that rats perceive these types of handling differently. When rats are repeatedly restrained, such as for an injection procedure, they secrete excessive porphyrins resulting in chromodacryorrhea ("red tears")[8]. Chromodacryorrhea occurs in response to acute stressors and causes the skin and fur around the eyes and nose to become stained a reddish color[23]. When repeatedly held down and restrained on the back as in a pin, rats showed less attraction to humans than when tickled[12]. These outcomes imply that the repeated brief restraints and releases during tickling are perceived differently than non-playful restraint. Thus, the precise technique used in tickling has important implications for generating a positive affective state as opposed to contributing to handling stress.

Monitoring the effect of tickling on rat affective state can be easily accomplished by attending to the rats' vocalizations. Rats typically produce two categories of ultrasonic vocalizations (USVs), at around 22-kHz and 50-kHz, in addition to audible vocalizations. The 22-kHz USVs typically occur when anticipating aversive events and are accompanied by elevated brain cholinergic activity[24]. Therefore, 22-kHz USVs are deemed to indicate a negative affective state of anxiety. The 50-kHz USVs, especially of the frequency-modulated "trill" type (**Figure 2**), are generally associated with rewarding situations and stimuli and are accompanied by increased brain dopaminergic activity[24,25]. Accordingly, expression of abundant 50-kHz USVs is considered to reflect a positive affective state[24,26,27,28]. Finally, audible vocalizations are usually associated with, and therefore used as markers of, physical pain and discomfort. The differential production of these three forms of vocalizations thus offers a reliable record of changes in the affective state of rats[26]. Rats produce USVs at around 22-kHz during exposure to predators, exposure to painful stimuli such as foot shock, and inter-male fighting[28]. Stroking has also been reported to elicit multiple 22-kHz USVs in naïve rats, inferred as indicating negative affect[2]. In contrast, when tickled by humans, as when engaged in rough-and-tumble play with other rats, rats produce numerous 50-kHz USVs. It follows that the rate of 50-kHz USVs provides a graded measure of the positive affective response generated by tickling[29,30].

Existing descriptions of "how to tickle a rat" are rather brief and can lead to variable results. Further, novice "rat ticklers" may be overly tentative in their attempts to mimic rough-and-tumble play. The words "tickle vigorously" are easier to understand when demonstrated visually. Therefore, the goal of this article is to provide a detailed description and demonstration of how to mimic rats' positive playful interactions in an effective and consistent manner that reliably elicits positive responses from most rats when handled. This protocol describes a standardized tickling technique, and explains how tickling can be combined with procedures such as restraint and injection to mitigate potentially unpleasant affective experiences.

Protocol

All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals[31,32] at facilities accredited by AAALAC International and were approved by the Washington State University Institutional Animal Care and Use Committee. The following protocol can expose personnel to laboratory animal allergens. Personal protective equipment should be worn as recommended by the institutional occupational health and safety program and health care providers. The protocol depicts procedures under typical laboratory housing conditions but could be adapted for use in higher-biosecurity facilities (e.g. by using a biosafety cabinet, etc.).

1. Preparation of Equipment and Setup

1. Handling box
    1. Prepare an animal's home cage or other enclosure for tickling, hereafter called the handling box.
    2. Make sure the handling box is large enough to provide space for the tickler's hand and for the rats to move around while preventing them from jumping out.
    3. Make sure the handling box is free of obstacles. Thus, if conducting tickling in the home cage, remove any objects such as huts, gnawing sticks, and crinkled paper.
    4. Line the handling box floor with bedding[33]. Other types of soft floor cover can be used but are not as effective at eliciting 50-kHz vocalizations[13].
    5. Place the handling box on a table or countertop at a height that allows the handler's hand to comfortably reach the entire floor area of the handling box.
    6. Minimize the effect of novelty by habituating the rats to the handling box prior to tickling, if needed.
2. Timer: To indicate the start and end of 15 s bouts, place a timing device near the handling box where it can be easily read. Alternatively, an auditory time signal may be transmitted to the tickler through earphones.
3. Vocalization recording
    1. If vocalizations are being recorded to quantify different vocalization types for research purposes, use a microphone capable of capturing 10-120-kHz broadband sound, connected to a device capable of recording high frequency sounds.
    2. Suspend or attach the microphone at the top or side of the box, low enough to clearly detect the calls and avoid confusion with calls from rats in nearby cages, but out of physical reach of the rats.
    3. If vocalizations only need to be monitored to confirm proper tickling technique, then use a bat detector and set it up on the side of the box. If using a simple, heterodyne detector, set the device at 50-kHz.
4. Laboratory gloves: Use a bare or thinly gloved hand when tickling. The tickling technique was developed using a bare hand. However, a thinly gloved hand is also effective at inducing playful behavior and 50-kHz USV production while allowing the handler to sense the amount of pressure being applied to the rat's body and feel the rat's responses through the glove.

2. Tickling Procedure

1. Put on the laboratory gloves, if required. Bring the home cage to a table or workbench. Gently transfer the rat into the handling box if tickling is not conducted in the home cage. Start the timer, and the audio recording if needed.
2. For consistent application, alternate between 15 s of hand contact and 15 s release from contact for a total of 2 min. This duration of interaction has been found sufficient for increasing attraction of rats to humans[11].
    1. To begin the tickling session with a 15 s release, place the hand motionless inside one side of the handling box. The presence of the hand allows rats to solicit interaction by directing licks and light nips (nibbles) towards the hand, indicating the rat's interest to interact[22]. When this occurs, move the hand away from the rat, which can solicit playful chasing of the hand by the rat. Alternatively, leave the hand out of the box during the 15 s release.
    2. Follow the release with 15 s of tickling, initiate the interaction with a dorsal contact (2-4 s). Using one hand, vigorously touch the rat's nape (dorsal surface of the neck) with the fingertips using rapid finger movements as commonly performed in human tickling. Avoid contact with the rump as this area of the body is targeted during aggressive encounters[34].
    3. Follow the dorsal contact with a pin. Place the thumb and middle finger under the rat's forelegs; lift the rat, just high enough to allow quickly turning the rat on its back while ensuring the tail does not get kinked.
    NOTE: It may help to lightly push on the back of the rat with the little finger when turning the rat on its back.
    4. Hold the rat loosely, but firmly; be assertive but not forceful. Once the rat is on its back on the floor, hold the body down loosely while moving the fingers quickly and vigorously but gently on the belly, as is commonly used in human tickling.
    5. Even though the stimulation is rapid and assertive, keep the pinning brisk (2-4 s/pin) to minimize negative responses. Allow the rat to right itself and rapidly follow with a dorsal contact.
    6. Continue to alternate between dorsal contacts and pins. On average, there should be 4 to 5 pins/15 s[35].
    7. Do not pick up the rat with both hands. During the 15 s of tickling, always keep the fingers moving and in contact with the rat's body, even in transitions between pins and dorsal contacts.
    8. Perform three additional 15-s tickles and 15-s releases for a total of 2 min.
3. If using a handling box, return the rat to its home cage. If using the home cage, place enrichment objects back in the cage. Clean the handling box with paper towels to remove feces and urine not absorbed by the bedding.
4. Repeat with the next cage of rats, as needed.

3. Tickling Multiple Rats Simultaneously

1. Rats can be tickled as a group[36]. If the handling duration is not relevant, alternate tickling each rat for 15 s. The duration of the tickling session can be extended beyond 2 min as needed so that each rat receives four bouts of tickling hand contact interspersed by release.
2. If the handling duration is relevant for research results, then use the following procedures:
    1. For pairs, tickle one rat for 15 s followed immediately by tickling the second rat for the next 15 s (*i.e.*, during the release of the first rat), and so on. The tickler does not have a rest period.
    2. For triplets, use the procedure described in 3.2.1 to tickle two of the rats and tickle the third one at the same time as one of the others using the other hand.
    3. For groups of 4 or more rats, tickle each rat or pair of rats for 15 s. The duration of the tickling session can be extended beyond 2 min as needed.

Representative Results

Factors affecting, or potentially affecting, responses to tickling are summarized in **Table 1**. Positive outcomes from rat tickling have been indicated by multiple measures of rat welfare and positive affect. Common findings include increased production of 50-kHz USVs, increased approach behavior, and increased ease of handling. Reported effects of rat tickling at the genetic and cellular level include altered gene expression in the hypothalamus[37], and increased neurogenesis in the dentate gyrus of the hippocampus[38,39]. Outcomes indicative of reduced generalized anxiety and fear of humans are also reported such as reduced latency to approach in 9 different experiments[13], less freezing after fear conditioning[40,41], and increased entries into the center of an open field arena by individually housed rats[36]. For further information see LaFollette *et al.*, who present a systematic review of the tickling literature that identifies 22 reports investigating the outcomes of rat tickling in comparison to other techniques[13].

Next, specific examples of results that can be obtained when the tickling technique is paired with restraint and intra-peritoneal (IP) injection procedures are outlined.

Sprague-Dawley rats (males; 85-94 days of age; n = 48) tickled for 2 min immediately before and after IP saline injection daily for 10 days, especially when combined with the juvenile tickling experience (2 min/day from 25-45 days of age), produced more 50-kHz USV just before (GLM ANOVA, $F_{3, 44} = 9.59$, $p < 0.0001$; **Figure 3A**) and just after ($F_{3, 44} = 8.74$, $p = 0.0001$; **Figure 3B**) injection (Tukey-Kramer, $p < 0.05$) compared to rats exposed to a passive hand[10]. During the actual brief delivery of the injection, production of 50-kHz USVs was curbed (mean ± SE, 15 ± 1.4 USVs/min) and handling treatment had no effect on their rate[10]. It was concluded that tickling, when applied both before and after an injection procedure, induced a positive affective state that moderated the aversiveness of the procedure, especially for those that had been exposed to tickling as juveniles.

To find out if tickling would be effective if reduced to before or after an injection rather than both and, if so, which time would be better, rat responses to tickling before, after, and before-after injection were compared. Rates of 50-kHz USVs and audible calls were measured as indicators of positive and negative affect, respectively[12]. Sprague-Dawley rats (males; 32-41 days of age; n = 96) were either restrained and given a daily IP saline injection (INJ) or restrained without receiving an injection (control, CON), and exposed to one of the following treatments: no tickling, the rats were not touched before and after the restraint procedure (TN); tickled for 2 min right before (TB), after (TA), or before and after (TBA) restraint[12]. In the 2 min before restraint, rats from treatments that received tickling before the restraint (TB, TBA) produced the most 50-kHz USVs (GLMM ANOVA, $F_{3,88} = 49.19$, $p < 0.0001$; **Figure 3C**). TA and TN rats did not differ in the rate of USVs produced before the restraint procedure. During the procedure, any rat that received tickling (TA, TB, TBA) produced more 50-kHz USV than those that were not tickled (TN; $F_{3,88} = 50.67$, $p < 0.0001$; **Figure 3D**). Among tickled rats, those receiving tickling prior to the procedure (TB, TBA) produced more 50-kHz USVs during the restraint compared to the ones tickled afterwards (TA; $p < 0.05$). In the 2-min period after the procedure, rats in all tickled groups (TA, TB, TBA) produced more 50-kHz USVs than those that were not tickled (TN; $F_{3,88} = 53.64$, $p < 0.0001$; **Figure 3E**). Furthermore, during the procedure, rats tickled before restraint (TB, TBA) produced fewer audible calls compared to rats that did not received tickling (TN), whereas rats tickled afterwards (TA) did not differ from those in the other groups ($F_{3,88} = 3.67$, $p= 0.02$; **Figure 3F**). The results demonstrate that tickling rats before restraining them for an IP injection produced signs of greater positive affect across the periods before, during, and after the procedure than tickling only after restraint[12].

Caretakers applied one of the following handling treatments to rats (Sprague-Dawley males; n = 32) from 57 to 74 days of age: (1) Minimal handling once a week at cage change; (2) Exposure to a passive hand for 2 min daily; (3) 2 min of tickling each day, to mimic social play; (4) 2 min daily of restraint on the rat's back, simulating unreciprocated pinning by a dominant[11]. In the minute following an IP injection, rats from the Tickled and Passive treatments spent more time rearing in the front half of the cage, close to where the researcher was standing, compared to rats from the Minimal and Restraint treatments (GLM ANOVA, $F_{3, 27} = 5.70$, $p = 0.004$; **Figure 3G**). Additionally, Tickled rats uttered more 50-kHz USVs than rats in the Passive treatment when exposed to a familiar or unfamiliar hand for 3 min in a Human approach test (Log-transformed mean ± SE calls/min, Minimal: 0.7 ± 0.26, Passive: 0.6 ± 0.18, Tickled: 1.8 ± 0.30, Restraint: 1.4 ± 0.28; $F_{3,28} = 4.04$, $p = 0.02$). Thus, the simple presence of a passive hand aided habituation of rats to humans relative to minimal handling, but tickling was the most effective method for producing a positive response towards humans[11].

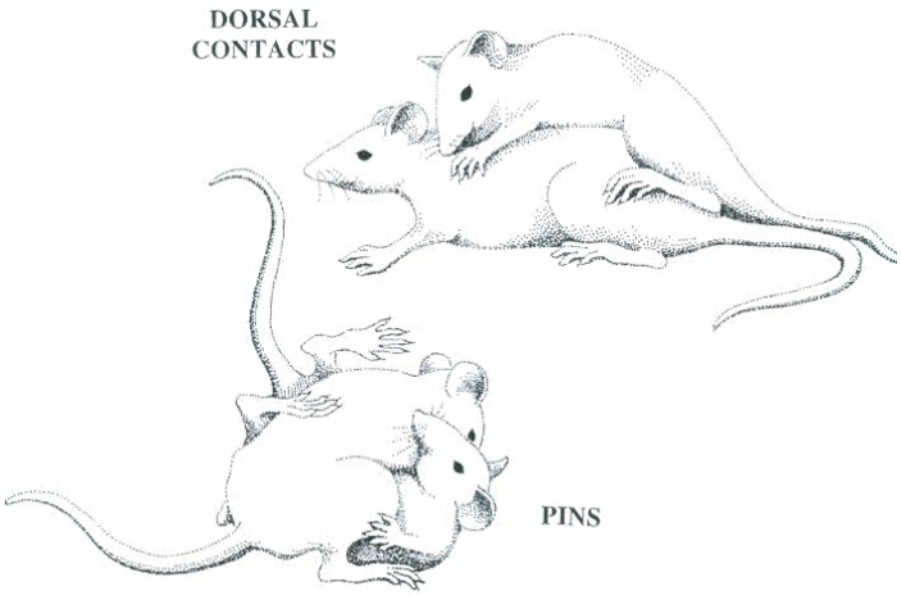

Figure 1: Rat rough-and-tumble play behavior. (**A**) Dorsal contact; (**B**) Pin[18]. Please click here to view a larger version of this figure.

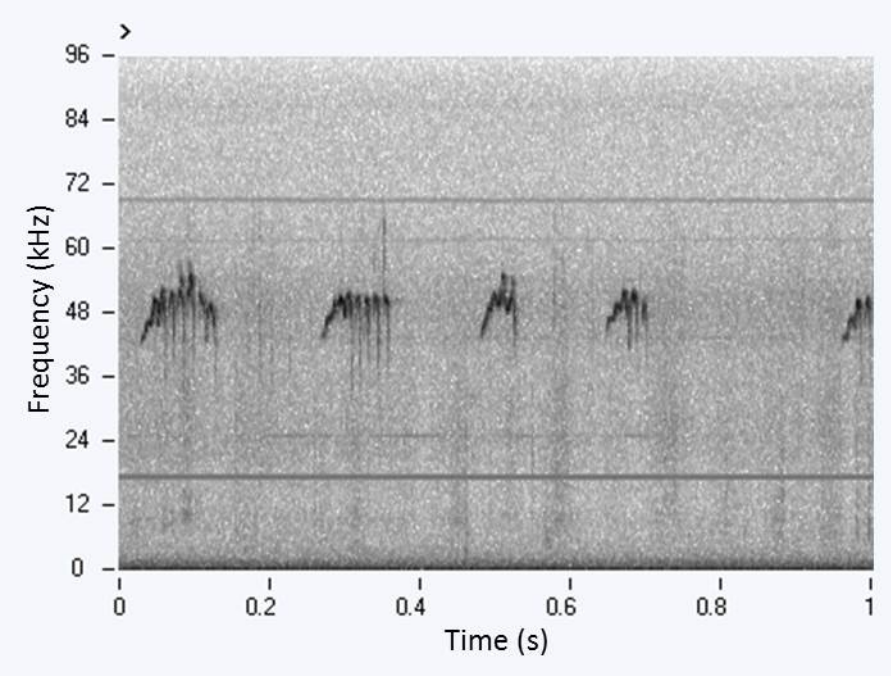

Figure 2: 50-kHz ultrasonic vocalizations of the frequency modulated "trill" type. Five trill vocalizations were produced in 1 s by a rat during tickling. The horizontal lines at 17-, 25-, and 70-kHz were produced by electronic equipment in the room (*e.g.*, computer, microphone, camera).

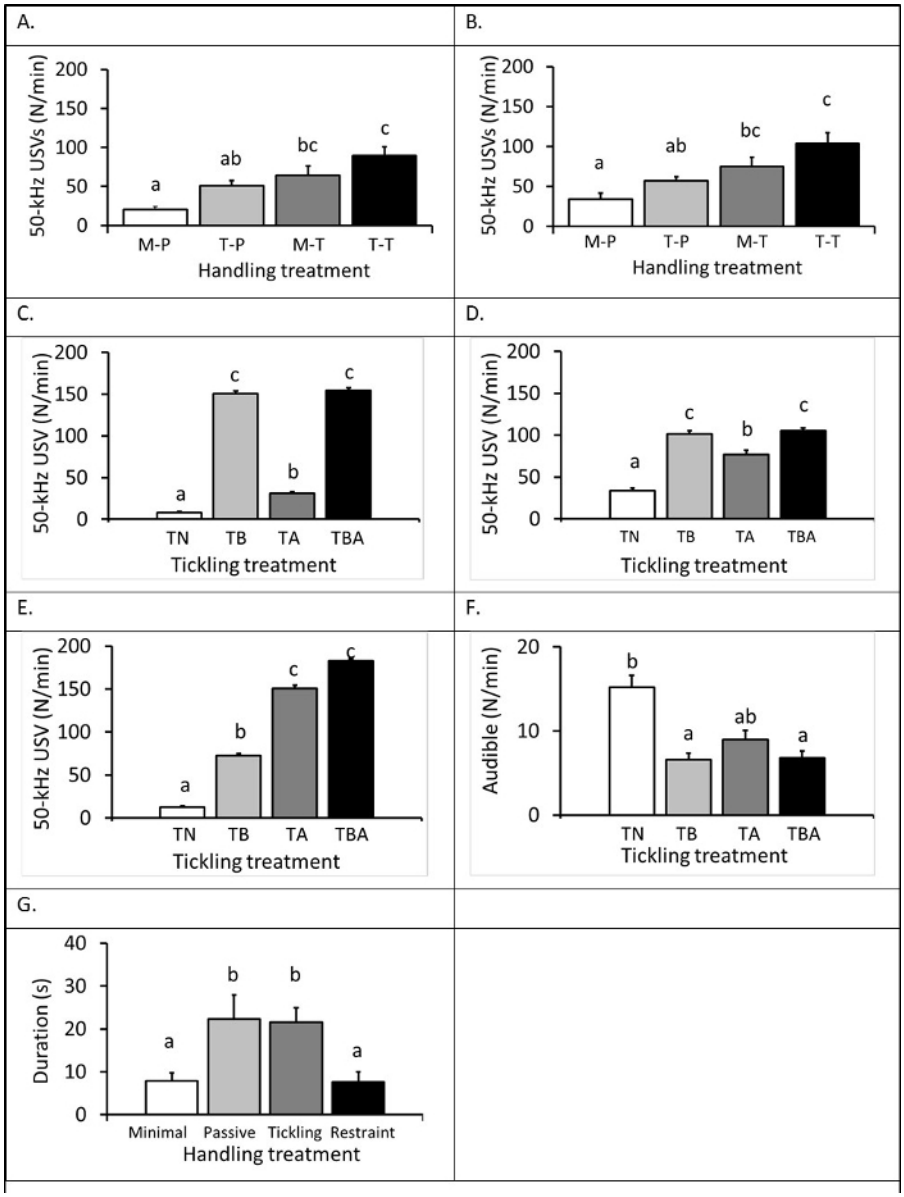

Figure 3: Responses of rats exposed to tickling prior to, or in combination with, experimental procedures such as intra-peritoneal (IP) injections. Rate (mean ± SE calls/min) of 50-kHz USVs produced (**A**) before and (**B**) after an injection procedure by adult male rats exposed to minimal handling (M) or tickling (T) as juveniles and a passive hand (P) or tickling (T) immediately before and after injection (yielding four treatment combination: M-P, T-P, M-T, T-T); from Cloutier *et al.*[10]. Rate of 50-kHz USVs (mean ± SE calls/min) produced (**C**) before, (**D**) during, and (**E**) after restraint (with or without an IP injection) once daily for 10 consecutive days by juvenile male rats that received no tickling (TN) or tickling before (TB), after (TA), or both before and after (TBA) the procedure; and (**F**) the rate of audible calls produced during restraint; from Cloutier *et al.*[12]. (**G**) Duration (mean ± SE s) of rearing nearby the researcher during the 1 min following an IP injection by male rats exposed to minimal, passive, tickle, or restraint handling by caretakers; from Cloutier *et al.*[11]. Bars with different letters (a, b, c) indicate differences in pairwise comparisons (Tukey-Kramer, $p < 0.05$). Please click here to view a larger version of this figure.

Factor	Effect	References
Intrinsic		
Age	No difference in 50-kHz ultrasonic vocalizations (USVs) between rats ages 24-44 and 89-92 days old	18, 29, 42
	Decrease in 50-kHz USVs for rats from 71 days old to 148 days old	29
	17 day old males made many more 50-kHz USVs than 7-9 month old males	43
	Rats tickled as both juveniles and adults showed the most positive results compared to rats only tickled as juveniles or adults.	10
Sex	Male rats produced more 50-kHz USVs at 44 and 71 days old, but females produced more 50-kHz USVs at 148 days old	29
	Male rats produced more 50-kHz USVs than females at 44 and 58 days.	43
	No sex differences in 50-kHz USVs in 96 juvenile Long-Evans rats or 52 juvenile Wistar rats	42, 44
	Female juvenile Wistar rats produced more 22-kHz USVs than males	44
Inter-individual differences	Rats that uttered more 50-kHz USVs showed decreased approach time to self-administered tickling, increased positive judgement bias after tickling, and increased hippocampal cell proliferation after tickling	27, 37, 45
	More playful rats produced more 50-kHz USVs	43
Strain	Not reported, but anecdotal evidence suggests rats from different strains and breeders differ in their response to tickling. Rat lines diverged when selected upon high vs. low 50-KHz USVs rate in response to tickling.	22, 26, 42, 46
Previous experience of tickling	Increased production of 50-kHz ultrasonic USVs	12
Extrinsic		
Bedding	Rats produced more 50-kHz USVs when tickled on familiar bedding vs no bedding	33
Stress	Tickling rats 1 hour, but not 23 hours, after restraint stress reduced 50-kHz USVs production	47, 48
Timing	Tickling rats before injection was more effective in increasing 50-KHz USVs than tickling after injection	12
Sex of handler	No effect found	Panksepp, J. 2014, Unpublished data
Familiarity of rat with handler	Rats produced more 50-kHz USVs and spent more time near a familiar compared to an unfamiliar tickler	11
Handler tickling experience / vigor of tickling by handler	Affected production of 50-kHz USVs but not preference and approach behavior	Cloutier, S. 2011, Unpublished data
Duration of pins by handler / frequency of alternation between dorsal contacts and pins	Lower frequency of nibbles (interpreted as play solicitation) was directed to a hand imposing 15-s pins (restraint) than a hand giving tickles with multiple dorsal contacts and pins per 15 s	11
Odorants (e.g., perfume, shampoo, shaving cream) used by handler	Not reported	
Social group size	Individual housing increased positive responses to tickling compared to group housing in most experiments. Tickling group-housed rats with cage mates in the home cage reduced this difference	7, 22, 27, 36
Light intensity	Tickling at 1000 lux reduced 50-kHz USVs compared to 25 lux.	43
Noise	Not reported	
Cage cleaning before tickling	Induced production of 22-kHz USVs in some rats	12

Time of day	No effect on 50-kHz USVs production when performed at the beginning or end of the light period. Tickling rats at the beginning/end of the light period vs middle of light or dark period has not yet been compared	Cloutier, S. 2011, Unpublished data

Table 1: List of factors affecting, or potentially affecting, the responses of rats to tickling, a playful handling technique intended to mimic rat rough-and-tumble play.

Discussion

This protocol describes a technique for rat tickling, a playful handling technique mimicking rat rough-and-tumble play behavior that is effective in generating vocalizations indicative of positive affect and increasing approach behavior when rats are handled for common husbandry or medical and research procedures such as IP injection.

Social play, a typically positive form of social contact, has been proposed to assist in the calibration of emotional reactions to frightening situations[42,43], and generate resilience to anxiety and depression[44]. By mimicking rat social play, tickling appears to have similarly beneficial effects[7]. Thus, for the application of tickling to be successful at inducing positive affective states, it is important to keep in mind that the hand is intended to act as "the play partner" during the tickling. The hand is attractive because it simulates the behaviors that rats expect and enjoy from another rat during rough-and-tumble play. Hence, lifting and holding the rat with both hands is not mimicking rat play, and may not have the same effect. In fact, laboratory rats will learn to avoid non-playful restraints in which a handler holds them with one hand and strokes them with the other hand[45]. During rat tickling, it is also important to move the hand confidently and assertively, while being lively and playful. Keeping the pinning brisk (2-4 s/pin) should minimize negative responses because in rough-and-tumble play between rats, repeated pinning leads to uttering of 22-kHz USVs[18].

Even though juvenile rats are more playful and show stronger responses to tickling than adult rats[18], tickling in adulthood also has benefits[10]. Tickling rats when young has also been found to have benefits later in life[36], suggesting that rats experience a prolonged positive effect on their emotional reaction to human presence. Sex differences in response to tickling are inconsistent, with reports of no sex differences in juvenile rats[46,47], juvenile males producing more 50-kHz vocalizations and fewer 22-kHz vocalizations than juvenile females[29,48,39], and older females producing more 50-kHz vocalizations than same-aged males[29], similar to findings for social play[49].

The effectiveness of the tickling technique can be assessed using rat movement patterns and vocalization. Rats will nibble and lick the handler's fingers during the 15-s periods of release following tickling[11]. Nibbles are light, non-injurious bites that have been described as play-solicitation behavior in young rats[22]. When the handler moves the hand around the handling box, the rat will follow and chase it. Rats, especially young ones, will also run, scamper around the box between tickling bouts, and show a distinct increase in activity after a tickling bout, similar to the running activity that characterizes rough-and-tumble play. If the rat remains immobile during releases, adjustment of the tickling technique, such as reducing pin duration or giving fewer pins, is probably needed. It is best to give pins of short duration for the first tickling session. Duration and number of pins can be increased as the rats get used to tickling. Rats utter 50-kHz USVs, including trills, during tickling and between tickling bouts. A gradual increase in the number of trills produced during the 15-s releases can be seen over the first two weeks of daily tickling before stabilizing[47]. Individual differences allow tickled rats to be divided into high and low callers that respond differentially to stress, anxiety, and anhedonia[46,47,50].

Rat tickling has been used successfully to induce positive affect as assessed based on production of 50-kHz USVs during tickling[13]. It has recently been implemented as a technique to reduce fear of humans in the rats and mitigate their aversiveness of medical and research procedures such as injection. Evidence suggests that, when provided for only 2 min daily per animal, tickling is an effective and practical method for reducing avoidance of humans[11]. Considering that juvenile rats will play together for about 60 min daily[51,52,53], tickling for 2 min daily is practical but presumably too brief to replicate the full effects of social play among rats. Pet rat owners, who generally have more time for handling their rats than laboratory caretakers, may provide rats with additional tickling as long as the rats continue to respond positively. Based on Burgdorf *et al.*[44,45], the first 15 min of rough-and-tumble play seems to be the most positive for rats. In turn, it may be more effective to tickle rats for periods of up to 15 min, especially if for a justified reason they cannot be kept in a social group. However, based on play as "training for the unexpected"[38], a more varied technique than the method described here might be needed to maintain the playfulness of longer tickling sessions. Additional tickling sessions each day may also be beneficial as long as other biologically important activities such as sleep and feeding are not impaired. As well as contributing to improving the well-being of laboratory and pet rats, the described standardized tickling technique can be used in continued research aimed at understanding the effects of social play and its components on behavioral, social, and brain development.

When tickling was performed surrounding injection, particularly when combined with previous tickling experience (ending 40-50 days earlier), it increased 50-kHz USV rates before and after injection, and reduced audible call rates during injection[10]. Thus tickling seems to have mitigated the adverse effects of IP injections, especially in rats accustomed to tickling as juveniles[10]. Even though tickling did not prevent discomfort associated with the injection as assessed based on production of audible calls, it increased 50-kHz USVs compared to the control condition, before, during and after the restraint procedure[12]. According to 50-kHz USV data, the most effective time point for applying tickling was just before rather than after the restraint procedure[12]. Tickling rats before the injection had a carry-over effect that elevated positive affect during the procedure[12]. Tickling can therefore minimize potential negative effects of routine medical procedures on rat welfare and behavior. More research is necessary to determine how the technique could be paired effectively with other injection techniques and other medical/experimental procedures.

Overall, we have demonstrated that rat tickling results in positive outcomes such as increased production of 50-kHz USV and approach behavior, as well as decreased avoidance of humans and resistance to handling. We recommend rat tickling as a viable method for improving rat welfare and modeling positive affective states.

Disclosures

The authors declare that they have nothing to disclose.

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