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Phenotyping and genetics of rodent grooming and barbering: utility for experimental neuroscience research

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Summary

Grooming and barbering (behavior-associated hair loss) are complex, ethologically rich behaviors. They are commonly observed in different animal species, and represent important phenotypes to study in experimental models utilizing rodent research. Due to sensitivity to alterations in activity and microstructure, grooming analysis has utility in the assessment of stress in individual animals, the testing of psychotropic drugs, phenotyping mutant or transgenic animals, as well as the selection of proper strains for experimental modeling of affective disorders. Similarly, barbering shows context- and strain-specific variations, and may serve as an indicator of social dominance or behavioral perseveration. While little is known about the genetics of barbering phenotypes, evaluation of this behavior has implications in neurophysiology and biological psychiatry, providing insight into trichotillomania, obsessive–compulsive disorder, aggression-related and other human brain disorders. Here, we discuss ethologically based approaches to the assessment of animal grooming and barbering activity. Additionally, we present examples of genetic variation leading to altered grooming and barbering phenotypes in rodents, and summarize the growing value of these two phenotypes for translational neurobehavioral research.

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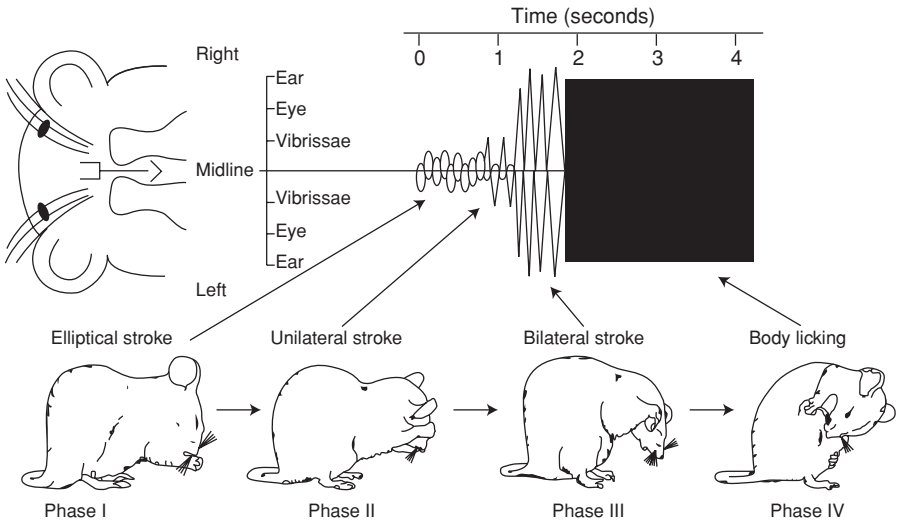

Figure 3.1 Prototypical syntactic grooming chain pattern in mice (K. Berridge, with permission). Phase I: series of ellipse-shaped strokes tightly around the nose (paw, nose grooming). Phase II: series of unilateral strokes (each made by one paw) that reach up the mystacial vibrissae to below the eye (face grooming). Phase III: series of bilateral strokes made by both paws simultaneously. Paws reach back and upwards, ascending usually high enough to pass over the ears (head grooming). Phase IV: body licking, preceded by postural cephalocaudal transition from paw/head grooming to body grooming.

Introduction

Grooming is an innate behavior shared across many animal species with remarkable homology (Fentress 1988; Sachs 1988; Spruijt *et al*. 1992). Common in laboratory and wild rodents, grooming occupies a substantial portion of their waking time, thereby representing an important phenotype to study (Bolles 1960; Hyman 2007; Kalueff *et al*. 2007a; Kalueff and Tuohimaa 2004b; Kalueff and Tuohimaa 2005b). Rodent grooming is a patterned behavior, which generally proceeds in a cephalo–caudal direction (Berridge *et al*. 2005; Fentress 1988). This pattern begins with paw licking, followed by washing of the nose and face, head, body, legs, and finally, the tail and genitals (Figure 3.1). Regulation of grooming behavior is mediated by multiple brain regions, especially the basal ganglia and hypothalamus (Aldridge *et al*. 2004; Berntson *et al*. 1988; Kruk *et al*. 1998; Roeling *et al*. 1993). Various endogenous and exogenous substances, such as the neuromediators dopamine, GABA (γ-amino butyric acid) or serotonin, as well as many hormones and psychotropic drugs, have been shown to modulate grooming activity (Barros *et al*. 1994; Bertolini *et al*. 1988; Dunn 1988; Dunn *et al*. 1987; Hill *et al*. 2007; Kalueff

Table 3.1 *Examples of grooming phenotypes in different genetically modified mice (data obtained from Mouse Genome Informatics and PubMed)*

Model	Background strain	Grooming behavior	References
Engrailed 2 (En2) gene knockout mice	129S2/SvPas × C57BL/6	Increased grooming	(Cheh *et al*. 2006)
Vitamin D receptor knockout mice	129S1	Increased grooming	(Kalueff *et al*. 2006a)
Homeo box (B8) gene knockout mice	129S1/Sv × 129 × 1/SvJ	Increased grooming	(Greer and Capecchi 2002)
Paired related homeobox protein-like 1 (Prrxl1) knockout mice	129S7/SvEvBrd × C57BL/6J × CD-1	Increased grooming	(Chen *et al*. 2001)
Cholinergic receptor, nicotinic, alpha polypeptide 4 (Chrna 4) knockout mice	129S4/SvJae × C57BL/6	Decreased grooming	(Ross *et al*. 2000)
D-aspartate oxidase knockout mice	129S4/SvJae	Decreased grooming	(Huang *et al*. 2006)
Oxytocin knockout mice	129S/SvEv × C57BL/6	Decreased grooming	(DeVries *et al*. 1997)
AT rich interactive domain 5B (Arid5b) knockout mice	129S4/SvJae× BALB/c	Decreased grooming	(Lahoud *et al*. 2001)

and Tuohimaa 2005c; Kruk *et al*. 1998; Navarro *et al*. 1995; Yalcin *et al*. 2007). Genes also play an important role in the regulation of this behavior (Greer and Capecchi 2002; Welch *et al*. 2007), and various genetic manipulations in animals have been reported to produce robust grooming phenotypes (Table 3.1).

Given the importance of grooming in animal phenotypes, it is reasonable to predict alterations in this domain would be seen in various experimental models of brain disorders. For example, as a displacement behavior, grooming is frequently displayed in animal models of stress, suggesting that it may simply be an anxiogenic response (Choleris *et al*. 2001). However, recent data show that higher stress in animals does not necessarily cultivate increased grooming activity, as it may also be increased under conditions of low stress (e.g., "comfort" grooming that occurs spontaneously as a transition between rest and activity) (Kalueff *et al*. 2007a; Kalueff and Tuohimaa 2004b, 2005b).

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Table 3.2 *Methodological approaches to animal grooming phenotyping, according to (Aldridge* et al. 2004; Berridge et al. 2005; Kalueff et al. 2007a; Kalueff and Tuohimaa 2004b; Kalueff and Tuohimaa 2005b; Piato et al. 2008)

Global assessment
Coat state
General cumulative measures
The latency to onset, the duration and the number of grooming episodes (bouts). Temporal patterning (e.g., per-minute distribution) of grooming duration and frequency may be recorded to examine habituation of this behavior.
The following patterns can be recorded for each bout: paw licking; nose/face grooming; head washing; body and leg grooming/scratching; tail/genitals grooming.
Additional cumulative indices: the average duration of a single grooming bout, total number of transitions between grooming stages, and average number of transitions per bout.
Patterning (sequencing)
The percentages of incorrect transitions, as well as interrupted and incomplete grooming bouts.
Regional distribution of grooming
Can be assessed as directed to the following five anatomic areas: forepaws, head, body, hind legs, and tail/genitals. Rostral grooming includes forepaw (preliminary rostral grooming) and head grooming. Body, legs and tail/genital grooming can be considered as caudal grooming. Each bout can be categorized as being directed to (i) multiple regions or (ii) a single region, and the percentages of grooming bouts and of time spent grooming can be calculated for both categories.
Additional useful indices of grooming
Probability of chain initiation (frequency of chain initiation per minute of grooming time), probability of pattern completion once initiated.

Due to this complexity, grooming phenotypes must be examined both qualitatively and quantitatively. While general cumulative measures provide a gross assessment of grooming activity, its patterning and regional distribution indices are also important for comprehensive evaluation of this behavior (Table 3.2). Understanding the cumulative, patterning, and regional alterations in grooming has implications for developing improved animal models of human brain disorders (e.g., anxiety, depression, obsessive–compulsive disorder [OCD], or Tourette's syndrome), behavioral phenotyping of mutant or transgenic strains, and the testing of psychotropic drugs (Berridge *et al*. 2005; Campbell *et al*. 1999; Kalueff *et al*. 2007a; Kalueff and Tuohimaa 2005c; Welch *et al*. 2007).

Like grooming, barbering (Figure 3.2) is a common phenotype in many different species. Representing a behavior-associated hair loss, it is also known in the literature as whisker-eating, whisker trimming, hair nibbling, hair pulling,

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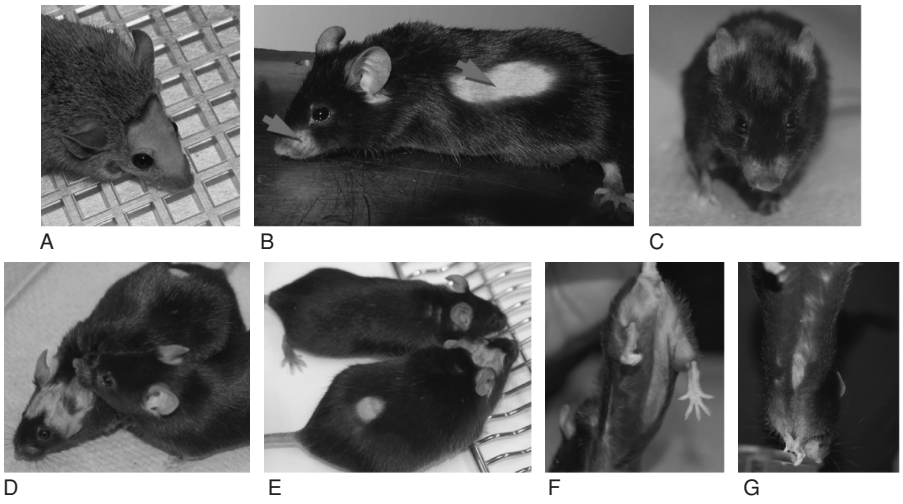

Figure 3.2 Examples of barbering phenotypes in different mice. A: hetero-barbering in 129Sv mice; B, C, D, E: hetero-barbering in C57Bl/6 mice; F, G: self-barbering in C57Bl/6 mice. (Photos: B. Dufour, J. Garner).

behavior-associated alopecia areata, and the Dalila effect (Garner *et al*. 2004a; Kurien *et al*. 2005; Long 1972; Sarna *et al*. 2000). Barbering behavior is frequently seen in laboratory mice, when an individual plucks or trims fur and/or whiskers from cage-mates and/or itself, leaving idiosyncratic patches of hair loss on the nose, head, shoulders, forearms, or elsewhere (Garner *et al*. 2004b; Hill *et al*. 2007; Kalueff *et al*. 2006b; Kurien *et al*. 2005; Sarna *et al*. 2000).

There has been a growing interest in barbering phenotype recently, both as a husbandry problem (De Luca 1997; Garner *et al*. 2004a) and as a behavioral assay in biomedical research (Garner *et al*. 2004b; Hill *et al*. 2007; Kalueff *et al*. 2006b). However, relatively little is known about how or why barbering occurs. The existence of barbering behavior is a biological paradox, since it is sometimes performed without an apparent adaptive benefit for the barber, and in spite of fitness costs associated with this behavior. Answers to this paradox are the most contentious issues within the barbering literature, to which several hypotheses have been developed (Garner *et al*. 2004a; Kalueff *et al*. 2006b; Kurien *et al*. 2005; Sarna *et al*. 2000). Briefly, the *dominance hypothesis* claims that mice pluck hair in order to establish their dominance over their cage-mates (Long 1972). For example, hetero-barbering may be a dominant behavior related to social hierarchy, since in mouse groups, there is often one individual with unbarbered whiskers who appears to play a dominant role in the cage (Kalueff *et al*. 2006b; Sarna *et al*. 2000). Thus, the adaptive value of barbering may be to facilitate murine

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social hierarchy development and/or maintenance, which will reduce the incidence of aggression and improve the health and survival of both the barber and its cage-mates.

The *coping hypothesis* suggests that barbering may represent a form of aberrant behavior developed to cope with inadequate housing conditions. Since coping is often invoked as a functional explanation for other abnormal behaviors seen in captivity (such as stereotypy), the adaptive value of barbering may be to reduce stress in individuals that pluck, which should improve the health and survival of laboratory mice. However, while plausible, there is no evidence that the performance of barbering behavior provides any anxiolytic or stress-reducing effects for barbers. The *pathology hypothesis* explains the barbering paradox by claiming that mice pluck hair as a result of abnormal brain function, which is induced by the unnatural environment in which they develop. In contrast to the other hypotheses, it implies that barbering behavior has no adaptive value, but instead occurs as a symptom of disturbed neurophysiology (Garner *et al*. 2004a, b).

Although the exact biological reasons for barbering remain unclear, many studies indicate that there may be a strong genetic component, as barbering occurs more frequently in some mouse strains than others (Figure 3.2), and because some genetic manipulations may robustly affect barbering phenotypes (Table 3.4). Thus, the *genetic hypothesis* of barbering may also be an interesting avenue for further research in this field. Finally, it is possible that barbering represents a more *complex, multifactorial* behavioral phenomenon, and several different context-specific factors play a role in this behavior (Kalueff *et al*. 2006b).

Interestingly, while appearing similar enough, animals' barbering profiles do not always correlate with grooming phenotypes (Kalueff *et al*. 2006b; Sarna *et al*. 2000), and the two activities are likely to represent related but distinct behavioral domains. For example, in hetero-grooming, the mouse licks the body surface, often in specific regions, and sometimes even gently bites the fur without pulling any hair out. In whisker plucking, some mouthing and licking may also take place, but hairs are often plucked out in the absence of these normal grooming behaviors after the cage-mate has been pressed down (Sarna *et al*. 2000). Overall, there are several reasons why domain-specific behavioral analyses may benefit neurobehavioral research. First, like grooming, barbering is an interesting behavior per se, which plays an important role in mouse activity (Garner *et al*. 2004b; Kalueff *et al*. 2006b; Sarna *et al*. 2000). Second, because barbering often affects the reception of essential sensory input from the whiskers, barbered whiskers may affect all rodent behaviors, including behavioral performance in experimental tasks. Third, barbering is observed more commonly in some strains than others (Carruthers *et al*. 1998; Garner *et al*. 2004a; Kalueff *et al*. 2006b; Sarna *et al*. 2000), enabling studies of different genetic contributors to the behavior. Finally, phenotyping rodent

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barbering could lead to ethologically oriented experimental models of many prevalent human disorders, such as trichotillomania, OCD, and aggression (Garner *et al*. 2004b; Hill *et al*. 2007; Kalueff *et al*. 2006b; Kurien *et al*. 2005). Therefore, further in-depth ethological analyses are necessary to achieve a detailed understanding of the nature, etiology, and genetics of rodent barbering.

Given the considerable amount of time animals spend on grooming and barbering (Bolles 1960; Fentress 1988; Garner *et al*. 2004b; Sarna *et al*. 2000), these behaviors are a noteworthy subject of research in behavioral neuroscience. This chapter will provide an updated information on the phenotyping and genetics of animal grooming and barbering behaviors, and how their analysis may foster further advances in translational biopsychiatry research.

Behavioral phenotyping

Animal grooming

Procedures

Coat-state assessment is a simple method to evaluate animal grooming activity (Piato *et al*. 2008; Yalcin *et al*. 2005; Yalcin *et al*. 2007), and may be performed in each individual rodent in eight separate body parts: head, neck, forepaws, dorsal coat, ventral coat, hind legs, tail, and genital region. For example, a score of 0 could be attributed to a coat in good form, and a score of 1 could be given to a dirty disheveled coat. The resulting score will represent the average (or the sum) of all body areas, and can also be compared across different experimental groups. Although this approach may lack some ethological sensitivity, poor coat state generally correlates with experimental depression. Indeed, chronically stressed "depressed" mice typically display poor coat status, whereas antidepressant treatments tend to reverse this phenotype (Piato *et al*. 2008; Yalcin *et al*. 2005, 2007). Therefore, coat-state assessment can be a useful tool in measuring animal brain pathology.

To induce acute stress-evoked grooming, researchers may use a brief mild stress, such as exposure to a novelty (Barros *et al*. 1994; Clement *et al*. 1994; Crusio *et al*. 1989; Crusio and van Abeelen 1987; Enginar *et al*. 2008; Kalueff and Tuohimaa 2004a). In addition, stronger stressors (e.g., a bright light, social aggression, a predator, or a predator's scent) will also generate stress-evoked grooming, which is highly relevant to emotionality and experimental modeling research.

While chronically applied mild stress may reduce animal grooming, stronger stressors (e.g., olfacto-bulbectomy or peripheral anosmia) produce pronounced activation of stereotypic grooming activity. This "pathological" grooming is

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generally focused on one specific area of the body (e.g., flanks), and is often accompanied by severe depression-like behaviors (such as anhedonia, hypoactivity, and aggression) (Kalueff *et al*. 2001; Makarchuk 1999; Makarchuk and Zyma 2002; Makarchuk 1998). In general, these observations seem to parallel clinical data showing overall increases in stereotypic behavior (grooming disorders, hair pulling) in depressed patients.

Unlike spontaneous stress-induced grooming, artificially induced grooming can be evoked by swimming or by smearing the animal with food (Audet *et al*. 2006; Burne *et al*. 2006). The splash test (in which a sucrose solution is squirted onto the dorsal region of the animal, and grooming is recorded for five minutes after solution vaporization) will also stimulate artificial grooming in rodents (Piato *et al*. 2008; Yalcin *et al*. 2005). Misting with water is another easy and reliable method to evoke artificial grooming behavior, and is widely used in neurobehavioral experiments (Audet *et al*. 2006; Hartley and Montgomery 2008). Since spontaneous and artificial grooming represent two different forms of this behavior, abnormalities in one type do not necessarily imply deficits in the other. Thus, a parallel assessment of stress-evoked and artificial grooming is necessary for an accurate characterization of animal behavioral phenotypes (Kalueff *et al*. 2005; Kalueff and Tuohimaa 2004a, 2005a).

In addition to these methods, a "smart battery" that combines several other behavioral tests may be used (Kalueff *et al*. 2008). For example, a five-minute open field test (to assess baseline anxiety and spontaneous novelty induced grooming) may be followed by the Porsolt's forced swim test to evaluate depression-related immobility or despair. In order to maximize the number of behavioral endpoints and domains per experiment, researchers may place animals into an observation cylinder (for five minutes) to investigate artificial, swim-induced grooming immediately following the forced swim test. Comparing the patterning and activity of the artificial post-swim grooming with the spontaneous pre-swim grooming may provide intriguing data regarding grooming phenotypes. In some instances, animals may also have a "fatigueability" phenotype that should be discriminated from other grooming behaviors, as it will often be a confounding factor in such studies (Kalueff *et al*. 2008).

Behavioral analysis

Table 3.2 summarizes a systematic and high-throughput approach to analyzing mouse grooming activity and microstructure. To accurately evaluate grooming, researchers may develop a standardized scale to represent specific grooming activity and use it consistently within each laboratory. A typical scale may be as follows:

- no grooming (0)
- paw licking (1)
- nose, face, and head wash (2)
- body grooming, including body fur licking and scratching with hind paws (3)
- leg licking (4)
- tail or genital grooming (5).

However, researchers may modify this scale to suit their individual needs (e.g., by including additional strain-specific grooming behaviors of interest or by simplifying this scale for better detectibility).

A "correct" bout is cephalo–caudal in direction and follows a (0–1) (1–2) (2–3) (3–4) (4–5) (5–0) pattern of transitions (Table 3.2). An "incorrect" bout can vary from the model in one of four ways:

- aborted or prematurely terminated bouts (2–0, 4–0)
- skipped transitions (1–3, 2–5)
- reversed bouts (4–3, 5–2)
- incorrectly initiated bouts (0–2, 0–5).

A "complete" bout consists of a strict (0–1–2–3–4–5–0) sequence and any other pattern is considered incomplete. Frequently, researchers will notice grooming interruptions. Any sequence that contains at least one interruption is deemed "interrupted." However, an interruption of six seconds or longer is judged to be an entirely separate bout.

Using this approach, researchers may assess the three primary ethological measures of grooming patterning: the percentage of incorrect transitions, interrupted bouts, and incomplete bouts. In addition, the duration of correct versus incorrect patterns, the number of interruptions during bouts, and the duration of complete versus incomplete bouts may be calculated. It is also useful to investigate the regional distribution of grooming patterning. For example, data may be collected based on five anatomic areas (forepaws, head, body, hind legs, and tail/genitals) or simply a rostral (forepaw and head) versus caudal (body, legs, and tail/genitals) distinction. Researchers may also classify each grooming bout as being directed to a single anatomic region or multiple regions, and calculate the percentage of grooming bouts and the percentage of time spent grooming for each category. Furthermore, the percentage of total grooming patterns, the percentage of time spent grooming, and the number of interruptions for each anatomic area may be assessed (Table 3.1).

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Animal barbering

Procedures and behavioral analyses

Barbering behavior can often be observed in individual mice (such as C57Bl/6 mice) that pluck whiskers from cage-mates, and can be broken into four distinct stages, see (Sarna *et al*. 2000) for details:

- Hold: the barber presses down on the back and neck of its cage-mate
- Grasp: the barber grasps a single hair from the victim with its incisors
- Pluck: the barber pulls its head away from the victim, removing the hair from the root
- Manipulation: the barber often manipulates the removed hair with its paws, sometimes ingesting the hair.

The process for plucking fur has not been described in such detail, but is presumably very similar. Over time, barbers pluck hair from focused areas on the body of the recipients, leaving idiosyncratic patterns of alopecia (Garner *et al*. 2004a, b; Long 1972; Sarna *et al*. 2000; Figure 3.2). The skin of these regions is nonpruritic, since barbering per se does not involve tissue damage. Each barber typically plucks a similar, matching pattern from all accessible cage-mates, and this pattern is referred to as the barber's "cutting style" (Sarna *et al*. 2000). For example, one barber may pluck the whiskers, between the ears, and around the tail of its cage-mates, while another barber may pluck only a spot on the left flank of its cage-mates. Cutting styles also differ between strains, as some only pluck whiskers and from the face, while others pluck their idiosyncratic pattern from any area that is accessible (Figure 3.2; Garner *et al*. 2004b; Kalueff *et al*. 2006b; Sarna *et al*. 2000).

Table 3.4 summarizes some approaches to behavioral assessment of rodent barbering phenotypes. Patterns of hair loss can be drawn on a standardized mouse map. Cage-mates of individuals with no dorsal and ventral hair loss are classified as "non-barbers," whereas animals with ventral or low-forelimb hair loss can be classified as "self-barbers." Mice with cage-mates having similar patterns of alopecia on the face, whiskers, or dorsal surface only, and with the indicated mouse missing that pattern are classified as "cage-mate barbers," and those showing both cage-mate and self-barbering as "both barbers." Mice with any type of hair loss are categorized as "barbered," and mice with no hair loss as "intact" for each time-point.

On the mouse maps, both nonpruritic alopecia (hair loss without any redness, tissue damage, or scabbing) as well as pruritis must be recorded and differentiated. Typically, patterns of hair loss due to barbering have smooth and well-defined borders (Figure 3.2), and are distinct from hair loss caused by other factors. Presence

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Table 3.3 *Examples of strain differences in mouse grooming behavior*

Measure	Strain ranking	References
Frequency	DBA/2J, F1 [C57BL/6J-DBA/2J] > C57BL/6J	(van Abeelen 1966)
	A/Ibg, BALB/cIbg > DBA2Ibg, C57BL/6Ibg	(Streng 1971)
	C57BL/6J > 129S1	(Kalueff and Tuohimaa 2004a)
Duration	DBA/2J > CPB-K-Nmg > C3H/St, C57BL/6J	(Crusio and van Abeelen 1986)
	C57BL/6J > FVB/N	(Mineur and Crusio 2002)
	C57BL/6J > 129S1	(Kalueff and Tuohimaa 2004a)
	BALB/c > 129S1, NMRI	(Kalueff and Tuohimaa 2004a)

or absence of pruritis can be recorded for each mouse on alopecia scoring days, and mice with skin/tissue damage consistent with *ulcerative dermatitis* must be categorized accordingly.

Genetics

Grooming behavior

Interesting data on the behavioral genetics of grooming is currently available in the literature (Tables 3.1 and 3.3). For example, increased grooming was found to be associated with the *pink-eyed dilution* (*p*) and *brown coat color* (*b*) loci on chromosomes 7 and 4, respectively (van Abeelen 1963a, b, c). The *p* locus is located close to a cluster of GABAergic genes, and because the GABAergic system regulates both grooming and emotional behaviors, it is possible that these genes play a role in grooming phenotypes. Indeed, as both *p* and *b* loci are associated with increased anxiety (Clement *et al*. 1994; Clement and Chapouthier 1998), they may modulate the interplay between grooming and anxiety at a genetic and behavioral level.

Several studies have examined strain differences in mouse grooming (Table 3.3). For example, when BALB/cIbg, C57BL/6Ibg, A/Ibg, and DBA/2Ibg were tested in the open field, their cumulative grooming scores showed a significant increase in time effect and time × strain effect (grooming increase over a test time, observed in all strains) (Streng 1971). Additionally, ABP/Le mice groomed significantly more in the open field than less anxious C57BL/6 mice, while F1 ABP/Le-C57BL/b mice groomed more than F1 C57BL/6-ABP/Le, indicating a possible maternal effect (Clement *et al*. 1994).

There were also strain differences between C57BL/6 and some 129 substrains. 129SvEm and 129SvHsD showed lower light and dark grooming, but grooming scores rose for C57BL/6J mice during the dark phase (Rodgers *et al*. 2002). In

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Table 3.4 *Assessment of animal barbering phenotypes*

The following five-point scale can be used to assess barbering: 0 – no barbering; 1 – whisker removal or shortening; 2 – snout/face denuding; 3 – individual bald patches on head and body; 4 – multiple alopecic areas on head and/or body; 5 – severe alopecia including complete snout denuding and large alopecic areas on head and body This scale may be modified if necessary (Kalueff *et al*. 2006b), depending on the requirements of the study, but must remain consistent within the laboratory.
Hair loss can be scored at baseline, and every two weeks thereafter through the completion of the experiment (Garner *et al*. 2004a, b). Mice can be inspected on both dorsal and ventral surfaces for hair loss. Within each pattern, the severity of hair loss can be recorded as follows: 0 – intact; 1 – slight; 2 – medium; 3 – heavy; 4 – completely nude.
Hair loss can be scored as barbering only if the hair lesion was nonpuritic, there was no scarring or scabbing around the lesion, and the animal was otherwise in good health and the fur (where present) was in good condition.
The following parameters of barbering can be assessed: the number (%) of cages in which the barbering occurred; the average severity of barbering in each cage; and the percentages of barbers and barbered animals (of total animals of each strain). Barber animals can be easily identified as the single intact mouse in the cage (see Garner *et al*. 2004b; Sarna *et al*. 2000 for details).
If necessary, self-barbering may be assessed in mice housed individually (to prevent hetero-barbering) for three to four weeks (see Kalueff *et al*. 2006b) for details). Note, however, that such isolation stress may trigger animal anxiety that can further provoke stereotypic behaviors, including self-barbering.
Sometimes, excessive grooming in mice (e.g., Greer and Capecchi 2002) may lead to pronounced barbering-like alopecia (homecage observations may be needed in such cases, to distinguish between the two behaviors).

a similar study, FVB/N and C57BL/6J mice displayed comparable frequencies of grooming, but exhibited differences in duration, confirming that grooming frequency and duration may vary independently in different mouse strains (Mineur and Crusio 2002). Interestingly, grooming behavior in that study did not correlate with open field horizontal and vertical activity (FVB/N > C57), suggesting that grooming represents a distinct dimension in the organization of rodent behavior.

Strain differences in grooming were also reported between NMRI, 129S1, and BALB/c mice (Kalueff and Tuohimaa 2005a). NMRI mice displayed a clear tendency to earlier onset of grooming than 129S1 and BALB/c strains; however, there was no correlation between grooming activity and anxiety. Anxious strains display high (BALB/c) and low (129S1) grooming profiles, and nonanxious mice showed moderate to high (NMRI, C57BL/6) grooming profiles. Thus, overall grooming

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activity cannot accurately measure anxiety in mice. In contrast, studies investigating grooming microstructure did reveal significant differences between anxious and nonanxious mouse strains; anxious 129S1 mice displayed higher percentages of incorrect transitions and interrupted grooming bouts (Kalueff and Tuohimaa 2005a).

Grooming responses also vary across selectively bred mouse strains. For example, female Turku Aggressive strain mice spent considerably less time grooming during predatory aggression than the Turku Non-aggressive strain (Sandnabba 1995). In contrast, anxious high-thigmotaxis strain exhibited fewer grooming bouts in the open field than did less anxious, low-thigmotaxis strain (Leppanen and Ewalds-Kvist 2005; Leppanen *et al*. 2006). When these strains were cross-fostered, data revealed similar grooming activity in both the high- and low-thigmotaxis strains, indicating that both genetic and epigenetic factors influence mouse grooming.

Barbering behavior

There is limited data on the behavioral genetics of barbering, particularly on genetic mapping and strain differences. We have recently (Kalueff *et al*. 2007b, 2006b; Kalueff 2006, unpublished data) assessed barbering in several strains and their F1 offspring, focusing on distinct domains of this behavior. These included social dominance barbering in same-sex cages (observed in C57BL/J6, A/J, 129S1, and NMRI but not BALB/c mice), barbering of males by females in breeding pairs (C57BL/J6, 129S1, and NMRI but not BALB/c mice), maternal barbering (removal of lactating dam's ventral fur by pups) (C57BL/6 and 129S1), and whisker barbering of pups by their mothers (129S1). Notably, the percentage of mice exhibiting barbered hair varies markedly from strain to strain. For example, BALB/c mice never exhibit barbering, while C57BL/6, A/J, A2G, and NMRI show frequent barbering behavior (Carruthers *et al*. 1998; Kalueff *et al*. 2006b; Sarna *et al*. 2000). Additionally, several studies have demonstrated that mice may have consistent individual (Sarna *et al*. 2000) or strain-specific (Kalueff *et al*. 2006b) "cutting styles"; see Figure 3.2 for more examples.

Rodent barbering has been shown to be associated with social dominance and low levels of aggression. For example, the strain ranking of barbering activity (NMRI, C57 > 129 >>> BALB/c) generally negatively correlated with that of aggressiveness (BALB/c >> 129, C57 >> NMRI), which suggests that barbering might emerge in rodents to minimize potential aggression (Kalueff *et al*. 2006b).

Further revealing the behavioral complexity and multifactorial nature of mouse barbering, four different outcomes have been observed following genetic and epigenetic barbering crosses. In the first case, one of the parental phenotypes can

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outcompete the other in a hybrid cross. For example, F1 hybrids derived from the BALB/c strain exhibit the low-barbering phenotype associated with that strain, regardless of maternal influence (Kalueff *et al*. 2007b). The second case results in a blending of the parental strain barbering phenotypes in the hybrid offspring that is independent of maternal influences, as in the different crosses (between 129S1 and C57 or NMRI) performed by Kalueff *et al*. (2006a, b, and unpublished data). The third case results from cross-fostering experiments in which offspring take up the barbering phenotype of their foster parents. For example, nonbarbering strains raised with barbering foster parents may develop barbering behavior, although only in a small percentage of animals (Carruthers *et al*. 1998). Finally, the fourth outcome occurs when the genotype overcomes the maternal influence in cross-foster experiments, as in the same study by Carruthers *et al*. (1998) in which pups of barbering strains raised by nonbarbering foster parents continued to develop a barbering phenotype.

Assessment of the phenotypes of nonbarbering strains may be another useful approach to understanding the behavioral genetics of mouse barbering. For example, over 800 BALB/c mice did not show barbering activity (Carruthers *et al*. 1997, 1998; also see similar data in Kalueff *et al*. 2006b). Strain differences in sociability have recently been suggested as underlying factors in barbering phenotypes (Brodkin 2007). If confirmed, this interesting hypothesis may explain the low barbering activity in "autistic" mouse strains like BALB/c, as well as the high intensity of barbering in "sociable" strains, such as C57BL/6. Thus, barbering emerges as an important part of mouse social behavior, and strain differences may reflect (or underlie) different aspects and strategies of animal socialization. These variations in socialization, in turn, may confound all other behavioral domains, implying that in-depth analyses of strain barbering phenotypes may be even more significant than has been previously recognized.

Conclusion

Overall, in-depth phenotyping of animal grooming and barbering offers clear benefits for neurobehavioral research. First, it allows assessment of these biologically-important behaviors per se. Second, grooming and barbering activity may reflect strain differences in activity, anxiety, sociability, motor activity, and behavioral patterning, in addition to data from existing methods for phenotyping emotionality. Third, given the sensitivity of rodent grooming and sequencing to various pharmacological and physiological manipulations, ethological analysis of grooming may be used in pharmacogenetics and neurophysiology – for example, in the dissection of brain substrates involved in behavior regulation. Fourth, altered

Table 3.5 *Examples of barbering phenotypes in genetically modified mice (data obtained from Mouse Genome Informatics and PubMed)*

Model	Background strain	Barbering behavior	References
Phospholipase C beta1 knockout mice	F1 C57Bl/6J(N8) × 129S4/SvJae(N8)	Lack of whisker trimming	(Koh *et al*. 2008)
Complexin II knockout mice	F1, F2 129Ola × C57Bl6	Lack of whisker trimming	(Glynn *et al*. 2003)
Disheveled gene 1 (Dvl1) knockout mice	129S/SvEv	Lack of whisker trimming	(Lijam *et al*. 1997)
Transgenic mice over-expressing G protein-coupled receptor 85	C57BL/6	Reduced whisker trimming	(Matsumoto *et al*. 2008)
Vitamin D receptor knockout mice	129S1	Reduced whisker trimming and fur barbering	(Kalueff *et al*. 2006a)
Transcription factor USF1 knockout mice	C57BL/6	Increased whisker trimming	(Sirito *et al*. 1998)
Aromatase knockout mice	C57B6J × J129	Increased whisker trimming and fur barbering	(Hill *et al*. 2007)

grooming and barbering profiles may indicate behavioral perseverations, which may originate from an animal's natural displacement activity. Therefore, profiling both grooming and barbering phenotypes may allow researchers to indirectly assess potential strain differences in "compulsivity." Finally, comprehensive coverage of animal grooming and barbering peculiarities (Tables 3.1, 3.5) may assist researchers in correct data interpretation and in selecting appropriate animal models for their studies.

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