The role of hair in swimming of laboratory mice: implications for behavioural studies in animals with abnormal hair

A V Kalueff[1] and P Tuohimaa[2]

[1]Department of Anatomy, Medical School; [2]Department of Clinical Chemistry, University Hospital, University of Tampere, Tampere 33014, Finland

Summary

Animal swimming tests, such as the forced swim test, are extensively used in biomedical research to study rodent behaviour. Hair and skin exposed to water may be an important factor affecting the performance in this test. Since various hair and skin abnormalities are not uncommon in genetically modified or drug-treated laboratory animals, this test may be inappropriate for these animals. Because on occasions it is necessary to screen their swimming behaviour, in the present study we aimed to assess the role of hair in swimming of laboratory rodents in the forced swim test, widely used in behavioural research. For this, we shaved laboratory mice (129S1 strain) and compared their swimming patterns with those of unshaven controls. Overall, shaving mice did not affect their swimming behaviours in the 5 min forced swim test. Our results indicate that hair condition is not an important factor in the forced swim test for this mouse strain, and suggest that this test may have wider utility for behavioural analyses of mice with abnormal hair.

Keywords Swimming; mice; hair; behavioural test

Animal swimming is extensively used in biomedical research to study normal and pathological behaviours in rodents, including their emotional reactivity, memory, spatial navigation, motor coordination and performance (Morris *et al*. 1982, Weiss *et al*. 1998, Porsolt 2000, D'Hooge & De Deyn 2001, Ho *et al*. 2002, Yoshikawa *et al*. 2002). Overall, there are two standard behavioural tests for rodents which rely on their swimming ability: the forced swim test and the Morris water maze (Porsolt 2000, D'Hooge & De Deyn 2001). However, there are several important differences between these tests. The forced swim test is widely used to study animal depression-like 'despair' behaviours induced by swimming for 5–10 min in a relatively small water tank with no possibility of escape. In contrast, the Morris water maze is extensively used to assess memory and spatial navigation in animals, and consists of several shorter (1 min) sessions in a larger water container with a hidden escape platform.

Since swimming for 5 min is more dependent on animals' swimming abilities *per se*, our present study focused on the forced swim test. This test possesses procedural simplicity and reproducibility, and is widely used to assess the effects of different drugs and genetic mutations on small laboratory rodents (Weiss *et al*. 1998, Ho *et al*. 2002, Kalueff *et al*. 2004). Since skin and hair are exposed to water during swimming and may therefore be an important factor in the swim tests, the

Correspondence: Allan V Kalueff, Department of Anatomy, Medical School, University Hospital, University of Tampere, Tampere 33014, Finland. Email: avkalueff@inbox.ru

Accepted 7 March 2005

significant area of research where this test may be problematic is the study of animals with abnormal hair and/or skin.

We shall note, however, that this situation is not rare in biomedical research, and the growing number of genetically modified laboratory mouse and rat strains with abnormal hair makes the swim tests important in the area of neurobehavioural research. For example, mice with genetically ablated vitamin D receptor gene have no hair due to secondary alopecia developed approximately one month after birth (Yoshizawa *et al*. 1997). Mutant mice lacking the Cathepsin L gene develop periodic hair loss, epidermal hyperplasia and hyperkeratosis (Roth *et al*. 2000, Benavides *et al*. 2002). Defolliculated mouse mutation in chromosome 11 produces abnormal hair follicles and short hair shafts (Porter *et al*. 2002), while genetic ablation of retinoid X receptor in mice results in progressive alopecia with destruction of hair follicles (Li *et al*. 2001). Alopecia-like hair loss has been recently reported in one congenic and seven inbred laboratory mouse strains, as well as in many other species, including dogs, cats, horses, cattle and non-human primates (McElwee *et al*. 1999, Garner *et al*. 2004a,b). In rats, dominant hairless gene (Ht) and recessive shorn *shn* mutation generate an almost complete absence of hair (Akimoto *et al*. 2000, Chrissluis *et al*. 2002). There are many other known rodent strains which display hair and skin abnormalities accompanying various systemic disorders.

In addition, there are numerous data on behaviour-associated alopecia in rodents. For example, socially housed male mice generally display alopecic areas on the head, over the shoulders, the back and the pelvic regions due to an increase in self-grooming and a dramatic increase in allogrooming by their female partners (Militzer & Wecker 1986). Bresnahan *et al*. (1983) reported facial alopecia in female Fischer 344 rats caused by hair barbering by a dominant female rat, Long (1972) described hair loss as an indicator of low social hierarchy in mice, while Garner *et al*. (2004a,b) demonstrated that alopecia in mice occurs due to abnormal repetitive barbering behaviours, highly sensitive to the environmental factors. Moreover, animals with various brain and behavioural anomalies often display specific skin damage and hair removal due to excessive self-destructive grooming (see, for example, Greer & Capecci 2002). Finally, many chemical agents and peptides, including all classes of psychotropic drugs, are known to produce hair loss (Warnock *et al*. 1991, Uno & Kurata 1993, Merckle *et al*. 2000). Taken together, this clearly shows that laboratory animals with abnormal hair are much more common than is traditionally recognized. Therefore, the use of these animals in biomedical research and their behavioural phenotyping, including behavioural screening in the swim tests, can be a necessary task.

However, the question arises as to how one can properly assess swimming behaviour in these animals? Indeed, it seems methodologically inappropriate to compare swimming of hairless experimental animals with normally haired controls. For example, our group experienced such a problem earlier when studying the behavioural phenotype of vitamin D receptor knockout mice (Kalueff *et al*. 2004): while the latter were hairless, animals from both heterozygous and wild-type control groups had normal hair. Clearly, other laboratories might also experience similar technical problems in their research.

To the best of our knowledge, there have been no published studies analysing the role of hair in rodent swimming. In order to overcome this methodological problem, our present work investigates whether hair plays an important role for animal swimming by examining whether mouse swimming patterns would change if animals had no hair. For this, we removed all body hair by shaving mice and compared their swimming patterns with those of unshaven controls. Since 129 mouse strain is the major progenitor strain in transgenic and null mutation techniques, and one of the most commonly used strains in biomedical research (Cook *et al*. 2002, Rodgers *et al*. 2002, Lipp & Wolfer 2003), we used this strain in the present study. 129S1 mice were chosen for our experiments as the most commonly used substrain of 129 mouse

strain (Kalueff & Tuohimaa 2004). An in-depth ethological assessment of mouse swimming was performed in the forced swim test, using the commonly used 5 min version of this test (Porsolt 2000).

Materials and methods

Animals

The study was carried out on 20 adult male 129S1 mice (25–30 g; University of Tampere, Finland). Animals were housed in fours in plastic cages (425 × 266 × 185 mm, Scanbur BK, Sweden) in an animal house with a 12 h light–dark cycle (lights on at 06:00 h) with *ad libitum* food and water. All animals used in this study were experimentally naïve, and kept in a controlled environment maintained at a constant temperature (24 ± 1°C), relative humidity of 50 ± 5% and a light intensity of 25 ± 10 lux. Bedding – 200 g aspen chips (4 × 4 × 1 mm, Tapbei Oy, Finland) – was changed once a week. Animal care procedures were conducted in accordance with guidelines set by the European Community Council Directives (86/609/EEC) and the guidelines of the National Institutes of Health.

Apparatus and procedure

Animals from both groups ($n = 10$ in each group) were anaesthetized with Hypnorm (Jannsen Pharmaceutica, Beerse Belgium; 0.315 mg fentanyl citrate and 10 mg fluanisone/mL, dose: 10 mL/kg subcutaneously), and the shaving of the control mice was performed using small electric shears. This procedure was performed very accurately, taking approximately 15 min per animal and did not cause any harm to the mice. Seven days later, behavioural testing was performed. Behavioural testing was always conducted between 14:00 and 18:00 h. On the day of the experiments both groups of animals were transported to the dimly lit room and left undisturbed for 3 h prior to testing. To assess animal swimming, we used a water tank consisting of a glass cylinder 50 cm tall and 30 cm in diameter filled with water (25°C) to a height of 15 cm from the top. The tank was illuminated by a 40 W light suspended 90 cm above the tank. The forced swim test was conducted by removing an animal from its home cage and placing it into the tank. An investigator observed the mice timing the duration and the number of motor activity bouts for a period of 5 min. During the testing sessions, the observer remained standing in front of (and 2 m away from) the water tank. The following behavioural measures were recorded: (1) struggling; vigorous movement of all paws or scratching the walls; (2) sliding; small limb movements to keep head above the water; (3) immobility; motionless floating with no limb movements except for small postural adjustments or minor head movements; (4) the number of diving episodes; and (5) the number of defaecation boli deposited. In addition, the average duration of a single immobility episode was calculated as the total time immobile divided by the number of immobility episodes. The water was changed after each animal was tested. If the mice appeared to be drowning, they were picked up immediately. All experimental procedures were conducted in accordance with the European legislation and the guidelines of the National Institutes of Health. All animal experiments were approved by the Ethical Committee of the University of Tampere.

Statistics

All results are expressed as mean ± SEM. Since all behavioural data were normally distributed, they were analysed by Student's *t*-test. A probability of less than 0.05 was considered statistically significant.

Results

Figure 1 shows the swimming performance of mice tested for 5 min in the forced swim test. Overall, the duration of struggling episodes was similar in both groups (shaven mice: ~49%; unshaven control mice: ~45% of the test time). In both groups, there was a considerable similarity in the duration of sliding behaviour (shaven mice: ~37%; control mice: ~39% of the recording period,

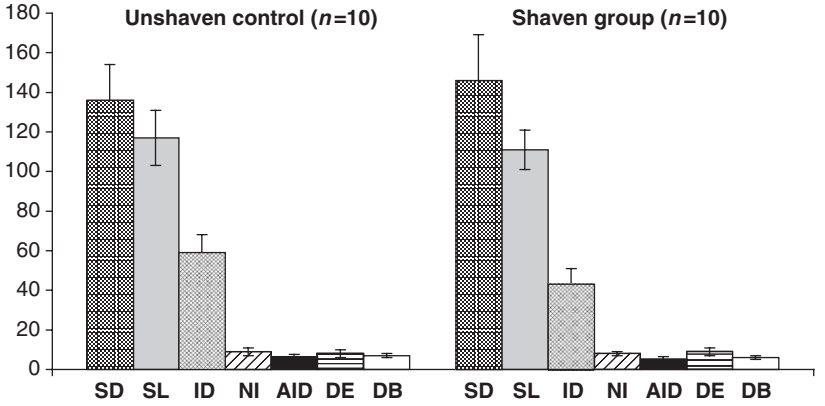

Figure 1 Diagram showing similar patterns of swimming behaviour of normal and shaven 129S1 mice subjected to a 5 min forced swim test. Data are expressed as mean ± SEM. $P>0.05$ (*t*-test). SD = duration of struggling (s); SL = duration of sliding (s); ID = duration of immobility episodes (s); NI = number of immobility episodes; AID = average duration of immobility episode (s); DE = number of diving episodes; DB = number of defaecation boli deposited

respectively). As can be seen in Figure 1, there were no differences in the number and duration of immobility episodes in both groups. Overall, shaven mice were immobile for ~14% of the recording time, compared with ~20% in the control group ($P>0.05$, *t*-test). In addition, both groups demonstrated similar average duration of a single immobility episode, as well as no significant differences in the number of diving behaviours (Figure 1). This figure also shows that both groups did not differ in the number of defaecation boli deposited.

Discussion

As already mentioned, measurement of the activity of rodents in a tank of water is widely used in animal behavioural research to establish behavioural phenotypes and to assess deficits in motor performance (Weiss *et al*. 1998, Kalueff *et al*. 2004). In the forced swim test, mice generally explore different behavioural strategies, including: (1) active swimming along the wall, scratching the wall (defined as struggling), (2) diving, (3) passive floating with only occasional paw movements (defined as sliding) and (4) immobility (Weiss *et al*. 1998, Porsolt 2000, Kalueff *et al*. 2004). Since there have been no published data on the role of hair in rodent swimming in the forced swim test, here we examined whether the presence or absence of hair may alter these key behavioural patterns in this paradigm.

Overall, several working hypotheses were examined in our study. First, it was possible to assume that hair *per se*, and especially air between hair, may provide mice with additional buoyancy and therefore assist swimming. This raises the possibility that animals without hair would have a poorer swimming performance. On the other hand, it seems possible that hair might increase the physiological costs of swimming and therefore should impede mouse performance in the forced swim test. If the latter hypothesis is correct, animals without hair will swim better than those with normal hair. Taking both possibilities together, the exact role of hair in the forced swim test seemed to be unclear and therefore experimental studies were needed to address this problem. A detailed ethological analysis of animal swimming demonstrates that the swimming of shaven mice is generally very similar to that of unshaven control animals. Indeed, as can be seen in Figure 1, there is a clear similarity between shaven and unshaven mice in all key swim test measures, including struggling, sliding, diving and

immobility, as well as in emotional reactivity index such as defaecation boli.

However, there are several interesting problems which were not directly addressed in our present study. Notably, shaven mice spent about 20% less time immobile (14% versus 20% of the recording time) compared with the control group animals. While support by hair, as already discussed above, may be unimportant to mice while swimming actively (struggling) or sliding, it may make it easier for mice to remain immobile (floating) and also complicate their diving. Although this interesting hypothesis seems theoretically reasonable, the lack of significant differences in both the number and average duration of a single immobility episode (Figure 1) allows us to discount this possibility. In line with this, there were no differences in mouse diving behaviours.

Another potentially important factor to consider is the thermoregulation in the swim tests. Since it does play a role in animal swimming (Iivonen *et al*. 2003), and shaving clearly can affect thermoregulation, this aspect would also require further investigation in detail. For example, it would be of interest in future studies to take body temperature measures in both groups and explore in more detail if and how swimming patterns correlate with body temperature. However, our observation that all swimming patterns were ethologically similar in both groups lends indirect support to the contention that possible changes in thermoregulation produced in mice by hair removal do not affect animal swimming performance over a 5 min test. Perhaps, future studies might better address these questions and determine if animal swimming would remain similar when tested over a longer period of time, under different water temperature conditions, and/or in a bigger water tank.

Several limitations of the present preliminary study (based on one strain, sex, age and one type of swimming test) indicate the need to further assess the generalizability of our findings. For example, it is interesting to know whether the results of our study may be extrapolated to another widely used swim test such as the Morris water maze (Morris *et al*. 1982), given shorter (i.e. less dependent on swimming *per se*) swimming sessions in this model. It will also be of interest to know if other mouse strains may demonstrate results similar to those of 129S1 mice. Taken together, this indicates that further experiments need to be done to extend our results to other experimental models based on animal swimming.

In general, our data suggest that hair is not a key physical factor that affects animal swimming behaviour in the 5 min forced swim test. Therefore, this test may be used to study swimming patterns of animals with and without hair. Importantly, there may be two potential practical implications from our findings. First, if a marked difference in swimming performance is found between haired and hairless animals in the forced swim test, this phenomenon may not be due to their hair-related phenotypical differences, and may indeed be attributed to some other factors, such as musculoskeletal anomalies, motor/behavioural deficits, sensorimotor dysfunctions or navigation difficulties (see, for example, Kalueff *et al*. 2004). Second, our data may be useful for other groups studying mice with abnormal hair, enabling more rational planning of their behavioural experiments. For example, this may allow researchers to reduce the number of animals that need to be used in animal experimentation – the important goal in laboratory animal science – by excluding 'shaven controls' from the experimental design (which would otherwise make hair removal in this group necessary for correct behavioural comparisons). Thus, the results of the present study may lead to more simple and less time- and animal-consuming experimental protocols that can be used to assess swimming in animals with different hair status.

Conclusion

The main conclusion of our study is that 129S1 mice without hair swim similarly to their normal counterparts in the forced swim test in its most widely used 5 min version. Since hair status, at least in small rodents such as mice, appears to have no

effect on their behavioural performance in this test, we may suggest that laboratory animals with and without hair can both be equally tested in this behavioural model. Given the growing number of mouse strains possessing various hair disorders, our data may represent a substantial methodological and practical contribution to the forced swim test when used as a tool in behavioural research, underlying its potential utility for behavioural screening of laboratory animals with various hair abnormalities.

Acknowledgements This research was supported in part by the grants from Tampere University and Tampere University Hospital (EVO).

References

Akimoto T, Suzuki H, Nakama K, Suzuki K (2000) Locus of dominant hairless gene (Ht) causing abnormal hair and keratinization maps to rat chromosome 10. *Experimental Animals* **49**, 137–40

Benavides F, Starost MF, Flores M, Gimenez-Conti IB, Guenet JL, Conti CJ (2002) Impaired hair follicle morphogenesis and cycling with abnormal epidermal differentiation in nackt mice, a cathepsin L-deficient mutation. *American Journal of Pathology* **161**, 693–703

Bresnahan JF, Kitchell BB, Wildman MF (1983) Facial hair barbering in rats. *Laboratory Animal Science* **33**, 290–1

Chrissluis RR, Stoklasek TA, Loman JA, Guariglia A, King TR (2002) The rat shorn mutation (shn) maps between D7Got143 and D7Rat94. *Molecular Genetics and Metabolism* **76**, 335–9

Cook MC, Bolivar VJ, McFadyen MP, Flatherty L (2002) Behavioral differences among 129 substrains: implications for knockout and transgenic mice. *Behavioral Neuroscience* **116**, 600–11

D'Hooge R, De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. *Brain Research Reviews* **36**, 60–90

Garner JP, Weisker SM, Dufour B, Mench JA (2004a) Barbering (fur and whisker trimming) by laboratory mice as a model of human trichotillomania and obsessive-compulsive spectrum disorders. *Comparative Medicine* **54**, 216–24

Garner JP, Dufour B, Gregg LE, Weisker SM, Mench JA (2004b) Social and husbandry factors affecting the prevalence and severity of barbering ('whisker trimming') by laboratory mice. *Applied Animal Behaviour Science* **89**, 263–82

Greer JM, Capecci MR (2002) Hoxb8 is required for normal grooming behaviour in mice. *Neuron* **33**, 23–34

Ho Y-J, Eichendorff J, Schwarting RKW (2002) Individual response profiles of male Wistar rats in animal models for anxiety and depression. *Behavioural Brain Research* **136**, 1– 12

Iivonen H, Nurminen L, Harri M, Tanila H, Puolivali J (2003) Hypothermia in mice tested in Morris water maze. *Behavioural Brain Research* **141**, 207–13

Kalueff AV, Lou Y-R, Laaksi I, Tuohimaa P (2004) Impaired motor performance in mice lacking neurosteroid vitamin D receptors. *Brain Research Bulletin* **64**, 25–9

Kalueff AV, Tuohimaa P (2004) Contrasting grooming phenotypes in C57BL/6 and 129S1/SvImJ mice. *Brain Research* **1028**, 75–82

Li M, Chiba H, Warot X, *et al.* (2001) RXR-alpha ablation in skin keratinocytes results in alopecia and epidermal alterations. *Development* **128**, 675–88

Lipp HP, Wolfer DP (2003) Genetical background problems in the analysis of cognitive and neuronal changes in genetically modified animals. *Clinical Neuroscience Research* **3**, 223–31

Long SY (1972) Hair-nibbling and whisker-trimming as indicators of social hierarchy in mice. *Animal Behavior* **20**, 10–2

Merckle Y, Sheng H, Khan T, Lippmann S (2000) Hair loss in psychopharmacology. *Annales of Clinical Psychiatry* **12**, 35–42

McElwee KJ, Boggess D, Miller J, King LE, Sundberg JP (1999) Spontaneous alopecia areata-like hair loss in one congenic and seven inbred laboratory mouse strains. *Journal of Investigative Dermatology Symposium Proceedings* **4**, 202–6

Militzer K, Wecker E (1986) Behaviour-associated alopecia areata in mice. *Laboratory Animals* **20**, 9–13

Morris RGM, Garrud P, Rawlins JNP, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. *Nature* **297**, 681–3

Porsolt RD (2000) Animal models of depression: utility for transgenic research. *Reviews in Neurosciences* **11**, 53–8

Porter RM, Jahoda CA, Lunny DP, *et al.* (2002) Defolliculated (dfl): a dominant mouse mutation leading to poor sebaceous gland differentiation and total elimination of pelage follicles. *Journal of Dermatology* **119**, 32–7

Rodgers RJ, Boullier E, Chatzimichalaki P, Cooper GD, Shorten A (2002) Contrasting phenotypes of C57BL/6JOlaHsd, 129S2/SvHsd and 129/SvEv mice in two exploration-based tests of anxiety-related behaviour. *Physiology & Behaviour* **77**, 301–10

Roth W, Deussing J, Botchkarev VA, *et al.* (2000) Cathepsin L deficiency as molecular defect of furless: hyperproliferation of keratinocytes and pertubation of hair follicle cycling. *FASEB Journal* **14**, 2075–86

Uno H, Kurata S (1993) Chemical agents and peptides affect hair growth. *Journal of Dermatology* **101**, 143S–7S

Warnock JK, Sieg K, Willsie D, Stevenson EK, Kestenbaum T (1991) Drug-related alopecia in patients treated with tricyclic antidepressants. *Journal of Nervous and Mental Disorders* **179**, 441–2

Weiss JM, Cierpial MA, West CHK (1998) Selective breeding of rats for high and low motor activity in a swim test: toward a new animal model of depression. *Pharmacology Biochemistry Behavior* **61**, 49-66

Yoshikawa T, Watanabe A, Ishitsuka Y, Nakaya A, Nakatani N (2002) Identification of multiple genetic loci linked to the propensity for 'behavioural despair' in mice. *Genome Research* **12**, 357–66

Yoshizava T, Handa Y, Uematsu Y, *et al.* (1997) Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. *Nature Genetics* **16**, 391–6