An Analysis of Affective Ultrasonic Vocalizations of Rats as a Function of Social Play and Tickling

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As advisor for Kate Fyrqvist, I have read this paper and find it satisfactory.

Signature

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PRÉCIS

Although young laboratory rats play vigorously when taken from isolation and presented with an acceptable play partner, their positive play behaviors gradually decline over the course of a prolonged (30-minute) play session. Additionally, some rats begin to make vocalizations in the 22-kHz range, which may indicate a negative emotional state. This study first took an observational approach to attempt to determine if the decline in play behaviors in these animals was due to the rise of a general negative-affective state. Animals were observed during 30-minute play sessions throughout adolescence to attempt to tease out relationships between vocalizations and corresponding play behaviors. Subsequently, a “psychoassay,” based around a standard tickle test attempted to determine the affective phenotypes of these animals.

Twenty-two kHz vocalizations generally arose while one animal was being pinned by the other; a natural action in the course of rat play, but also one that may have expressed brief dominance of one rat over the other. Although the occurrence of these vocalizations possibly represented momentary “complaints,” it is unlikely that the associated negative emotions were sufficient to differentially affect the overall pattern of play. Although complaining animals tended to pin each other more often than non-complainers, they did not show different patterns in other play behaviors. They generally recovered quickly after a bout of 22-kHz USVs and continued to play until satiated. Furthermore, a standard tickle assay demonstrated that many of these “complaining” animals in fact had overall positive-affective phenotypes. This phenotype may have allowed this swift recovery from brief
complaint to resume to play. The strength of this assay was demonstrated by consistent results across days and experimenters, and was further supported by follow-up experiments in which animals expressed their desire to be tickled.
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INTRODUCTION

What is social play and why is it such fun? Historically, play has been considered a rather frivolous activity, with no scientifically well-established end. However, humans and other mammals play at strikingly high rates and often devote more time to playing than to fighting, having sex or even eating (Burghardt, 2010). In recent years, play research has become more prevalent, as links between play deficits and societal problems such as childhood obesity and Attention Deficit Hyperactivity Disorder (ADHD) have been revealed (Burghardt, 2010; Panksepp, 2007). Of the three generally accepted categories of play, social play has been more thoroughly studied than its two counterparts: locomotor and object play. Indeed, social play seems to be the most rewarding, particularly when it is the rough-and-tumble (RAT) variety (figure 10.1, Panksepp & Biven, 2012). As we will see, simple and reliable measures of this kind of play have been devised, such as dorsal contacts and pinning behaviors, vastly facilitating the empirical study of such social interactions (as first reported by Panksepp & Beatty, 1980).

Play is easily recognized, whether manifested as ponies prancing over a field, children playing with dolls or puppies wrestling. However, scientists have had difficulty conceptualizing what specifically defines play. In his book, The Genesis of Animal Play (2005), Gordon Burghardt proposed five criteria, all of which should be met in order for behavior patterns to be classified as play. First, play is functionless in that it involves activities that require energy and do not immediately contribute to survival. Second, play is a rewarding process and so is performed voluntarily by the participants. Third, although play generally resembles serious behavior such as
hunting or fighting, it is incomplete or exaggerated to indicate to all participants that it is not the real thing. Fourth, the behavior is spontaneously repeated in a flowing, non-rigid manner. Finally, play only occurs when the animal is otherwise taken care of in body and mind. Hunger, fear and extreme stress all drastically reduce play. These criteria can be used to define a vast array of behaviors as play, including human activities such as gourmet cooking! However, as previously mentioned, rough-and-tumble play is the most rewarding, and is practiced universally by young mammals.

Pellis et al. (2010) proposed two hypotheses for the function of play. One is that when young animals play, they prime their motor systems, namely the muscles and associated nervous system, for later performance in adult behaviors. The other is that play exposes young animals to unpredictable situations, thus equipping them with greater flexibility and capabilities for dealing with adversity and unanticipated events (for a full development of this idea, see Spinka, et al., 2001). The two hypotheses are not mutually exclusive; play may be responsible for both and possibly a variety of other bodily and brain benefits. At any rate, it is becoming increasingly clear that play is necessary for proper development (Panksepp, 2007). For instance, it has been demonstrated in the laboratory rat that if the opportunity to play with others is denied to juvenile rats, the animals show various social deficits later in life. For example, the adult animal may overreact to normal social behaviors, such as investigatory sniffing, which results in an increased likelihood for the encounter to escalate to fighting (Pellis & Pellis, 2007; Potegal & Einon, 1989).
Here is what play in rats looks like: given the opportunity, healthy juvenile rats play together vigorously, taking turns chasing, pouncing upon and wrestling with each other. This type of rough-and-tumble play fighting is the most common variant of play in the laboratory rat. It can generally be described as a “game” where the objective is to gain access to the partner’s nape (Pellis & Pellis, 2009). Each rat enthusiastically tries to pounce upon its partner’s back and nuzzle the nape with its forepaws and snout. These “dorsal contacts” have been suggested to reflect “play solicitation,” as they are usually followed by chasing and wrestling and often end in a “pin.” Pins are considered to be the consummatory phase of play, and are characterized by one animal lying on its back, with the other on top (Siviy & Panksepp, 2011). After a dorsal contact, the “attacked” rat may use one of several tactics to attempt to defend its nape. These tactics include standing on the hind legs and assuming a “boxing” posture to fend off the attacking rat or flipping over into a supine position so the nape is against the ground (Pellis & Pellis, 2009). The latter tactic is more common and allows the attacking rat to hold the defending rat in this pinned position just briefly or up to a few seconds. Generally, as the rats become experienced players, consistent dominance-submission patterns emerge, where one rat reliably pins the other more often (Panksepp, 1981). However, play is rewarding to both submissive and dominant rats since both will run through a maze for an opportunity to play equally quickly (Normansell & Panksepp, 1990; Panksepp, 1998). These pins and dorsal contacts are simple and reliable indications of rat playfulness and, therefore, are used as the main empirical measures in the study of play (Panksepp & Beatty, 1980).
Young rats in a bout of rough-and-tumble play seem to exude a sense of child-like, uninhibited joy. Indeed, RAT play is routinely accompanied by a series of brief ultrasonic vocalizations (USVs) in the 50-kHz range that have been empirically associated with a positive affective state since they arise from rewarding brain networks (Panksepp & Biven, 2012). In adults, these calls, albeit at much lower levels, commonly accompany a variety of other gratifying situations (anticipation of tasty food, sex, and even addictive drugs) (Burgdorf et al. 2000; Knutson et al. 1999). Their production has been related consistently to arousal of the Medial Forebrain Bundle (MFB); most specifically activity of the dopaminergic SEEKING or “reward-circuitry” of the brain that courses through the MFB (Burgdorf et al., 2007). It has been suggested that these 50-kHz vocalizations (commonly called “chirps”) are related to human laughter, either in function or evolutionary origin (Panksepp & Burgdorf, 2000). Generally, 50-kHz chirps are correlated with the appetitive phase of play, specifically occurring more often during dorsal contacts (Knutson, Burgdorf & Panksepp, 1998).

When human children participate in rough-and-tumble play, colloquially called “roughhousing,” it usually begins with high levels of activity and constant bursts of laughter. As the play progresses, disputes may arise, marked often by brief bouts of complaining or crying. Young rats show a similar pattern, starting with original high levels of 50-kHz USVs that decline over time, as “negative-affect” 22-kHz USVs become more prominent later in the play session (figure 1- Burgdorf, 2006). The relatively long (0.2 seconds to 3 seconds in duration) 22-kHz calls generally start at a frequency in the 30-kHz range and settle to a sustained flat
frequency in the 20-kHz range. They are typically elicited by a variety of stressful situations, for example when a rat is anticipating a negative stimulus such as foot-shocks, after social defeat during aggressive encounters, or upon detection of a predator’s odor (Antoniadis & McDonald, 1999; Panksepp et al., 2004; Blanchard et al., 2005). However, these vocalizations do not express the experience of acute physical pain. Rather, the calls may indicate anxiety about a potential painful event, and are not made during actual painful events. When in pain, animals instead commonly squeal at an audible level (Jourdan et al. 2002).

It is possible that the pattern of diminishing 50-kHz USVs and increasing 22-kHz USVs marks the decline of the positive-affective state and the rise of an aversive emotional state. The specific behaviors associated with the decline of the 50-kHz USVs and the appearance of 22-kHz USVs have not been thoroughly studied. Thus, the purpose of Experiment 1 of this study was to identify the relationship between dorsal contacts and pins to the corresponding ultrasonic vocalizations. This was done by ethologically describing rat ultrasonic vocalizations in the context of these play behaviors, and by using observational analysis to distinguish relationships. Subsequently, a series of experiments attempted to dissect how these vocalizations and their accompanying play patterns varied with the affective phenotype of
animals in play pairs. This was performed using a short tickle-based “psychoassay” for positive social affect.

In the absence of a rat playmate, a vigorously socially-interactive human hand (aka “tickling”) can present a satisfactory play surrogate for socially-deprived rats (Panksepp & Burgdorf, 2000). Tickling a rat normally results in a burst of 50-kHz USVs, but the quantity of these chirps that animals emit during tickling varies among individuals. This response has been suggested to be an aspect of the rat’s affective phenotype, which is genetically determined and stable throughout an individual’s life (Panksepp & Burgdorf, 2000), and may index susceptibility to depression (Harro, 2010). Rats that naturally emit high quantities of 50-kHz USVs in response to tickling generally exhibit behaviors that are reflective of a positive affective state, while those that respond less strongly to tickling behave in ways demonstrative of a negative affective state (Mällo et al., 2009). For example, when allowed an opportunity to play, high tickle-responders exhibited more overall playful behavior and positive vocalizations compared to low-responders, at least in some breeding experiments, but not others (Panksepp & Burgdorf, 2000; Webber et al., 2011).

Experiment 1B of this study determined the affective phenotypes of each of the rats from Experiment 1A by tickling them. It thereby categorized all rats as either high-, medium- or low- tickle responders. This allowed integration of the information gleaned from the animals’ play behaviors in Experiment 1A with their affective phenotypes. Based on the previous literature, it was hypothesized that the
animals that showed the strongest response to tickling would be the ones that had played the most, while those that showed weaker responses to tickling might have played less. Those findings set the stage for the collection of additional data concerning the motivation of these animals to be tickled in Experiment 2: namely, all animals were allowed an opportunity to emerge from a safe place to be tickled (Exp. 2A), or to escape from tickling (Exp 2B). These follow up studies directly evaluated the motivation of animals to be tickled or to avoid tickling.

**METHODS**

*Subjects*

Thirty Long-Evans rats (18 male and 12 female) from 10 different litters were used. Rats were born and bred in-house and were weaned at 21 days of age. To ensure a maximal play response, animals were individually housed in 25 x 45 x 20 cm transparent home cages at 25-29 days of age, two days before the start of testing (as in Panksepp, 1981). Throughout testing, all animals had free access to food and water *ad libitum*, and were housed and tested in temperature-controlled adjacent rooms. The housing facilities were on a 12:12 light/dark cycle, with lights on at 8:00 am. All testing was done during the light phase of the cycle under low illumination.

**Experiment 1A**

This longitudinal study of juvenile rat play was conducted with the intent of assessing the specific play behaviors involved in the decline of “positive-affect” (50-
kHz) USVs and emergence of “negative-affect” (22-kHz) indicative USVs over the course of a prolonged play session. Animals were between the ages of 28-31 days at the start of testing and 48-51 days of age at completion.

The apparatus

All play testing took place in a 30 x 30 x 50 cm high clear Plexiglas chamber with three of the sides covered with brown paper. One half inch of corn-cob bedding covered the floor of the chamber. Fecal pellets were removed after each play session, but the bedding was not changed throughout the course of the study. A video camera was set up to record each session with Pinnacle Studio software. Ultrasonic vocalizations were recorded with SeaPro (CIBRA) software, which produces visually scorable sonographic representations of vocalizations that occur at frequencies outside of the human hearing range.

Experimental procedure

Animals were assigned to a same-sex, weight matched sibling play pair that remained constant for the duration of the study. Play pairs were habituated twice to the test boxes for 5 minutes on the two days preceding the start of testing. During all testing, play pairs were carried together in a transfer box to the testing room and were placed in the test chamber. Their social interactions were recorded for 30 minutes. This was repeated at five-day intervals for a total of four test days distributed across adolescence.
Frequencies of play behaviors (pins and dorsal contacts) during the 30-minute testing period were scored on-line by an experimenter in the room with a manual counter. Vocalizations were scored off-line and the audio files were synchronized to the video files so that relationships between USVs and specific play behaviors could be determined. During each of the test days, data was harvested for six successive 5-minute play periods during the 30-minute play opportunities.

To increase familiarity with their play partners, on the two days prior to each testing day, animals were allowed one unscored 30-minute play session per day. These play sessions were conducted in a cage similar to their home cage but were lined with corn-cob bedding and did not provide access to food or water. Each rat pair played in the same cage throughout the experiment.

Statistics

Analysis of Variance (ANOVA) was used to assess the differences in frequency of pins, dorsal contacts and vocalizations across the testing days and within 30-minute play sessions. Student’s t-tests were used when appropriate. Dominance patterns were determined by calculating the percentage of pins each animal made out of the total pins made by the pair. Only data from test days 3 and 4 were used for this analysis to allow the animals time and experience to develop true dominance patterns. It was also on these days that 22-kHz USVs were most evident.
**Experiment 1B**

This experiment was conducted as a psychoassay to elucidate the affective phenotypes of the animals used in Experiment 1A. Two days after the final day of play, subjects were exposed to a standard 2-minute tickle assay to reveal each individual’s baseline response to tickle treatment.

*Experimental Procedure*

On the day before animals were tested, they were habituated to the test chamber; a 50x25x30cm glass aquarium lined on the bottom with 0.5 cm thick carpeting. For habituation, animals were tickled for the first 15 seconds after being placed in the box, allowed to explore freely for two minutes, then tickled again for an additional 15 seconds. Tickling procedure consisted of one experimenter quickly moving his or her fingers right hand across the rat’s dorsal side with movements concentrated on the nape. Then the rat was quickly flipped onto its back and the ventral surface was vigorously tickled for a few seconds. The rat was released and the process was repeated in a playful fashion until 15 seconds had passed (Panksepp & Burgdorf 2000). All USVs were recorded on-line by another experimenter.

To evaluate the reliability of the assay, two experimenters, experienced with rat tickling, each tested half of the rats, counterbalanced on each of two test days. Our standard tickle test consisted of 15 seconds of no stimulation followed by 15 seconds of tickle stimulation. This was repeated four times for a total of two minutes (Panksepp & Burgdorf 2000). Subjects were tickled again 24 hours later. To test the strength of this psychoassay, on the second day, each experimenter tickled the half
set of animals that they had not tested the previous day. USVs were recorded on-line using SeaPro (CIBRA) software by a separate experimenter using a manual counter. Vocalizations occurring in each 15-second interval were counted separately.

Statistics

Correlation analyses were conducted to assess consistent vocalization responses during the tickle stimulation intervals and no-stimulation intervals. ANOVAs were used to determine consistency between experimenters and test days. Animals were ranked based on 50-kHz USV responses during off-tickle periods and categorized as high-, medium- and low- responders (Panksepp & Burgdorf, 2000).

Experiment 2A

To further reveal the playfulness of subjects, animals were allowed to demonstrate their desire to be tickled in a test that allowed the animal to decide whether or not to expose itself to this stimulation. Animals were between 59-65 days of age when testing was conducted.

Experimental Procedure

The glass aquarium from Experiment 1 B was used again. However, this time a glass dome was placed at one end of the box. The dome was 11.5 cm tall and 16.5 cm in diameter across the bottom. There was a 6 cm hole in the top for rats to enter and exit. On the day before testing, rats were exposed to a two-part habituation process. For the first part, they were brought into the testing room and placed in the dome (gently held with both hands, head first, through the hole in the top). Two minutes
were allowed for the rat to emerge, after which it was tickled for 15 seconds and then returned to its home cage. For the second part of habituation, a glass dome was placed in each rat’s cage for five minutes and the animal was allowed to explore it freely during that time.

On the day of testing, each subject was placed in the dome and their latency to emerge into the open field was monitored. When the animal exited the dome with all four paws (coming down either to the floor, or simply sitting on top of the dome), it was quickly picked up and either tickled or pet for 15 seconds on the floor of the test chamber and then placed back into the dome for a second trial. Half the animals were tickled and, as a control, half were pet gently but firmly with the palm of the right hand on the dorsal side of the rat. Petting treatment has been characterized as a reliable control to tickling, as it presents physical interaction between the experimenter and subject, but does not possess the reward value of tickling (Burgdorf & Panksepp, 2011). If the animal did not emerge after two minutes, the dome was lifted and the animal was picked up to be either tickled or petted before being placed back into the dome for a second trial. After the second emergence or manual removal, the animal was again tickled or petted and returned to its home cage. Two days later, animals were tested again and treatment was counterbalanced so the animals that were tickled on the first day were petted on the second test day, and those that had been petted were tickled.
Experiment 2 B

Subjects indicated their motivation to escape from tickling in a test that allowed the animal to hide in the protective zone of the dome after being tickled. They were then allowed two minutes to exit from the dome for another tickle. Animals were between 63-67 days of age when testing was conducted.

Experimental Procedure

The same experimental set-up was used in this test as in Part B. However, this time, upon arrival at the test chamber, the animal was immediately tickled for 15 seconds. It was then released and allowed to roam freely throughout the box for 15 seconds. If the rat had not entered the dome within the 15 seconds of no-stimulation, the procedure was repeated (15 seconds of tickle followed by 15 seconds of no-tickle). This was done up to four times, resulting in a maximum testing time of two minutes for a rat that did not enter the dome. However, if the animal escaped into the dome, it was allowed to stay there until it emerged (with all four paws) on its own or until it had been inside the dome for two minutes. When the animal came forth from the dome, it was given the same procedure of 15 seconds of tickling followed by 15 seconds of no-tickle. This was again repeated until the animal escaped to the dome a second time or two minutes had elapsed. If the animal entered the dome a second time, it was allowed to stay there until it exited on its own or until it had been inside for two minutes. The test was concluded after the rat’s second exit or termination of the session by manual removal.
RESULTS

Experiment 1 A

Overall, when animals were taken from isolation and presented with a play partner for 30 minutes, they played vigorously. Longitudinal age- and gender- related play activity, averaged per day across the four testing days, is characterized in Figure 2, parts a and b. A 4 (day) x 2 (gender) ANOVA revealed a significant main effect of testing day and a significant interaction. Both males and females made more pins and dorsal contacts on day 2 than on day 1 (Bonferroni post hoc, males: t(17)=5.07, p<0.001, females: t(11)=2.74, p<0.05). Numbers of pins and dorsal contacts did not vary within males or within females between test days 2, 3 and 4(p’s>0.05), except that on day 4, females made more pins and dorsal contacts than they had on day 2 (Bonferroni post hoc t(11)=2.74, p<0.05). It is possible that the low level of pins and dorsal contacts on day 1 was due to the habituation nature of this day. Since it was the first day of 30-minute testing, the animals may not yet have been comfortable

![Figure 2](image-url). Dorsal contacts (a) and pins (b) across testing days, averaged for males and females (±SEM). The x-axis shows rat ages on each testing day (PND= Post-Natal Day).
enough to play as robustly as they did on days 2, 3 and 4. Thus, this day will be excluded from further analysis. Although ANOVA revealed overall gender difference, individual t-tests indicated that males made more pins than females on day 2 (t(28)=-1.89, p<0.05), but that females made more pins on day 4 (t(28)=2.39), p<0.05). As pins are regarded to be the consummatory portion of play, this suggests that females express more playfulness than males later in adolescence and into early adulthood. This is examined further in Experiment 2, parts A and B.

Within a given test session, animals played energetically when first placed in the chamber but activity decreased over time. Indeed, 6 (5-minute time period) x 2 (gender) ANOVAs revealed a significant main effect of time on pins (F(5,28)=8.78, p<0.001), dorsal contacts (F(5,28)=27.96, p<0.001) and 50-kHz USVs.

![Figure 3](image-url)

**Figure 3.** Average (±SEM) dorsal contacts (a), pins (b) and 50-kHz USVs per 5-minute time period across play session. For example, the data point for "10 mins" represents the behaviors performed between 5 and 10 mins. Each data point represents an average from testing days 2, 3 and 4. (Note the different y-axis scale on the 50-kHz USVs graph).
(F(5,28)=49.41, p<0.001). (figure 3, parts a, b and c). Thus, these animals played according to the traditional pattern with declining play behaviors as a factor of time (Burgdorf et al., 2006).

Five pairs of rats (three male, two female) made 22-kHz USVs on test days 3 and 4 (Post-natal day (PND) 38-41 and PND 43-46, respectively). These five pairs will be referred to as “complainers” while pairs that did not express 22-kHz USVs will be referred to as “non-complainers.” On test days 3 and 4, significantly more 22-kHz USVs were made during the final 10 minutes of play than during the first 10 minutes of play (F(2, 19)=4.76, p<0.05) (figure 4). In male pairs, sixty-five percent of the 22-kHz USVs were made during pins. However, often the “complaints” continued for a few moments after a pin. Indeed, an additional 15% of 22-kHz USVs occurred within 15 seconds after a pin had ended. Thus, 80% of all males’ 22-kHz USVs occurred either during or immediately after a pin. In females, the percentages

![Figure 4](image)

**Figure 4.** Average (±SEM) 22-kHz USVs per 5-minute period across play session for males and females.
were slightly lower, with 55% of 22-kHz USVs occurring during pins and 70% occurring either during or immediately after a pin. Therefore, it appears that 22-kHz USVs are typically elicited when pins occur and it may be hypothesized that it is the pinned animal that is making these vocalizations. In previous research, it was found that these vocalizations were generally made by submissive animals when adult resident-intruder aggression tests are conducted (Panksepp et al., 2004), but this issue needs further analysis using devocalized dominant animals.

On days 3 and 4, “complainers” made significantly more pins than “non-complainers” (F(9, 19) = 7.84, p < 0.01), but the groups did not vary in rates of dorsal contacts or 50-kHz USVs (p = 0.5, 0.7, respectively) (figure 5). If 22-kHz USVs are indicative of the rise of an overall aversive emotional state, it would be expected that dorsal contacts, which represent motivation to play, and 50-kHz USVs, which represent a positive affective state, would decline at a sharper rate in complainers.

![Figure 5. Average (±SEM) from entire 30-minute play session, across test days 3 and 4, of pins, dorsal contacts (a) and 50-kHz USVs (b) for complainers vs. non-complainers.](image)
than in non-complainers. However, the data does not follow this pattern. This lends support to an alternative hypothesis that the decline in play behaviors within a play session may be more likely due to play satiety in non-complaining as well as complaining animals (as reflected in the declining play in Figure 3). Animals that have been housed in isolation play much more vigorously when given an opportunity than those that have been previously socially housed (Panksepp, 1980). Isolate-housed animals are “hungry” for play and, as they participate in this activity, they may experience satiation. This evidence supports that this is true for both complaining and non-complaining rats.

Additionally, dominance patterns were analyzed to assess the possibility that the play patterns in complaining pairs were defined by one hyperactive animal. If this animal essentially “bullied” the other by constantly pinning it and never allowing reciprocity of play, it might not have been reflected in the previous quantifications of play behavior. Of the 15 pairs of rats, 10 showed marked dominance, meaning that starting on test day 3, one member of the pair consistently made at least 60% of the pins. Overall, complaining animals did not show significantly greater dominance patterns than non-complainers (p=0.2). Dominant animals in complaining pairs on average made 60% (SD=6.3) of the pins while dominant non-complainers made 62% (SD=9.7) of the pins. By accounting for within-pair variation in play behavior, this analysis strengthens the previous conclusion that the decline in play with time is probably due to a satiety effect rather than a prominent negative emotional state.
Experiment 1B

When exposed to the tickle psychoassay, all rats made 50-kHz chirps during the two-minute session, with more chirps during tickle-stimulation than during no-stimulation periods (F(1,29)=207, p<0.001) (figure 6). No variation in tickle responses was found between experimenters (F(1,29)=1.04, p=0.3) or between testing days (F(1,30)=1.16, p=0.28) or between males vs. females (F(1,29)=1.18, p=0.3). The consistency of the data reveals that this test is a powerful psycho-assay, producing consistent results across testing days as well as different experimenters. Indeed, even throughout individual test sessions, subjects’ responses to tickle or no-tickle stimulation were very consistent. The frequency of 50-kHz USVs were highly correlated during both on-tickle periods (r(28)=0.70, p<0.001) and off-tickle periods (r(28)=0.72, p<0.001). The consistency of the data allowed for confident determination of the animals as phenotypically high-, medium-, or low- responders.

![Figure 6. Average 50-kHz USVs made by males and females across two-minute standard tickle assay Eight 15 s intervals. 15 s no-tickle followed by tickle (odd numbers are no tickle, even numbers are tickle).](image-url)
Six out of the ten animals that were “complainers” were categorized as high-responders. One complainer was a medium responder and three were low responders (table 1). Both submissive and dominant animals were found in the low and high categories (table 1). This supports the likelihood that it was a satiety effect rather than the rise of an aversive emotional state that caused complaining animals’ play to decline. These animals’ high scores in the tickle psychoassay appear to reflect a general state of positive affect. It is unlikely that the animals with positive-emotional phenotypes reverted to a negative phenotype within a 30-minute play session. As the complainers also made more pins than non-complainers, this data is consistent with previous findings that animals that are more playful tend to have higher responses to the tickle test (Panksepp & Burgdorf, 2000).

**Experiment 2 A**

This test examined the animals’ motivation to present themselves to be tickled by choosing to emerge from a “safe place” to receive tickling. All animals except one

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<th>tickle phenotype</th>
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**Table 1.** Dominance phenotypes and tickle phenotypes of “complaining” rats. “Dominant” or “submissive” means the animal achieved >60% or <40% (respectively) of pins on test days 3 and 4. “Mildly dominant” and “mildly submissive” means the animal achieved >50%, but < 60% or <50% but >40% (respectively) of pins on test days 3 and 4.
male emerged from the dome at least once. Overall, females exited faster than males (figure 8) \( F(1,29)=4.72, p<0.05 \). However, within sexes, neither males nor females emerged faster for tickling treatment than for petting treatment. Previous experiments have found that tickle-treatment is preferred to petting (Burgdorf & Panksepp, 2001), and indeed, animals in this experiment made substantially less 50-kHz USVs in response to petting than to tickling \( t(29)=15.16, p<0.001 \). However, it is possible that the animals’ dome-emergence latencies were biased in some way by prior experience in the tickle aquarium and a few experiences of petting were not enough to overcome this established association (an issue that cannot be evaluated from the present data). The females’ faster emergence rate suggests that tickling is more rewarding for female than male rats, perhaps because of a general higher level of playfulness in females at older ages. This supplements the evidence from Experiment 1A that found that in late adolescence, females participated in the consummatory phase of play (namely, pinning), at higher rates than males.

![Figure 7](image)

*Figure 7.* Latency to emerge from dome. Average (±SEM) of second latency on day 1 and day 2.
However, it must be noted that the males were significantly larger than the females at the time of this test. Though both sexes appeared to be able to emerge with ease, the process of exiting the dome may have been slightly more difficult for the males due to their larger size.

Experiment 2 B

When animals were first tickled then given the opportunity to enter the dome to escape the treatment, only 17 out of 30 rats entered the dome at least once during the experiment. More males entered the dome than females (13 males vs. 4 females). The first dome entrance generally occurred during the first minute of testing (M=50.3 seconds, SD=28.7) and the animal typically stayed inside for about a minute (M=61.1 seconds, SD=38.9). After remerging from the dome, only six of the 13 males (46%) escaped a second time, while three of the four females (75%) escaped again. However, the animals that entered the dome a second time, especially the females, popped back out again much faster than they had after the first escape (t(7)=3.67, p<0.01) (figure 9). This further supports the conclusion that

![Figure 8](image)

**Figure 8.** Eight out of 12 animals that escaped once, escaped a second time. This figure compares the average (±SEM) second latency to escape of these eight to their first latency to escape.
tickling is a positive affective experience for the rats, even if they sometimes elect to briefly take a break from the procedure. The animals that exhibited avoidance behavior by entering the dome subsequently came out again for another dose of tickling. Many of them then chose to stay out to receive the full two minutes of tickling. The few animals that escaped twice quickly popped out the second time, seemingly in anticipation of another tickle. The escape-emergence behavior shown during this test seemed to be a component of the “tickling game” for the rat. Rather than being an indication of aversion, it is possible that having the opportunity to briefly escape enhances the rat’s positive experience and makes the tickling process even more fun.

DISCUSSION

The evidence provided in this study supports the theory that rat rough-and-tumble play diminishes within a prolonged play session as a result of play satiety, rather than because of the rise of a general aversive emotional state. In some animals, the emergence of 22-kHz USVs may indicate an increasing incidence of social interactions with a negative affective edge. Complaining animals tended to pin each other more frequently than non-complainers, and it was found that the appearance of 22-kHz USVs was associated with the act of pinning. However, motivation to play (as indexed by rates of dorsal contacts) and overall positive affective state (indicated by 50-kHz USVs) did not vary between complainers and non-complainers. Thus, it appeared that complaining animals might have experienced brief bouts of negative emotions while being pinned, but quickly recovered and
continued participating in play until satiated. Future devocalization studies may shed light on the distinct circumstances under which 22-kHz USVs arise. They would allow determination of specifically which animal of a given pair was making the 22-kHz USVs, thus providing further insight to the affective state associated with these calls. However, the issue that may arise with devocalization studies is that ultrasonic vocalizations are vital to coordinating social play. Therefore, the character of the play may be altered if one rat is not able to communicate in such a fashion. Overall, the findings from this study and possible future devocalization studies have important implications in play research, as understanding the emotional state of the subjects is often crucial to drawing reliable conclusions from data.

Additionally, when animals were exposed to a standard tickle test, “complainers” often were categorized as high-responders, indicating an overall positive affective phenotype. Although these animals may have experienced brief bouts of negative affect, as indicated by the appearance of 22-kHz USVS, it is possible that the resilience of their positive-affective phenotype allowed them to quickly recover, causing minimal disruption to their play. The current study also provided evidence supporting the use of this standard tickle test as a powerful psychoassay, as the animals’ behavioral responses were consistent throughout testing days and experimenters. This allowed confident determination of the affective phenotypes of the animals. Finally, the use of a simple glass dome changed the tickle test from a “treatment” to a game-like situation for the animals, allowing
them to express their desire to be tickled and to indicate the reward value of partaking in this activity.
REFERENCES


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