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Relationships between play and responses to tickling in male juvenile rats

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Declarations of competing interest: none

Highlights

- Solitary but not social play increased prior to and potentially in anticipation of tickling sessions
- There were substantial differences between cohorts in their tickling responses and play behaviour.
- Taking account of cohort there was evidence that tickling showed rebound and emotional contagion effects
- Cohort effects may be explained by differences in physical condition prior to tickling.

Abstract

Play is a putatively positive experience and of key interest to the study of affective state in animals. Rats produce 50kHz ultrasonic vocalisation (USVs) during positive experiences, including social play and tickling. The tickling paradigm is intended to mimic social play resulting in positively valanced ultrasonic vocalisation (USV) production. We tested two hypotheses on the relationship between tickling and play: that tickling would increase play behaviour or that play behaviour would increase in anticipation of tickling, and that tickling would share some specific properties of play (rebound and emotional contagion of unexposed cage mates). Male Wistar rats (N=64, with 32 rats/cohort) of 28 days of age were housed in pairs with one rat assigned to be tickled and one as the non-tickled control. Production of 50kHz USVs and hand-following behaviour was measured. Prior to handling, solitary and social play was recorded for 5 minutes in the home cage. A two-day break in tickling was used to assess a potential rebound increase in responses to tickling. Only one rat within each cage was handled to assess emotional contagion through changes in the behaviour of the cage-mate. Solitary but not social play increased prior to tickling relative to controls ($p = 0.01$). There were marked differences between cohorts; tickled rats in C2 produced less 50kHz USVs than those in C1 ($p = 0.04$) and overall, C2 rats played less than rats in C1 (social $p = 0.04$ and solitary $p < 0.001$) and had a lighter start weight on arrival ($p = 0.009$) compared with cohort 1 (C1). In C1, there was evidence of rebound in USV production ($p < 0.001$) and a contagious effect of tickling reflected by increased hand-following in cage mates ($p = 0.02$). We found a positive relationship between start weight and USV responses to tickling ($R_s = 0.43$, $p < 0.001$), suggesting that the divergence in USV production may be due to developmental differences between cohorts. The results suggest that the relationship between tickling and play is complex in that tickling only affected solitary and not social play, and that tickling responses showed rebound and contagion effects on cage-mates which were specific to cohort responses to tickling.

Keywords Tickling; Play; Male rats; Positive affective states; Positive animal welfare

1. Introduction

Play behaviour has been proposed as an indicator of positive affective states (Boissy et al., 2007). In human children, play is important within developmental psychology, being a key indicator of cognitive and physical development, as well as positive affect (Piaget, 1952). More recently, play has become of key interest to the study of positive affective states in animals (for a recent review, see Graham and Burghardt, 2010), a timely change as the vast majority of animal welfare research has been, and continues to be, focused on more negative aspects of animals' lives (Lawrence et al., 2017). This focus on negative affect has led to a relative lack of quantifiable models of positive affective state in animals.

Absence of play is widely recognised as an indicator of negative psychological and environmental conditions (e.g. Ahloy-Dallaire et al., 2017, Bateson, 2014; Boissy et al., 2007; Burghardt, 2005; Held and Špinka, 2011; Lawrence, 1987). This sensitivity of play to the environment, e.g. food availability, social conditions and experiencing pain (reviewed in Held and Špinka, 2011), suggests that play only occurs when primary survival needs are met and immediate fitness is secure (Boissy et al., 2007; Lawrence, 1987). However, of more significance to positive animal welfare is the neurobiological and behavioural evidence that play is thought to represent a positive psychological state (Siviy, 2016), and is often used to reduce anxiety and negative emotion in children (Li et al., 2016).

Play behaviour is thought to be self-rewarding (Trezza et al., 2010), occurring spontaneously in all mammalian species and perhaps also in other animal classes (Graham and Burghardt, 2010). Indeed, animals place such value on social play that in a social discrimination task, they will show preference for access to social interactions involving play (Humphreys and Einon, 1981). An increase in play performance is also seen after a period of temporary social or locomotor deprivation (Hole, 1991). This rebound effect occurs when a new opportunity for play is presented, either through increased space or presentation of a play partner, with examples in rats (Ikemoto and Panksepp, 1992), as well as pigs (Wood-Gush et al., 1990) and calves (Jensen, 1999). For example, calves under confinement will conduct more locomotor-rotational play (bucks and leaps) than unconfined calves upon release (Jensen, 1999) with social isolation commonly used to increase subsequent social play in rat studies (e.g. Panksepp and Beatty, 1980). Play can also be stimulated in others by simply seeing another animal play (Bekoff, 2001), with exposure to more playful partners increasing levels of play in rats (e.g. Pellis and McKenna, 1992). As the behaviour of one rat was changed by the behaviour of another, it can be assumed that emotional contagion has occurred, whereby the emotional state of one animal is transferred to another (Held and Spinka, 2011). Although this contagious property of play has been documented, it has received little attention despite having the potential to spread a positive emotional state (Held and Spinka, 2011).

In rats, the production of 50kHz ultrasonic vocalisations (USVs) are associated with positive behaviours, such as feeding and mating (Schwarting et al., 2007). Juvenile rats will also emit these vocalisations during social play, as well as in anticipation of social play (Knutson et al., 1998). It is well established that social behaviour (including social play) is regulated by the actions of endogenous opioids (Vanderschuren, 2010). The play response to playback of 50kHz USVs can be negated by administration of the opioid antagonist naloxone and enhanced by administration of the opioid agonist morphine (Schwarting et al., 2007). These positive USVs may be one method by which play facilitates emotional contagion in rats (Schwarting et al., 2007). Although the true function of play is still unclear from an evolutionary perspective, play is likely to provide an animal with psychological benefits through an opioid-mediated pleasurable experience (Vanderschuren et al., 1995) while improving skills such as social interaction (Pellis and Pellis, 2007).

The heterospecific tickling model aims to mimic the ‘rough and tumble’ aspects of play without the need to wait for the spontaneous occurrence of play (Panksepp and Burgdorf, 2003). During play, rats partake in a series of chasing and wrestling movements, with rapid movement ceasing when one rat allows the play partner to pin it onto it’s back with the play partner on top (Pellis and McKenna, 1992). Tickling aims to mimic this interaction by stimulating areas which are contacted during play, as well as replicating pinning behaviour by turning the rat onto it’s back (Panksepp and Burgdorf, 2003). Some rats produce plentiful positive (50kHz) USVs during tickling and will actively seek interaction with the experimenter (Burgdorf and Panksepp, 2001). As with play, tickling is thought to activate neural pathways associated with positive affect, particularly the mesolimbic dopaminergic system or reward system (Ishiyama and Brecht, 2016; Burgdorf and Panksepp, 2006). For example, Hori et al., 2013 found that tickling increased dopamine release in the nucleus accumbens from baseline levels, with no increase found in rats which received light-touch stimulation. Activation of the mesolimbic pathway is also induced by anticipation of a reward (e.g. Spruijt et al., 2001; Schultz, 1997). Following the logic of Dudink et al. (2006), if tickling activates the mesolimbic dopaminergic system, rats should learn to anticipate the rewarding experience of tickling leading to an increased expression of play behaviour which is also known to depend on this system (Held and Spinka, 2011; Dudink et al., 2006).

Given this the aims of this study were: (a) To investigate whether the predicted positive experience of tickling would increase play behaviour. Considering that tickling is intended to mimic social play and that they share neural substrates, we hypothesised that a) tickled rats would show an increase in social play prior to tickling and b) tickling responses, as with play, would show rebound following a period without tickling and also have contagious effects on cage-mate vocalization production and approach behaviour.

2. Materials and Methods

All work was carried out at the Roslin Institute, Edinburgh, U.K., in accordance with the U.K. Animals (Scientific procedures) act 1986. Ethical approval was granted by the Roslin Institute Animal Welfare and Ethical Review Body (AWERB) and the Royal (Dick) School of Veterinary Studies Veterinary Ethical Review Committee (VERC).

2.1 Subjects, housing and husbandry

Male Wistar rats (N=64; 2 cohorts of 32) were obtained from Charles River Laboratories, Tranent, Scotland. Adolescent males (28 days old) were used, as social play in rats is seen to peak between 30- and 40-days post-partum before declining until puberty at around 60 days, with males typically expressing higher absolute frequencies of play fighting (Pellis and Pellis, 2013). Studies investigating the tickling paradigm typically also use adolescent males with adolescents robustly showing stronger tickling responses than adults (Burgdorf and Panksepp, 2001). The Wistar strain is the 3rd most commonly used rat strain for tickling experiments (23%, n = 13) (LaFollette et al., 2017). On arrival, rats were housed in pairs, randomly allocated to home cages, then left to acclimatise for 6 days prior to handling. Home cages were made of clear plastic with a metal mesh open-top lid (l x d x h: 48cm x 26.3cm x 20.5cm; Techniplast, Italy) with aspen chip shavings topped with wood fibre bedding (Estonia, England), a chewable wooden block (Datesand, England), and with *ad libitum* access to food (Teklad Global Rodent Maintenance Diet (14% protein); Envigo, England) and tap water. Clean bedding was provided on the morning of day 10 of the 32-day-long study, 3 days before the experimental phase began. Cages were assigned to being tickled or neutrally handled controls with one tickled cage and one control cage together as a “block”. Within the cage, one rat was randomly allocated as the handled rat (to be tickled or neutrally handled), with their paired cage mate not being handled until the final day (Section 2.2). Cages were distributed across four tiers of a standard rodent rack (Techniplast, Italy) with lux levels, measured using an Isotech digital light meter Lux-1337, varying across each tier due to the design of the rack and cages (top tier: 44 lx; 2nd tier: 25 lx; 3rd tier: 45 lx; bottom tier: 6 lx). To account for this variation in light, treatment and control cages were balanced across all rows. Rats were kept under a 12:12 light: dark cycle (light from 7:00 a.m. to 7:00 p.m.), with an average room temperature of 22.4 ± 0.3˚C and relative humidity of 43 ± 5%. The allocation of treatment or control of each cage was inverted for the second cohort of rats. This aimed to control for effects of tier level and distance from human activity on behaviour (Cloutier and Newberry, 2010). All handling was conducted by a single female experimenter (TH) to reduce handling stress and create consistency in tickling.

2.2 Handling procedures

Habituation was conducted over 5 days prior to the experimental phase, aiming to expose the handled rat gradually to test conditions, first as pairs (5 minutes exposure to the arena and handler; days 1, 2 and 3), then individually (5 minutes; days 4 and 5) (**Figure 1**).

Figure 1.

Handling took place every weekday for 2 weeks, with a 2 day break after 5 days to study rebound effects (**Figure 1;** see below for details). Trial order was randomised for each day. All handling was conducted in a procedure room away from the main holding room, during the light phase in the afternoon (12.00 h to 17:00 h). Rats were transported to the procedure room on a trolley in the home cage without being individually handled. During travel, the cage was covered by a large dark towel to prevent exposure to bright light as the rats were tested in their dark photoperiod.

Red lighting produced a low-intensity illumination of the procedure room (4.5 lx). Rats, especially albinos, prefer lower light intensities due to high visual sensitivity (see Burn, 2008, for a full review), with positive USV production being reduced during exposure to bright light (Knutson et al., 1998).

The handling arena was a transparent, open top box (l x d x h: 51cm x 42cm x 23.2cm; VetTech Solutions Ltd, England) with the base covered with disposable and absorbent non-slip bench liner (LabMat, LabLogic Systems Ltd., England) secured with masking tape to provide a non-slip surface which would not disturb vocalisation recordings by producing excessive background noise, as found with litter during in-house pilot studies. At the end of each test day, the arena was cleaned with 70% ethanol and new matting secured.

Tickling involved the experimenter using one hand, covered by soft knitted glove, to touch, tickle, chase and pin the rat in a manner that mimics rough and tumble play (Bombail et al., 2019). Rats were tickled in this way for repeated bouts of 20 seconds alternated with 20 second “pauses” lasting for a total of 3 minutes (adapted from Panksepp and Burgdorf, 2010). This allowed for 4 periods of active tickling (total 1min 20 secs) interspersed with 5 periods of pauses. For control animals, during the active tickling phases the gloved hand rested in the centre of the arena. For all rats, during pauses the hand moved in slow circles around the arena while wiggling the fingers to gain the attention of the rat and to measure approach behaviour, as the duration and counts of hand-following events (Lampe et al., 2017, Melotti et al., 2014) (see section *2.4*).

2.3 Recording and analysis of vocalisations

Vocalisations produced during handling in the arena were recorded using a high-quality condenser microphone designed for recording ultrasonic vocalisations produced by bats (Pettersson M500-384 USB Ultrasound microphone, Pettersson Electronik; Sweden) and a free recording software (Audacity, Version 2.1.3, Pennsylvania, United States of America). The microphone was placed over the centre of the arena, pointing downwards 61.5 cm from the arena floor. Vocalisations were manually counted from spectrograms produced using Audacity software (Version 2.1.3, Pennsylvania, United States of America). Spectrograms were generated with a fast Fourier transform length of 512 points with a Hanning window (50% overlap frame). Only 50kHz vocalisations (peak frequency between 30 and 80 kHz and a duration between 10–150 ms) were counted as the production of 50kHz USVs are associated with positive behaviours and were used as an indicator of positive experience, (Brudzynski, 2009; Wright et al., 2010; LaFollette et al., 2018). Overlapping calls were counted as one call (Wright et al., 2010), with only clearly categorizable vocalisations counted.

2.4 Recording and analysis of hand-following behaviour

The behaviour of tickled and control rats in the arena was recorded using a second Sony HD camcorder (HDR-CX405). Observer XT 11 software was used to analyse the duration of hand-following (HF) events during the pause section of each handling session to gain information on approach behaviour (Lampe et al., 2017). The duration (in seconds) and counts of hand-following events were calculated for each rat using focal observation sampling with continuous recording. Rats were deemed to be hand-following when the nose was oriented towards the hand and was actively moving towards the experimenter's hand. The experimenter moved the hand in circles in the same manner for all rats, standardised by counting the number of circles made by the hand, to help assess whether movement towards the hand was intentional (Melotti et al., 2014).

2.5 Recording and analysis of play behaviour in the home cage

Prior to handling and immediately after being taken to the procedure room, behaviour in the home cage was recorded using a Sony HD camcorder (HDR- CX405) for 5 minutes to measure anticipation of interaction with the handler. Videos were analysed using Observer XT 11 software (Noldus Information Technology, Wageningen, the Netherlands). The frequency of solitary play and the frequency and direction of social play were scored using focal observation sampling with continuous recording (see **Table 1** for full ethogram of behaviours scored). Solitary play was scored as a measure of individual play with an event being defined as finished when the rat ceased movement or engaged in behaviours other than the leaps and running described in **Table 1,** with the behavioural unit as the number of solitary play events per 5 minutes. Initiation of social play was scored as a measure of the appetitive motivation for social play. A bout of social play was deemed to be finished when rats had no contact with each other for 2 or more seconds, with the behavioural unit as the number of initiations of social play events per 5 minutes. Intra-observer reliability was high (Cohen's kappa = 0.93), with a percentage of agreement of 98.7% - only one observer scored all behaviours.

Table 1.

2.6 Test of rebound effects

To explore potential rebound effects, after 5 days of continuous handling all rats were given a 2-day break, then handled for another 5 days. A rebound effect was defined as an increase in USV and hand following responses to tickling (relative to day 5) on the first day after the 2-day break (day 6).

2.7 Test of emotional contagion

To explore whether there was a contagious effect of tickling, we investigated the behaviour of the paired (previously unhandled) cage mate on the final test day (day 10) after being housed with a rat which was tickled or control handled for 10 days. During the 10 days of handling, cage mates remained in the home cage (fitted with a wire lid) and were placed on the bench next to handling arena. This allowed the cage mate to have auditory and visual contact when their paired rat was handled. After the handled rat had experienced tickling or control handling, all cage mates were subjected to the same procedure and conditions as control rats to investigate whether treatment (i.e. being housed with a tickled or neutrally handled rat) influenced USV production or hand-following of the cage mate. Cage mates had 2 minutes of contact with their paired handled cage mate in the home cage prior to being moved to the arena and tested. As such, emotional contagion could be facilitated by a long-term mechanism in which the behaviour of the previously unhandled cage mate could be altered by being exposed to another rat whom was routinely tickled. Responses were recorded and measured as in Sections 2.3 and 2.4.

2.8 Body weight measurement

Following handling, body weight of both handled rats and cage mates was measured weekly by placing the rat in a box on an electronic scale (OHAUS Adventurer Pro AV2101).

2.9 Statistical analysis

All data from the first and fifth day of consecutive handling were used in the analyses. Following a two-day break from handling, data were also collected from the sixth and tenth day (see **Figure 1**). Basic descriptive statistics and correlations were calculated using Minitab 18. For the majority of analyses, Generalised Linear Mixed Models (containing both fixed and random effects) were fitted in Genstat (16th Edition) using the REML algorithm with a log link function, a Poisson error distribution and dispersion parameter fixed at one. To investigate differences in play and handling responses (USV production and hand following) in handled rats between cohorts the fixed effects part of the model comprised treatment, cohort, day, treatment X cohort x day interaction effects. The random effects part of the model reflected the fact that cages were nested within pairs of cages and they were all crossed with the two runs in which different cohorts were tested, as well as the fact that there were four repeat observations over 2 weeks on each cage per cohort. As there was a significant interaction between responses to tickling and cohort, each cohort was also analysed independently to investigate rebound and contagion properties where effects may have been masked when fitted together. To investigate a potential rebound effect, we looked at the difference between handling responses on day 5 compared to day 6 within each cohort. Fixed effects within the REML model were treatment and day and the interaction between treatment and day. The random effects part of the model reflected the fact that cages were nested within pairs of cages. Cage mate handling responses (USVs and hand following) during a single test were used to investigate contagion. When fitted for both cohorts together, the fixed effects part of the model comprised treatment, cohort and a treatment X cohort interaction effects. The random effects part of the model reflected the fact that cages were nested within pairs of cages and they were all crossed with the two runs in which different cohorts were tested. Due to the difference in cage mate responses between cohorts, responses were fitted independently, with treatment as the only fixed effect. Predicted means and associated standard errors of the mean (SEM) reported were back transformed to the original scale produced by the REML output. As weight data met the assumptions of normality, two ANOVAs were used to investigate a potential difference in start and final weight in all rats between cohorts, with cohort fitted as a factor. To investigate the potential influence of physical condition on responses to tickling we ran a Spearman’s rank correlation on tickled rats in both cohorts between start weight and tickling responses across the four test days. For all tests, the level of statistical significance was set at $p < 0.05$.

3. Results

3.1 Cohort effects on USV production in response to tickling

Overall, combining both cohorts, tickled rats produced more 50kHz USVs than control rats (tickled vs. controls $M \pm SEM$ = 82.3 vs. 32.8 ± 5.91; $F_{1,14} = 8.18$, $p = 0.013$; **Figure 2a**). Tickled rats also showed increased HF compared with controls (tickled vs. controls $M \pm SEM$ = 5.9 vs. 1.9 secs ± 1.22; $F_{1,14} = 19.38$, $p = 0.004$; **Figure 2b**).

Figure 2

However, a significant interaction was observed between treatment and cohort in USV production; tickled rats in cohort 1 performed more 50kHz USVs than those in cohort 2 (C1 vs. C2 $M \pm SEM$ = 125.9 vs. 53.8 ± 4.59; $F_{1,14} = 5.18$, $p = 0.039$). There was also an interaction between cohort and day within tickled rats ($F_{3,14} = 42.4$, $p = 0.003$), with USV production increasing across days in cohort 1 (day 1 vs. day 10 $M \pm SEM$ = 114.6 vs. 186.8 ± 5.9) and decreasing in cohort 2 (day 1 vs. day 10 $M \pm SEM$ = 92.5 vs. 43.6 ± 5.9; **Figure 3**). This interaction was not seen in USV production of control rats, with no significant difference between cohort (C1 vs. C2 $M \pm SEM$ = 85.1 vs. 97.5 ± 4.68; $F_{3,14} = 0.6$, $p = 0.439$) and no effect of day (day 1 vs. day 10 $M \pm SEM$ = 78.4 vs. 112.6 ± 3.35; $F_{3,14} = 1.99$, $p = 0.121$; **Figure 3**). As such, the effect of tickling on USVs was dependent on cohort. There was no cohort effect in HF duration ($F_{1,14} = 1.84$, $p = 0.202$).

Figure 3

3.2 Tickling (and handling) induce play behaviour

In handled rats in both cohorts, there was a significant effect of tickling on solitary play, with tickled rats playing more than controls in the 15 minutes before handling (tickled vs. controls; $M \pm SEM$ = 1.01 vs. 0.63 ± 0.18; $F_{1,31} = 6.86$, $p = 0.01$; **Figure 4**). In both cohorts, there was no effect of tickling observed on social play ($F_{1,14} = 2.58$, $p = 0.11$). Across 4 days of testing, both tickled and control rats in cohort 1 consistently performed more play events than cohort 2 (solitary: C1 vs. C2 $M \pm SEM$ = 0.99 vs. 0.47 ± 0.89 play events per 5 minutes, $F_{1,14} = 4.19$, $p = 0.042$; social play initiations: C1 vs. C2 $M \pm SEM$ = 2.62 vs. 2.10 ± 1.08 play initiations per 5 minutes, $F_{1,14} = 14.79$, $p < 0.001$) with no significant effect of day (solitary: $F_{1,14} = 3.18$, $p = 0.369$; social: $F_{1,14} = 0.73$, $p = 0.867$).

Figure 4

3.3 Tickling responses show cohort-dependent evidence of rebound and contagion properties

To investigate rebound, we examined the difference in handling responses (USVs and HF) before and after a two-day break. Due to the previously observed cohort/day interaction (Section 3.2), cohorts were tested independently to investigate rebound and contagion properties where effects may have been masked when fitted together. In cohort 1, there was a significant interaction between day and treatment in USV production ($F_{1,14} = 132.16$, $p < 0.001$), but not HF ($F_{1,14} = 2.95$, $p = 0.106$), with tickled rats showing an increase in USV production on day 6 compared to day 5 (day 5 vs. day 6 tickled rats *M* ± *SEM* = 105.5 vs. 141.5 ± 5.91; **Figure 3**). This effect was not seen in cohort 2 in either USV production ($F_{1,14} = 0.16$, $p = 0.69$; **Figure 3**) or HF ($F_{1,14} = 0.36$, $p = 0.55$).

Evidence of a contagious effect of tickling on cage mate behaviour was also specific to cohort. Between cohorts there was a significant difference in cage mate performance of both 50kHz USVs (C1 vs. C2 M ± SEM = 131.97 vs. 13.31 ± 1.17; $F_{1,14} = 16.71$, $p < 0.001$) and HF (C1 vs. C2 M ± SEM = 7.19 vs. 154.69 secs ± 1.11; $F_{1,14} = 19.30$, $p < 0.001$) when tested on day 10. When cohorts were fitted independently, cohort 1 cage mates of tickled rats showed increased HF compared with cage mates of control rats (cage mates of tickled vs. controls *M* ± *SEM* = 14.51 vs. 1.27 secs ± 1.17; $F_{1,14} = 0.59$, $p = 0.023$; **Figure 5**). This was not seen in USV production ($F_{1,14} = 0.07$, $p = 0.79$). There was no treatment effect on cage mate behaviour in cohort 2 (50kHz USVs: $F_{1,14} = 4.87$, $p = 0.90$; HF: $F_{1,14} = 0.04$, $p = 0.84$).

Figure 5.

3.4 Potential influence of physical condition on response to tickling

Differences in physical condition were observed between cohorts as rats in cohort 2 were lighter on arrival (C1 vs. C2 *M* ± *SEM* = 103.0g vs. 89.54g ± 3.40; $F_{1,63} = 7.83$, $p = 0.009$) and at the end of the experiment (C1 vs. C2 *M* ± *SEM* = 264.87 vs. 235.94g ± 5.07; $F_{1,63} = 16.25$, $p < 0.001$). To investigate the potential influence of physical condition on responses to tickling we ran a Spearman's rank correlation on tickled rats in both cohorts between start and final weight and tickling responses across the four test days. There was a statistically significant positive correlation between start weight and average USV production across all days ($R_s = 0.43$, $p < 0.001$; **Figure 6**) with no relationship between start weight and HF ($R_s = 0.19$, $p = 0.14$). No relationships were found between final weight and tickling responses (USVS; $R_s = 0.23$, $p = 0.07$: HF; $R_s = (-) 0.04$, $p = 0.73$).

Figure 6.

4. Discussion

4.1 General discussion

Tickling aims to mimic rough and tumble social play between rats (Cloutier et al., 2018; Panksepp, 2000) with evidence suggesting that tickling activates the same reward mechanisms as play. However, considering the proposed relationship between these hedonic experiences, there has been little investigation into the relationship between tickling and play. We found that tickling male juvenile rats increased solitary play but not social play before a predicted tickling experience. There were also substantial differences between cohorts in their responses to tickling and play. Taking account of these cohort effects, we found evidence that tickling and play share similar properties, inducing rebound and having a contagious effect on cage mates. Differences between cohort responses may be explained by the divergence in physical condition between cohorts on arrival and throughout the study when taken as an indicator of early life experience.

4.2 Vocalisations as an indicator of affective state

Quantification of 50kHz USVs are the most commonly used measure to assess responses to tickling and are often used to infer a positive affective state (e.g. Panksepp and Burgdorf, 2000). Overall, the production of USVs were consistent with the idea that tickled rats in this study were in a more positive state, with tickled rats producing over twice the average of 50kHz vocalisations of control rats. A recent review supports these findings, with 94% (n=15/16) of all tickling experiments reporting that tickled rats produced more 50kHz USVs than controls (LaFollette et al., 2017). However, we found differences in USV production in response to tickling between cohorts; our first cohort produced significantly more 50kHz USVs than the second cohort, with production increasing across days in cohort 1 but decreasing in cohort 2.

4.3 Approach behaviour as an indicator of motivation to be tickled

In our other measure of response for tickling, we found no effect of cohort with tickled rats following the experimenter's hand for longer than controls in both cohorts. This suggests that tickled rats in both cohorts were as equally motivated to interact with the handler. Following the same protocol as Lampe et al. (2017), approach behaviour was assessed by the duration and number of hand-following events in the time gap between each handling stimulation. Approach tests in general have received criticism for their lack of sensitivity in discriminating between emotions and arousal, for example, enjoyment and curiosity (Waiblinger et al., 2006). Although supporting a treatment effect between tickled and control rats, approach behaviour protocols such as the one used here may not be sensitive enough to pick up cohort differences in responses to tickling. Supplementation with an evidenced indicator of emotions during approach behaviour, such as use of facial indicators (Finlayson et al., 2016; Sotocinal et al., 2011) would aid discrimination. This highlights the need to use multiple measures to provide complementary information on emotional state, as well as, the need for a comparison of different approaches of assessing how rewarding rats find tickling.

4.4 The effect of tickling on play behaviour

Tickling has been shown to be a positive experience for some rats (e.g. Panksepp and Burgdorf, 2003) with evidence that lines bred for a high USV tickling response display more play behaviour (Panksepp and Burgdorf, 2000). As tickling is intended to mimic social play (Panksepp and Burgdorf, 2003), and both social play and tickling activate the mesolimbic dopaminergic system, we expected that tickled rats would show increased social play behaviour prior to a scheduled handling experience compared with controls. We found more solitary play being performed by tickled rats within the home cage prior to treatment. However, in contrast to our prediction, we found that social play initiation was unaffected by tickling. This distinction between types of play in rats was also noted by Melotti et al. (2014), who found that solitary and social play were unrelated when measured in the home cage. These results correspond with Burghardt's (2005) theory that different types of play may have evolved independently, with solitary play perhaps reflecting anticipation for a rewarding positive experience, in this case tickling.

Expectation of a reward, like play and tickling, activates the mesolimbic system and often results in expression of anticipatory behaviours (e.g. Spruijt et al., 2001; Schultz, 1997). Anticipatory behaviour has been suggested to reflect the value an animal places on a reward state (van der Harst and Spruijt, 2007). It has been suggested that spontaneous behavioural frequencies can be used to assess the current affective state (van der Harst and Spruijt, 2007). We suggest that frequency of solitary play could act as an indicator of anticipation, with solitary play reflecting the increase in reward stimulated by expectation of tickling. Dudink et al. (2006) found that announcement of opportunity to access an environmentally-enriched area facilitated locomotory solitary play behaviour in weaned pigs prior to access. As such play behaviour could reflect anticipation for access to environmental enrichment. Interestingly Dudink et al. (2006) only measured solitary play, suggesting that solitary and social play are differently affected by the reward system.

4.5 Evidence of a rebound effect in tickling

Providing an opportunity for play following a period of deprivation induces an amplification of play known as the rebound effect (Held and Špinka, 2011). Social isolation is commonly used to increase the motivation for social play (e.g. Panksepp and Beatty, 1980) and to induce a behavioural rebound in social play (Held and Špinka, 2011; Loranca et al., 1999; Ikemoto and Panksepp, 1992). Rebound effects in social play in rats occur both with short (a few hours) (Siviy, 2016) and longer periods (up to 14 days) of deprivation (Ikemoto and Panksepp, 1992; Holloway and Suter, 2004), with Niesink and Van Ree (1989) suggesting that 24h of social isolation is required to maximize rebound in social play. We found that a two-day cessation of tickling led to a cohort-specific increase in USV production in cohort 1. Considering the higher USV production by tickled rats in the first cohort and the presence of a rebound effect in the first, but not second, cohort, this suggests that a rebound effect in tickling is dependent on responsiveness to tickling as reflected by USV production. Further, this finding suggests that isolation and the associated complete absence of play is not necessary to bring about the rebound effect, as previously suggested because our rats were group housed (Holloway and Suter, 2004).

4.6 Evidence of tickling-induced contagion

We also found a cohort-specific effect of contagion related to tickling. Play behaviour is proposed as a contagious activity in that the observation of animals playing can induce play in others (Bekoff, 2001). Further, play is also thought to represent an example of emotional contagion through the transfer of a positive emotional state, assumed to be present during play, between play partners (Held and Špinka, 2011). Play has specific cues and signals which may influence others (such as play bows in canids; Rooney et al., 2001), with more playful individuals inducing play in another animal through increased play cues (Pellis and McKenna, 1992). Along with the pinning, scampering and leaping movements which characterize rat play (Pellis and Pellis, 1991), USV production may be a key signal by which the transfer of positive emotions between individuals occurs. We expected that cage-mates of tickled rats would show increased production of 50 kHz USVs and increased hand-following behaviour when exposed in a single test to the control conditions of handled animals (i.e. placed in the handling arena with human hand in the neutral position). This would indicate a more positive emotional state transferred to them by their tickled cage-mates.

We found that in the first cohort, cage mates of tickled rats were more motivated to interact with the experimenter than control rats. This discrepancy between cohorts provides further evidence that additional effects of tickling (e.g. rebound and contagion) are reliant on responsiveness to tickling as measured by USV production. Although there was no effect of treatment on USVs, cage mates in cohort 1 produced almost ten times the number of USVs than cohort 2 which may have influenced their cage mate. This is consistent with the idea that contagion is spread through greater USV production from the tickled animal in the home cage (Saito et al., 2016). USVs have already been found to evoke cognitive bias by Saito et al. (2016) with rats responding to ambiguous cues as positive after hearing FM 50-kHz USVs and negative after 22-kHz USVs. Further investigation should investigate what is signaling the change in behaviour in cage mates, whether it is USVs or some other signal.

4.7 Potential influence of early life on play and tickling responses

As well as an attenuated response to tickling in terms of USVs and HF, the second cohort of rats, including handled rats and cage mates, also showed reduced overall play behaviour (both solitary and social) compared to the first cohort. Play behaviour is known to be affected by early life conditions. In animal models, prenatal stress has long been known to cause a number of long-term disturbances including enhanced anxiety and a reduction in social play during adolescence (Ward and Stehm, 1991). Further, post-natal manipulations, such as prolonged maternal separation (Arnold and Siviy, 2002), and early fostering or handling (Maccari et al., 1995; Wakshlak and Weinstock, 1990) can directly affect the interaction between mother and pup and consequently influence early life development

(Morley-Fletcher et al., 2003). There are also multiple lines of evidence that rats handled between birth and weaning (approx. 0 – 28 days post-partum) exhibit less negative emotionality (i.e. anxiety) than rats handled later in life (for a review see Hertenstein et al., 2006). As such, unknown early life experiences may have resulted in a reduced propensity to partake in hedonic experiences in rats within cohort two, indicated by reduced play and tickling responses.

In order to investigate these unexpected cohort effects on tickling, we used start and final weight as indicators of development in early life. Rats in the second cohort were lighter on arrival and at the end of the experiment. As an exploratory investigation, rats who were heavier on arrival across both cohorts showed more pronounced responses to tickling as measured through 50kHz USV production. As we found no relationship between final weight and tickling responses, this suggests there is an influence of physical condition from before the experiment rather than concurrent with the experiment. In several species, low birth weight shows an association with reduced total play (rats; Morley-Fletcher et al., 2003, pigs; Brown et al., 2015; Litten et al., 2003 and horses; Cameron et al., 2008). As also noted by Brown et al. (2015), our findings fit well with Burghardt's (2005) surplus resource theory, which proposes that play evolved to occur only when juveniles were provided by the parent(s) with periods of sufficient resource availability and protection. As such, the second cohort of animals may have needed to allocate more resources towards growth, resulting in a decrease in the motivation to play, both with another rat and with a human during tickling, as indicated by 50kHz USV production.

Cohort effects are rarely discussed within animal behaviour literature. However, differences between supposed replicates have important implications for many rodent studies using sensitive behavioural assays. Although sourced from the same breeder, of the same age, sex and strain, we found differences between cohorts in USV response to tickling and play behaviour. This is one of few studies to report significant cohort-to-cohort differences in rat behaviour and physical condition. The one other study known to the authors reported significant cohort variability in the acquisition and performance of a skilled reaching task in Long-Evans rats (O'Bryant et al., 2011). The scarcity of evidence may be due to a lack of replicates across cohorts or because of reporting bias towards positive results (e.g. Dickersin, 1990). Ultimately, testing for differences between cohorts is an important consideration in attempts to control for within experiment variability. It also has the potential to yield understanding of mechanisms underlying behavioural responses in this case the relationship between physical condition and responses to tickling.

5. Conclusion

In conclusion, our results show a previously unfound relationship between tickling and play. The positive affect induced by tickling was specific to solitary play and may reflect a positively valanced anticipation to be tickled. Like play, responses to tickling increased following a short break, with tickling having a contagious effect on cage mate responses. However, rebound and contagion effects were dependent on cohort, requiring augmented responses to tickling and a baseline level of play. Overall, our results suggest that the effectiveness of tickling as a positive experience for rats may be influenced by early life experience.

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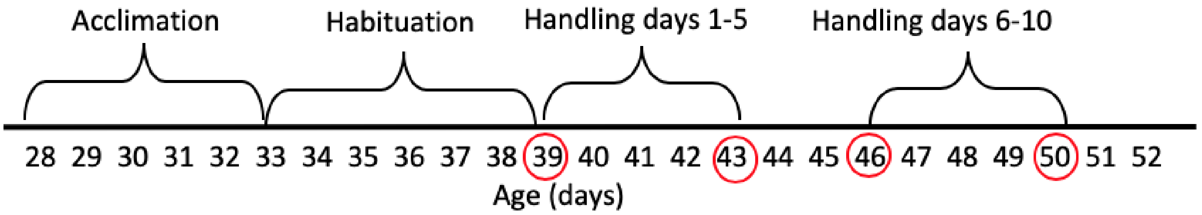

Figure 1. Experimental timeline according to the age of the rats. Red circles indicate days from which data were collected and analysed.

Table 1. Ethogram describing play behaviour of pair-housed juvenile male Wistar rats (N = 64 split into 2 equal cohorts; aged between 39-50 days old) in the home cage 5 minutes prior to experiencing either tickling or control handling.

Behaviour	**Description**
Solitary play	Seemingly spontaneous burst of motion involving at least two hops, where hops involve all four paws leaving the ground at the same time; can occur from stationary of during locomotor movement; not in the direction of a play partner during a play bout or as an evasion response to being chased by a play partner (adapted from Lampe et al., 2017)
Social play	One rat pounces or rubs on the partner, resulting in the partner either chasing the soliciting rat, rearing (in which pairs make rapid pawing movements at each other) or rotating to where one rat is pinned onto its back with the other standing over it (van Kerkhof et al., 2013)

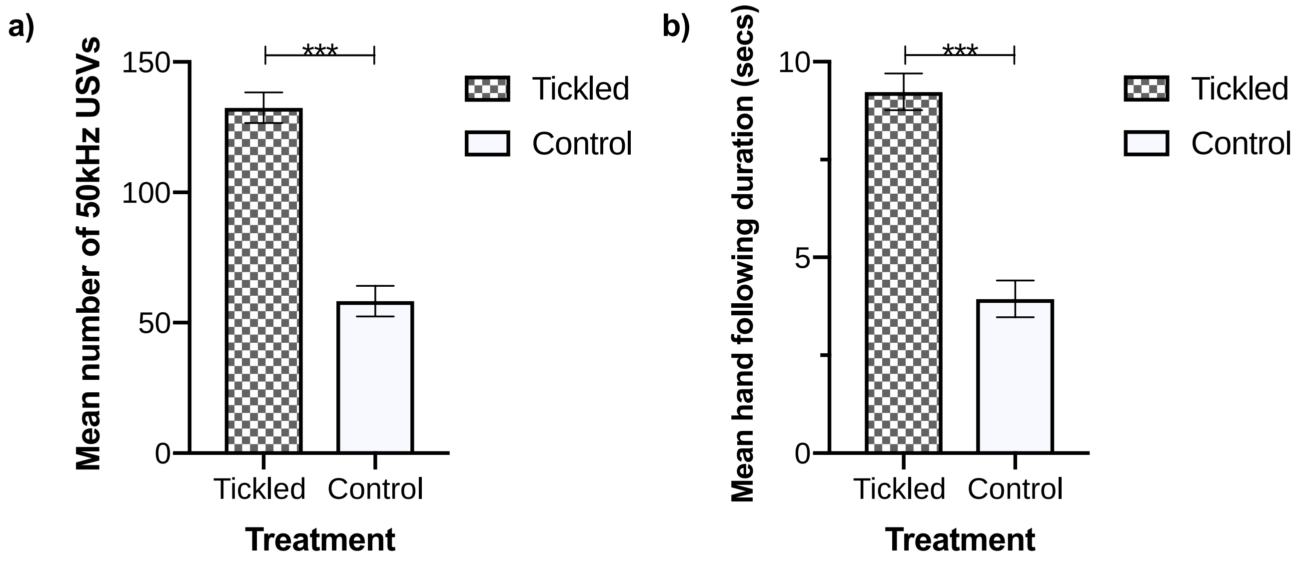

Figure 2. Bar graphs showing the mean number of a) 50kHz USV production and the mean duration of b) hand following across two cohorts of juvenile male Wistar rats (N = 32 split into 2 equal cohorts; aged between 39-50 days old) when experiencing tickling or control handling. Tickled rats produced more 50kHz USVs and followed the experimenter's hand for a longer duration than control rats (USV production; tickled vs. controls; Mean ± SED = 132.45 vs. 58.31 ± 5.91 and hand following duration; tickled vs. controls; Mean ± SED = 9.23 vs. 3.94 secs ± 0.47). Data were analysed using a Generalised Linear Mixed Model in Genstat. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

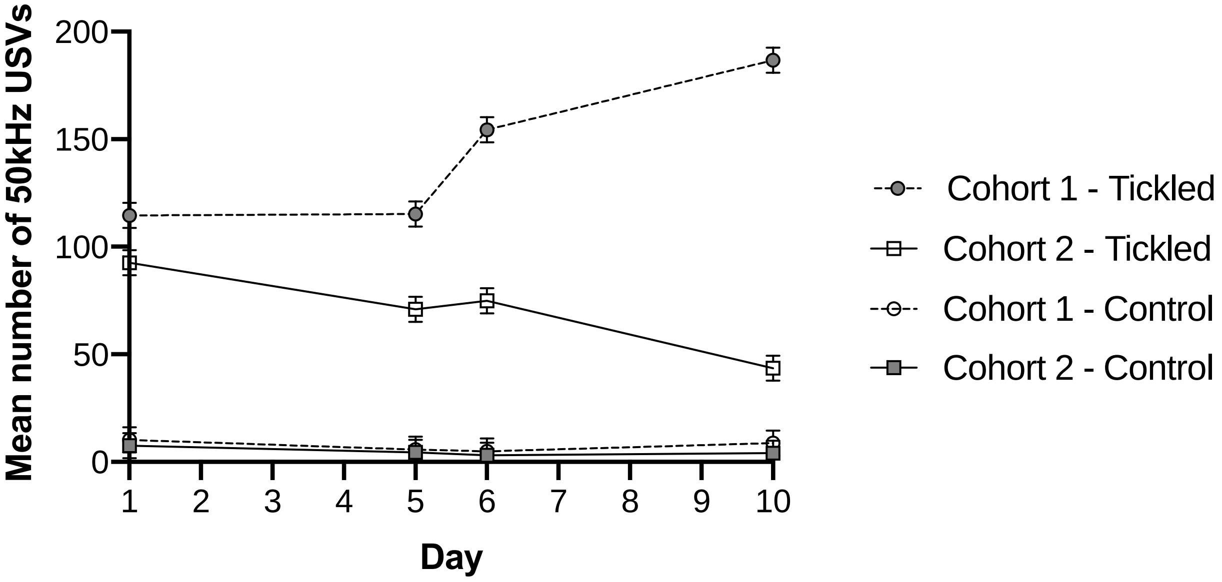

Figure 3. The mean number of 50kHz USVs produced by two cohorts of juvenile male Wistar rats (N = 32 split into 2 equal cohorts; aged between 39-50 days old) when experiencing tickling or control handling across the 10-day experiment. Data were from the first and fifth day of consecutive handling and following a two-day break from handling, data were also collected from the sixth and tenth day. The legend indicates the symbols associated with each cohort and treatment within cohort, with the top two lines showing 50kHz USV production of tickled rats and the bottom two lines showing controls. 50kHz USV production increased in cohort 1 (day 1 vs. day 10 M ± SEM = 114.6 vs. 186.8 ± 5.9) and decreased in cohort 2 (day 1 vs. day 10 M ± SEM = 92.5 vs. 43.6 ± 5.9). In cohort 1 only, tickled rats showed an increase in USV production on day 6 compared to day 5 (day 5 vs. day 6 tickled rats M ± SED = 109.19 vs. 146.33 ± 5.91): an indication of a rebound effect. Means and

standard errors are reported from analyses conducted using a Generalised Linear Mixed Model in Genstat. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

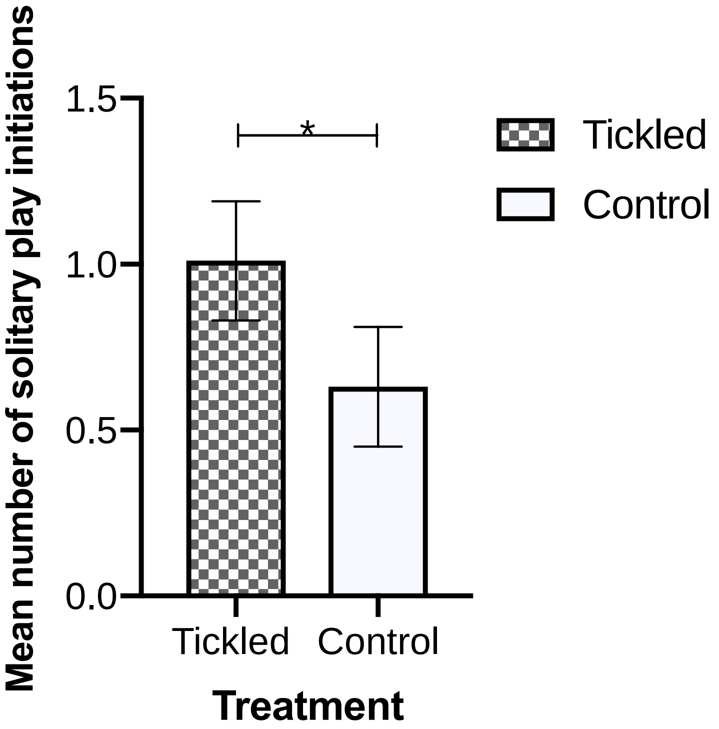

Figure 4. Bar graph showing the number of solitary play events by juvenile male Wistar rats (N = 32 split into 2 equal cohorts; aged between 39-50 days old) in the home cage 5 minutes prior to experiencing either tickling or control handling. Rats were pair housed with one rat deemed as a handled rat and the other as an unhandled cage mate (total N = 64 split into two equal cohorts). Only the handled rat play events are reported here. Solitary play involved fast locomotor movement involving at least two hops; not in the direction of a play partner. Tickled rats conducted more solitary play events than control rats (tickled vs. controls; Mean ± SED = 1.01 vs. 0.63 ± 0.18). Data were analysed using a Generalised Linear Mixed Model in Genstat. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

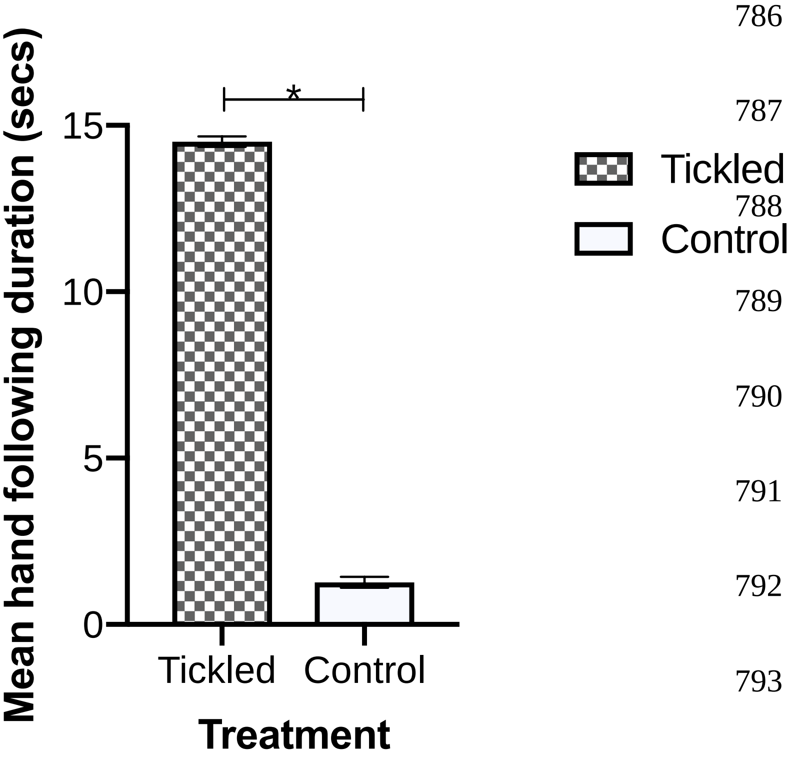

Figure 5. Hand following duration (secs) of cage mate juvenile male Wistar rats when placed the handling arena on day 10 (N = 32 split into 2 equal cohorts; aged between 39-50 days old). Rats were pair housed with one rat deemed as a handled rat and the other as an unhandled cage mate (total N = 64 split into 2 equal cohorts). Handled rats were either tickled or not-tickled (control). On the last day of the experiment, cage mates were placed into the arena and experienced the same conditions as control handled rats; for a total of 3 minutes, the experimenter’s hand alternated each 20 seconds between resting motionless in the centre of the arena and moving in slow circles around the arena. Cage mates of tickled rats followed the hand for a longer duration than cage mates of control rats when the hand moved in slow circles around the arena (cage mates of tickled vs. cage mates of control rats; Mean ± SED = 14.51 vs. 1.27 secs ± 0.16). Data were analysed using a Generalised Linear Mixed Model in Genstat. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

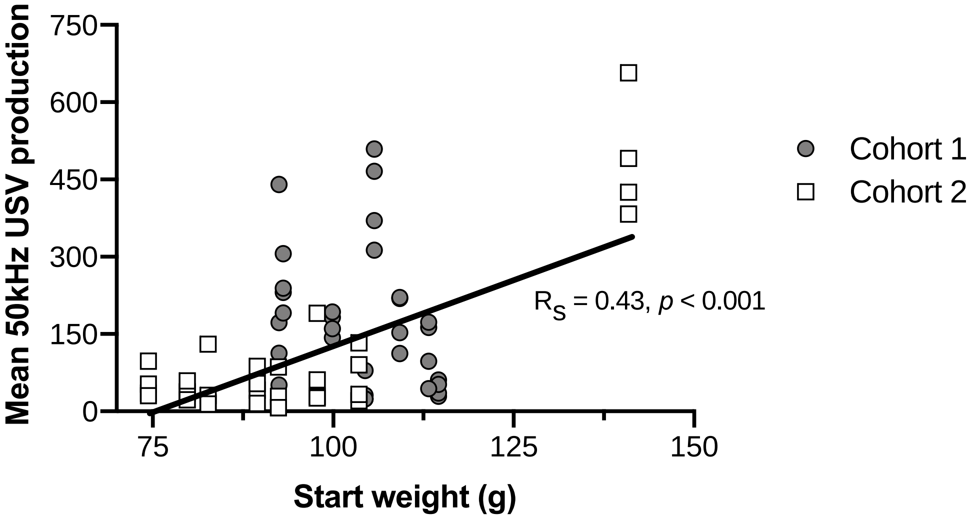

Figure 6. Relationship between start weight(g) and mean 50kHz USV produced across 4 days of tickling juvenile male Wistar rats (N = 32 split into 2 equal cohorts; aged between 39-50 days old). Start weight was taken on the first day of handling (rats aged 39 days old). 50kHz USV production during tickling was recorded on first and fifth day of consecutive handling and following a two-day break from handling, data were also collected from the sixth and tenth day. Cohort 1 data points are filled grey circles and cohort 2 data points are in clear filled squares. Data were analysed using Spearman's Rank Correlation in Minitab 17. The line was fitted by Prism 8 (GraphPad) software using $R_s = 0.43$ as the slope.