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Research Articles

Synaptic Rearrangement During Postembryonic Development in the Cricket

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Synaptic rearrangement during development is a characteristic of the vertebrate nervous system and was thought to distinguish vertebrates from the invertebrates. However, examination of the wind-sensitive cercal sensory system of the cricket demonstrates that some identified synaptic connections systematically decrease in strength as an animal matures, while others increase in strength over the same period. Moreover, a single sensory neuron could increase the strength of its synaptic connection with one interneuron while decreasing the strength of its connection with another interneuron. Thus, rather than being a hallmark of the vertebrate nervous system, synaptic rearrangement is probably characteristic of the development of many if not all nervous systems.

ONE IMPORTANT DISCOVERY IN NEUROBIOLOGY HAS BEEN that neural circuits are refined as an animal matures. One manifestation of this phenomenon is called "synaptic rearrangement," and can be observed in a variety of neural subsystems (*1*). In some situations, such as the visual cortex and the neuromuscular junction, synaptic rearrangement refines an initially diffuse set of connections (*2*). In others, such as the visual system of lower vertebrates, it compensates for differential patterns of growth in the retina and tectum (*3*). Invertebrate nervous systems, in contrast, are thought to be under a more rigid, presumably genetic, control and therefore are not likely to exhibit synaptic rearrangement or any other refinements as the animal matures (*4*).

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However, a new view is emerging which suggests that the mechanisms for nervous system assembly are much the same in vertebrates and invertebrates (*5*). One invertebrate nervous system that is receiving considerable attention in this regard is the wind-sensitive, cercal sensory system of orthopteran insects: crickets, cockroaches, and locusts (*6*). This neural circuit triggers escape from approaching predators, and the first-order interneurons encode a number of features of the wind stimuli, including wind direction, velocity, and acceleration. The transducers are wind-sensitive hairs each of which is innervated by a single sensory neuron. In the adult cricket these sensory neurons synapse with the first-order interneurons in very selective ways, conferring on the interneurons particular response properties. Once established, these synaptic connections were assumed to be constant (*7–9*). However, examination of synaptic connections in crickets of different ages demonstrates continual changes in synaptic connections as an animal matures.

Change of synaptic strengths during development. By focusing our attention on synapses that could be identified at a number of different developmental stages, we found that synaptic connections varied in strength with the age of the specimen. For example, the synaptic connection between a sensory neuron (4x) and the medial giant interneuron (MGI) decreased in strength as the animal matured (Fig. 1A, see legend for methods). We used two measures of synaptic strength: first, the average amplitude of the EPSP (excitatory postsynaptic potential) and second, the probability of detecting an EPSP. The results from 126 synapses recorded in 84 specimens showed that there was a steady decrease in the average EPSP amplitude as the animal matured (Fig. 1B). Three different receptors showed the same trends, although afferents from older hairs, such as 3x (born in the third instar), showed the decrease first followed by successively younger receptors such as 4x and 6x (Fig. 1B). Apparently each newly formed afferent made a strong connection with the MGI, and this synaptic connection then gradually faded away as the animal matured and the hair grew longer. Younger afferents, associated with smaller hairs, were born later in development and took over the function of exciting MGI.

We saw a parallel change in the probability of observing a synaptic connection for these receptors. Synaptic connections are probabilistic in the sense that not all specimens exhibit a particular synaptic connection (*10, 11*). When this probability was assessed as a function of developmental stage, a monotonic decrease was revealed (Fig. 1C). For example, the synaptic connection between sensory neuron 3x and the MGI decreased steadily from a frequency of 80 percent in the eighth instar specimens to zero in the adult. This could not be attributed to loss of the axonal arbor nor to shifting of its location because it was present throughout development, and the location did not change in any manner detectable with the light microscope (*11, 12*).

We considered the possibility that differences in the dissection or recording situation could account for the observations. However, we included only those specimens in which the intracellular recording from the interneurons was judged to be excellent. This decision was based on resting potential size (greater than −50 mV), action potential shape and amplitude (greater than 5 mV), and the amplitude of spontaneous synaptic activity (Table 1). Thus, there is no evidence that the recordings are systematically different in juvenile and adult specimens.

In individual crickets, the age of a receptor was correlated with its synaptic strength. The birth date of each of the receptors was known (*10, 11*), and when sensory neurons of different ages were compared within a single specimen, the synaptic strengths for receptors of different ages were nearly always (95 percent of the specimens) arranged in the same order—the youngest receptor made the strongest connections (Table 2). This comparison of the synaptic strengths in individual giant interneurons provided independent support for the idea that synapses of different ages had consistently different strengths. It also eliminated interanimal variability and differences in the quality of the intracellular recording as factors in assessing the data.

Changes in efficacy are specific to particular interneurons. One important question was whether all afferent synapses showed a similar decline in efficacy. Shimozawa and Kanou (*9*) demonstrated that different interneurons received input from receptors of different lengths. For example, interneuron 10-3 (10-3) received strong input from large receptors, and this suggested that synapses on 10-3 might be functionally distinct from those on MGI. We therefore examined the synaptic connections of a large receptor, 2c, whose axonal arborizations overlap both the MGI and 10-3 (Fig. 2D). We showed that the 2c-to-MGI synapse exhibited the same maturational changes as did the 3x-to-MGI synaptic connection. Receptor 2c always synapsed with MGI in the eighth instar specimens but, as the hair it innervated grew to be longer than 800 μm, neuron 2c lost the ability to excite MGI (Fig. 2) and there was no detectable connection in adults.

In contrast, interneuron 10-3 received powerful synaptic input from this same receptor and the EPSP amplitude increased as the specimen matured (Fig. 2). Thus, a single sensory neuron simultaneously lost synaptic contact with MGI and strengthened its synaptic contacts with 10-3.

These results eliminate the possibility that old sensory neurons are

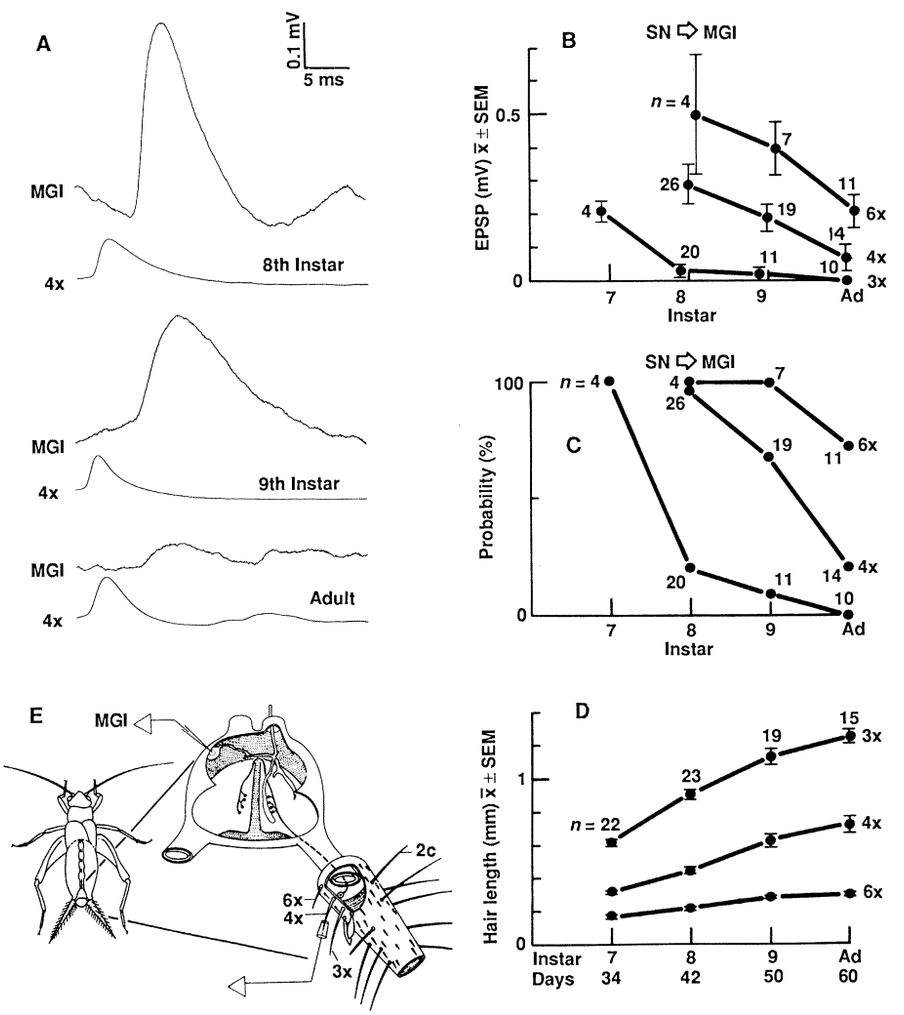

Fig. 1. Changes in synaptic strength with maturation. (**A**) Sample recordings from an identified synaptic connection in specimens of different ages. The records were obtained from the MGI and afferent 4x in all three specimens, and the records were selected to represent the average EPSP sizes obtained at each age. The activity of the sensory neuron, identified by the size, location, and birth date of the hair it innervates (*10*), was recorded by cutting a hair to about half its length, slipping a large-bore, saline-filled pipette over the cut hair, and recording the action potentials through a conventional high impedance recording apparatus (*8*). Spontaneous activity in the afferent was maintained at frequencies below 0.2 Hz by passing hyperpolarizing current through the hair, thereby eliminating any effects of habituation from our analysis (*10*). Correlated synaptic activity was recorded by triggering the oscilloscope on the sensory neuron action potential and recording simultaneously from the soma of the interneuron and averaging 30 events (*10, 11*). (**B**) The decrease in synaptic strength as measured by the average size of the EPSP, mean ($\bar{x}$) ± standard error of the mean (SEM). The number of recordings represented at each point is indicated (n). (**C**) The decrease in synaptic strength as measured by the probability of finding a particular synaptic connection. (**D**) The increase in receptor hair length as the animal matures (mean ± SEM). The receptor complex, consisting of four cells, goes through its terminal mitosis in the instar before it appears on the body surface. The instar in which the receptor is first visible on the body surface is indicated by the number in the sensory neuron label. Sensory neurons 3x, 4x, and 6x were previously assigned the names x, px, and vpx, respectively (*11*). (**E**) A schematic diagram of the structure of the cercal sensory system.

simply losing their ability to transmit, since the same sensory neuron can lose synaptic contact with one interneuron while increasing the strength of its synaptic connection with others. The results also suggest that the preference of the afferent for particular interneurons changes as the sensory structures change their transducer properties. Based on the available evidence, one cannot attribute this change in preference exclusively to either the pre- or postsynaptic cell.

Regeneration does not alter the specificity of synaptic connections. There was a close correlation between the strength of the synaptic connections and their age, and we set out to disturb this correlation by cutting the cercal nerve (a pure sensory nerve containing all axons from that cercus) and allowing these axons to regenerate. If either the order of arrival of the axons or the absolute age of the synapses determined synaptic strength, then this maneuver should alter the relative synaptic strengths of the various sensory neurons. The sensory cells regenerate their axons and restore the afferent connection in 10 to 25 days (*13*). We concentrated our attention on MGI and the sensory neurons 3x, 4x, and 6x. The results showed that both the rank order and the absolute level of synaptic efficacies were restored after regeneration (Fig. 3). Neuron 3x seldom made a synaptic connection with MGI in these experimental animals, neuron 4x excited MGI in 26 percent of the animals, and neuron 6x excited MGI in 83 percent of the experimental animals. In those animals where more than one synapse was detected in an MGI, the rank order of synaptic strengths was similar to that seen in controls (Table 2). Further, as soon as synaptic connections could be detected, 10 days after nerve cut, the rank order of EPSP amplitudes was correlated with receptor age, just as in the controls.

These results show that the chronological age of the synapse is not crucial to the ordering of synaptic strengths; all of the regenerated synapses are similar in age, but they vary systematically in synaptic strength. The results also suggest that the order of synaptic strengths is not dependent on the order of arrival of the axons in the central nervous system, since they all arrive at the same time after nerve cut. Our interpretation is that the sensory neurons have specific preferences for particular interneurons, and the strength of this preference is unchanged by regeneration.

Mechanisms controlling the changing synaptic strengths. It is important to distinguish between active and passive mechanisms that might lead to changes in synaptic strength (as measured by EPSP size). For example, the changes in synaptic strength might be the result of growth of the postsynaptic cell. The linear dimensions of MGI increase by about 18 percent (*14*) and this might lead to a decrease in input resistance which could account for the decrease in

Table 1. Comparison of the interneuron recordings in specimens of different ages.

Interneuron	Recordings (mV)					
	8th Instar		9th Instar		Adult	
	Mean ± SEM	*n*	Mean ± SEM	*n*	Mean ± SEM	*n*
MGI AP*	6.6 ± 0.6	12	6.9 ± 0.4	20	6.7 ± 0.8	10
RP†	64.2 ± 1.6	12	62.5 ± 1.9	12	63.0 ± 2.9	10
10-3 AP	6.4 ± 0.6	5	7.0 ± 0.6	8	6.8 ± 0.8	9
RP			60.0 ± 4.1	4	62.1 ± 3.9	7

*The action potential (AP) amplitude was measured at the soma (mV). No significant differences were detected by the *t* test. †The resting potential (RP), in mV, was measured at the end of the experiment when the electrode was removed from the soma.

Table 2. Relative synaptic strengths for the three receptors of different lengths as measured in the MGI. The relative strengths were compared between pairs of sensory neurons synapsing on MGI. More than 20 traces were averaged to obtain the EPSP amplitudes. The relative synaptic strengths are expressed as "greater than" (>), "equal to" (=), or "less than" (<), on the basis of comparisons of such average EPSP amplitudes. The data are a subset of those shown in Figs. 1 and 3, namely, those specimens in which we obtain data for two or three afferents in the same specimen.

Item	Short > long % (*n*)	Short = long % (*n*)	Short < long % (*n*)	Neither connected*
Control	95 (42)	0 (0)	5 (2)	13
Axon regeneration	88 (16)	6 (1)	6 (1)	10

*There was no detectable connection for either afferent in these specimens; thus, a comparison could not be made. These cases are not included in calculating the percentages shown.

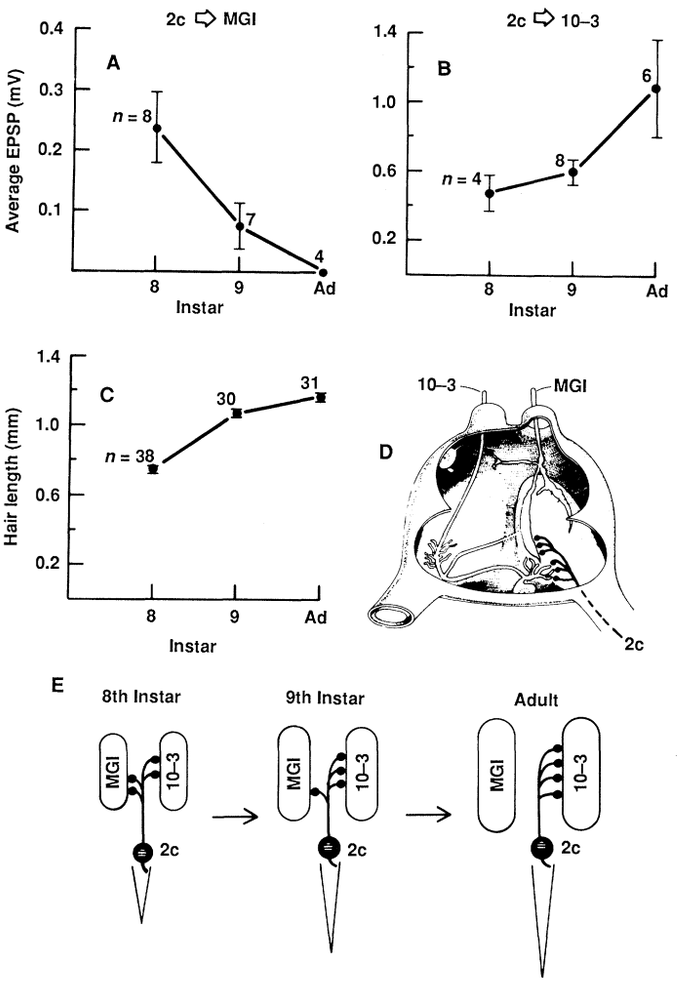

Fig. 2. Differential changes in synaptic strength between one sensory neuron and two different interneurons. (**A**) The average size of the EPSP produced by afferent 2c when recorded in MGI (mean ± SEM). Just as with the afferents considered in Fig. 1, the average size of the EPSP decreased with age of the specimen. (**B**) The average EPSP size produced by afferent 2c and recorded in interneuron 10-3 ($\bar{x}$ ± SEM). The difference between the slopes for the two interneurons was significant at $P < 0.001$, by analysis of variance. (**C**) The change in length of hair 2c as a function of age ($\bar{x}$ ± SEM). (**D**) A schematic diagram of the afferent and the two interneurons described earlier. (**E**) Schematic diagram of our interpretation of the results. In the eighth instar an afferent like 2c is connected to both interneurons. As the specimen matures and the hair grows longer the connection with MGI decreases in strength while the connection with 10-3 increases in strength. Finally, in the adult there is no detectable contact with MGI but a very strong contact with 10-3. The diagram is intended to schematize our interpretation of the physiological results. The anatomical implications of the results have yet to be established, although available light microscopic observations make it likely that the changes will be detected only at the ultrastructural level.

EPSP size (*15*). As one measure of input resistance, we examined action potential amplitude in the inexcitable soma of MGI (*16*) and found no significant change with age (Table 1). Interneuron 10-3 also grows and it is likely that its input resistance would decrease as well. However, the observed increase in the size of the EPSP's in 10-3 is incompatible with this idea. Thus the available evidence indicates that a change in input resistance does not account for the observed age-dependent changes in synaptic strength.

It is our conclusion that the physiological observations reflect a true synaptic rearrangement, similar to that seen during the maturation of a variety of vertebrate nervous systems, but unlike anything previously reported for the invertebrates. As in the vertebrates, there are various mechanisms that may produce these synaptic rearrangements. There is a strong three-way correlation between the age of a receptor, the size of the hair, and the strength of the connection. Thus, the age of the receptor might directly control connectivity by an age-dependent change in the chemoaffinity of the presynaptic cells for different postsynaptic cells. One aspect of such a model, namely control of synaptic strength by the age of the presynaptic terminal, is eliminated by the regeneration experiments. However, age of the soma may be the important variable, and this has not been assessed.

It is also possible that age is indirectly controlling the strength of various synapses through its control on afferent activity levels. Large (old) receptors have lower thresholds and exhibit considerable spontaneous activity, while small (young) receptors have higher thresholds and no spontaneous activity (*9*). In that the development of the cercal system can be influenced by activity levels (*17*), it is possible that the synaptic rearrangements observed in our study may be brought about by differential activity levels in sensory neurons of different ages.

Still another possibility is that the sensory neurons shift their axonal arborizations from an initial, effective location on the dendrites to a less favorable location (farther from the spike initiating region and farther from the somatic recording site). In other experiments (*12*), neuron 3x was examined morphologically at various stages bracketing the time covered by the physiological data. There is no qualitative evidence to support the idea that the axonal arborizations change their locations with respect to the MGI. The shape and location of the arbor appear very similar in young and old specimens and the density of varicosities changes very little as the animal matures (*12*).

Finally, this rearrangement may be under the direct control of hormones. During metamorphosis the moth *Manduca* drastically reorganizes its nervous system to accommodate changes in its life style and the strength of many synapses is altered (*18*). Our results may be a less extreme form of this hormonally driven rearrangement.

One advantage of the cricket as a model, which will allow us to understand more fully the mechanisms underlying the synaptic rearrangement, is our ability to manipulate the peripherally located sensory cells. For example, by transplanting sensory cells from young to old animals we will be able to show whether the pre- or postsynaptic cell controls the age-dependent choice of connectivity. We can also assess the role of sensory neuron activity in this process by blocking the afferent activity with tetrodotoxin for a part of postembryonic development and then assessing the resulting synaptic connectivity diagram.

Changing synaptic connections may compensate for changes in the properties of the transducer. One function of the cercal sensory system is to trigger escape behaviors that protect crickets and cockroaches from approaching predators. These animals are exquisitely sensitive to the acceleration, velocity, and direction of a wind stimulus and these stimuli are important cues for the escape behavior (*19*). The wind-sensitive hairs are found on the youngest

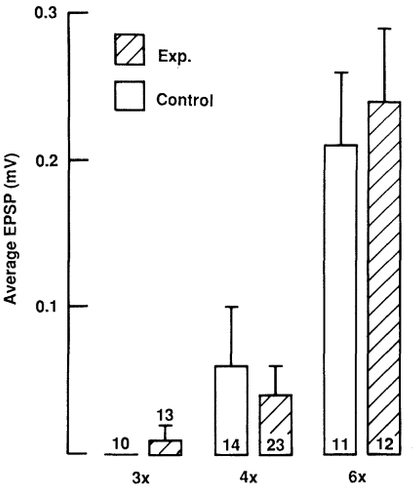

Fig. 3. The strength of synaptic connections with MGI after the sensory axons were cut and then allowed to regenerate. The nerve sections were done in the seventh or eighth instar and all recordings were obtained from adults, 10 to 25 days after the nerve was cut. Both the rank order (Table 2) and the absolute values of the synaptic strengths for different afferents are nearly perfectly restored by the regeneration process. The number of synapses tested is indicated at the base of each column. Error bars indicate SEM.

crickets and they too escape successfully from predators. However, as the animal grows the hairs grow longer, and in order to successfully avoid predators these changes in the transducer must be compensated for. Since the giant interneurons, like MGI and 10-3, are important components of this escape circuit it seems likely that the synaptic rearrangements described here are an important element in this process.

In adult crickets the largest interneurons are maximally sensitive to the acceleration of the stimulus, while others are more sensitive to wind velocity. This differential sensitivity can be traced to the transducer: shorter hairs are more sensitive to acceleration while longer ones are more sensitive to velocity (*9*). The filiform hairs grow as the animal grows (Fig. 1D), and each time the animal molts a new, larger hair is secreted and the associated sensory neuron is thought to become less acceleration-sensitive as a result of this growth (*9*). In addition, new receptors are continually being generated, and these new receptors are associated with the smallest, presumably most acceleration-sensitive, filiform hairs (*10*). Thus, for an interneuron to maintain a constant sensitivity to acceleration and velocity throughout postembryonic development, the pool of afferents innervating that interneuron must be constantly changing. This is analogous to the synaptic rearrangement seen in the retino-tectal system where ganglion cell synapses are continually rearranged to accommodate the differential patterns of growth in the retina and tectum (*3*). The observed synaptic rearrangements in crickets may compensate for changes in the transducer properties in an analogous manner, matching the transducer properties of the receptors to the appropriate interneurons.

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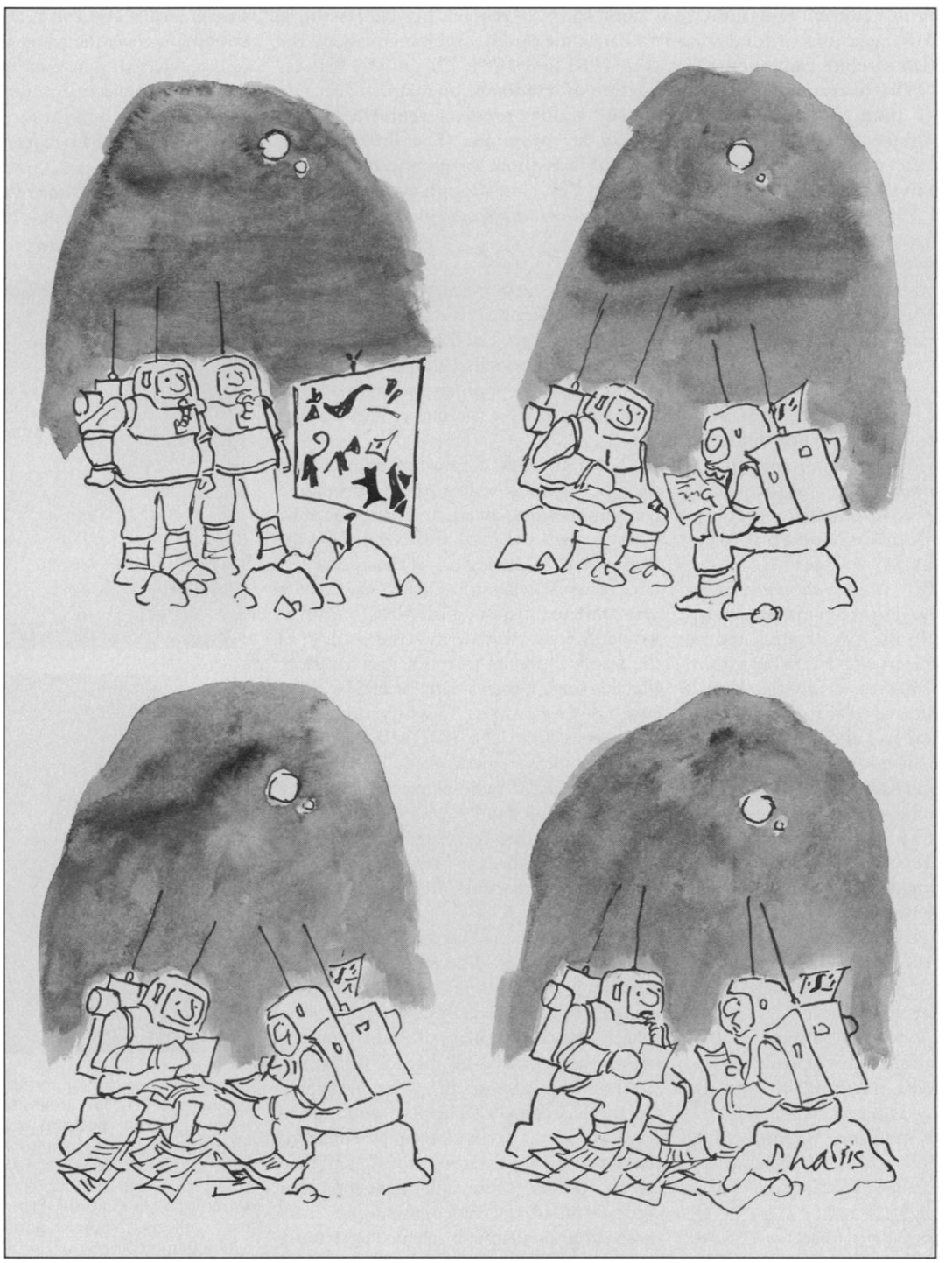

It says, "Keep off the rocks!"