LINK TO ORIGINAL ARTICLE
LINK TO INITIAL CORRESPONDENCE

'Stressing' rodent self-grooming for neuroscience research

Cai Song, Kent C. Berridge and Allan V. Kalueff

We appreciate the thoughtful Correspondence by Fernández-Teruel and Estanislau on our Review (Neurobiology of rodent self-grooming and its value for translational neuroscience. *Nat. Rev. Neurosci.* **17**, 45–59 (2016))[1], which raises the issue of the relationship between stress and self-grooming (Meanings of self-grooming depend on an inverted U-shaped function with aversiveness. *Nat. Rev. Neurosci.* http://dx.doi.org/10.1038/nrn.2016.102 (2016))[2]. We agree that the effect of stress on self-grooming can often be described as an inverted U-shaped function: self-grooming typically occurs spontaneously at low arousal (as a maintenance behaviour), becomes longer (and may alter in pattern) during moderate arousal (as a 'displacement activity') and can be inhibited by high-stress states that elicit freezing, fight or flight responses[1–4].

However, despite the usefulness of this view, caution is needed because the relationship between stress and self-grooming can be more complex, and self-grooming duration measures in relatively mild stress (the main behavioural measures and situations discussed in the Correspondence[2]) alone may be insufficient for adequate neurobehavioural analyses of rodent self-grooming[1,3,4]. For example, high-frequency, short bouts of self-grooming can yield a cumulative duration that is similar to that of fewer, longer bouts of such behaviour. Moreover, rats that exhibit different self-grooming durations may show no differences in anxiety-related behavioural or neuroendocrine parameters[5]. In addition, as self-grooming frequency (the rate of initiation) and bout length (execution) under stress probably have differential neural underpinnings, these aspects of self-grooming may differentially change during stress (BOX 1). Even when different groups of rodents show similar times spent self-grooming under conditions of stress, they may exhibit altered self-grooming body targets (that is, rostral face versus caudal body and tail regions)[1]. Indeed, mounting evidence suggests that the behavioural microstructure of rodent self-grooming may serve as a sensitive marker of stress levels[1] (BOX 1). Therefore, a more detailed measure of self-grooming behaviour — incorporating the average bout duration, the transitions between stages, the number of interrupted or incomplete bouts and other ethologically derived parameters[1] — can help to provide significant insights into the nature of self-grooming phenotypes under different levels of stress or arousal.

It may also be important to recognize that low–moderate–high arousal and self-grooming continuums in various behavioural contexts may not 'flow' as tightly as can be assumed[3,4]. For example, self-grooming bouts can occur immediately in anticipation of, or right after, exposure to a stressful stimulus (for example, self-grooming in voles occurs first after predator fright, before locomotion[3,4]; BOX 1). Thus, this raises the possibility of rethinking the acute stress response in rodents as 'freeze, fight, flight and groom'. Namely, self-grooming evoked by high-stress situations may differ considerably — both behaviourally and mechanistically — from low-arousal 'comfort' and moderate-arousal (for example, novelty-evoked) self-grooming[1]. Moreover, although high-stress self-grooming is often associated behaviourally with freezing, fight or flight[2] (BOX 1), it is currently unclear whether all of these behaviours are mediated by shared 'high-stress' neural circuits or compete with each other and with self-grooming for circuitry and motor movements.

In summary, we agree that stress modulates rodent self-grooming behaviour in ways that often follow an inverted-U relation[2], but we also note that this crucial relationship may be more complicated. Given the emerging relevance of self-grooming in the modelling

Box 1 | **The emerging complexity of rodent self-grooming during stress**

Rodent self-grooming frequency and duration may differentially change during stress

- Restraint-induced stress increases the duration but not the frequency of self-grooming in wild-type mice, although such stress elevates both the frequency and duration of such grooming in mice lacking period circadian clock 1 (*Per1*), an acute stress response effector gene[6]
- Alcohol-preferring (AA) rats, which show low levels of anxiety-like behaviour, initiate more self-grooming bouts than more anxious non-alcohol preferring (ANA) rats[7]

Rodent stress and anxiety may be poorly correlated with self-grooming duration

- Rat subcohorts selected on the basis of self-grooming duration show no differences in anxiety-like behaviours or neurochemical and neuroendocrine parameters[5]
- Acid-sensing (proton gated) ion channel 3 (*Asic3*)-knockout mice show reduced anxiety-like behaviour but increased self-grooming duration compared with wild-type mice[8]
- SH3 and multiple ankyrin repeat domains 3 (*Shank3*)-conditional-knockout mice show increased self-grooming duration compared with wild-type mice; the duration decreases following SHANK3 re-expression without affecting anxiety levels[9]
- BTBR T+tf/J mice show increased self-grooming duration and frequency but normal baseline anxiety and higher stress resilience compared with C57BL/6J mice[10]

Rodent self-grooming behavioural patterning is affected during stress

- The anxiolytic drug clonazepam potently alters both self-grooming activity and sequencing parameters in rats but causes only mild anxiolytic-like effects on other (non-grooming) behaviours[11]
- Overt correlations exist between the number and percentage of correct cephalocaudal transitions of self-grooming and the expression of non-grooming anxiety-related behaviours[11]
- In rats, grooming microstructure is highly sensitive to sleep deprivation-related stress[12]
- Anxious 'high-yawning' rats show a higher frequency of rostral self-grooming in novel environments than less anxious 'low-yawning' rats[13]

Rodent self-grooming activation in high-arousal, potentially life-threatening stress

- Voles exposed to predator-like overhead stimuli display self-grooming after predator fright, before locomotion[3]
- Saline injection or electric shock evokes elevated self-grooming in mice[14]
- *Asic3*-knockout mice in the resident-intruder test often display stereotypical repetitive self-grooming after fighting[8]
- Mutant mice lacking histidine decarboxylase (*Hdc*) exhibit an increase in tic-like repetitive self-grooming in the conditioned fear paradigm[15]

of various affective brain disorders, the analysis of this important relationship will benefit from focusing on multiple (rather than single) self-grooming behavioural measures, an appreciation of a wider spectrum of specific biological contexts in which self-grooming occurs and an in-depth analysis of its underlying neural circuitry[1].

Cai Song is at the Research Institute for Marine Drugs and Nutrition, Provincial Key Laboratory of Aquatic Product Processing and Safety, College of Food Science and Technology, Guangdong Ocean University, Zhanjiang, 524088, China; and at the Graduate Institute of Neural and Cognitive Sciences, China Medical University, Taichung 000001, Taiwan.

Kent C. Berridge is at the Department of Psychology, University of Michigan, 525E University Street, Ann Arbor, Michigan 48109, USA.

Allan V. Kalueff is at the Research Institute for Marine Drugs and Nutrition, Provincial Key Laboratory of Aquatic Product Processing and Safety, College of Food Science and Technology, Guangdong Ocean University, Zhanjiang, 524088, China; at the Key Laboratory of Advanced Processing of Aquatic Products of Guangdong Higher Education Institution, Zhanjiang, 524088, China; at the Neuroscience Research Laboratory, ZENEREI Research Center, Slidell, Louisiana 70458, USA; at the Institute of Translational Biomedicine, St. Petersburg State University, St. Petersburg 199034, Russia; and at the Institutes of Chemical Technologies and Natural Sciences, Ural Federal University, Ekaterinburg 620002, Russia.

Correspondence to A.V.K.
avkalueff@gmail.com

doi:10.1038/nrn.2016.103
Published online 28 Jul 2016

1. Kalueff, A. V. *et al.* Neurobiology of rodent self-grooming and its value for translational neuroscience. *Nat. Rev. Neurosci.* **17**, 45–59 (2016).
2. Fernández-Teruel, A. & Estanislau, C. Meanings of self-grooming depend on an inverted U-shaped function with aversiveness. *Nat. Rev. Neurosci.* http://dx.doi.org/10.1038/nrn.2016.102 (2016).
3. Andrew, R. J. Arousal and the causation of behavior. *Behaviour* **51**, 135–164 (1974).
4. Fentress, J. C. Interrupted ongoing behaviour in two species of vole (*Microtus agrestis* and *Clethrionomys brittanicus*). *Anim. Behav.* **16**, 135–153 (1968).
5. Reimer, A. E. *et al.* Rats with differential self-grooming expression in the elevated plus-maze do not differ in anxiety-related behaviors. *Behav. Brain Res.* **292**, 370–380 (2015).
6. Zhang, J. *et al.* Deficiency of antinociception and excessive grooming induced by acute immobilization stress in *Per1* mutant mice. *PLoS ONE* **6**, e16212 (2011).
7. Roman, E., Meyerson, B. J., Hyytia, P. & Nylander, I. The multivariate concentric square field test reveals different behavioural profiles in male AA and ANA rats with regard to risk taking and environmental reactivity. *Behav. Brain Res.* **183**, 195–205 (2007).
8. Wu, W. L., Lin, Y. W., Min, M. Y. & Chen, C. C. Mice lacking *Asic3* show reduced anxiety-like behavior on the elevated plus maze and reduced aggression. *Genes Brain Behav.* **9**, 603–614 (2010).
9. Mei, Y. *et al.* Adult restoration of *Shank3* expression rescues selective autistic-like phenotypes. *Nature* **530**, 481–484 (2016).
10. Silverman, J. L. *et al.* Low stress reactivity and neuroendocrine factors in the BTBR T$^+$tf/J mouse model of autism. *Neuroscience* **171**, 1197–1208 (2010).
11. Nin, M. S. *et al.* Anxiolytic effect of clonazepam in female rats: grooming microstructure and elevated plus maze tests. *Eur. J. Pharmacol.* **684**, 95–101 (2012).
12. Pires, G. N., Tufik, S. & Andersen, M. L. Grooming analysis algorithm: use in the relationship between sleep deprivation and anxiety-like behavior. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **41**, 6–10 (2013).
13. Moyaho, A. & Valencia, J. Grooming and yawning trace adjustment to unfamiliar environments in laboratory Sprague-Dawley rats (*Rattus norvegicus*). *J. Comp. Psychol.* **116**, 263–269 (2002).
14. Goto, A. *et al.* Circuit-dependent striatal PKA and ERK signaling underlies rapid behavioral shift in mating reaction of male mice. *Proc. Natl Acad. Sci. USA* **112**, 6718–6723 (2015).
15. Xu, M., Li, L., Ohtsu, H. & Pittenger, C. Histidine decarboxylase knockout mice, a genetic model of Tourette syndrome, show repetitive grooming after induced fear. *Neurosci. Lett.* **595**, 50–53 (2015).

Acknowledgements
A.V.K. is supported by the Russian Foundation for Basic Research (grant 16-04-00851).

Competing interests statement
The authors declare no competing interests.