

INTRODUCTION

- Rats have a well-developed social life, they produce ultrasonic vocalisations (USVs) to communicate their emotional states [1].
- There are two basic types of ultrasonic vocalisations:
 - The 22kHz USVs have negative value and are elicited by alarm or distress situation.
 - The 50kHz USVs have positive value and are emitted in reward states including during social interactions [2].
- Current preclinical assays have limitations in measuring anhedonia, a key feature of depression.
- The female urine sniffing test (FUST) is a quantitative non-operant test designed to monitor hedonic behaviour in male rodents [3].
 - Based on the interest in pheromonal odour from the opposite sex.
 - Consists in measuring the time spent sniffing oestrus female urine which have intrinsic rewarding properties.
- The Wistar-Kyoto (WKY) rat strain is hyperresponsive to stress and reveal behaviours that reflect endogenous depressive phenotype [4,5].

Aim: to develop a new assay to measure anhedonia by simultaneously measuring sniffing and 50kHz USVs in the endogenous “depressed” WKY rat compared to Sprague Dawley (SD) as “healthy” control strain when exposed to either male or female urine.

METHODS

FUST protocol: Six month old WKY (n=10,350-300g) and SD (n=8,350-400g) were singly housed 7 days before the test to increase social motivation. Urine collected from animals of the same strain was kept frozen at -80°C until use.

- One hour before the test the animals were habituated to a cotton tip inserted into their home cage.
- They were then transferred to a dimly-lit (3lux) room where they perform the three phases of the test:
 - They were exposed for 3 min to a cotton tip infused with 60µl sterile water;
 - They were exposed for 3 min to a cotton tip infused with 60µl oestrus female urine;
 - They were exposed for 3 min to a cotton tip infused with 60µl male urine (used as control odour).
- Each phase was preceded by 5 min of habituation in the test room and separated by an interval of 45 min.
- Sniffing was taped and measured in seconds while USVs were recorded and analysed with the SONOTRACK system.
- Data were analysed with two-way ANOVA for repeated measures followed by Fisher’s LSD test.

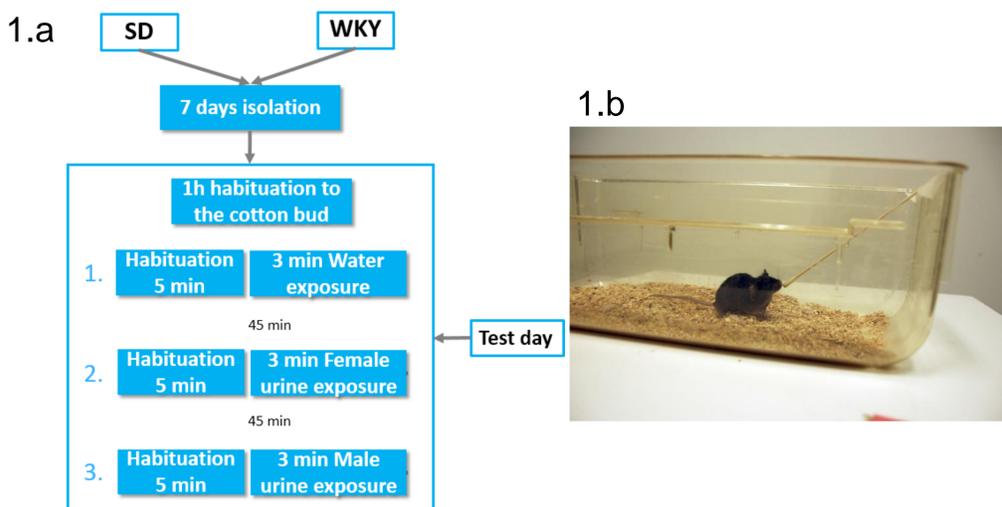


Figure 1.a Study design; 1.b Illustration of the female urine sniffing test

CONCLUSION

- Our results indicate that both WKY and SD rats found female urine more attractive than water.
- WKY rats demonstrates a weaker positive response a rewarding stimulus such as oestrus urine which might be analogous to the anhedonia observed in depressed patients.
- In this experiment the male urine could represent an attractive social stimulus for SD, due to the social isolation they experience before the test, but not rewarding as the oestrus urine.
- We aim to further investigate the validity of our assay in measuring depression-like behaviour by recording 50kHz-USVs after antidepressant treatment.

RESULTS

Vocalisations

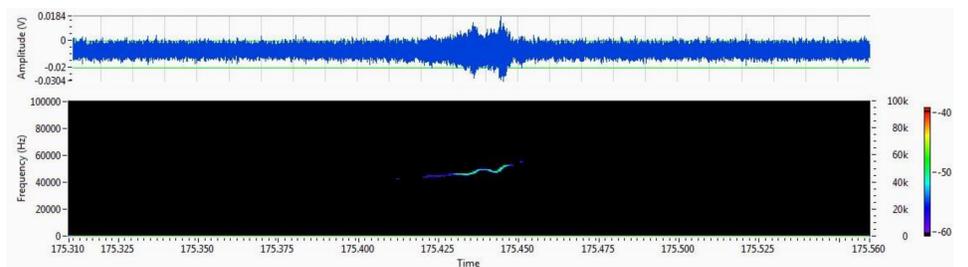
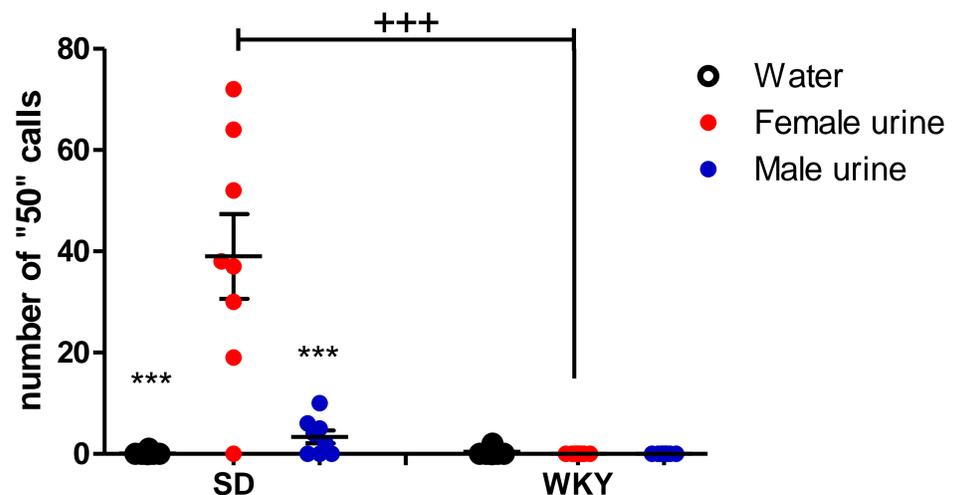


Figure 2. Spectrogram of a 50kHz positive call.

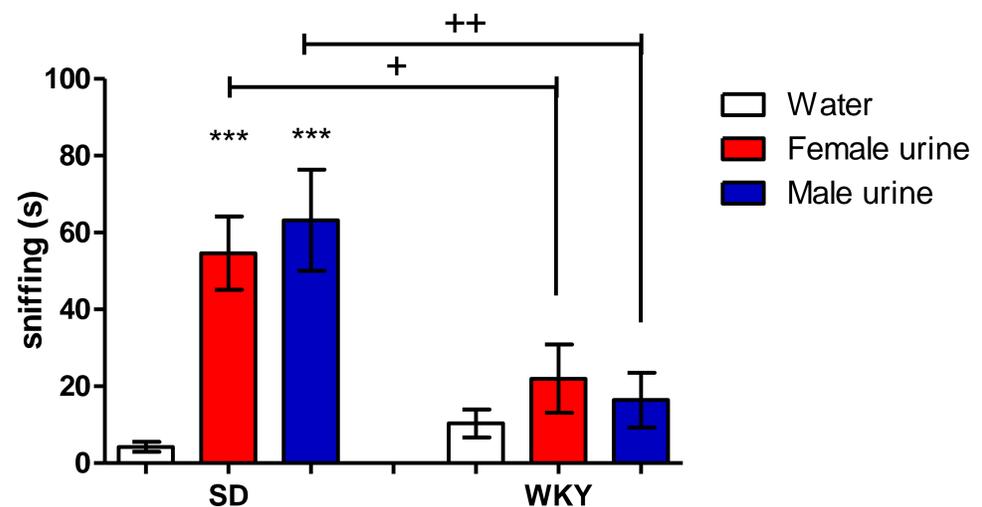


n=8-10 per group, mean±SEM. ***p<0.001 vs female urine; +++p<0.001 Female urine SD vs WKY.

Two-Way ANOVA for repeated measures followed by Fisher’s LSD post-test. Factor strain ***p<0.001, factor exposure ***p<0.001, interaction ***p<0.001.

- We found that SD emitted significantly more 50kHz USVs during the exposure to female urine than when exposed to other stimuli.
- When comparing the two strains a significantly higher number of 50kHz USVs was emitted by SD during the exposure to female urine.

Sniffing



"n=8-10 per group, mean±SEM. ***p<0.001 vs Water; ++p<0.01 Male urine +p<0.05 Female urine SD vs WKY.

Two-Way ANOVA for repeated measures followed by Fisher’s LSD post-test. Factor strain *p<0.05, factor exposure ***p<0.001, interaction *p<0.05."

- Comparing the SD with the WKY we found a significant difference in the amount of time spent sniffing male urine.
- The sniffing does not match with the amount of vocalisation, in fact rats do not vocalise during male urine despite sniffing male urine for a longer time period than female urine.

REFERENCES

- Brudzynski SM. Ethotransmission: communication of emotional states through ultrasonic vocalization in rats. (2013) *Curr Opin Neurobiol.* 23(3):310-7.
- Manduca A, Campolongo P, Palmery M, Vanderschuren LJ, Cuomo V, Trezza V. (2014) Social play behavior, ultrasonic vocalizations and their modulation by morphine and amphetamine in Wistar and Sprague-Dawley rats. *Psychopharmacology (Berl).* 231(8):1661-73.
- Malkesman O, Scattoni ML, Paredes D, Tragon T, Pearson B, Shaltiel G, Chen G, Crawley JN, Manji HK. The female urine sniffing test: a novel approach for assessing reward-seeking behavior in rodents. (2010) *Biological Psychiatry.* 67 (9): 864-71.
- Paré WP, Redei E. A biobehavioral profile of an ulcer susceptible rat strain. (1995) *Neuroendocrinology of Gastrointestinal Ulceration*, Plenum, New York, pp. 201–208
- Rittenhouse PA, López-Rubalcava C, Stanwood GD, Lucki I (2002) Amplified behavioral and endocrine responses to forced swim stress in the Wistar-Kyoto rat. *Psychoneuroendocrinology.* 27(3):303-18.