Rat 50kHz calls reflect tickling-induced positive emotion as quantified by the affective bias test

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Positive animal emotion (affect) is increasingly viewed as a key component of good animal welfare [1] and as playing an important role in stress-coping and resilience [2]. Methods for reliably inducing and measuring positive affect are critical for progress, but both have been limited in availability. In rats, one promising way of inducing positive affective states is by human-simulated rough and tumble play or 'tickling' [3,4]. However, in humans tickling induces both pleasure and displeasure, and neither an established non-verbal indicator of positive affect, the Duchenne smile, nor laughter detects this variation [5,6]. Rats also show individual differences in response to tickling [7] and this variation needs to be readily quantified if we are to ensure that tickling is only implemented where it generates positive affect. Here we use a new validated and objective measure of affective valence, the Affective Bias Test (ABT) [8,9], to show that 50kHz USVs provide a quantifiable and graded measure of positive affect that accurately reflects the positive state induced by this human-rat interaction.

Reliable induction of positive affect in animals is critical if we are to successfully improve animal welfare [1] or generate model systems to investigate the putative benefits of such states [2]. In laboratory rats 'tickling' is a widely advocated approach ([3]; see: https://nc3rs.org.uk/news/tickling-rats-social-enrichment-improve-rodent-welfare). Evidence indicates that it has beneficial effects but also that individuals vary in their response [7]. Blanket recommendations to implement tickling thus risk having unintended detrimental effects. This can be avoided if easy-to-use quantifiable and graded measures of positive affect allow accurate real-time monitoring of the response to tickling. Here we ask whether, unlike Duchenne smiles and laughter in humans [5,6], 50kHz rat USVs fulfil this role.

Answering this question requires a validated measure of affective valence to provide a ground truth against which 50kHz USV responses to tickling can be gauged. Previous studies have been limited to either measures of motivation such as approach to the tickler's hand [4], or analysis of affective decision-making in rats who either do or don't vocalize when tickled [10]. Here we use the ABT which has demonstrated the predicted affective valence for a wide range of pharmaceutical, hormonal, immune, and environmental manipulations [8,9]. Moreover, dose-dependent bias data in drug studies indicate that it provides a highly sensitive, graded measure of the induced state [8,9].

16 male Lister Hooded rats (c.300-350g) were tested using a standard 5-day ABT protocol (see supplemental information for details) in which each animal experienced four independent associative learning sessions (finding a food reward in a specific digging substrate) under either an affect manipulation (substrate A) or control condition (substrate B), followed by a choice test (A vs B). Each learning session involved presentation of two bowls, one containing a rewarded substrate (A or B, plus food pellet) and the other being unrewarded (C plus a crushed food pellet to prevent odour-guided choices). A learning session was completed when the rat chose the rewarded substrate on six consecutive trials. Daily training sessions involved choosing between substrates A and C (e.g. days 1,3) and, on alternate days, between B and C (e.g. days 2,4). Prior to A vs C sessions, rats were exposed to 30s of tickling (see Fig.1a) [3,4,10] and the mean number of 50kHz USVs emitted was calculated. The control condition prior to B vs C sessions was to remain in the home cage. All factors were fully counter-balanced. Day 5 involved 30 trials in which rats chose between substrates A and B. Choice bias score was calculated as: [(choices for A/(choices for A + B)) x 100] - 50. In the week following the ABT, each rat's latency to approach the experimenter's hand was measured in 30 x 10s trials separated by 5s intervals [4] (see supplemental information for details).

Rats showed a positive bias for substrate A (one sample t-test against null hypothesis of 0: t_{15}=4.753, p=0.0003, Fig. 1b), indicating that tickling conferred greater reward-value to the digging experience and hence generated a more positive state than the control procedure. Moreover, there was a strong positive correlation between the choice bias score and the number of 50kHz calls emitted during tickling sessions (Pearson's correlation, r=0.8911, p<0.0001, Fig. 1c), whilst no correlation was found between approach latency to the experimenter's hand and either the number of 50kHz calls emitted (r=-0.3337, p=0.206, Fig. 1d) or the ABT choice bias score (r=-0.4130, p=0.112, Fig. 1e).

Our findings show that, at a population level tickling induced a more positive affective state. However, rats varied in how strongly they preferred substrate A (Fig. 1b), just as drug dosage affected preference strength in previous ABT studies [8,9], implying that tickling induces stronger positive affect in some individuals than others. Therefore, not all rats like to be tickled [7] and when employing tickling to enhance affect and welfare, care should be taken to identify these animals. To this end, we show that 50kHz USVs provide a real-time indicator of (tickling-induced) positive affect and, importantly, that the rate of calling reflects how positively valenced the state is (Fig. 1c). 50kHz USVs could thus be used (e.g. with a bat detector) to monitor the affective state induced by tickling. Latency to approach the experimenter's hand did not reflect the strength of positive affect induced by tickling (Figs. 1d,e), suggesting that interaction with the handler alone is not related to affective state and/or that hand approach is not a particularly sensitive measure of positive affect.

Overall, our findings demonstrate that 50kHz USVs provide an easy-to-use, graded, and real-time measure of positive affect in response to a short-term event (tickling). Our results support the use of tickling to induce positive affect and welfare in rats, whilst confirming that rats, like humans, vary in how rewarding they find it. They also indicate that 50kHz vocalisations may not be as directly related to tickling-induced human laughter as previously suggested. Unlike human smiles and laughter [5,6], 50kHz vocalisations directly reflect the animal's affective experience when being tickled. We suggest that future studies should use the ABT to investigate whether this generalises to other contexts in which 50kHz vocalisations have been recorded e.g. juvenile play, mating, aggression [3]. Because the ABT is sensitive to both positive and negative affective states [8,9], hence addressing a long-standing challenge in studies of animal affect, and especially positive emotion, of establishing a ground truth state against which methods for inducing and measuring affect can be validated, it could also be used to investigate whether rat 22kHz alarm calls provide a similarly graded measure of negative affective state.

SUPPLEMENTAL INFORMATION

Supplemental information including methods and additional results can be found with this article online. Data from this study are available online at: https://figshare.com/s/ab8f75ecfdeaea5153b0

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DECLARATION OF INTERESTS

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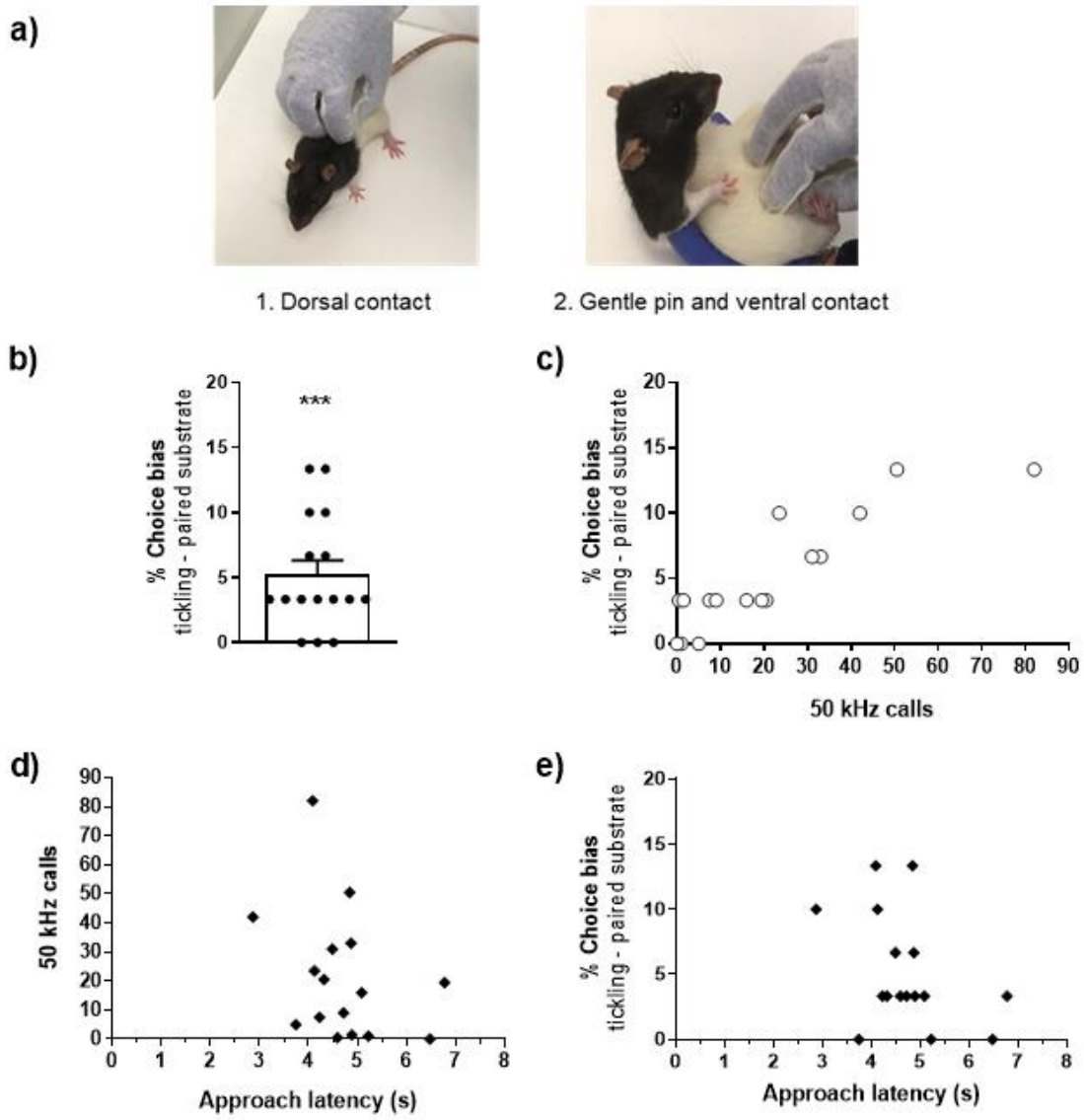

Figure 1. (a) Example of the tickling stimulation method used as described by Panksepp and co-workers (Burgdorf et al., 2008). Photographs by Justyna Hinchcliffe. (b) Rats show a positive bias towards the digging substrate experienced following tickling relative to that experienced following the control condition indicating induction of a relatively positive affective state by tickling at that time. Data shown as mean % choice bias ± SEM; one sample t-test against a null hypothesised mean of 0: $p=0.0003$ (*** on graph). (c) Scatter plot of relationship between % choice bias and the mean number of 50kHz vocalisations emitted during tickling sessions prior to two substrate-reward training sessions; Pearson's correlation: $p<0.0001$. There was no correlation between approach latency to the experimenter's hand and (d) the mean number of 50kHz calls emitted during tickling sessions prior to two substrate-reward training sessions or (e) % choice bias; Pearson's correlations: $p>0.05$.