Modulation of Synaptic Effectiveness of Ia and Descending Fibers in Cat Spinal Cord

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FRANK AND FUORTES (10) described a depression of monosynaptic group Ia EPSPs in extensor motoneurons with conditioning stimulation of group I afferents in nerves to flexor muscles which did not appear to alter the excitability of postsynaptic neurons. Later, Frank (9) assumed that the interaction between the conditioning volley and the EPSP occurred at loci remote from the motoneuron cell body, and suggested that such "remote" inhibition could be due either to interaction between EPSPs and postsynaptic IPSPs in electrotonically distant dendritic sites or to a reduction in synaptic effectiveness caused by a mechanism operating directly on the presynaptic terminals.

In order to assess whether the Ia EPSP depression is entirely explained by presynaptic inhibition, the presence of remote postsynaptic inhibition must be ruled out. Detection of remote dendritic conductance changes by injecting current pulses into the motoneuron soma is not a very satisfactory test (25, 34); variations in cell firing to injected steady currents appears to be a more sensitive index of remote conductance changes and this method has been used to demonstrate the presence of remote postsynaptic inhibition during some conditioning inputs which may also depolarize the Ia terminal arborizations (12, 13, 18, 19).

Another index of the presence of effective remote inhibition in alpha motoneurons is the time course of the falling phase of the composite Ia EPSP itself, which largely represents EPSP components generated in the distal dendrites (25). Local inhibitory conductances in remote dendritic sites should attenuate particularly these components causing an apparent faster rate of decay of the composite Ia EPSP (3, 24, 25, 36). Accordingly, the observations that Ia EPSPs can be depressed by conditioning inputs without alteration of their falling phases (3, 8) have been interpreted as being due to a presynaptic mechanism.

More recently, Eide et al. (8) reported that monosynaptic EPSPs produced in motoneurons by stimulation of descending tracts were unaffected by conditioning volleys to flexor group I afferents which depressed Ia monosynaptic EPSPs. This was taken as evidence that the observed Ia EPSP depression was not due to remote postsynaptic inhibition because, if this were the case, the descending EPSP would also have been reduced by the conditioning volley. This conclusion implicitly assumes that the active terminals producing the two EPSP species end in electrotonic proximity to one another in the dendritic tree, an assumption not tested in the experiments of Eide et al. (8). Although there is suggestive evidence that both species of terminals do overlap in space over the motoneuron surface (15, 16, 23) the extent of such overlap is unknown and could vary from one motoneuron to the other, as is the case with different Ia fibers (2, 21). If it is assumed that remote inhibitory conductance changes are restricted to the vicinity of Ia fiber synaptic loci, EPSPs produced by other, electrotonically distant, synapses (e.g., of descending fibers) would be unaffected (see ref 2, 26).

In the present series of experiments we studied the effects of conditioning volleys to sensory nerves on the mean and
variance of monosynaptic EPSPs in gastrocnemius motoneurons produced by Ia fibers and by axons descending in the ventral funiculus (VF). The degree of spatial overlap of the active terminals of the two species of fibers was estimated from the amount of nonlinear addition between the corresponding monosynaptic EPSPs (2, 21, 26). The results obtained support the conclusion that Ia EPSP amplitude changes can be produced by conditioning inputs without noticeable contamination from postsynaptic conductance changes and may, therefore, be attributed to a presynaptic mechanism. Analysis of the fluctuations of Ia and VF monosynaptic EPSPs suggests that Ia EPSP variability is introduced, at least in part, through the segmental pathways mediating primary afferent depolarization. A preliminary account of some of these observations has been published elsewhere (32).

METHODS

General procedures
Cats (between 2.5 and 3.5 kg wt) were initially anesthetized with ether. The trachea was cannulated and both carotid arteries tied. The right lateral gastrocnemius plus soleus (GL), medial gastrocnemius (GM), biceps semitendinosus (BST), and sural (SU) nerves were dissected free, sectioned distally, and their central ends prepared for stimulation. Thereafter, the lumbosacral and lower thoracic spinal cord was exposed by laminectomy, the right L5–S1 ventral roots were sectioned and their central ends prepared for stimulation. The spinal cord was completely sectioned at an upper thoracic level (T3–T4) and a second section was made four segments caudally, leaving most of the right ventral funiculus (VF) intact (see Fig. 1B–E). The VF contains, among other pathways, vestibulospinal and reticulospinal fibers synapsing directly with spinal interneurons and motoneurons (15, 16, 23, 38).

On completion of the dissection, the cat was fixed to a heavy metal frame and decerebrated by intercollicular section. The anesthetic was then discontinued. Pools of liquid paraffin covered the exposed cord and hindlimb nerves. Cord pool temperature was kept at 37°C by means of radiant heat. The animal was paralyzed with gallamine triethiodide (Flaxedil) and maintained by artificial respiration. End-tidal CO₂ concentration was held throughout the experiment between 4.5 and 5%. Arterial blood pressure was monitored in all experiments and mean blood pressure ranged between 80 and 120 mm Hg. At least 3 h elapsed between cessation of ether anesthesia and beginning of recording.

FIG. 1. A: diagram of the experimental method. VF, ventral fasciculus; VR, ventral root; BST, biceps semitendinosus; GS, gastrocnemius soleus; SU, sural. B, C, D, and E: drawings from the histological sections of the thoracic spinal cord obtained from four different experiments. The hatching shows the extent of the ipsilateral lesion made to spare the VF. The contralateral side of the spinal cord was completely sectioned. Further explanations in text.
Stimulation and recording

Tungsten electrode pairs (50 μm) insulated up to the tip were placed on the intact right VF, rostrally to the partial section of the thoracic spinal cord (Fig. 1A). The stimulating electrode pair was usually placed as medially and ventrally as possible, in the region where the threshold for the descending volley recorded from the cord dorsum was lowest (14–16, 22, 23, 38–40).

Synaptic responses of spinal motoneurons were intracellularly recorded with glass micropipettes filled with 2 M potassium citrate. The stimuli to the VF and to the sensory nerves were automatically alternated by computer control to have the conditioned responses interposed between unconditioned responses. This procedure reduced possible effects due to slow drift in the synaptic responses and allowed accurate comparison of conditioned and test responses (for instance, see Fig. 5). The intracellular as well as the cord dorsum potentials were recorded on magnetic tape and later played back for analysis. Each data series, consisting of 100–200 responses, was processed separately using a PDP12 computer. The method employed for calculation of the mean and variance of the synaptic responses as a “continuous” function of time has been described elsewhere (28). The data presented in this paper were selected from a population of 80 gastrocnemius cells which could be held for a length of time sufficient for data collection (15 min to 1 h) with a membrane potential ranging from about 50 to 70 mV.

RESULTS

Synaptic potentials produced by VF stimulation

The synaptic potentials produced in gastrocnemius motoneurons by stimulation of the ventral funiculus will be a mixture of excitatory and inhibitory actions, depending on the relative proportion of vestibulospinal and reticulospinal axons activated (among others; see ref 38, 40). In our hands, VF stimulation generally produced excitatory synaptic potentials, usually with two components (Fig. 2B). The first component had a latency ranging from 0.4 to 0.6 ms (mean 0.43) after the arrival of the descending volley at the corresponding segmental level, and probably represents the EPSP produced by activation of vestibulospinal fibers synapsing directly with motoneurons (14–16, 23, 38–40). The second component varied in size and time course from one motoneuron to the other. Sometimes, as in Figs. 2B and 6E, only a hump was evident in the rising phase of the EPSP, 1.0–1.6 ms after the arrival of the descending volley. In other instances, the second component appeared well after the peak of the monosynaptic EPSP (Figs. 3D and 4D). In contrast with the early monosynaptic EPSP, the second component was increased greatly with repetitive stimulation, and most authors agree it is due to motoneuron depolarization via one
Effects of BST conditioning on EPSPs produced by stimulation of the Ia and VF fibers. A, C, and E: recordings made from the dorsal root entry zone. B, D, and F: corresponding intracellular recordings. B: EPSPs produced by supramaximal stimulation of the group I fibers in the GL nerve. D: EPSPs produced by stimulation of the ipsilateral VF at the midthoracic level (2 x T strength of the most excitable fibers in the tract). Continuous traces in B and D are the control responses. Dotted traces represent responses obtained 30 ms after BST conditioning stimulation (3 shocks at 300/s, 1.3 times threshold of the most excitable fibers in the nerve). The continuous trace in F is the synaptic potential obtained during stimulation of both the Ia and descending fibers at times indicated by arrows, with the same parameters as in B and D. The dotted trace in F shows the synaptic potential obtained by algebraic addition of the two unconditioned EPSPs illustrated in B and D. The vertical interrupted line was traced at the peak of the monosynaptic component of the descending EPSP. All are averaged records of 200 responses elicited once every 6 s. Calibration: 1 mV, 1 ms. Further explanations in text.

Interposed interneuron (14-16, 22, 23, 38-40), although more slowly conducting fibers synapsing directly with motoneurons may also contribute to this component (cf. ref 15, 23). Additional evidence supporting the polysynaptic nature of the second component will be discussed below when analyzing EPSP variability.

VF stimulation could also produce a prolonged hyperpolarization following the initial monosynaptic EPSP (Fig. 7E). Sometimes this late IPSP, which probably arises from activation of inhibitory interneurons by reticulospinal fibers in the VF, could be reduced by decreasing the stimulus strength or by shifting the VF stimulating electrode toward the midline (see ref 15, 16). The appearance of relatively large polysynaptic PSPs could be related to the use of unanesthetized spinal cords. After sodium pentobarbital (10-30 mg/kg), polysynaptic PSPs following the VF monosynaptic EPSP were less frequently observed (see ref 38). However, since the background primary afferent depolarization (PAD) of Ia terminal arborizations and the action of cutaneous conditioning on such background PAD are very sensitive to barbiturates (see ref 31), the present experiments required the use of unanesthetized spinal cords. This interfered with a detailed analysis of the changes induced by sensory conditioning on the falling phase of both the Ia and the VF EPSPs (see ref 3 and 17).

Effects of sensory conditioning on mean of Ia and VF monosynaptic EPSPs

GROUP I FLEXOR NERVE CONDITIONING. It has been reported that volleys in group I BST nerve fibers depolarize the Ia terminal arborizations ending within extensor motor nuclei (7, 29, 31) and reduce the corresponding monosynaptic Ia EPSPs (see ref 3, 6). In the present series of experiments we analyzed the effects of BST conditioning on the Ia and VF monosynaptic EPSPs elicited in the same motoneuron. Typical results are illustrated in Figs. 3 and 4. Every other Ia and VF EPSP was conditioned by short trains to the group I fibers in the BST nerve (1.3 x threshold of most excitable fibers). Such a low-intensity stimulus to the BST nerve was selected to avoid, as far as possible, long-latency postsynaptic conductance changes produced in motoneurons by stimulation of higher threshold fibers (see Fig. 5 and ref 3). During BST conditioning the Ia EPSPs (Figs. 3B and 4B, dotted traces) were depressed to about 87% of their control size (Figs. 3B and 4B, con-
PRESYNAPTIC MODULATION OF Ia TERMINALS

However, the monosynaptic components of the EPSPs produced by VF stimulation were virtually unaffected by the same BST conditioning stimulus (Figs. 3D and 4D, dotted traces).

The lack of change of the monosynaptic component of the VF EPSP following low-threshold BST conditioning volleys, which effectively depress Ia EPSPs, is also illustrated in Figs. 6 and 7. In the example of Fig. 7, the VF stimulation produced a monosynaptic EPSP followed by a late IPSP. The BST conditioning volley reduced the Ia EPSP (Fig. 7B, dotted trace), also without affecting the monosynaptic component of the descending EPSP (Fig. 7E, double-headed arrow).

A summary of the data obtained comparing the effects of BST conditioning on monosynaptic Ia and VF EPSPs is presented in Table 1. Only data where interaction between EPSPs was also analyzed and where the conditioning volleys produced no significant change in the Ia EPSP falling phase are included in this table. In most cases, BST conditioning depressed the Ia EPSP amplitude over a wide range (up to 66%) without affecting the monosynaptic VF EPSP by more than 2%. Only in two cases were the descending EPSPs depressed more than 2% (up to 5.9%), perhaps because of underlying postsynaptic conductance changes produced by the conditioning stimulus (see below).

When the BST conditioning stimulus intensity was increased, usually above 1.4 times threshold strength, the descending EPSPs could be reduced in size and their rate of decay increased, as expected from increased postsynaptic conductances (see ref 3). Figure 5 shows the effects of graded stimulation of BST afferents on the monosynaptic VF EPSPs. This set of data was obtained from a motoneuron where stimulation of group I fibers in the gastrocnemius nerve produced no monosynaptic EPSPs. It is illustrated because it was one of the few cases where the VF EPSPs appeared not to be contaminated with large polysynaptic components. In this cell the BST stimulus produced a slow depolarization followed by hyperpolarization. With increasing BST volleys the depolarization was increased in size and duration and the hyperpolarization became less obvious. As shown in Fig. 5, the peak of the VF EPSPs tested about 17 ms after the first of the three BST shocks was not importantly changed until the BST stimulus was above 1.66 times threshold strength. With larger intensities, the VF EPSPs were reduced in size and their falling phase shortened. It should be noticed that the effects of BST stimulation of 2.5 and 3.3 x T strength on the VF EPSP size are not due to the changes in the membrane potential trajectory caused by the BST stimulus itself. Since at the time of the VF EPSP the cell membrane potential had practically recovered its control value, the shortening in the falling phase of the VF EPSP is probably produced by the same mechanism that reduced the VF EPSP size (see discussion).

CUTANEOUS CONDITIONING. In the unanesthetized spinal cord, stimulation of the low-threshold afferent fibers in the
FIG. 5. Effects of BST conditioning on the monosynaptic EPSP produced by stimulation of the VF. Recordings from an unidentified motoneuron. Stimulation of group I afferent fibers from the gastrocnemius nerve produced no monosynaptic EPSPs. In all cases, from A to G, two traces are superimposed: one showing only the unconditioned VF EPSPs and the other showing the VF EPSPs preceded by the BST conditioning stimulus at various strengths, expressed as multiples of the threshold of the most excitable fibers in the BST nerve (see values in each group of traces). Traces in the left-hand column are at slow sweep to display both the potential changes produced by the conditioning and by the test stimulus. Traces in the right-hand column show only the unconditioned and conditioned test responses. For comparison purposes, the base line of the conditioned responses was shifted in the vertical axis to match the base line of the unconditioned response. H: recording from the dorsal root entry zone obtained with a BST conditioning volley of 1.66 times T strength. Note that with BST conditioning stimuli up to 1.33 times threshold there was no change in the test EPSP peak amplitude. With larger strengths there was a clear reduction in the EPSP amplitude and a shortening in EPSP falling phase. Each trace is the average of 100 responses taken every 3 s. Note from H to A, which is the order in which the series were taken, that the test VF EPSP gets progressively smaller, probably because of cell deterioration. Calibration: 2 mV, 1 ms.
FIG. 6. Effects of BST conditioning on EPSPs produced by stimulation of Ia and VF fibers. A and D: recordings from the dorsal root entry zone. B and E: intracellular recordings. C and F: variances of the intracellular potentials. A, B, and C: supramaximal stimulation of group I fibers. D, E, and F: VF stimulations (3 × T strength of the most excitable fibers in the tract). Continuous traces, control responses. Dotted traces, responses obtained after BST conditioning (3 shocks at 300/s, 1.35 × threshold, 30 ms before test stimulus). Double-headed arrows show the time of occurrence of the monosynaptic Ia and VF-EPSPs, respectively. Single-headed arrows indicate stimulus artifacts. Note that the conditioning stimulus reduced the amplitude (B) and the variance (C) of the Ia EPSP, but had practically no effect on the mean (E) and variance (F) of the monosynaptic component of the descending EPSP. Calibration for intracellular traces, 2 mV, 1 ms. Variance calibration in mV². Note that the Ia EPSP has a relatively short duration compared with EPSPs illustrated in Figs. 3 and 4, perhaps because of contamination with late EPSPs due to the supramaximal stimulation of group I fibers (5), although shortening of membrane time constant because of cell deterioration cannot be excluded.

Sural nerve may increase Ia EPSPs, presumably because of a relative hyperpolarization (PAH) of the Ia fiber terminals (31). It was, therefore, interesting to compare the effects of SU conditioning both on the Ia and on the descending monosynaptic EPSPs. As shown in Table 1, SU conditioning could increase the amplitude of the Ia EPSPs up to 110% of the control size without importantly affecting the amplitude of the monosynaptic descending EPSPs. The effects of high-threshold (above 2 × T) cutaneous afferents on Ia and VF monosynaptic EPSPs were not explored in the present series of experiments.

**Summation between Ia and VF EPSPs**

The degree of spatial overlap of the Ia and VF fibers impinging onto a
### Table 1. Effects of conditioning volleys on Ia and VF monosynaptic EPSPs

<table>
<thead>
<tr>
<th>Cell No.</th>
<th>Peak Amplitude, mV</th>
<th>Percentage of Control Following Conditioning</th>
<th>Summation Between Ia and VF EPSPs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ia EPSP</td>
<td>VF EPSP</td>
<td>Ia EPSP</td>
</tr>
<tr>
<td>BST Conditioning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.50</td>
<td>0.92</td>
<td>100.0</td>
</tr>
<tr>
<td>2</td>
<td>1.32</td>
<td>1.08</td>
<td>98.8</td>
</tr>
<tr>
<td>3</td>
<td>3.71</td>
<td>2.26</td>
<td>95.2</td>
</tr>
<tr>
<td>4</td>
<td>2.00</td>
<td>0.90</td>
<td>87.5</td>
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<td>5</td>
<td>4.62</td>
<td>0.95</td>
<td>85.3</td>
</tr>
<tr>
<td>6</td>
<td>1.25</td>
<td>0.60</td>
<td>82.5</td>
</tr>
<tr>
<td>7</td>
<td>2.25</td>
<td>1.35</td>
<td>82.2</td>
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<tr>
<td>8</td>
<td>0.85</td>
<td>0.93</td>
<td>81.5</td>
</tr>
<tr>
<td>9</td>
<td>3.66</td>
<td>1.66</td>
<td>73.5</td>
</tr>
<tr>
<td>Mean</td>
<td>2.24</td>
<td>1.18</td>
<td>87.2</td>
</tr>
<tr>
<td>10</td>
<td>2.86</td>
<td>1.45</td>
<td>95.9</td>
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<td>11</td>
<td>3.55</td>
<td>2.24</td>
<td>92.7</td>
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<tr>
<td>12</td>
<td>3.18</td>
<td>1.89</td>
<td>76.6</td>
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<tr>
<td>13</td>
<td>1.26</td>
<td>0.89</td>
<td>66.6</td>
</tr>
<tr>
<td>Mean</td>
<td>2.7</td>
<td>1.60</td>
<td>82.9</td>
</tr>
<tr>
<td>Total mean</td>
<td>2.39 ± 1.3</td>
<td>1.51 ± 0.5</td>
<td>85.9 ± 10.2</td>
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**SU Conditioning**

<table>
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<tr>
<th></th>
<th>Ia EPSP</th>
<th>VF EPSP</th>
<th>Ia EPSP</th>
<th>VF EPSP</th>
<th>Control</th>
<th>Conditioned</th>
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<td>14</td>
<td>1.26</td>
<td>2.95</td>
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<td>100.0</td>
<td>88.8</td>
<td>88.8</td>
</tr>
<tr>
<td>15</td>
<td>1.92</td>
<td>0.62</td>
<td>104.0</td>
<td>100.0</td>
<td>91.0</td>
<td>82.0</td>
</tr>
<tr>
<td>16</td>
<td>2.21</td>
<td>1.00</td>
<td>109.5</td>
<td>103.1</td>
<td>78.7</td>
<td>69.9</td>
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<tr>
<td>17</td>
<td>1.05</td>
<td>1.21</td>
<td>110.0</td>
<td>100.0</td>
<td>86.0</td>
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<tr>
<td>18</td>
<td>2.93</td>
<td>1.90</td>
<td>110.7</td>
<td>100.0</td>
<td>73.6</td>
<td>73.6</td>
</tr>
<tr>
<td>Mean</td>
<td>1.87 ± 0.7</td>
<td>1.54 ± 0.9</td>
<td>106.8 ± 4.6</td>
<td>100.2 ± 1.7</td>
<td>85.6 ± 7.2</td>
<td>79.7 ± 7.8</td>
</tr>
</tbody>
</table>

BST conditioning: 3 shocks at 300/s, 1.4 × T, 30–40 ms before test stimulus. SU conditioning: 1 shock, 2 × T, 40–60 ms before test stimulus. Cells 1–13 were separated in two groups depending on the effects of BST conditioning on summation between EPSPs.

The motoneuron can be estimated from the amount of nonlinear addition between the corresponding monosynaptic EPSPs (see ref 2 and 26 for a detailed discussion of this issue). The procedure used for testing addition between the monosynaptic Ia and VF EPSPs is illustrated in Fig. 2. Trace B in Fig. 2 shows the EPSP produced by VF stimulation and trace C, the Ia EPSP produced by stimulation of the gastrocnemius nerve. The continuous line in D shows the EPSP obtained during simultaneous stimulation of both pathways. The timing between the two shocks was adjusted so that both EPSPs had their onset at about the same time, because this provides the optimal conditions for interaction (2). The dotted trace in Fig. 2D shows the EPSP obtained after the algebraic addition of the two EPSPs shown in B and C. It can be seen that the two synaptic potentials are essentially of the same size, i.e., that the EPSPs summed linearly. This suggests that in this particular case both EPSPs were generated by sets of fibers ending in electrotonically distant synaptic sites, one relative to the other (2). In most cases, however, there was a measurable nonlinear addition between the monosynaptic VF and Ia EPSPs (Figs. 3F and 4F), as expected from sets of fiber terminals ending in electrotonically close synaptic sites (see ref 2 and 21).

Table 1 gives additional information on summation between Ia and VF EPSPs. Both EPSPs were evoked simultaneously as well as individually (as in Figs. 2, 3, and 4). Measurements were made at the time of the peak of the monosynaptic VF EPSP (as indicated by the vertical interrupted lines in Figs. 2–4). In the table, EPSP summation is expressed as percentage of the value expected from linear addition between the two EPSPs. Perfect linearity in EPSP summation is expressed as 100%. It can be seen that in most cases summation of the potentials was less than linear. The average EPSP summation for this set...
FIG. 7. Effects of BST conditioning on synaptic potentials produced by Ia and VF stimulation. A and D: recording from the dorsal root entry zone. B and E: intracellular recordings from a GI motoneuron. C and F: corresponding variances. B and C: after stimulation of group I afferent fibers (see trace A); E and F: after stimulation of the VF tract at 3 × T strength (see trace D). Continuous traces, unconditioned responses. Dotted traces, after BST conditioning (3 shocks at 300/s, 1.3 × threshold, 30 ms before test stimulus). Voltage calibration: 1 mV, 1 ms. Variance calibration in mV². Double-headed arrows indicate the time of occurrence of the peak of the monosynaptic EPSP components. All traces are averages derived from 200 responses.

of data was 90.3 ± 6.4% of the predicted value, which is somewhat smaller than the average obtained from a comparable set by Burke (2) for interaction between pairs of Ia EPSPs (96%). As shown in Table 1, during BST conditioning, summation between Ia and VF monosynaptic EPSPs was unchanged in three cases, increased in six cases, and reduced in four cases. On the other hand, SU conditioning reduced the amount of linear summation between the Ia and VF EPSPs in three of the five cases where this was tested, and had no effect in two cases.

Variance of monosynaptic Ia and VF EPSPs

Rudomin et al. (28) have suggested that in the unanesthetized spinal cord, in addition to the random process which governs transmitter release intrinsic to a given synaptic terminal, there is another stochastic process affecting, in a correlated manner, transmitter release in large sets of Ia synaptic terminals. Such correlation in transmitter release seems to be achieved by membrane potential fluctuations imposed onto the Ia terminal arborizations by ongoing activity of the segmental mechanism mediating PAD. Conditioning volleys to muscle and to cutaneous nerves may reduce these correlated fluctuations, thereby affecting the information transmitted from Ia fibers to motoneurons (28, 30).

Since the experiments described in previous sections suggested that the VF fibers synapsing with motoneurons, unlike the Ia fibers, are not subjected to presynaptic modulation by PAD (Figs. 3 and 4), it was of interest to compare the changes produced by BST conditioning volleys in the fluctuations of monosynaptic Ia and VF EPSPs elicited in the same motoneuron. VF EPSPs were generated by supramaximal stimulation of the descending tract. Care had to be taken to avoid changes in stimulus strength because of movements.

The continuous traces in Fig. 6B and C show the mean and variance of the Ia EPSP elicited in a gastrocnemius motoneuron (see ref 28 for a full description of the method for the variance computation). During the Ia EPSP, the variance of the motoneuron membrane potential usually increased above baseline levels with a time course approximately matching the Ia EPSP itself (see also Fig. 7 and ref 28).

In most cases, during the VF EPSP, the variance was also increased above baseline levels, usually with two well-defined components. The first component was
generally smaller than the second and corresponds in time with the monosynaptic component of the mean VF EPSP (Fig. 6E and F, double-headed arrow). The second component appears during the delayed hump in the VF EPSP and probably reflects the variability of EPSPs generated through polysynaptic pathways (see also ref 38). In a few cases, the variance was not increased above base-line levels during the monosynaptic component of the VF EPSP. Substantial variance changes (either increases or decreases) appeared later, during the development of polysynaptic PSPs (see, for instance, Fig. 7 where there is a marked reduction in variance coincident with a prolonged IPSP; see also ref 28).

As reported previously (28), conditioning volleys in group I afferents in the BST nerve may reduce the fluctuations of Ia EPSPs elicited in gastrocnemius motoneurons. This effect seems to be to some extent independent of the changes in the mean size of the Ia EPSP and in base-line variance (i.e., the fluctuations due to ongoing postsynaptic activity) produced by the conditioning volley. The above observations have been confirmed in the present series of experiments (Figs. 6C and 7C, dotted traces). As shown in Table 2 (columns 3 and 4), in five gastrocnemius cells where this was tested, BST conditioning volleys reduced the variance of the Ia EPSP peak (measured as variance increment above base-line levels) to a mean value of 43% relative to control values. In contrast with this effect, the same conditioning volleys either had no effect (two cases) or slightly increased (five cases) the variance at the time of the VF monosynaptic EPSP. The mean value of this change was of 110.0%.

**DISCUSSION**

**Presynaptic origin of Ia EPSP amplitude changes produced by conditioning volleys**

The observations presented in this paper show that conditioning volleys to sensory nerves may change the amplitude of the monosynaptic Ia EPSPs without affecting the amplitude of the monosynaptic component of the EPSP produced by stimulation of the ventral funiculus in the thoracic spinal cord. Evidence is provided also that in many instances the monosynaptic EPSPs produced by stimulation of the two pathways added less than linearly.

According to Burke (2), the fact that less than linear EPSP summations are seen (e.g., Figs. 3 and 4 and Table 1) implies that at least one of the monosynaptic EPSPs is generated by a transmembrane conductance change, as expected from chemical synapses. The nonlinearity of summation is viewed as the result of mutual interaction of perturbations (conductance changes and attendant potential

| Table 2. Effects of BST conditioning on mean and variance of Ia and VF monosynaptic EPSPs |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                | Percentage Change in Mean EPSP | Ratio of Variance Increments at Peak of EPSP | Base-Line Variance Ratio |
| Cell No. | Ia EPSP | VF EPSP | (P - B) Conditioned (P - B) Control x 100 | (σ² - σ²) Conditioned (σ² - σ²) Control | (σ²) Conditioned (σ²) Control |
| 1 (11)  | 92.7  | 100.0  | 0.13 | 1.00 | 0.41 |
| 2      | 89.5  | 100.7  | 0.43 | 1.08 | 0.67 |
| 3 (9)  | 73.5  | 99.2  | 0.23 | 1.10 | 0.75 |
| 4 (12) | 76.6  | 98.1  | 0.40 | 1.13 | 0.80 |
| 5      | 100.0 |       | 0.96 | 1.24 | 1.44 |
| 6      | 100.7 |       | 1.15 | 1.45 | 1.98 |
| 7 (13) | 66.6  | 100.0 | 0.96 | 1.00 | 2.51 |
| Mean   | 79.8 ± 11.0 | 99.8 ± 0.9 | 0.43 ± 0.3 | 1.10 ± 0.1 | 1.22 ± 0.8 |

B, base-line voltage. σ², base-line variance. P, voltage at peak of EPSP. σ², variance at peak of EPSP. BST conditioning, 3 shocks at 300/s 1.4 × T, 30–40 ms before test. Numbers in parentheses are for identification of cells listed in Table 1.
changes). Some proportion of the conductance changes generating the EPSPs must be located spatially and electrically close enough to one another to permit such mutual interactions. Therefore, if the amplitude changes of the Ia EPSPs induced by the conditioning volleys were of a purely postsynaptic origin, one would expect similar effects on the monosynaptic VF EPSPs produced by fibers ending in electrotonically close synaptic loci (see ref 8).

The results presented in this paper (see Table 1) suggest that there is only a partial overlap between Ia and VF synaptic loci. Comparison of the rise times of the monosynaptic Ia and VF EPSPs has suggested that the VF synaptic loci are more proximally located than the Ia synaptic loci (17, 26). Therefore, it could be argued that the Ia EPSPs were depressed by postsynaptic inhibitory synapses acting on the nonoverlapping, presumably distal Ia synaptic loci, still without affecting the EPSPs generated by the VF fibers in more proximal loci. It can be shown that postsynaptic inhibition acting on the nonoverlapping Ia distal synaptic loci or presynaptic inhibition acting on the Ia fibers terminating on such loci will reduce the amount of linear interaction between the monosynaptic Ia and VF EPSPs. Table 1 shows examples of results consistent with this possibility (cells 10–13). There are, however, several cases where BST conditioning increased the linear summation between the two species of EPSPs (cells 2–4, 6, 7, and 9). This implies that the inhibitory action reduced the Ia EPSPs generated in the synaptic loci overlapping with VF fibers. If this effect were postsynaptic, the VF EPSPs generated in these same loci would be expected to be reduced to the same extent as the Ia EPSPs, but this was not observed. Hence, it is reasonable to assume that the depression of the Ia EPSPs produced under such conditions was due to a presynaptic mechanism. As shown in Table 1, SU conditioning could increase the Ia EPSPs without changing the VF EPSPs while decreasing the linear summation between both EPSPs. This action is also compatible with a presynaptic mechanism.

In summary, the simplest explanation for the differential action of conditioning volleys to sensory nerves on Ia and VF EPSPs is that afferent volleys change the synaptic effectiveness of the Ia fiber terminals by a presynaptic mechanism without changing the effectiveness of neighboring VF fibers. Low-threshold afferents in the BST nerve would produce PAD and presynaptic inhibition of the Ia fiber terminals (3, 8), whereas large cutaneous fibers would produce PAH and presynaptic facilitation as suggested previously (see ref 31). The mechanism involved in such a presynaptic modulation is envisaged as being rather specific since it affects group Ia synaptic endings without affecting nearby VF synaptic endings. This is more consistent with the hypothesis that the presynaptic depolarization is mediated by interneurons releasing a specific transmitter substance than with accumulation of potassium ions in the extracellular space as a result of increased activity of nearby neuronal elements (1, 4, 20, 33, 35, 37). However, it might be argued that electrotonic proximity does not necessarily imply physical proximity when synapses end on a given dendritic branch (26). Although interacting terminals are probably pretty close physically, at least within 10s or at most 100 or 200 μm, depending on local dendritic diameters and branching (R. E. Burke, personal communication), a final word on this issue must await detailed information on the spatial distribution and modes of termination of Ia and VF fibers, and their possible relationships with glial cells (see ref 1). The lack of modulation by PAD of the descending fibers ending on spinal motoneurons documented in this paper has been also demonstrated in the frog spinal cord (11), and this appears to be a general feature of the spinal cord organization.

As stated in the introduction, a very effective index for the presence of remote inhibition in alpha motoneurons is the falling phase of the composite Ia EPSP itself. Local inhibitory conductances in remote dendritic sites should attenuate the EPSP, causing an apparent faster rate of decay (cf. Fig. 5). If the depression of the Ia EPSPs illustrated in Figs. 3 and 4 were
exclusively presynaptic, one would expect the Ia EPSPs to be reduced without a change in their falling phase. Although this was so in the example of Fig. 4, it is not certainly the case in Fig. 3 where it can be seen that the latter part of the depressed Ia EPSP is slowed down. In the absence of postsynaptic inhibitory conductances, reduction in EPSP amplitude without changes in the EPSP falling phase will be obtained only if the Ia fibers are uniformly depolarized and subjected to about the same degree of presynaptic inhibition. If presynaptic inhibition were to affect Ia synapses in a differential manner, depending on their spatial distribution over the motoneuron surface, because of the composite nature of the EPSP, one could obtain changes in either direction in the EPSP falling phase. For instance, presynaptic inhibition restricted to the Ia fibers synapsing with the distal dendrites, would generate depressed composite Ia EPSPs with a shortened falling phase. Therefore, depression of Ia EPSPs with shortening of their falling phase may not be used as an unequivocal criterion to distinguish between pre- and postsynaptic (remote) inhibition. Clearly, additional criteria are necessary to establish the possible contribution of postsynaptic inhibitory conductances in the distal dendrites (see ref 3).

Possible sources of Ia and VF EPSP variability

The finding that BST conditioning may reduce the variance of the Ia EPSPs either without affecting or by increasing the variance of the monosynaptic VF EPSPs (Fig. 6 and Table 2) provides additional evidence for the existence of a presynaptic mechanism controlling the synaptic effectiveness of the Ia fiber terminals. In agreement with previous suggestions (27, 28, 31) we believe that in the spinal cat, Ia EPSP fluctuations are mostly generated by correlated fluctuations in transmitter release imposed onto the Ia terminal arborizations via the segmental pathways mediating the PAD. Volleys to sensory nerves would reduce the contribution of the PAD pathways as sources of correlated fluctuations, thereby reducing Ia EPSP variability (28).

Since the VF fiber terminals appear not to be subjected to presynaptic modulation by PAD, VF EPSP variability would be expected to reflect fluctuations generated by other mechanisms, i.e., by fluctuations in the transmitter release mechanism (unrelated to the PAD pathways) and/or fluctuations due to postsynaptic membrane potential or conductance changes. In this regard, the observation that BST conditioning slightly increased the VF EPSP fluctuations implies that in those cases (cf. Table 2) the conditioning stimulus introduced additional fluctuations which were partly correlated with the mechanism generating the VF monosynaptic EPSP fluctuations. This effect could result from changes in the VF EPSP driving potential due to membrane potential fluctuations generated by the polysynaptic PSPs produced by the conditioning stimulus itself (cf. appendix in ref 28).

Regardless of the mechanisms generating the EPSP fluctuations, it is important to point out that BST conditioning volleys changed the fluctuations contributed by the Ia and VF EPSPs in opposite directions (cf. Table 2, columns 3 and 4). The extent to which variance changes in EPSP amplitude will affect cell firing will also depend on the concomitant base-line variance which was sometimes increased and sometimes decreased by the conditioning volleys (Table 2, column 5). With the available evidence, which is rather limited, it is difficult to envisage what would be the overall firing behavior of the motoneuron pool, even in this particular set of conditions. However, it may be suggested, as a working hypothesis, that in some conditions a given sensory input may affect in a differential manner the information conveyed by the cell ensemble (see ref 30), depending on whether the routes utilized to monosynaptically activate the cells are subjected to a mechanism of presynaptic modulation. The functional consequences of such a differential modulation of information flow are rather interesting, and it is hoped that in the future better methods of analysis will be available to examine this possibility.
SUMMARY

1. In the unanesthetized spinal cord, conditioning stimulation of low-threshold afferents (below 1.3 times threshold strength) in the biceps semitendinosus (BST) nerve often reduced the peak amplitude of the monosynaptic Ia EPSPs evoked in gastrocnemius motoneurons without affecting the monosynaptic component of the EPSPs evoked by stimulation of the ipsilateral ventral funiculus (VF) in the thoracic cord.

2. Volleys to the BST nerve comprising higher threshold afferents (usually above 1.4 times threshold strength) reduced the peak amplitude of the monosynaptic Ia and VF EPSPs and shortened their falling phase.

3. Conditioning volleys to low-threshold cutaneous afferents often increased the Ia-EPSP peak amplitude, sometimes without affecting the monosynaptic component of the VF EPSP.

4. In most cases the Ia and VF monosynaptic EPSPs elicited in a given motoneuron summed nonlinearly. The amount of nonlinear summation between Ia and VF monosynaptic EPSPs was often reduced by low-threshold BST conditioning volleys. These observations suggest that in many instances, both species of fibers end in "electrotonically close" synaptic loci over the motoneuron surface. Therefore, amplitude changes of monosynaptic Ia EPSPs produced by conditioning afferent volleys without concomitant changes of monosynaptic VF EPSPs do not appear to result from postsynaptic remote conductance changes and may be attributed to a presynaptic mechanism.

5. At the time of occurrence of the Ia and VF monosynaptic EPSP the variance of the motoneuron membrane potential may be increased above base-line levels with a time course approximately matching the EPSP itself. Conditioning stimulation of BST afferents usually reduced Ia EPSP variance, often without affecting or even increasing the variance of the monosynaptic VF EPSPs. These observations provide additional evidence that Ia EPSP variability is introduced, at least in part, through the segmental pathways mediating primary afferent depolarization.

6. The possibility of a differential control of the information flow transmitted through two independent channels converging on a given cell ensemble is discussed.

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