International Journal of Comparative Psychology, 2003, **16**, 237-246.

SHORT COMMUNICATION

Does Changing Levels of Stress Affect the Characteristics of Grooming Behavior in Rats?

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Twenty seven experimentally naïve adult female rats were exposed to a novel arena with shelters for a period of 15 min. Various measures of their pelage-cleaning behavior were analyzed. Bouts of grooming were shown to increase in duration and complexity and decrease in their rostral content over the span of the measurement period. Simultaneously, decrease in risk-assessment activity, as measured by the stretched attend posture, was noted in association with consecutive bouts. The effect on risk assessment appears to demonstrate that the aforementioned changes in grooming bout parameters resulted from a decrease in the level of stress. Differences in the characteristics of the early and the late grooming bouts suggest that bout initiation and bout continuation are affected by two relatively independent mechanisms involved in the shaping of pelage-cleaning behavior.

A variety of stimuli, both acutely and chronically administered, have been shown to stimulate sympathic-adrenomedullary and pituitary-adrenocortical responses characteristic of physiological stress. A wide range of physiological and metabolic changes are indicative of this stimulation in laboratory rodents: fluctuations in heart-beat frequency, increased blood pressure, and elevated plasma levels of catecholamines, ACTH, corticosterone, and glucose are all regarded as objective measures of stress (Armario, Montero, & Balasch, 1986; de Boer et al., 1990; Dobrakovova et al., 1993; Fokkema et al., 1988; Pfister, 1979; Rodgers et al., 1999; Walker et al., 1992; Warburton, 1991). The magnitude of these responses is dependent on type and the intensity of the stressor that is being applied (Armario et al., 1986; de Boer et al., 1990; Fokkema et al., 1988).

Exposure to novelty, particularly in the form of open, illuminated spaces, has been well documented as a stress-inducing factor (Armario et al., 1986; Choleris et al., 2001; de Boer et al., 1990; Holmes, 2001; Pfister, 1979; Spruijt, van Hooff, & Gispen, 1992). Rats and mice placed in such conditions exhibit a repertoire of behaviors believed to be indicative of stress and stress-related states of conflict, anxiety, and fear (Choleris et al., 2001; Escorihuela et al., 1999; Suchecki, Palma, & Tufik, 2000; Walsh, & Cummins, 1976). Unfortunately, many such ethological variables are sensitive to procedural modifications and stimulus parameters (e.g., Rodgers et al., 1997), thus their usefulness in the assessment of stress-related

The first author wishes to thank Sergio Pellis for a critical review of the manuscript. Correspondence concerning this article may be addressed to Joanna Komorowska, Department of Psychology and Neuroscience, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4 (j.komorowska@uleth.ca).

states is questionable (e.g., Choleris et al., 2001; Rodgers, 1997; Walsh, & Cummins, 1976).

One type of risk-assessment behavior, however, described as stretched attend posture (SAP), has been identified as a robust correlate of stressor-evoked anxiety in laboratory rodents (Rodgers, 1997; Rodgers et al., 1997). Evidence points to SAP as associated with two reliable measures of stress: high plasma levels of corticosterone and elevated blood pressure (Dielenberg & McGregor, 2001; Rodgers et al., 1999). Additionally, for moderately threatening conditions of novelty exposure, when high baseline level of risk assessment is present, the incidence of SAP can effectively be reduced by the administration of anxiolytics (benzodiazepines and 5-HT$_{1A}$ receptor agonists; Blanchard et al., 1990; Blanchard et al., 1997; Kaesermann, 1986; Rodgers, 1997; Wall, & Messier, 2001) and by repeated exposure to novelty (Kaesermann, 1986). This reduction is observable across a number of experimental paradigms (Choleris et al., 2001; Kaesermann, 1986; Rodgers et al., 1997). It appears then that SAP may serve as a valuable ethological marker indicative of stress, useful in examining the effects of stress on other behavioral indices.

Both the theoretical assumptions and the empirical evidence show that the elevated level of stress gradually decreases with increased habituation to novel environments (de Boer et al., 1990; Kant et al., 1984; Pfister, 1979; Pleskacheva, 1996; Suarez, & Gallup, 1981; van Erp et al., 1994; Roth & Katz, 1979). This effect renders exposure to novelty as a convenient testing paradigm in which differences in stress level can be comfortably produced and the influence of stressors on behavior can be studied.

One behavior clearly associated with neurophysiological stress is grooming. It can be induced by the experimental stimulation of the paraventricular nucleus of the hypothalamus and the central administration of neuropeptides (ACTH and MSH; Del Angel-Meza et al., 1996; Spruijt et al., 1992; van Erp et al., 1994). Grooming is easily triggered by various stressors including novelty exposure (Spruijt et al., 1992; van Erp et al., 1995).

Interestingly, temporal profiles of the amount of grooming in the novel environment demonstrate a consistent pattern: Initially low grooming scores increase with prolonged exposure. This pattern has been reported by independent researchers for a number of novel settings in laboratory rats (Horvath, Kirby, & Smith, 1971; Hughes, 1991; Pleskacheva, 1996; van Erp et al., 1994) and mice (Choleris et al., 2001), and appears to be relatively unaffected by procedural modifications. Therefore, the total amount of grooming can be viewed as a relatively reliable marker of changing stress levels, at least when exposure to novelty in concerned. Fine-tuning of such a stress-detecting tool may be of practical value to research in which the presence of stress needs to be assessed with a high degree of precision.

Pelage cleaning is not a uniform behavior. It consists of a variety of activities that occur in bouts and are performed in an orderly cephalocaudal progression (Del Angel-Meza et al., 1996; Golani, & Fentress, 1985; Richmond, & Sachs, 1980; Spruijt et al., 1992). Given their complexity, bout characteristics may provide valuable information regarding the changing level of stress in the grooming animals. They may also shed light on the internal mechanisms involved in the execution of the pelage cleaning activity. Both these issues were investigated in the present study.

Method

Subjects

Twenty-seven naïve, adult female rats of a Wistar strain were obtained from the breeder (Szostak; Warsaw, Poland) at 40-50 days of age and used as subjects. They were housed in groups of 3 or 4 in wire mesh cages (44x28x28 cm) at the University of Warsaw Psychology Department laboratory, and maintained under standard laboratory conditions on a 12:12 h light: dark cycle (lights on at 08:00 h), with free access to rat chow and water at all times except for the period of testing. The animals were tested at 80-85 days of age. Preliminary observations determined that animals of this age were fully capable of expressing all the behaviors of interest in the context of this experiment. No attempt was made to control for the stage of estrus.

Apparatus and Procedure

An open arena 120 cm wide and 150 cm long covered with gray linoleum was used as the testing enclosure. It was illuminated with a 60 W incandescent light source suspended over its center. This provided moderate light intensity in the amount of 40 lx as measured on its surface. Two shelters made of transparent glass (14x20x16 cm) positioned in the center of the arena, at a distance of 42 cm from each other, were provided as hiding places to facilitate the reduction of stress during the course of the trial (e.g., Holmes, 2001). It was in these shelters that all the grooming behavior took place.

Experiments were conducted during the light phase of the light: dark cycle (between 16:00 and 18:00 h). Each subject was carried into the experimental room in an opaque plastic container and left undisturbed for a period of 10 min. Then the animal was picked up by the experimenter and placed in the testing apparatus. Every subject was placed directly in one of the shelters in order to standardize the initial conditions. Each observation period lasted 15 min. The surface of the arena and the interior of shelters were thoroughly cleaned with soapy water and then clean water and allowed to dry between subjects.

The rat's behaviour was videotaped with a camera (Panasonic) mounted on a tripod positioned at a distance of 2 m from the front edge of the arena. The experimenter (person familiar to the subjects) was present in the room during filming and sat unobtrusively in the far corner of the room, facing the arena. All behavioral measures were transcribed from the videotaped recordings.

Analyses

Out of all pelage-directed activities performed by the rats the authors selected those bouts of grooming which included a stereotypical face wash (SFW): rapid, circular and bilaterally symmetrical forepaw strokes over the area of the mystacial vibrissae (Berridge, 1989; Berridge, & Fentress, 1987). Episodes of pelage manipulation that lacked the SFW component were not considered, since there is reason to believe that they are regulated by mechanisms other than and independent of those mechanisms that control typical novelty-induced grooming bouts (Bressers et al., 1995b; Pleskacheva, 1996; Richmond, & Sachs, 1980; Spruijt et al., 1992). Pauses in grooming activity in excess of 8 s in duration were treated as a cut off point between separate bouts. The 8 s cut-off period was established on the basis of preliminary observations indicating that pauses shorter than 8 s tended to be followed by the same grooming activity that immediately preceded the pause. Such outcome suggested that bouts interrupted by shorter pauses should be treated as temporarily halted rather than terminated.

The following parameters of consecutive bouts were recorded: Duration, rostral content (RC), and number of grooming actions. RC of each grooming bout was calculated as a ratio of 3 s intervals spent on face and head washing to all 3 s intervals spent on grooming. In order to assess the number of grooming actions, all grooming activities were organized into distinct categories based on their focus on the subjects' body and the type of the grooming action. Nine such categories were identified. (For information purposes, brief descriptions of the categories follows, although these descriptions have no further relevance for the present paper.) The categories included: *Face wash* (bilateral or unilateral forepaw strokes over the area of the snout, including the stereotypical face wash); *head groom* (broad, slow, bilateral or unilateral strokes of the forepaws over the area of the head, including the ears); *side and back groom* (rubbing and licking of the fur of dorsal area); *chest and belly groom* (rubbing and licking of the ventral area, excluding the anogenital area); *anogenital groom* (licking of the anogenital area); *hind paw groom* (rubbing and licking of a hind foot); *tail groom* (rubbing and licking of the tail); *nibbling of the fur,* irrespective of the location on the body; *scratching with the hind paw*, irrespective of the location on the body.

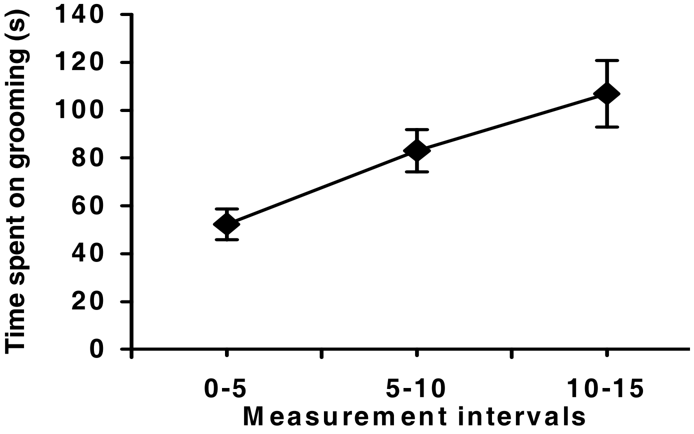

Figure 1. Changes in the duration of grooming over the course of the measurement period. Data represent means ± *SEM*.

The total amount of grooming in three consecutive 5-min intervals was calculated in order to test whether temporal changes in the general grooming scores complied with a pattern typical for prolonged exposure to novelty (see introduction for details). All subsequent analyses were performed on the first three grooming bouts, since further bouts of grooming were too infrequent to warrant their statistical comparison.

Presence of stress-induced anxiety was evaluated on the basis of risk assessment activity associated with consecutive bouts of grooming (Dielenberg, & McGregor, 2001; Rodgers et al., 1997; Rodgers et al., 1999). For this reason, the frequency of SAP was counted during a period of 60 s immediately preceding each grooming bout. In the SAP, the subject would poke its head, or both its head and its trunk, out of the shelter with its hind feet still retaining contact with the surface of the shelter.

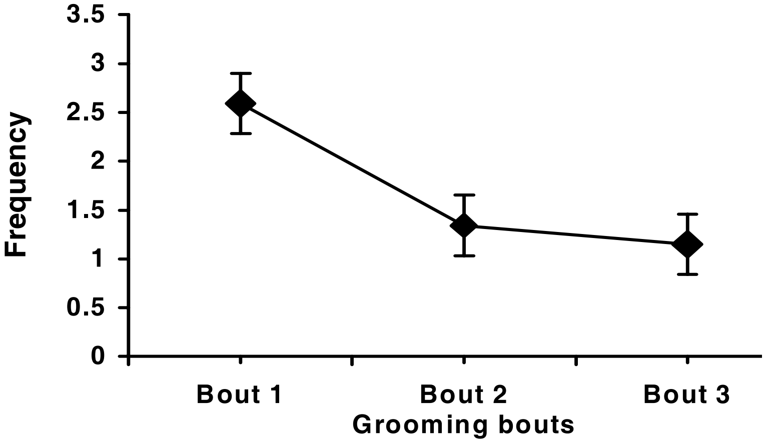

Figure 2. Frequency of SAP associated with consecutive grooming bouts. SAP = stretched attend posture. Data represent means ± *SEM*.

Friedman's one-way analysis of variance (ANOVA) was chosen for statistical analyses because of lack of homogeneity between sample variances. For significant results, pairwise comparisons were performed using the Wilcoxon matched-pair signed-rank test. An alpha of 0.05 was used in all statistical tests.

Results

The amount of time spent grooming was significantly different between consecutive 5-min intervals, χ_r^2 (2) = 10.40 with rats grooming the least during the initial measurement interval and the most during the final interval (Figure 1). Significant differences were revealed between intervals 1 and 2, and intervals 1 and 3, but not between intervals 2 and 3. The frequency of SAP behavior which occurred in association with grooming was significantly different between the analyzed bouts, χ_r^2 (2) = 7.38. First bouts were preceded by the highest number of SAP episodes, while last analyzed bouts were preceded by the lowest number of SAP episodes (Figure 2). Pairwise comparisons revealed significant differences between bout one and bout two and between bout one and bout three. No significant differences were detected between bout two and three.

Grooming bouts differed in their duration, χ_r^2 (2) = 27.88, Figure 3A. Bout one was significantly shorter than both subsequent bouts but the difference between bout two and bout three was not significant. Differences in bout duration were accompanied by differences in other bout characteristics. The number of grooming actions turned out to be the smallest within the first bout and reached its maximum for the last bout, χ_r^2 (2) = 19.34, Figure 3B. There was also a decrease in the rostral content for consecutive bouts, χ_r^2 (2) = 20.23, Figure 3C. All pairwise comparisons for the last three measures revealed significant differences between bouts.

Discussion

A time-related pattern of changes was revealed in the amount of grooming over the span of the trial: Animals engaged in progressively more grooming in consecutive time intervals. This result is consistent which other studies (Choleris et al., 2001; Horvath et al., 1971; Pleskacheva, 1996; van Erp et al., 1994). Clearly, despite certain procedural modifications implemented in the present study, the overall amount of grooming changed in a way that is typical during prolonged exposure to novelty. This effect suggests that other findings regarding the characteristics of individual bouts are representative and may be generalized across a variety of similar conditions.

The SAP behavior observed immediately prior to the first bout was most pronounced, yet its frequency turned out to be gradually reduced in association with the following bouts. This effect closely parallels the pattern of changes in SAP scores reported for a prolonged exposure to the open field in the laboratory mice (Choleris et al., 2001). Direct measurements of physiological and metabolic correlates of stress (plasma levels of adrenaline, corticosterone, glucose, and free fatty acids), demonstrate that exposure to novelty produces stress of low to moderate intensity (Armario et al., 1986; de Boer et al., 1990). Ethological data show that moderately stressful conditions result in the initial activation of defensive responses involving SAP (Choleris et al., 2001; Kaesermann, 1986), and that a high incidence of SAP is associated with elevated levels of plasma corticosterone (Rodgers et al., 1999). High baseline SAP scores are effectively reduced by the

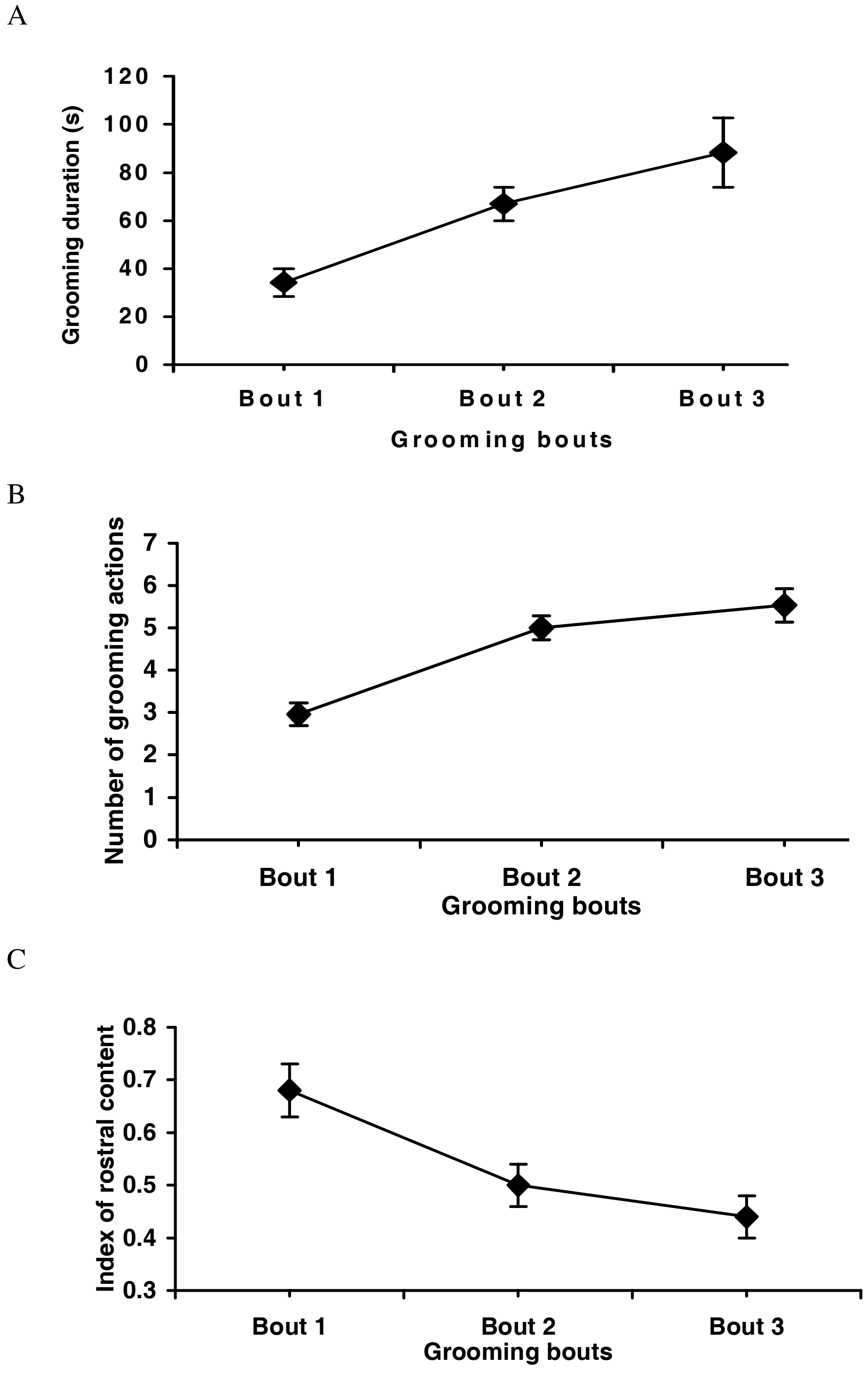

Figure 3. Characteristics of the consecutive grooming bouts. (A) duration. (B) number of grooming actions. (C) Rostral content (RC). RC = ratio of 3 s intervals devoted to face and head washing to all 3 s intervals spent on grooming. Data represent means ± *SEM*.

administration of anxiolytics as well as the prolonged duration of exposure to the novel environment (Blanchard et al., 1990; Choleris et al., 2001; Kaesermann, 1986; Rodgers, 1997). Thus, the observed pattern of changes in the frequency of

SAP is consistent with the interpretation that the first grooming bouts were associated with higher level of stress than the subsequent bouts.

Simultaneously, the duration of consecutive grooming bouts changed over the span of the trial: Initial bouts were relatively brief and later bouts increased in their length. Clear and unidirectional changes were also observed in the structural characteristics of the grooming bouts. The initial, shortest bouts included the smallest number of grooming actions and were focused predominantly on the face and the head. The final, longest bouts on the other hand, involved a much higher number of grooming actions and their rostral component declined substantially. (It is worth mentioning that the identified tendencies were not limited to the first three bouts but continued for the following bouts of grooming which, due to their infrequent occurrence, were excluded from the formal statistical evaluation.)

High rostral content of grooming has been shown to be associated with stress. It was suggested as a result of "emotional disturbance" (Doyle & Yule, 1959) and observed by Cohen and Price (1979) in rats exposed to an auditory stressor. Similarly, van Erp et al. (1995) reported a high proportion of face washing following physical restraint, but not in a fur-moistening paradigm. These data support the authors' interpretation that the high rostral content of the first grooming bouts may be a reflection of a high level of stress.

The proximal causes of the high rostral content of the early grooming bouts may be found in the structure of these bouts. Rats and other rodents are reported to start their grooming with face and head directed strokes and gradually shift their grooming actions towards the trunk and the extremities. In other words, grooming bouts typically progress in a cephalocaudal direction (Del Angel-Meza et al., 1996; Golani & Fentress, 1985; Richmond & Sachs, 1980; Spruijt, van Hooff, & Gispen, 1992). When this stereotypical order of grooming is considered in combination with features of the first grooming bouts (their short duration and a small number of grooming actions), it becomes clear that the high rostral orientation of those bouts resulted from their premature termination. Apparently, rats stopped grooming before the chain of activities progressed toward posterior parts of the body.

The short duration of the early bouts may be the result of the generally inhibitory and interruptive effect of stress on a variety of behaviors. In its extreme form, stress inhibits activity, not only in rodents (e.g., Doyle & Yule, 1959; Walsh & Cummins, 1976), but also in other, more distantly related vertebrate species (Suarez & Gallup, 1981). Shorter spurts of activity interspersed with periods of alert attention have been documented in novel, unfamiliar, and potentially threatening situations. For example Eilam and Golani (1990) reported that the exploration of a novel arena by amphetamine-treated rats was characterized by a high frequency of stopping and a short duration of the stopping episodes. Additionally, the number of stops performed by rats on the way out of a home base has been shown to be higher than the number of stops performed on their way back (Eilam & Golani, 1989). In unfavorable conditions (e.g., under high illumination), the animals engaging in a nondefensive behavior, such as eating, tend to interrupt their activity and scan the environment (Whishaw, Gorny, & Dringenberg, 1991). Threatened animals are also easily startled by sudden stimuli even of a relatively low intensity—this last tendency is successfully implemented as a model of anxiety (Rodgers, 1997). The above data suggest that the short duration of the early grooming bouts may have been caused by a rather general effect of stress, observable in a number of behavioral responses.

Viewed from a functional perspective, the relatively short duration of initial grooming bouts is not surprising. Protracted and complex grooming activity is likely to slow the animal's responses and decrease its chances of quick and successful defense. Involvement in high intensity nondefensive activities appears to be maladaptive under conditions of potential danger. The presence of grooming in association with novelty, as well as the increasingly long duration of subsequent bouts are more difficult to explain in functional terms. These two tendencies may be related to the dearousing properties of the grooming activity, as has already been suggested by some authors (see Spruij, van Hooff & Gispen, 1992, for overview). Further studies are required in order to adequately investigate the functional value of the observed changes in novelty induced grooming.

The present data also shed light on the processes, which were involved in the shaping of consecutive bouts. The fact that the early bouts were terminated prematurely suggests that the tendency to continue grooming was negatively affected during the execution of these bouts. Clearly, since grooming was still present, there was no negative impact on bout initiation. In the case of the final, longest bouts, this dichotomous effect disappeared and both the tendency to initiate and the tendency to continue grooming converged to produce protracted and diverse pelage cleaning behavior. Final bouts reached a mean duration of 88.33 s (± 14.53 *SEM*), which approximated bout duration reported for the relatively stress free home-cage conditions (Bolles, 1960).

These results suggest the involvement of two relatively independent mechanisms in the shaping of grooming behaviour. One would be responsible for the initiation of grooming, the other for its continuation. It appears that both of these mechanisms are differentially affected by the changing levels of stress. Indeed, other research shows that the lesioning of the paraventricular nucleus of the hypothalamus produces pelage cleaning, which, in its spatial distribution, resembles the late bouts of grooming observed in the present study (Bressers et al., 1995b; van Erp et al., 1995). In contrast, Bressers et al. (1995a) demonstrated that manipulations of the PVH involving direct electrical stimulation and injections of ACTH and related neuropeptides, increase the rats' tendency to initiate grooming bouts and, at the same time, decrease face-to-body transition rates. As a result, such artificially induced grooming is characterized by a high rostral component, an effect, which was typical of the first grooming bouts in the present study.

More research is required before the proposed relationship between stress and grooming can be firmly established. If confirmed, the present results suggest the characteristics of the grooming bouts as a promising ethological marker of stress and a useful tool in the investigation of the mechanisms of patterned behavior.

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Received February 4, 2003.
Revision received July 31, 2003.
Accepted August 18, 2003.