An Ethological Analysis of Excessive Grooming in Young and Aged Rats

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INTRODUCTION

Age-related behavioral changes have been studied predominantly in learning paradigms. The declines in memory and cognitive performance are among the most salient effects of aging and dementia.[1–5] The changes in cognitive performance of aging animals have been interpreted as deficits in acquisition and retention of learned skills.[6–13] However, age-related changes in cognitive functioning are not necessarily caused by deficits in mechanisms involved in the retention and acquisition of new behavioral patterns. To assess the contribution of possible other causes the changes in physical strength and motor coordination have been investigated. It appeared that perception also markedly changes with age.[14] In addition, senescent animals showed a decrease in tests measuring motor performance.[14–17] The main interest of these studies on noncognitive behavioral changes concerned their possible contribution to the impaired performances of aged subjects in learning tests. As a consequence, current concepts such as the Jacksonian principle[18] of age-related behavioral alterations is restricted to behavior displayed in problem-solving tasks. In order to extend the spectrum of behavioral changes in aging animals and to evaluate the relevance of current aging models, other behavioral systems should be studied as well. In this paper the excessive grooming behavior of the rat is used as an appropriate noncognitive behavior.

EXCESSIVE GROOMING

Grooming or maintenance behavior is a common species characteristic movement pattern with readily definable components. In the laboratory rat, grooming behavior may occupy as much as 25–40% of the awake time, depending on the housing conditions, with most of the behavior seen just prior to and after the diurnal sleep period.[19] The grooming of the rat and the cat, the preening or sandbathing of the bird, the rubbing and sweeping of the fly, all serve a role in the care of the body surface. Therefore, this class of behaviors is also designated as care of body surface.[20] Since it is a very reproducible and easily measurable behavior, it has been used extensively in experimental studies, especially peptide-induced excessive grooming. Peptide-induced excessive grooming has been applied for different purposes: (1) structure-activity studies were

used to identify where the crucial information is hidden in peptide-inducing peptides (for reviews see refs. 23 and 24 and other papers in this volume), and (2) local application of peptides in specific brain areas in combination with dopaminergic and GABAergic agents were applied to unravel the underlying neural circuitry, *i.e.*, the modulating dopaminergic and GABAergic systems and the possible site of action of the peptide.[23-25]

The application of ethological methods in experimental studies yields a more sensitive tool in studying changes in behavior. In peptide-induced grooming the distinction between different grooming elements showed that different peptides induce grooming patterns with different distribution of frequencies of elements.[26,27] Even pharmacological manipulation of peptide-induced grooming with haloperidol and naloxone showed a differential suppression of, respectively, grooming and scratching.[28] An ethological method was also used to compare ACTH-induced excessive grooming with spontaneously occurring grooming in order to assign biological relevance to ACTH-induced grooming. The meaning of behavior is not only determined by the frequency and/or the durations of its components but to a great extent by the serial ordering of the different elements.

A sequential analysis of ACTH-induced excessive grooming demonstrated the similarity in the sequential structure of this behavior to the structure of saline-induced excessive grooming.[29] In the present study the combined use of ACTH-induced excessive grooming and a sequential analysis of the behavior were used to show in detail subtle differences in the sequential organization of grooming behavior between young and aged rats. In addition, on a higher level of behavioral organization the occurrence of grooming in relation to other behavioral systems such as sociosexual behavior in young and aged rats was studied. For this purpose a situation was created that elicited, apart from grooming behavior, sexual and social behaviors.

GROOMING BEHAVIOR IN YOUNG AND AGED RATS

Firstly, the grooming behavior of 10 young (3 months) and 12 old (24 months) WAG (rij) rats was observed in a setting in which almost no other behavior was induced. The experiment was carried out according to conditions described extensively elsewhere.[30] Fifteen min after the intracerebroventricular injection of ACTH (0.3 μg/0.3 μl) the animals were individually observed for 55 minutes. A complete record of the behavior displayed by each animal was made using the following behavioral elements: forepaw vibration (VI), face washing (FW), body grooming (BG), anogenital grooming (AG), tail sniffing (TS), scratching (SC), body shake (BS), and stretching and yawning (SYS). Exploratory behaviors, sleeping, and other nongrooming elements were taken together into one rest category (RE). These elements have been extensively described by Gispen and Isaacson[21] and Spruijt and Gispen.[22]

GROOMING DURING AN ENCOUNTER WITH A SEXUALLY RECEPTIVE FEMALE

Eighteen young (3 months) and 20 old (24 months) Brown Norway rats were exposed to estrous females of a similar age, according to standard procedures of Meyerson.[31] The sexual and social behaviors displayed in a 25 min encounter were registered. After the males had the opportunity to explore the cage (80 cm × 40 cm × 40 cm) the female entered the area. Ovariectomized females were treated with estradiol benzoate

(25 μg/kg) and 48 h later with progesterone (1 mg/rat) and used as stimulus objects 4 h after the injection.

Apart from the differences in sexual abilities, which will be described elsewhere, special attention was paid to the role of grooming behavior. Twenty-two behaviors, 11 for the male and 11 for the female, have been observed in this test. Since the behavior of the females is not discussed here we will mention only the behaviors of the males. They are: (1) approach towards the female, (2) mounting (clasping the flanks of the female and performing pelvic thrusts), (3) intromission (mount with a vigorous backward lunge followed by genital licking), (4) ejaculation (prolonged mount with intense clasping of the female followed by a slow dismount and subsequent genital licking), (5) exploration, (6) genital licking, (7) grooming, (8) aggression, (9) resting (lying down), (10) allogrooming, and (11) crawling over the female.

It must be emphasized that genital licking was clearly distinguished from grooming, since genital licking in this context is closely associated with sexual behaviors such as intromission, which is always followed by genital licking. The sociosexual behaviors of the male rat have been described by Meyerson and Hoglund.[31]

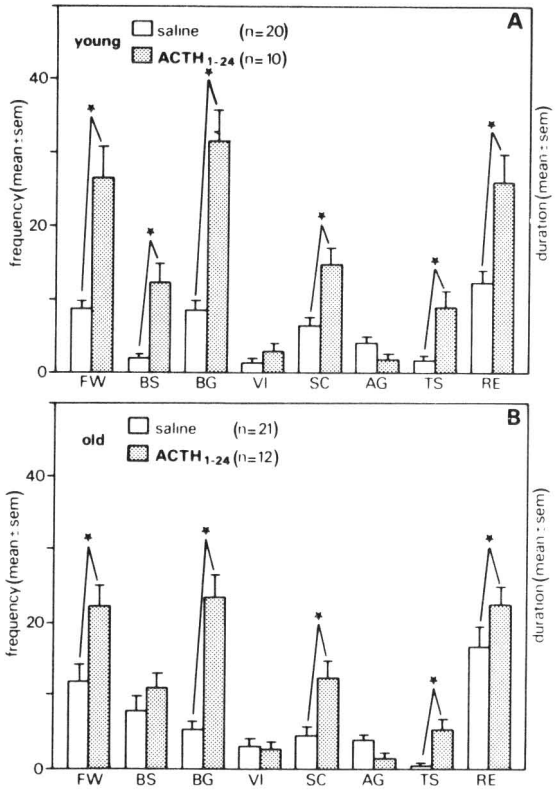
DATA ANALYSIS

In both experiments frequencies, durations, and in case of the sexual test, the latencies of each behavior were registered. The analysis of sequences was based on the assumption that the display of a given act is only determined by the immediate preceding act of the animal itself or in a social situation of its partner. For the observations and the analysis of the data previously described programs were used.[29] The sequential analysis was performed by comparing the frequencies of all combinations of two behavioral elements of both age classes. The combinations of elements were coded in so-called transition matrices, in which each cell represents the frequency of the concerning combination of elements. The next step was to sum the matrices per age class over all individuals. The two resulting total matrices were subjected to a chi-square followed by the calculation of the adjusted residual per cell. The adjusted residual per cell represents a parameter indicating the significance of occurrence of that combination of behavioral elements. All values exceeding ± 1.96 are supposed to have a higher frequency of occurrence than can be expected according to a random expectation.

ACTH-INDUCED EXCESSIVE GROOMING IN YOUNG AND AGED RATS

The scores of the different grooming elements for young and aged rats have been depicted in FIGURES 1A and 1B. The increase in the grooming scores and the level of ACTH-induced excessive grooming in aged rats is not different from the behavior displayed by young animals. Moreover, young and old rats show a similar distribution of elements as far as the frequencies are concerned. With respect to the durations of the defined elements only a difference is noticed in the amount of tail sniffing (FIGURE 1E). Thus, possible aged-related deficits in motor performance are not reflected in the durations and frequencies of different grooming behaviors. This similarity at first sight formed an excellent base for the sequential analysis, since apparently aged animals have the ability to display all components of grooming behavior.

In FIGURE 2 significant combinations of elements are indicated by arrows connecting the different behaviors. Each arrow can be read as either "is specifically followed by"


young
saline (n=20)
ACTH1-24 (n=10)
A
frequency (mean ± sem)
duration (mean ± sem)
40
20
0
FW
BS
BG
VI
SC
AG
TS
RE
old
saline (n=21)
ACTH1-24 (n=12)
B
frequency (mean ± sem)
duration (mean ± sem)
40
20
0
FW
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BG
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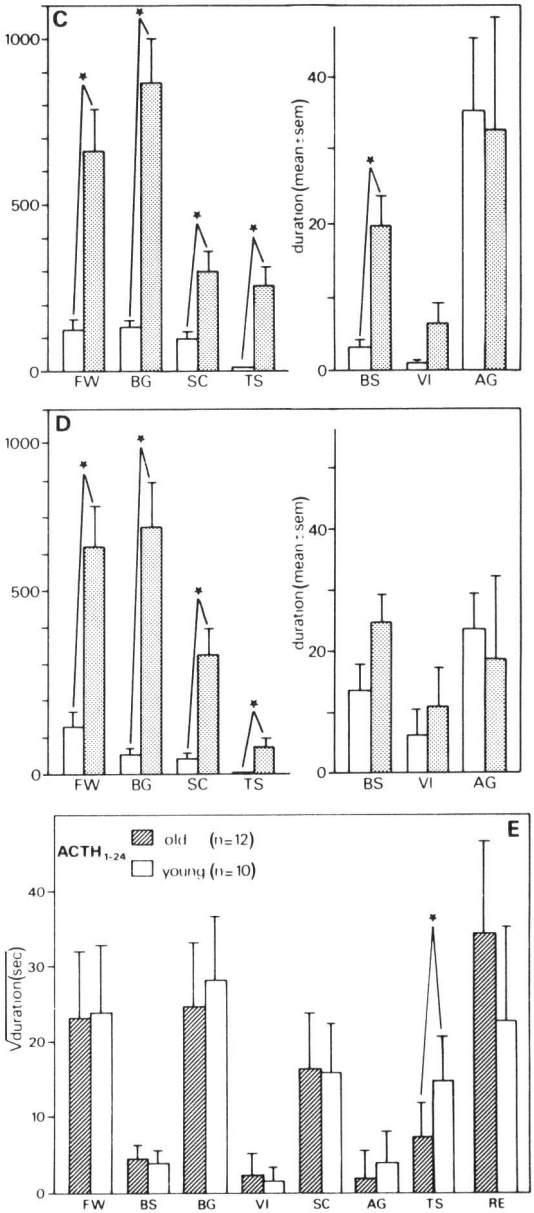

FIGURE 1. Excessive grooming in young and old rats. The frequencies and durations of face washing (*FW*), body shake (*BS*), body grooming (*BG*), vibration (*VI*), scratching (*SC*), anogenital grooming (*AG*), tail sniffing (*TS*), and other behaviors (*RE*) of young rats after saline and ACTH treatment are presented in, respectively, **A** and **C**. In **B** and **D** frequencies and durations of aged rats are shown. In **E** the durations of young versus old rats both treated with ACTH is compared; an *asterisk* indicates $p < 0.05$.

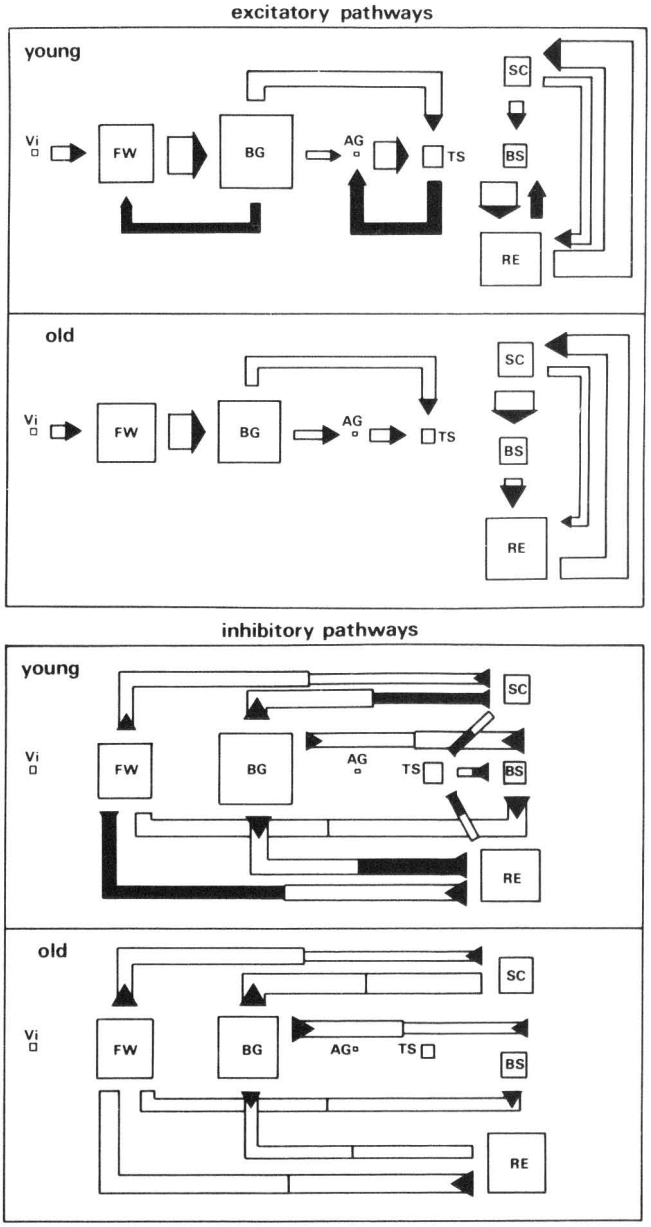

FIGURE 2. Differences in the structure of ACTH-induced grooming in young and old rats (indicated by *black arrows*). The area of each square represents the duration of the respective element (for the explanation of abbreviations see legend to FIG. 1). The width of the arrows is related to the transition frequency in excitatory pathways, in contrast to inhibitory pathways, in which arrowheads show the combination of elements not followed by each other. For further explanation see text.

(pointing outwards) or as "is certainly not followed by" (pointing inwards) representing, respectively, excitatory and inhibitory pathways. In the pathway diagrams of both the young and old animals, the main characteristic is the division into two distinct clusters of elements. One consists of the grooming elements head grooming, body grooming, anogenital grooming, and tail sniffing, and seems to be displayed in this cephalocaudal order. The order consists of scratching, body shake, and all other behavior, mostly exploration and lying down. Apparently, scratching does not fit in this cephalocaudal order of expression of acts. The special position of scratching in the expression of grooming has also been noticed in the ontogeny of grooming behavior.[32] There are more reasons to make a distinction between scratching and grooming. Lesions in the periaqueductal gray showed that grooming behavior was far more affected than scratching.[25] Pharmacological suppression of bombesin-induced scratching by naloxone showed that scratching was preferentially affected by the opioid antagonist.[28] Based on these findings it was hypothesized that there may be differences in the neural substrate of grooming and scratching.

COMPARISON BETWEEN YOUNG AND OLD

With respect to positive residuals—the excitatory pathways—the difference between young and old rats lies in the number of arrows and the difference in the width of one arrow. Young animals seem often to display the combination body grooming and face washing in both directions, while this reversed order is not present in aged animals, at least not more than can be expected according to a random model. In addition, the combination anogenital grooming and tail sniffing is displayed in the reversed order in the young animals only (see FIG. 2). The preference to perform tail sniffing after anogenital grooming is in young animals much greater than in aged animals (compare the widths of the two arrows).

Regarding the negative adjusted residuals, the inhibitory pathways of young animals again show more specific arrows (inhibitions). The combinations, which will certainly not be performed by young animals, are combinations of elements from either of the two clusters. In senescent animals, however, grooming of the body may be followed by scratching or resting, and tail sniffing may be followed by body shake. The aged animals may also shift from resting or scratching to body grooming or tail sniffing, whereas young animals always start grooming with vibration or face washing (notice the inhibitory connections to the other grooming elements). One must keep in mind that the absence of any arrow between two behavioral elements means that these behaviors may be "neighbors" in a string of elements according to a random expectation.

It is inferred from these findings that triplets of grooming elements occur more frequently in young animals than in old animals. TABLE 1 shows the relative frequency of some triplets of elements. Series of three grooming elements are more frequently seen in the young animals (see TABLE 1, a,c), whereas a combination of two elements of one cluster with one of the other cluster is seen more in the older animals (see TABLE 1,b,d,e,f). The cephalocaudal display seems more often interrupted in the older animals by behaviors such as body shake, rest, and scratching, probably resulting in a diminishment in duration of the terminating tail sniffing behavior. From these grooming patterns disrupted by other behaviors it is inferred that the nongrooming behaviors, displayed in the same test, are also more interrupted by grooming. To investigate the effect of a scattered grooming behavior on the sequential organization of nongrooming behaviors a setting was chosen in which other well-known behaviors and grooming are known to occur, namely a situation in which sociosexual behaviors will be performed.

TABLE 1. Triplets of Grooming Elements

	Young Rats	Old Rats
a. FW→BG→FW	53.8%	38.4%
b. FW→BG→SC	5.4%	14.0%
c. FW→BG→TS	13.4%	9.1%
d. BS→RE→FW	2.1%	13.5%
e. BS→RE→SC	15.6%	9.7%
f. RE→BG→RE	9.7%	15.1%

The relative frequencies of several triplets of grooming and nongrooming elements. FW: Face washing; BG: body grooming; SC: scratching; BS: body shake; TS: tail sniffing; RE: all other behavior.

The sexual behavior of rats has a known characteristic pattern and grooming bouts are seen after the sexual performance has taken place.

GROOMING BEHAVIOR IN SOCIOSEXUAL CONTEXT IN YOUNG AND AGED RATS

The sexual ability of young and old Brown Norway rats will be extensively described elsewhere. In brief, the time required to reach ejaculation is much shorter for young animals; moreover, the number of ejaculations reached in 25 min is lower in aged rats.[33] However, during the first 5 min no major differences between the two age classes could be assessed (see FIG. 3). In order to base the comparison of the sequential organization of behavior on similar distributions of frequencies, the analysis was performed on the first 5 min of the session. In the pathway diagram in FIGURE 4 the sequential structure of the male performance is depicted.

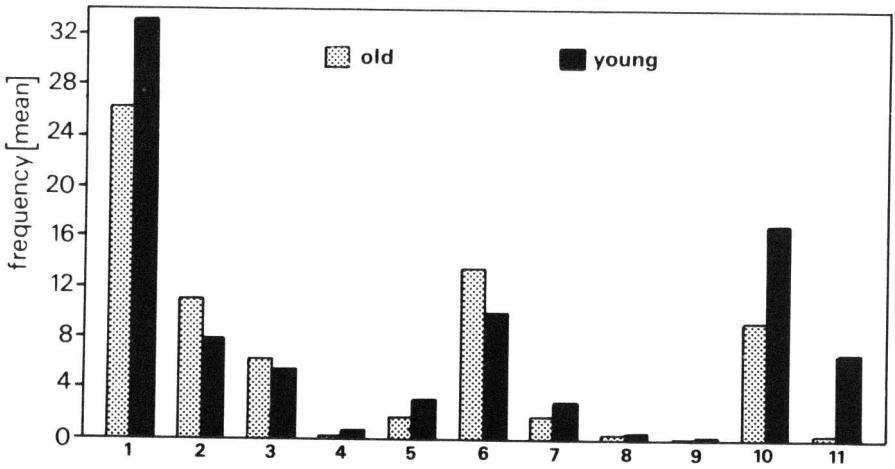

FIGURE 3. The frequencies of 11 behavioral elements measured during an encounter with an estrous female. Behaviors: *1*, approach; *2*, mounting; *3*, intromission; *5*, exploration; *6*, genital licking; *7*, self-grooming; *9*, resting, lying; *10*, grooming the female; *11*, crawling over. The only significant differences are found in exploratory behavior and crawling over.

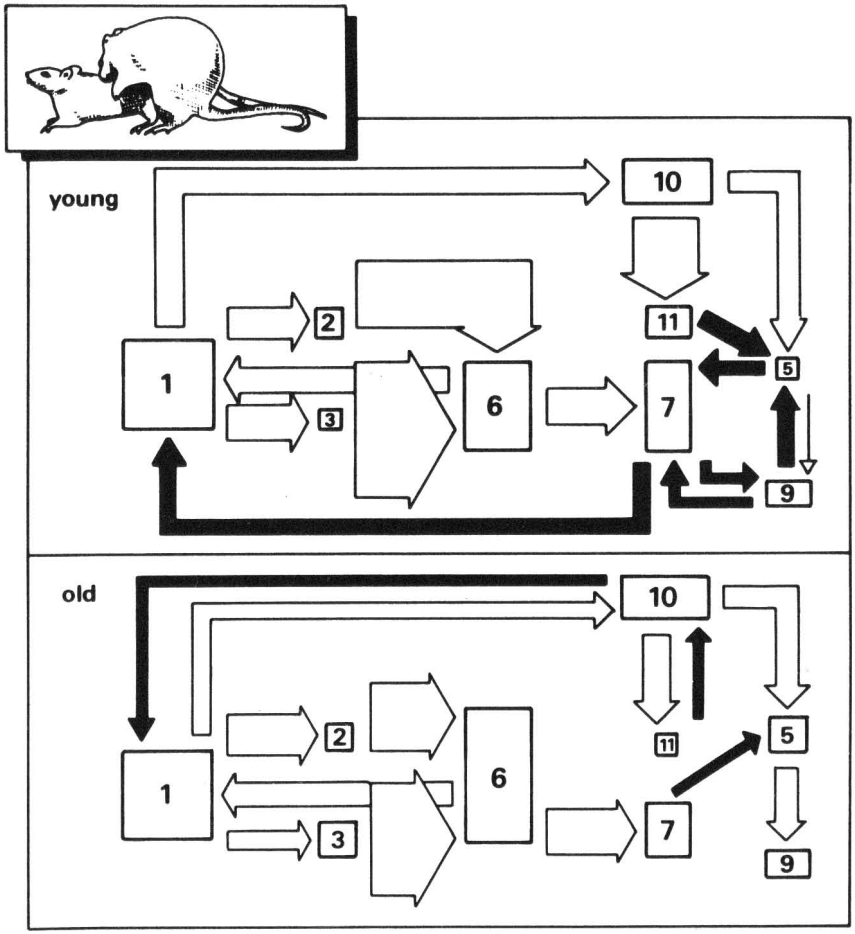

FIGURE 4. Sexual behavior of Brown Norway rats. The width of the arrows is related to the transition frequencies (see legend to FIG. 2). The area of the squares represents the duration of the respective behaviors. Differences between young and old rats are indicated by *black arrows*. See FIGURE 3 for an explanation of the numbers.

The well-known order of sexual behavior is clearly visible in both age classes: approach followed by mounting-intromission, and then genital licking and self-grooming. The black arrows, representing the differences between young and aged rats, are predominantly concentrated around the grooming behavior. Grooming behavior in young animals is specifically seen after genital licking, exploration, and resting. A lack of arrows towards grooming behavior can be noticed in old rats, whereas the frequency of grooming is similar in both age classes. This lack of arrows towards grooming (despite an equal frequency for the occurrence of grooming), and with an equal frequency for the transition from genital licking to grooming (see the width of the arrow from 6 to 7) means that the specific transitions from 5 to 7 and from 9 to 7 seen in young animals are divided over all behavioral elements in the older ones.

After self-grooming the young animals show a preference either for resting or for approaching the female. The aged rats show no specific preference at all for a certain following behavior, which means that any behavior may follow self-grooming. Thus, taken together a difference between the behavioral profiles of young and aged animals emerges. In young rats grooming has a more specific place in the profile, it is primarily performed after the sexual activity or exploratory activity, and it precedes either resting behavior or approaching the female again. In older animals it is also displayed after sexual activity, but more than in young animals it may precede or follow any other behavior.

A more random display of grooming in this sociosexual context leads to a sexual performance, which is more often interrupted by grooming behavior. Since no major differences are noticed either in the duration or in the frequency of grooming behavior, the grooming bouts must be of shorter duration.

Generally an encounter of a sexually experienced young male and a receptive female starts with copulatory behaviors and afterwards exploratory behaviors, grooming, and resting will be displayed. It can be seen in old animals that the fixed pattern of approach, mounting or intromission, and genital licking is interrupted by grooming. This phenomenon resembles the similar finding in grooming behavior, which was discussed above. In both behavioral systems a rather fixed pattern of elements was interrupted in aged individuals by a behavior to be performed at the end of a string of elements in young individuals. In addition, the interruption of grooming behavior by other behaviors—the rest category in the previous experiment—presumes the interruption of those "rest behaviors," in this case sociosexual behaviors, by grooming behavior, provided that the frequencies of the different behavioral elements are similar. In this sense the interruption of grooming is in agreement with the interruption of sociosexual behaviors by grooming.

The behavior studied in both settings demonstrates a drop in the number of specific combinations of behavioral elements in aging animals. The loss in sequential organization in grooming behavior is most clearly reflected in a decreased display of tail sniffing. Interestingly, tail sniffing is ontogenetically the last appearing element of the grooming repertoire. Such behavioral breakdown resembles the behavioral breakdown seen in learning paradigms (last learned, first forgotten). This so-called Jacksonian principle of behavioral degeneration is seemingly not confined to learning paradigms. The change in the sociosexual performance is probably the result of an interaction of many age-related deficits varying from degenerating peripheral organs, genitals, peripheral and central hormonal changes, testosterone, LH, LHRH, etc. But a loss in behavioral organization such as described for grooming behavior contributes to the above-described change in sexual performance. To summarize, in a variety of behavioral systems changes in aging animals may be characterized as Jacksonian degeneration. Ethological methods may provide appropriate tools to reveal such principles and to conceive possible consequences for other behavioral systems.

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