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### **Methods of inducing and assessing positive affective states in juvenile male Wistar rats.**

**Citation for published version:**

Hammond, T, Brown, S, Meddle, S, Nielsen, BL, Lawrence, A & Bombail, V 2023, 'Methods of inducing and assessing positive affective states in juvenile male Wistar rats.', 56th Congress of the International Society for Applied Animal Ethology, Tallin, Estonia, 1/08/23 - 5/08/23.

**Link:**

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**Document Version:**

Peer reviewed version

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### **Methods of inducing and assessing positive affective states in juvenile male Wistar rats.**

Across animal welfare science there is a critical lack of validated models of positive affective states. With over 1.9 million rats used for research purposes in the UK, USA and EU per annum as of 2018, contribution towards this gap in knowledge on positive affective states is vital to improving their welfare. The first aim of this research was to develop models of inducing a positive affective state in rats. In response to playful handling and playback of acoustic stimuli, rats showed increases in indicators of positive affect, including increased production of 50-kHz ultrasonic vocalisations (USVs), a type of vocalisation associated with positive affect. The successful induction of a positive affective state permitted investigation of whether play is sensitive to positive affective state as a promising indicator of positive animal welfare. After five days, there was an increase in play in the home cage prior to the experience in response to both playful handling and the playback of 50-kHz USVs. As increases in play occurred prior to the positive experience, this suggests that play may in part reflect a form of anticipatory behaviour. In the playback paradigm, the absence of a human-rat interaction also allowed the assessment of play during the presentation of acoustic stimuli. After five days of presentation with 50-kHz USVs, rats expressed more social play than those exposed to control stimuli. Overall, this work supports the use of two innovative methods to induce a positive affective state in rats, achieved through a total of six independent experiments. This work also contributes to the currently limited body of evidence that suggests that positive affect stimulates play. Of most significance is the fundamental evidence provided by this work for the capacity of rats to experience positive affective states and the relative ease by which positive affective states can be induced in this species. This further highlights our responsibility to incorporate positive animal welfare for rats as a prerequisite for their use.