lates the sulfation of cholesterol (*16*).

The relationship of the concentration of cholesterol in serum and vitamin C intake has been demonstrated in humans (*17*). If our conclusions are applicable to the human organism, we shall probably find that latent hypovitaminosis C can cause hypercholesterolemia, and that it may also play some role in the pathogenesis of atherosclerosis.

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10 July 1972; revised 10 October 1972

Development of Grooming in Mice with Amputated Forelimbs

Abstract. *Face grooming sequences that involve coordination of the shoulders, tongue, and eyes develop remarkably normally in inbred mice with one or both forelimbs amputated from birth. This indicates endogenous control with a strong genetic component. Evidence for the maturational expression of "sensory expectations" was also obtained.*

The extent to which the development and integration of species-characteristic movements depend on endogenous versus exogenous programming has received much attention in recent years (*1*). Experiments on a variety of invertebrate and vertebrate species suggest that central motor programs which are under strong genetic control may be more important than previously supposed (*2*). However, other data indicate that peripheral and exogenous feedback are often critical for the development, if not the maintenance, of integrated movement (*3*), and generalizations must be of a limited nature at this time. Most strikingly, there are few data on the emergence and functional coupling of complex yet relatively stereotyped species-characteristic movement patterns in mammals deprived of normal feedback experience from infancy.

Previous studies have suggested that face grooming in rodents might be usefully studied in this respect. Face grooming is a common species-characteristic (and strain-characteristic) movement pattern with readily definable components (*4*). Systematic maturational stages can be plotted from the abbreviated and rudimentary single face swipes of neonatal and fetal animals (*4, 5*), and in adults the basic patterning demonstrates considerable, although not exclusive, central control

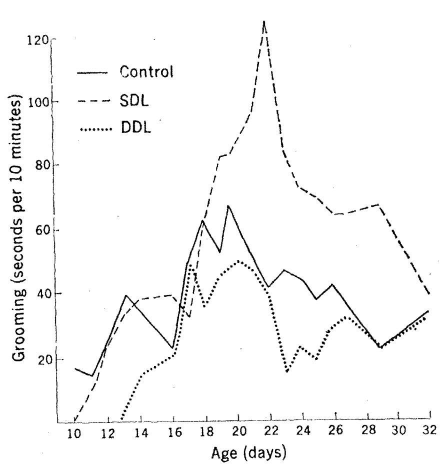

Fig. 1. Development of grooming in an observation cage by a litter of DBA/2J mice; *SDL*, single forelimb removed ($N = 2$); *DDL*, both forelimbs removed ($N = 1$); control ($N = 3$).

(*4*). Since mature face grooming involves a complex sequential pattern of contacts of the forepaws with the face and tongue it might reasonably be anticipated that experience resulting from these contacts is critical for the development, if not the maintenance, of the adult behavior. Therefore, I performed a series of experiments in which one or both forelimbs in inbred mice were painlessly amputated at birth, and observed the developmental patterning of grooming activities. The data indicate a high degree of endogenous control.

Three litters of DBA/2J and three litters of C57/6J mice were studied (seven to nine mice per litter). In each litter one to three infants had one forelimb amputated on postnatal day 1 [single delimb (SDL)], one to three infants had both forelimbs amputated [double delimb (DDL)], and one to three infants served as controls. Surgery was performed by anesthetizing the mice with ice and a small dose of Metofane (Pitman-Moore), sectioning one or both forelimbs between the elbow and the shoulder with a scalpel, and treating the limbs with ferric subsulfate to block bleeding. I removed an entire litter from the mother at one time and except for the actual surgery treated them identically, including the application of ferric subsulfate. They were returned to the mother simultaneously. In this way none of the infants was rejected, and only two of the operated animals died before the termination of the experiment.

Each mouse was observed at regular intervals (1 to 5 days) until it was 30 days old. Periodic observations were then continued over a period of 3 to 5 months. Sample films made at 32 feet/sec (1 foot = 0.3 m) permitted detailed single frame analysis of grooming and related movement patterns. Grooming movements could be separated without ambiguity from other behaviors by a combination of the animal's characteristic sitting posture, tongue movements, and pattern of shoulder and upper arm movements (*4*). Each mouse observed displayed obvious grooming behavior; its basic postnatal development is thus not dependent on normal contacts between the paws and face.

The broad profile of grooming probability during postnatal development was also similar for control and operated animals. For example, normal mice show a progressive increase and peak in grooming during the third week after birth. This trend was also seen in operated animals (Fig. 1). There is a considerable variability in total grooming shown by individual animals (*4*), and to date, no consistent differences in total grooming have been found between the groups. Individual DDL and SDL animals may exhibit either more or less grooming than individual control animals.

A striking illustration of endogenous coupling between the limbs and tongue is seen in Fig. 2. Normal mice lick their forepaws by bringing them in front of the face, and then extending the tongue as the paws are moved toward it by caudal rotation of the shoulders. Amputated animals also developed characteristic coupling between shoulder and tongue movements, even though with the caudal shoulder rotations this meant that the limb stubs were moving away from the tongue at the time of its extension. Similarly, forelimb grooming movements of large amplitude in normal mice cross over the ipsilateral eye, which closes just before contact. This might be attributed to conditioning, except that in amputated animals the ipsilateral eye also closes when characteristic large-amplitude shoulder rotations are made. Thus, normal experiential mechanisms that operate during postnatal ontogeny do not provide a sufficient explanation; endogenous control factors must be postulated.

Normal face grooming in mice consists of a variety of recognizable components that follow one another by sequential rules (*4*). For example, periods of licking behavior typically lead into a series of horizontal single strokes that involve movements of the forepaws along the snout at a frequency of 10 sec^{-1}, in turn followed by overhands of longer duration in which the limbs more or less alternate in making large semicircular movements over the top of the head. When it was possible to discern basic stroke types by the shoulder and upper arm movements, these strokes occurred in a sequence similar to that seen in normal mice. Thus, the switch from one grooming component to another does not depend solely on normal peripheral feedback. This conclusion is in direct contrast to previous generalizations offered on the basis of research on grooming in other vertebrate species (*6*). Endogenous mechanisms must again be postulated.

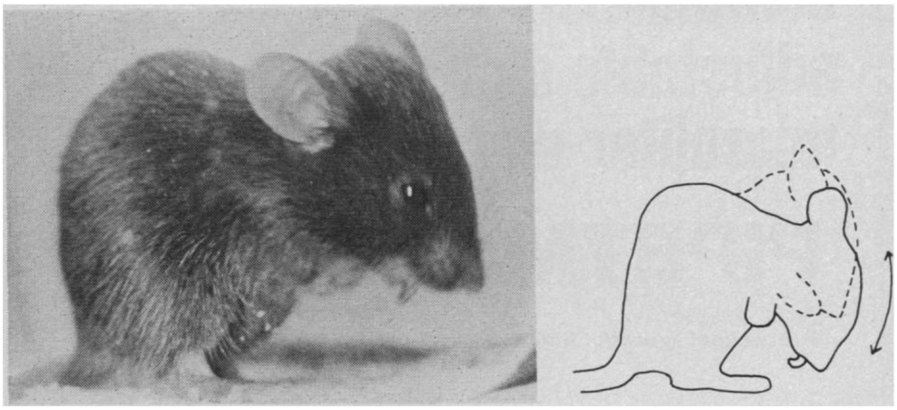

Fig. 2. Tongue extension during grooming shoulder rotation in a bilaterally amputated C57/6J mouse.

One further striking, and unexpected, characteristic of licking occurred reliably in the amputated animals. These animals, deprived of normal contact between the forepaws and the tongue during face grooming, would often introduce, interrupt, or conclude a grooming sequence with periods of licking the cage floor, cage sides, or even another mouse. The animals thus acted as if they were genetically predisposed to certain feedback "expectations" and, lacking normal tongue-paw contacts, obtained tactile input on the tongue from this special licking behavior. Although there are few data on genetic predispositions to seek specific sensory inputs, research on the development of bird song is compatible with this hypothesis (*7*).

This licking of course represents a form of adjustment as a consequence of the lesions, and indicates the subtle interplay between endogenous and exogenous mechanisms common to most if not all behaviors (*1*). Some other adjustments were also seen, such as an occasional exaggerated "tucking" posture during grooming in the amputated animals as if they were attempting to reach the face with the limb stubs. Also, during licking the upper arms were occasionally held in a more horizontal position than ever observed in normal mice. There may, in addition, be some subtle alterations in the precise duration and hierarchical structure of grooming components (*4*) that current analyses have not revealed. However, the basic point made clear by this investigation is that in postnatal mice there is an endogenous substrate that is of major importance in the maturation and performance of a complex species-characteristic movement sequence.

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8. Supported in part by PHS grant MH 16955. F. Stilwell assisted with data collection and preparation of figures.

18 August 1972; revised 22 November 1972