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Unit Activity of Neurons of the Dorsal Spinal Gray as Influenced by Quantitative Mechanical Stimulation of Elements Within Hairy Skin.

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MASTER

A major focus of research in my laboratory within the last few years has been the study of the central processing of activity from hairy skin of cat. The emphasis has been on precise control of afferent activity from the skin by selective activation of specific receptor systems rather than by electrical stimulation of nerve trunks or skin. By use of quantitative stimulus methods and computer averaging procedures to control afferent input, we have been exploring the dynamic organization of the cutaneous receptive fields of dorsal horn cells. This report concerns the responses of these neurons, in laminae IV and V, to activity in the tactile pad receptor system (1).

The tactile pad receptor system is an ideal vehicle for quantitative studies of central interactions because it is relatively easy to confine evoked activity to single Type I afferent fibers. The epidermal pads in which the receptors are embedded, the haarscheibe, are readily visualized in depilated skin under low power magnification, thus facilitating precise apposition of stimulating devices. The distribution of the pads is particularly well illustrated in skin of the new-born kitten (Figure 1A) where the density is higher, but the diameter of the structures is only slightly smaller than in adult skin (2). The number of tactile pads per animal appears to be constant throughout life, since, as the animal grows their density is reduced as if they were being pulled apart by stretching of the skin. More than 95% of the pads are innervated by only one large myelinated fiber (Figure 1B). A single axon may innervate as many as seven pads while the vast majority terminate in two or three pads (Figure 1C) (3). The area of skin occupied by a cluster of pads from one sensory unit does not intersect the area occupied by a neighboring unit (Figure 1D). From these considerations, it is clear that the family of Type I afferents emerges from a non-overlapping receptor mosaic which, by its spatial distribution, can portray a relatively unambiguous

two-dimensional representation of the hairy skin surface. In addition, the receptors are very sensitive to mechanical stimuli applied directly to the tactile pads but insensitive to stimuli applied to immediately surrounding structures. A single short duration mechanical pulse with a rise time of 2-3 msec and an amplitude between 2 and 20 μ is sufficient to produce a single action potential if applied to the pad (Figure 1D). In contrast, a displacement of 100 μ just a fraction of a millimeter away from the pad is an ineffective stimulus.

Single unit activity in Lamina IV and V cells at S_1 was observed in anesthetic-free decerebrate-low spinal preparations of cat. Extracellular action potentials were most often recorded with tungsten microelectrodes. Since many of these cells are spontaneously active under these conditions, we could study both inhibition and facilitation of propagated postsynaptic activity. The dynamic interactions of these cells with one another and with the periphery were studied by perturbing the central neuronal pool with single action potentials in single Type I afferents. The afferent impulses were produced by single short duration mechanical pulses applied to tactile pads within and peripheral to the grossly determined receptive field in clipped depilated skin.

When studying postsynaptic receptive field organization by specific receptor activation, it is necessary to know which receptor is activated and the extent of stimulus spread to adjacent structures. Two approaches have been used in achieving stimulus control. For some purposes, we have used an on-off pad stimulus paradigm and have looked for differences in the response of the central cell according to presence or absence of tactile pad input. A brief 10 micron displacement of a tactile pad will evoke a single action potential in the Type I afferent with high probability.

Although there is little chance of stimulus spread with this displacement amplitude, there is some small degree of uncertainty. Since stimulation of skin immediately adjacent to the pad will not elicit a Type I response, it may serve as a control for spread from the on-pad stimulus. The difference in response can be a measure of the effect of Type I input when no response is elicited by the off-pad stimulus. An example of this approach will be shown below (Figure 3).

The second approach to stimulus control involves the direct extraction of unit activity from the relevant whole dorsal root by signal averaging methods (4). The activity was recorded with a hook electrode and the signals were amplified and led to a seven bit (including sign) signal averaging device which summed the digitized potentials in 100, 200, 300 or 400 successive bins. A sampling rate of 8 KHz was used. An example of the procedure is illustrated in Figure 2. The data were obtained using displacements repeated every 60 msec for 5000 repetitions. A display of an averaged stimulus monitor wave form is shown at the bottom of the figure. As the displacement amplitude increased, a characteristic sequence of changes in the averaged response occurred. At or near threshold, 8.5μ , the potential was small and slightly spread out due to jitter in the response time of the receptor. With 10μ , the potential became sharper and larger and occurred slightly earlier. With 20μ or more, multiple peaks appeared which could be attributed either to a doubling from the tactile pad receptor or to recruitment of other afferents. It has been our experience that when a stimulus of less than 20μ is used, one rarely discerns more than the single action potential from the Type I afferent. For study of postsynaptic response, the amplitude of the mechanical pulse was adjusted for the sharpest single peak in the dorsal root record, for each tactile pad stimulated. As a practical rule of thumb, we have observed that a good approximation of the amplitude necessary to consistently produce a

single afferent impulse is often obtained with a displacement which is just threshold for a postsynaptic response. For almost all of the data presented in this paper, single impulse activity in Type I afferents was verified by dorsal root monitoring. It should be emphasized, however, that such stringent stimulus control is not always required.

The basic observation which has enabled this study to be pursued was that one or several action potentials can be evoked in some dorsal spinal neurons by single action potentials in single Type I afferents (5). An example is shown in Figures 3 (top). A bursting postsynaptic discharge was elicited. In this instance, a tactile pad displacement of less than $7\ \mu$ was a sufficient stimulus. The response of another neuron to an on-off pad stimulus paradigm is shown in the post-stimulus histograms (Figure 3 [bottom]). One hundred stimuli of $10\ \mu$ displacement were applied at each site once per three seconds. A clear postsynaptic discharge with a minimum latency of approximately 5 msec was obtained from the on-pad stimulus; no response occurred from stimulation of skin immediately surrounding the pad, in this region of the receptive field. In other regions of the skin, minute movements of hair and non-pad skin evoked a discharge in this cell. In both cases, it is highly probable that the response from on-pad stimulation was solely due to activation of the Type I afferent since, in our experience from dorsal root monitoring, a $10\ \mu$ displacement will only elicit a single action potential.

A variety of postsynaptic discharge patterns has been observed in response to single Type I afferent impulses. Some of the temporal patterns we have encountered are shown in Figure 4. These include: (a) a short latency prolonged inhibition of ongoing activity, which may last for more than 50 msec (Figure 4A), (b) a short latency bursting discharge which can be sustained for 30 msec or more (Figure 4B), (c) a single impulse after a short latency

which may be followed by a prolonged reduction of ongoing activity (Figure 4C) and (d) a long latency bursting discharge (Figure 4D). Long latency inhibitions have also been observed.

Of particular relevance to the subject of central cell interactions is the fact that all of the responses illustrated in Figure 4 were observed in the same spinal neuron. The differences in temporal patterns of discharge were determined by the site of stimulation, that is, by which tactile pad was stimulated. Clearly, this result demonstrates that these cells do not have a stereotyped response pattern. It can just as clearly be demonstrated that the response of the central cells is not determined solely by the particular tactile pad which is activated. This is illustrated by the responses of two neighboring cells, judged electrophysiologically to be approximately 50 μ from one another. The cells had overlapping receptive fields so their discharge patterns from stimulation of the same tactile pads could be compared (Figure 5). In some instances the responses were very similar, such as, for pads 21 and 14. In others, they were completely different. For example, stimulation of pad 18 produced a short latency single impulse response in unit 5 and a short latency prolonged response in unit 6. Stimulation of pad 25 evoked a short latency excitation in unit 5 and a short latency prolonged inhibition in unit 6. A single action potential from pad 19 also produced a short latency short duration response from unit 5 but a longer latency and more prolonged inhibition in unit 6 than did pad 25.

From these results, we conclude that the different types of response reflect activity in the whole neuronal pool with which the monitored cell is connected, and, that each temporal pattern produced by activation of a specific Type I afferent portrays an interaction sequence of high probability. By perturbing this neural network with very small inputs, we can obtain characteristic temporal patterns of discharge which depend upon the connectivity of

the monitored cell to the others within the network, and, upon the specific connectivity of the stimulated afferent which enters the network. As a corollary, we suggest that the variety of discharge patterns seen for one central cell when stimulated at various points on the skin is an indirect indicator of the simultaneous activities of a large number of the neurons within the network which are influenced by a given tactile pad.

Further information concerning the organization of these neuronal pools and their interactions with Type I afferents may be obtained by examining the response latencies and time courses of the discharges from stimulation of many different tactile pads in the receptive field. This is illustrated by an expanded plot of four very different responses of the same neuron over the first 60 msec (Figure 6). The inhibitory response appears to occur first, between 4 and 6 msec; the two early excitatory discharges start between 6 and 8 msec; the late excitatory response did not start for approximately 30 msec. While these postsynaptic latencies reflect the response initiation times as the cell sees the skin, they do not accurately represent central delays because of the variation in conduction times from different regions of the receptive field, particularly from large receptive fields.

Central delays can be computed from values of conduction time to the dorsal root recording site and from the postsynaptic response latencies. The array of these three measures from single impulse activation of Type I afferents of one receptive field is shown in Figure 7. Conduction times to dorsal root (left) were determined by average response computing of the whole S_1 root. Not all of the tactile pad activity was recovered in this root, especially those in the periphery of the receptive field near the heel. We assume that their fibers were in the L_1 root since this is a region of overlapping dermatomes. The distribution of dorsal root latencies shows the predicted proximal-distal trend, with a few exceptions. Postsynaptic

latencies were determined from the poststimulus histograms (middle). The time at which the histogram departed significantly from the background discharge level, that is, when the value was outside the range of background discharge, was selected as the postsynaptic latency. The profile of central delays (right) was constructed for those points where data for the previous two measures were available. Values are given for excitation, inhibition, and mixed effects where discernible. They have been rounded to the nearest 0.5 msec. Many early excitatory responses consistent with monosynaptic activation were obtained. Thus far, no delays of less than 1.0 msec have been observed for inhibitory effects. However, our sample is as yet small.

It is clear from the data which has been presented thus far, that the temporal patterns of the responses contain important information relating to the mechanisms of interaction of these dorsal spinal neurons. A statement of whether or not a response occurs, and a designation of the number of spikes occurring during a response period of arbitrarily selected length, is an inadequate description of the dynamics of these interactions. We have attempted to represent these events with a series of response magnitude contour maps. Three of these are illustrated in Figure 8. Each map is a description of the response of the central cell to single action potentials in each of the array of Type I afferents connected to the cell during some 5-msec interval after a short duration displacement of each tactile pad.

The contours are complex; excitatory regions are mingled with inhibitory regions. Loci of peak excitation and inhibition shift with time. During the 5-10 msec interval (Figure 8A) three excitatory peaks (stipled) and several patches of inhibition (slanted lines) were found. By 15-20 msec (Figure 8B) the regions of excitation had shifted to the right and inhibitory activity on the left side had moved toward the center. During the 30-35 msec interval (Figure 8C), a single excitatory peak remained and it was surrounded by patches of inhibition.

3

This method of representing the receptive field appears to have many advantages in studying the connectivity of the neuronal pools and their interactions with cutaneous afferents. The order in which peaks of activity slide around the receptive field tells us about the relationship between central connectivity of these afferents and the spatial distribution of their terminals on the skin. For example, if the peaks in excitatory activity move smoothly from one primary afferent receptive field to another, a much different connectivity must occur than if the excitatory peaks are seen to rise and fall independently. This type of 3-dimensional analysis from single impulse input is being used for study of other skin receptor systems and for evaluation of submodality interactions, coupling of sites in the receptive field and recovery cycles.

At present we don't have sufficient data to describe the central organization of these neural network in quantitative terms. We don't know whether there are one or several classes of cell according to the variety of response. We can't correlate these results with neuroanatomical observations of this region. However, we believe this approach will eventually lead to an understanding of these factors and that, in the meantime, testable models can be constructed in pursuit of these goals.

Figure 1. Properties of the tactile pad receptor system. A. The tactile pad or haarscheibje, in depilated skin of the new-born kitten. B. The receptive fields of the primary sensory unit are non-intersecting. A single tactile pad is innervated by only one large myelinated fiber. A single Type I afferent terminates in 1-7 of the tactile pads (C) but the cluster of pads of one unit do not intermingle with those of neighboring units. D. A displacement of the tactile pad with a short duration mechanical pulse (middle record: monitor of probe movement) of less than 10 microns will evoke a single action potential in the Type I afferent.

Figure 2. Averaged responses to single short duration displacements of a single tactile pad at increasing amplitudes extracted from whole dorsal root, S₁. 5000 repetitions once per 60 msec. With amplitudes of less than 20 microns a single impulse was evoked. Threshold amplitude: 8.5 microns. Bottom trace: wave form of an averaged stimulus monitor.

Figure 3. Response of dorsal spinal gray neurons to short duration displacements of structures in hairy skin. Top: Postsynaptic discharge (upper trace) of central cell in response to a 2-micron displacement (lower trace: stimulus monitor) of a tactile pad receptor. Bottom: Poststimulus histograms of 100 responses of another central cell to 10 micron displacements on and just adjacent to a tactile pad receptor. Bin width: 5 msec.

Figure 4. Responses of a single dorsal spinal gray neuron to single short duration displacements of four tactile pads at different sites within the receptive field of the neuron. Upper traces: dot patterns of 50 responses to stimuli applied once per three seconds. First dot is stimulus onset.

A. Short latency prolonged inhibition of ongoing activity. B. Short latency bursting discharge. C. Short latency short duration excitation followed by a reduction in ongoing activity. D. Long latency bursting discharge.

Figure 5. Comparison of responses of two neighboring dorsal spinal gray neurons with overlapping receptive fields to single impulses mechanically evoked in shared tactile pad receptors. Post stimulus histograms of responses to stimuli applied once every three seconds for a total of 50 stimuli. Units 5 and 6 responded to stimulation of pad 21 with a short latency short duration discharge and to stimulation of pad 14 by a long latency more sustained discharge. Stimulation of the remaining pads produced different responses in the two neurons.

Figure 6. Responses of a dorsal spinal neurons to single action potential in single Type I afferents evoked by short duration mechanical pulses applied to tactile pads within the receptive field of the neuron. Bin width: 2 msec. Same responses as shown in Figure 4.

Figure 7. Response latencies. Conduction times from stimulus site, i.e., the tactile pad receptor, in the receptive field to the recording site of the dorsal root. The dorsal root response was determined by average response computing (see Figure 2) [left]. Postsynaptic response latencies. Values above bar were excitations and below bar, inhibitory latencies [middle]. Central delays; dashed dotted area is region of grossly determined receptive field [right]. Times in msec. Triangles, some inhibitory activity; filled circles, excitations. Central delays and postsynaptic responses rounded to nearest 0.5 msec.

Figure 8. Response contour maps." Peaks of excitation are stippled; inhibitory regions are shaded with slanted lines. see text for explanation.

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A

1mm

B

D

C

NUMBER TACTILE UNITS

TERMINALS PER UNIT

1 7 μ

1 msec

12.5 msec.

UNIT-5

PAD
21

PAD
14

PAD
18

PAD
25

PAD
19

0 50 100 msec

UNIT-6

0 50 100 msec

5-10 msec

15-20 msec

30-35 msec