Available online at www.sciencedirect.com

SCIENCE DIRECT

Brain Research Protocols 13 (2004) 151–158

BRAIN RESEARCH PROTOCOLS

www.elsevier.com/locate/brainresprot

Protocols

Grooming analysis algorithm for neurobehavioural stress research

Allan V. Kalueff*, Pentti Tuohimaa

Medical School (Anatomy Department) and University Hospital, University of Tampere, Tampere 33014, Finland

Accepted 13 April 2004
Available online 1 June 2004

Abstract

Since rodent self-grooming behaviours are elicited by both comfort and stressful conditions, traditional measures such as duration, latency of onset and the number of bouts may be not suitable to dissociate between these opposite conditions. The aim of the current study was to improve and optimize ethological measurement of self-grooming in neurobehavioural stress research enabling differentiation between stress and no-stress situations. This protocol assists in the correct interpretation of animal grooming behaviours and detection of stress by measuring alterations in grooming microstructure in different test situations. While a general pattern of self-grooming uninterrupted cephalocaudal progression is normally observed in no-stress (comfort) conditions in mice and other rodents, the percentage of ''incorrect'' transitions between different stages and the percentage of interrupted grooming bouts may be used as behavioural marker of stress. The protocol can be a useful tool in neurobehavioural stress research including modelling stress-evoked states, pharmacological screening of potential antistress drugs or behavioural phenotyping of genetically modified animals.

Theme: Neural basis of behaviour
Topic: Stress

Keywords: Grooming behaviour; Mice; Ethological analysis; Stress; Behavioural models

1. Type of research

Algorithm to perform ethological analysis of stress-evoked self-grooming behaviours in laboratory rodents.

1.1. Introduction

Self-grooming is an ancient innate behaviour that is represented across most animal species [19] and is a particularly important part of rodent behavioural repertoire [2–5,29]. In rodents, a general pattern of self-grooming cephalocaudal progression is observed as follows: paw licking, nose and face wash, head wash, body wash and fur licking, leg licking, tail/genitals licking and wash [5,14,15,26]. Many neuromediators and hormones as well as multiple regions in the brain appear to be involved in the regulation of self-grooming behaviours [2,3,6,7,11,13,28,29]. Grooming is also known to be bidirectionally sensitive to various psychotropic drugs and genetic manipulations [12,13,21], and is often studied in mice [6,18,19,27].

It has long been known that rodents' grooming activity can be generally increased in two opposite situations: in high and low stress [20,21,24]. Low-stress comfort grooming is a spontaneous body care ritual which occurs as a transition from rest to activity; it is a typical behavioural marker of low or no stress and usually goes in a ''relaxed fashion'' from paw licking to tail/genitals wash [17,20,21]. Similarly, stress has long been known to induce grooming in rodents [14,25,28]. However, this stress-evoked grooming is ethologically different from low-stress grooming, and characterised by frequent bursts of rapid short grooming [17,25,28].

Since rodents' self-grooming is increased by both stress and comfort conditions [14,20,25,26], the major problem with grooming behavioural analysis is that its traditional cumulative ''gross'' measures (the latency to onset, the number of bouts and the duration [16]) may not be valid for correct data interpretation and analysis. As such, there is a great need for a tool which will make it possible to

* Corresponding author. Tel.: +358-3-2156640; fax: +358-3-2156170.
E-mail address: avkalueff@inbox.ru (A.V. Kalueff).

1385-299X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainresprot.2004.04.002

ethologically dissect different types of grooming activity. For this, we have designed the present grooming analysis algorithm based on the abovementioned ethological differences between comfort- and stress-evoked groomings. Our approach is based on differential registration and analysis of grooming behavioural microstructure, including (1) transitions between self-grooming patterns and (2) interruptions of grooming bouts in mice exposed to different stressors [20,21]. In the present study, we first used this approach to assess grooming behaviours of C57BL/6 mice subjected to several different stressors, and demonstrated that our protocol works consistently in several different models of stress. In the second part of our study, since the use of genetically modified mice is becoming increasingly common in behavioural neuroscience [9,10,18], and there is a growing interest in their grooming behaviours [6,12,19,27], we wanted to know if our protocol can be used to assess grooming behaviours in mutant mice. Using "anxious" mice lacking neurosteroid vitamin D receptor (VDR) gene [22] as an example, we demonstrated that our algorithm is able to detect stress-evoked differences in grooming of genetically modified animals. Overall, in the present study we show that the protocol allows detection of stress by measuring alterations in grooming patterns (microstructure), and could be extensively used in neurobehavioural stress research and behavioural phenotyping of genetically modified animals.

2. Time required

The time required for this protocol was calculated taking into account standard experiments with 10–15 animals per group and two groups:

(a) Handling of naïve animals: 5 days, 5 min/mouse/day.
(b) The open field, horizontal rod tests, the elevated plus maze, water misting and social encounter with another male mouse require 5 min each. Measures of self-grooming behaviours in the actimeter require 5 min.
(c) Animals are to be allowed at least 7 days between the tests, if a battery of tests is used.
(d) Analyses of the ethological data: 4–10 days, depending on the amount of data collected.

3. Materials

3.1. Animals

Experiments were carried out on 30 adult C57BL/6 male mice (25–30 g) bred in the University of Tampere, Finland. Experiments with genetically modified animals were carried out on 10 wild type (129S1) and 10 VDR gene null mutant adult male mice (25–30 g) bred in the University of Tampere from a line generated in the University of Tokyo, Japan [23]. These mice were littermates produced by heterozygous crosses for four generations. All animals used in this study were experimentally naïve, housed three to four per cage and kept in a controlled environment maintained at a constant temperature (24 ± 1 °C) and humidity ($50 \pm 5\%$), with free access to food and water. The animals were maintained on a 12:12-h light/dark cycle (lights on at 0600 h and off at 1800 h). Behavioural testing was always conducted between 1400 and 1800 h. Animal care procedures were conducted in accordance with guidelines set by the European Community Council Directives. The procedures used in this study were in strict accordance with the European legislation and the guidelines of the National Institutes of Health on the use and care of laboratory animals. All animal experiments reported here were approved by the Ethical Committee of the University of Tampere.

3.2. Special equipment

The open field arena is a square plastic box ($45 \times 45 \times 45$ cm) with Plexiglas front and the floor divided into nine squares (15×15 cm). The actimeter is a transparent plastic box ($30 \times 30 \times 30$ cm). The elevated plus maze is made from Plexiglas and consists of two open arms (30×10 cm) and two enclosed arms ($30 \times 10 \times 10$ cm) extending from a common central region (10×10 cm) elevated to a height of 70 cm. The horizontal rod is a 20-cm metal rod 1 cm in diameter, fixed to a platform elevated to a height of 20 cm. All equipment used in our study was constructed by a local manufacturer (TAYS Workshops) according to our specifications.

4. Detailed procedure

(a) Expose the mice to different stressors for 5 min, including placing mice in the open field, the elevated plus maze and on the horizontal rod. To induce artificial grooming, mist the mice in a plastic box individually with water (25 °C) using a hand spray. Induce social stress by placing an individual mouse in a cage with an unfamiliar "host" male mouse for 5 min. All test apparatus are thoroughly cleaned (wet and dry cloth) before each animal.
(b) After the exposure to stress, observe animal self-grooming behaviours in the actimeter for 5 min. Record the number and the duration of grooming bouts. Include paw licking, nose/face grooming (strokes along the snout), head washing (semicircular movements over the top of the head and behind the ears), body grooming/scratching (body fur licking and scratching the body with the hind paws), leg licking and tail/genitals grooming (licking of the genital area and tail) as components of grooming behaviour, according to Refs. [2–4,11,12,15]. Record all instances of interruption of

Table 1
An example of grooming transitions matrix for two mice

Stages of grooming	Control mouse # 3							Mouse # 12 (exposed to an unfamiliar male mouse)						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6
0	-	3	2	1	1			-	2	1		1	2	
1	2	-	2					2	-		1		1	
2	1		-	1				3	1	-				
3				-	1						-	1		
4		1			-	1			1			-		1
5	1					-	1	1	1			1	-	2
6	0						-	0	1	1			1	-
Interruption		1							1	2				

Data summary. Mouse 3 (Control): incorrect transitions—9 (50%), total—18; interrupted grooming bouts—1 (12.5%), total—8. Mouse 12: incorrect transitions—16 (76%), total—21; interrupted grooming bouts—3 (50%), total—6.
Rows are preceding stages; columns are following stages (see detailed procedure for the grooming scaling system used in this protocol). "Correct" transition fields are marked by grey colour.

grooming activity; interruptions longer than 5 s determine separate grooming bouts.

(c) Identify and register separately all grooming patterns and transitions between them using the following scaling system: no grooming (stage 0), paw licking (stage 1), nose and face wash (stage 2), head wash (stage 3), body grooming (stage 4), leg licking (stage 5), and tail/genitals grooming (stage 6).

(d) Analyse all "correct" and "incorrect" transitions between grooming stages, as well as interruptions in grooming activity using the transition matrix (Table 1). Correct transitions between grooming stages include the following progressive transitions: 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–0. Incorrect transitions are chaotic and characterised by skipped (e.g. 0–6, 1–6, etc.) or reversed (e.g. 3–2, 4–1, 5–2, etc.) stages. The percentage of incorrect transitions (of total transitions) and the percentage of interrupted grooming bouts (of total number of bouts) are used as behavioural indices of stress.

(e) Statistics. All results are expressed as mean ± S.E.M. Data are analysed by Mann–Whitney test for comparisons between control and experimental groups. A probability of less than 0.05 is considered statistically significant.

5. Results

5.1. Grooming behaviours of C57BL/6 mice after exposure to stress

Fig. 1 shows grooming activity of C57BL/6 mice after their exposure to different types of stress. Following exposure to a relatively mild stress evoked by the open arena + novelty of the open field test [9,10], the traditional gross measures (the number and the duration of grooming bouts) in the actimeter increased significantly compared to non-stressed control mice. In addition, the present grooming analysis algorithm shows that this procedure produced a shift in grooming patterns, slightly increasing the percentage of incorrect grooming transitions and interrupted bouts. However, after exposure to the elevated plus maze (known as a relatively high-stress anxiety model [9,10]), the number and the duration of grooming bouts were dramatically increased (Fig. 1). As expected, the use of the grooming analysis algorithm also revealed a marked shift in grooming behavioural patterns, significantly increasing the percentage of incorrect transitions and interrupted bouts. Stress induced by social encounter with an unknown male mouse also increased the number and the duration of grooming bouts (Fig. 1) in a fashion similar to that produced by the elevated plus maze. Grooming microstructure was also affected by social stress, manifest in the increased percentage of incorrect transitions and interrupted bouts, similar to that following the elevated plus maze exposure (Fig. 1). Together, this shows that the present protocol is particularly effective in detection of stress-evoked abnormal grooming patterns in mice tested in a battery of models of stress.

5.2. Grooming behaviours in VDR null mutant mice

In order to show that our protocol can be used to detect stress-evoked differences in grooming of genetically modified animals, we compared generally more anxious VDR null mutant mice [22] to their wild type 129S1 littermates in two different test situations: following misting with water, and exposure to the horizontal rod test. Numerous studies [5,19,28] have demonstrated that misting with water artificially stimulates grooming behaviours in rodents. Overall, the VDR null mutants spent more time engaged in water-induced self-grooming behaviours than did their control littermates (Fig. 2). A detailed ethological analysis using our algorithm shows that these anxious mutant mice demonstrated a trend towards more incorrect transitions and significantly higher percentage of interrupted bouts compared to the wild type controls. Similarly, after the horizon-

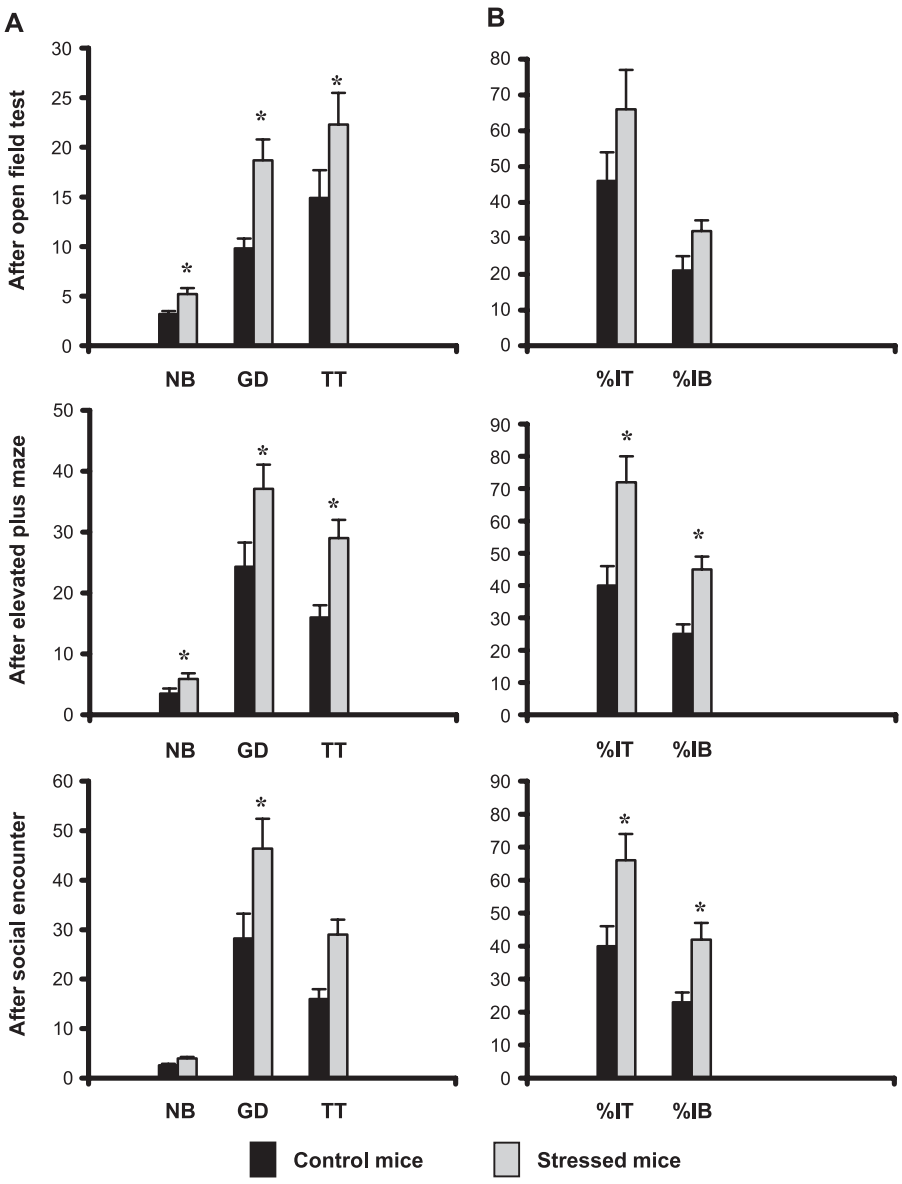

Fig. 1. Exposure to different stressors (the open field, the elevated plus maze and an encounter with an unfamiliar male) activates grooming gross measures (A) and produces changes in grooming behavioural microstructure (B) in C57BL/6 mice compared to non-stressed controls ($n = 15$ in each group). NB—number of grooming bouts; GD—total duration of grooming, s; TT—number of transitions between grooming stages; %IT—percent of incorrect transitions; %IB—percent of interrupted bouts. Data are expressed as mean ± S.E.M. $P < 0.05$, compared to the control group (*U*-test).

tal rod test, these mutant mice engaged in more self-grooming behaviours which, as predicted, were characterised by higher percentages of incorrect transitions and interrupted bouts (Fig. 2). Together, this shows that the present protocol is effective in detection of specific abnormal grooming patterns in mutant mice, including both artificial and spontaneous stress-evoked types of their grooming.

5.3. Grooming behaviours of C57BL/6 and 129S1 mice tested in a low-stress situation

In a separate experiment, we wanted to test our protocol by applying it to C57BL/6 and 129S1 mice (two most commonly used mouse strains) tested in low-stress situations. Would it still reveal alterations in grooming microstructure, and, if so, how would this correlate with changes observed through traditional measures of grooming? For this, we used the algorithm to analyse grooming of C57BL/6 and 129S1 mice following a 5-min exposure to a plastic box ($30 \times 30 \times 30$ cm, similar to the actimeter), which was familiar to the mice following three subsequent exposures (30 min each) 1 day prior to testing. As can be seen in Fig. 3, the grooming microstructure was not affected in this low-stress situation, although an increase in the duration of grooming was detected in both C57BL/6 and 129S1 mice, compared to the controls without exposure to the familiar box. This shows that in situations when traditional measures

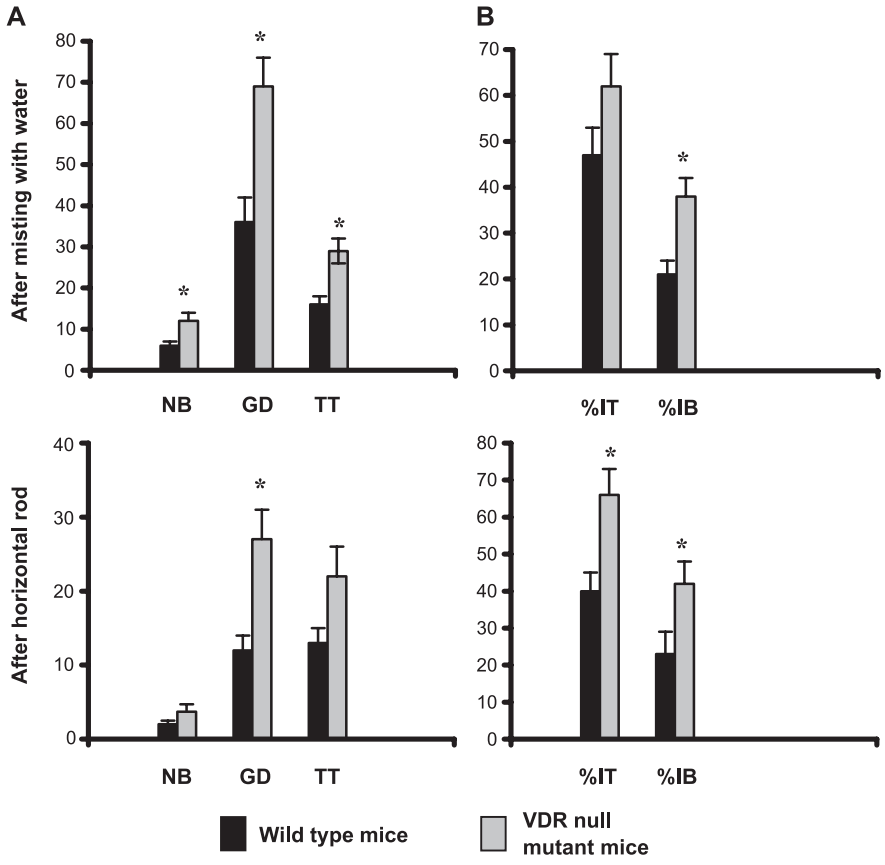

Fig. 2. Misting with water and exposure to the horizontal rod test activate grooming gross measures (A) and affect grooming behavioural microstructure (B), producing more incorrect transitions and grooming interruptions in the VDR null mutant compared to the wild type 129S1 mice ($n = 10$ in each group). NB—number of grooming bouts; GD—total duration of grooming, s; TT—number of transitions between grooming stages; %IT—percent of incorrect transitions; %IB—percent of interrupted bouts. Data are expressed as mean ± S.E.M. $P < 0.05$, compared to the control group (U-test).

of grooming show alterations which can be mistaken for stress markers, our protocol does not falsely detect stress in low-stress situations. This represents a clear advantage of the protocol, which appears to be more reliable in distinguishing between stress and no-stress situations.

6. Discussion

6.1. General assessment of the protocol

Overall, there were two major questions in the present research: (1) Is our protocol able to detect stress in mice tested in different stress situations? (2) Can the protocol be effective in detecting behavioural differences in genetically modified mice compared to their wild type littermates? The results presented here answer on both questions positively, and we will next discuss why this protocol can be a novel alternative method of behavioural analysis, a valuable tool in behavioural research, used to complement or even replace more traditional gross measures.

(i) The idea of analysing grooming separately after exposure to stressors (i.e. testing mice in the open field or elevated plus maze) instead of focusing on grooming demonstrated only during these tests (as done in the majority of studies [16,25]) can be a clear advantage for several reasons. Grooming scores taken during these tests are generally low (being masked by alterations in other, non-grooming behaviours), and their analysis can therefore be extremely difficult. In contrast, more specific analysis of mouse grooming in an actimeter test following testing in these paradigms not only enables higher grooming scores, but also the identification of more "pure" stress-evoked grooming, not confounded by other (non-grooming) behaviours. This approach allows obtaining several parallel sets of data, including: (a) behavioural measures taken in these initial behavioural tests, and (b) grooming measures obtained in the subsequent testing using this current protocol, which can then both be used for a detailed behavioural analysis.

(ii) The data presented in Figs. 1 and 2 show that in all models of stress (except for the open field, where the tendency did not reach significant level) our algorithm was able to detect stress as assessed by increased percentages of incorrect transitions between different stages and interrupted bouts. This shows that alteration in grooming microstructure

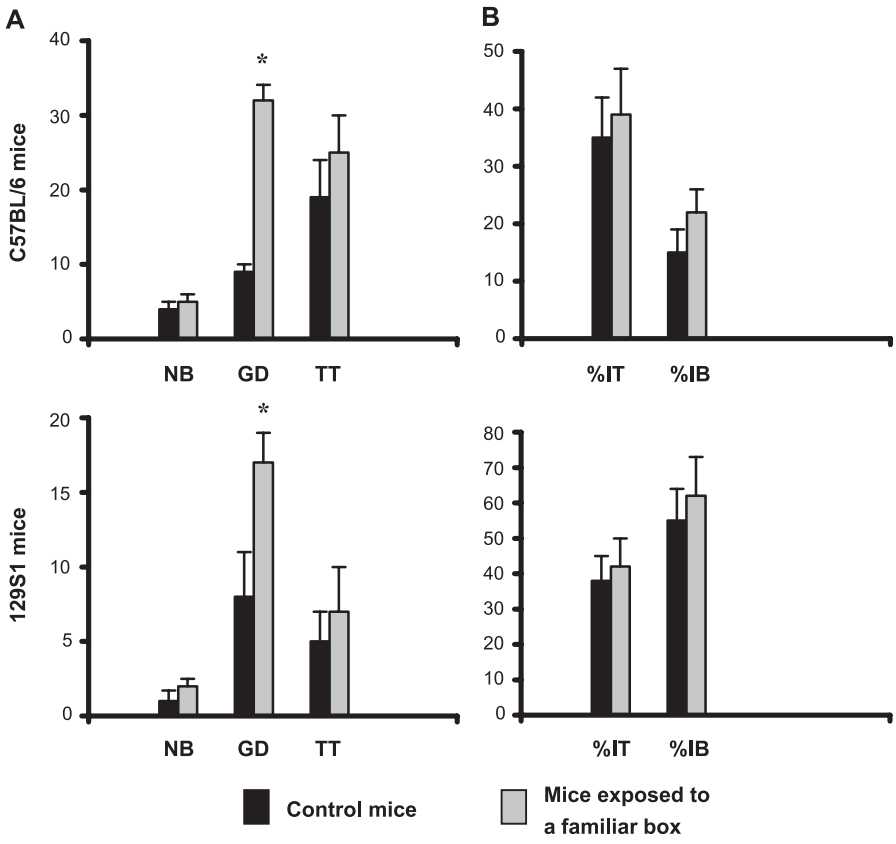

Fig. 3. Low-stress situation (exposure to familiar box for 5 min.) affects grooming gross measures (the duration of grooming) (A) but has no effects on its behavioural microstructure (B) in C57BL/6 and 129S1 mice compared to non-stressed controls ($n = 10$ in each group). NB—number of grooming bouts; GD—total duration of grooming, s; TT—number of transitions between grooming stages; %IT—percent of incorrect transitions; %IB—percent of interrupted bouts. Data are expressed as mean ± S.E.M. $P < 0.05$, compared to the corresponding control groups (U-test).

itself can be used as a sensitive behavioural marker of stress, which is at least as useful as traditional grooming duration measures (also altered in all these tests, Figs. 1 and 2). Importantly, the protocol works consistently in several different types of stress, including novelty stress (elevated plus maze), social stress (social encounter), psychophysical stress (horizontal rod test) and physical stress (misting with water). This observation further confirms the utility of the protocol as a universal method for analysing potential changes in grooming produced by different types of stressors.

(iii) In contrast, the use of our protocol in low-stress situations following exposure to a familiar box (Fig. 3) shows that grooming sequencing in C57BL/6 and 129S1 mice was not affected, despite an increase in the duration of grooming in both strains, compared to unexposed controls. This shows that, in line with our knowledge of the dual nature of grooming increase, the grooming duration measure cannot distinguish between stress and no stress, and therefore can result in false positive findings if taken alone. However, the lack of false positive findings using our protocol in two most popular mouse strains shows its potential for more correct behavioural data interpretations and analysis.

(iv) Using mutant mice lacking the VDR gene (Fig. 2), we analysed their grooming microstructure and showed that the present algorithm is able to detect stress-evoked alterations in grooming in these genetically modified animals, recently reported to have increased anxiety phenotype [22]. This clearly proves the utility of our approach to study behavioural phenotypes of various mutant mice, especially those displaying abnormal emotional behaviours. In addition, since some mutant mice may have abnormal sequencing of behavioural patterns including grooming [12,27] (which may occur with or without changes in grooming gross measures), our protocol based on sequential analysis of grooming may be a useful tool in the detection of such behavioural abnormalities.

(v) In general, our results show that a detailed analysis of grooming behaviour according to this protocol appears to be a useful tool for the study of mouse behaviours in the field of stress research and behavioural neurogenetics. Furthermore, we suggest that the protocol is also suitable for use with rats and other laboratory rodents given the similarity in their grooming behaviours [2,17]. The protocol can also be used in psychopharmacology research, in the search of novel anxiolytic antistress drugs. Given the results of our study, this protocol will be especially useful in assisting with more accurate interpretation of other (non-grooming) behavioural data, especially in situations

when conflicting data have been obtained, and there is a need for more detailed and in-depth behavioural analysis. For example, it may be extensively used when studying different manipulations with mixed or unclear effects, interpreting the behaviour of novel mutant mice with unknown or unclear behavioural phenotype, or screening drugs with unclear profile.

(vi) Finally, although not directly tested in this study, it is possible to speculate that our protocol may not only detect stress-evoked alterations in grooming microstructure, but also (after some modification) actually measure the degree of stress evoked by different tests. To some extent, our data confirm this possibility, since no difference in grooming microstructure measures was found for the low-stress familiar box, a tendency to increased grooming sequencing error rates for the mild-stress open field test, and predictable increase in grooming microstructural parameters for the high-stress elevated plus maze (Figs. 1 and 3). Clearly, this promising research direction will almost certainly require further investigation, and may represent an important potential application of our protocol.

6.2. Troubleshooting

(i) Numerous studies confirm that grooming is the initial behavioural response to stressful situations and is used by the animals to lower arousal [15,24,25,28]. These specific behaviours have long been studied in many researches [7,14,25,26]. Here, we show that not only traditional gross measures of grooming activity but also its behavioural microstructure (the percentages of incorrect transitions and interrupted bouts) are markedly affected by stress. The finding that grooming microstructure is sensitive to stressors suggests good predictive validity for the current protocol as a powerful tool to detect stress in animal behaviour. However, we should point out that stress-evoked non-grooming behavioural activity (e.g. risk assessment, freezing, and urination/defecation) can be a possible confounding factor, because alterations in these behaviours may reciprocally affect animal grooming [21]. Since all non-grooming behaviours are more obvious when mice are first exposed to the actimeter test, their grooming is easily affected by minimal changes in environmental conditions. Thus, it is crucial that the experimenters handle the mice for 5 min/day for 5 days and expose them to the actimeter for 5 min/day two to three times before the first experiment, in order for them to become familiar with environmental stimuli.

(ii) Since grooming response is known to habituate after repeated exposures to stress [15,21], we have noted that experimentally naïve animals on Test 1 usually show clearer and more robust results. If, however, a battery of several stressors has to be used in the same animals (which is nowadays the most common situation in behavioural research [9,10]), the mice should be allowed at least 7 days for acclimation between the tests. We have also noted that the present grooming analysis algorithm works better if the battery includes three or fewer tests. The timetable and intensity of the experiments is another related practical issue which may be critical for obtaining correct and reliable data [1,8–10]. Since rodents are very sensitive to the rhythm of activity of the researchers and animal house personnel (usually higher from Monday to Friday), all their behaviours can be affected by this factor [1]. This also relates to grooming: for example, we have noted that animal grooming scores are different on Monday than during the rest of the week. Although this is a common problem with all behavioural studies [1], it is advisable to consistently avoid, or, alternatively, prefer, weekends when scheduling the experiments aiming to assess animals' grooming behaviours.

(iii) Another common behavioural problem that may be encountered during the experiments is that mice sometimes display an unusually low activity level. This also relates to specific grooming activity. For example, some mice may fail to groom during the test, while others may display frequent extra-short (one to two stages) bouts or have unusual long latencies to start grooming [21]. Although usually the percentage of these animals is $<5–10\%$, which usually does not affect the results, this potential problem should be considered when performing analysis of animal grooming. There is neither a simple explanation nor a simple solution for this phenomenon. As one possible solution, it can be recommended to carefully check homecage behaviours before the testing and, if necessary, exclude mice with very low general motor and/or grooming activity from the experiments. In addition, the observation room conditions (ventilation, temperature, humidity, soundproofing, lights, etc.) and even experimenter identity may affect animal grooming performance, known to be very sensitive to all environmental conditions. As such, these factors have to be carefully controlled in the experiments.

7. Quick procedure

(a) Expose the mice to different stressors for 5 min and then assess their grooming patterns in the actimeter for 5 min.
(b) Identify and register separately all stages of a grooming ritual as follows: no grooming (stage 0), paw licking (stage 1), nose and face wash (stage 2), head wash (stage 3), body wash and fur licking (stage 4), leg licking (stage 5), and tail/genitals grooming (stage 6). Interruptions >5 s determine separate bouts.
(c) Analyse data using the transition matrix as in Table 1. The percentages of incorrect transitions and interrupted grooming bouts are used as behavioural markers of stress.

8. Essential literature references

Refs. [2–4,11,12,15,25,26,28].

Acknowledgements

This study was supported by the grants from CIMO, Tampere University Hospital (EVO) and the Academy of Finland. The authors thank Professor S. Kato, PhD, MD (University of Tokyo, Japan) for donating the initial VDR null mutant mice, and Dr. I. Laaksi, MD and Ms. Y.-R. Lou (University of Tampere, Finland) for breeding the mutant mice for our research.

References

[1] C. Belzung, Measuring rodent exploratory behavior, in: W.E. Cruzio, R.T. Gerlai (Eds.), Handbook of Molecular Genetics for Brain and Behavior Research, Elsevier, New York, 1999, pp. 738–749.
[2] K.C. Berridge, J.W. Aldridge, Super-stereotypy I: enhancement of a complex movement sequence by systemic dopamine D1 agonists, Synapse 37 (2000) 194–204.
[3] K.C. Berridge, J.W. Aldridge, Super-stereotypy II: enhancement of a complex movement sequence by intraventricular dopamine D1 agonists, Synapse 37 (2000) 205–215.
[4] K.C. Berridge, I.Q. Whishaw, Cortex, striatum and cerebellum: control of serial order in a grooming sequence, Exp. Brain Res. 90 (1992) 275–290.
[5] K.C. Berridge, J.C. Fentress, H. Parr, Natural syntax rules control action sequence of rats, Behav. Brain Res. 23 (1987) 59–68.
[6] V.J. Bolivar, W. Danilchuk, J.C. Fentress, Separation of activation and pattern in grooming development of weaver mice, Behav. Brain Res. 75 (1996) 49–58.
[7] W.M. Bressers, M.R. Kruk, A.M. Van Erp, D.C. Willekens-Bramer, P. Haccou, E. Meelis, The hypothalamus: cross-roads of endocrine and behavioural regulation in grooming and aggression, Neurosci. Biobehav. Rev. 23 (1998) 163–177.
[8] Y. Clement, F. Calatayud, C. Belzung, Genetic basis of anxiety-like behaviour: a critical review, Brain Res. Bull. 57 (2002) 57–71.
[9] J.N. Crawley, Behavioural phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioural tests, Brain Res. 835 (1999) 18–26.
[10] J.N. Crawley, J.K. Belknap, A. Collins, J.C. Crabbe, W. Frankel, N. Henderson, R.J. Hitzermann, S.C. Maxson, L.L. Miner, A.J. Silva, J.M. Wehner, A. Wynshaw-Boris, R. Paylor, Behavioural phenotypes of inbred mouse strains: implications and recommendations for molecular studies, Psychopharmacology 132 (1997) 107–124.
[11] H.C. Cromwell, K.C. Berridge, Implementation of action sequences by a neostriatal site: a lesion mapping study of grooming syntax, J. Neurosci. 16 (1996) 3444–3458.
[12] H.C. Cromwell, K.C. Berridge, J. Drago, M.S. Levine, Action sequencing is impaired in D1A-deficient mutant mice, Eur. J. Neurosci. 10 (1998) 2426–2432.
[13] A.J. Dunn, C.W. Berridge, Y.I. Lai, T.L. Yachabach, CRF-induced excessive grooming behavior in rats and mice, Peptides 8 (1987) 841–844.
[14] J.R. Eguibar, A. Moyaho, Inhibition of grooming by pilocarpine differs in high- and low-yawning sublines of Sprague–Dawley rats, Pharmacol. Biochem. Behav. 58 (1997) 317–322.
[15] J.R. Eguibar, J.C. Romero-Carbente, A. Moyaho, Behavioral differences between selectively bred rats: D1 versus D2 receptors in yawning and grooming, Pharmacol. Biochem. Behav. 74 (2003) 827–832.
[16] E.F. Espejo, Structure of the mouse behaviour on the elevated plus-maze test of anxiety, Behav. Brain Res. 86 (1997) 105–112.
[17] J.C. Fentress, The tonic hypothesis and the patterning of behaviour, Ann. N.Y. Acad. Sci. 290 (1977) 370–394.
[18] R. Gerlai, J.F. Henderson, J.C. Roder, Z. Jia, Multiple behavioural anomalies in GluR2 mutant mice exhibiting enhanced LTP, Behav. Brain Res. 95 (1998) 37–45.
[19] J.M. Greer, M.R. Capecci, Hoxb8 is required for normal grooming behaviour in mice, Neuron 33 (2003) 23–34.
[20] A.V. Kalueff, Measuring grooming in stress and comfort, Proc. Meas. Behav. 3 2000, pp. 148–149.
[21] A.V. Kalueff, Grooming and Stress, KSF, Kiv, 2002, 148 pp.
[22] A.V. Kalueff, Y.R. Lou, I. Laaksi, P. Tuohimaa, Increased anxiety in mice lacking Vitamin D receptor gene, NeuroReport 15 (2004) 26–31.
[23] Y. Kamei, T. Kawada, T. Fukuwatori, T. Ono, S. Kato, E. Sugimoto, Cloning and sequencing of the gene encoding the mouse vitamin D receptor, Gene 152 (1995) 281–282.
[24] R.J. Katz, K.A. Roth, Stress-induced grooming in the rat—an endorphin mediated syndrome, Neurosci. Lett. 13 (1979) 209–212.
[25] A. Moyaho, J. Valencia, Grooming and yawning trace adjustment to unfamiliar environments in laboratory Sprague–Dawley rats (*Rattus norvegicus*), J. Comp. Psychol. 116 (2002) 263–269.
[26] A. Moyaho, J.R. Eguibar, J.L. Diaz, Induced grooming transitions and open field behaviour differ in high- and low-yawning sublines of Sprague–Dawley rats, Anim. Behav. 50 (1995) 61–77.
[27] C. Strazielle, R. Lalonde, Grooming in Lurcher mutant mice, Physiol. Behav. 64 (1998) 57–61.
[28] A.M. van Erp, M.R. Kruk, W. Meelis, D.C. Willekens-Bramer, Effect of environmental stressors on time course, variability and form of self-grooming in the rat: handling, social contact, defeat, novelty, restraint and fur moistening, Behav Brain Res. 65 (1994) 47–55.
[29] A.M. van Erp, M.R. Kruk, D.C. Willekens-Bramer, P.C. Fermont, M.J. Nijsen, PVH lesions do not inhibit stressor-induced grooming in the rat, Physiol. Behav. 57 (1995) 887–892.