Five Sources of a Dorsal Root Potential: Their Interactions and Origins in the Superficial Dorsal Horn

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Wall, Patrick D. and Malcolm Lidierth. Five sources of a dorsal root potential: their interactions and origins in the superficial dorsal horn. J. Neurophysiol. 78: 860–871, 1997. The dorsal root potential (DRP) was divided into six components (Lloyd 1952). A single shock to the myelinated fibers in the sural nerve produced a DRP on the L₆ dorsal root after the arrival in the cord of the afferent volley. The shape of this DRP was similar to that produced by dorsal root stimulation. Repetitive stimulation of the myelinated fibers in the gastrocnemius nerve also produced a prolonged negative DRP on the L₆ dorsal root. When a single stimulus (<5 μA; 200 μs) was applied through a microelectrode to the superficial Lissauer Tract (LT) at the border of the L₂ and L₃ spinal segments, a characteristic prolonged negative DRP (LT-DRP) began on the L₂ dorsal root after some 15 ms. Stimulation of the LT evoked DRPs bilaterally. Recordings on nearby dorsal roots showed this DRP to be unaccompanied by stimulation of afferent fibers in those roots. The LT-DRP was unaffected by neonatal capsaicin treatment that destroyed most unmyelinated fibers. Measurements of myelinated fiber terminal excitability to microstimulation showed that the LT-DRP was accompanied by primary afferent depolarization. Repetitive stimulation through a microelectrode in sensorimotor cortex provoked a prolonged and delayed negative DRP (recorded L₁–L₅). Stimulation in the cortical arm area and recording on cervical dorsal roots showed that the DRP was evoked more from motor areas than sensory areas of cortex. Interactions were observed between the LT-DRP and that evoked from the sural or gastrocnemius nerves or motor cortex. The LT-DRP was inhibited by preceding stimulation of the other three sources but LT stimulation did not inhibit DRPs evoked from sural or gastrocnemius nerves on the L₆ dorsal root or from motor cortex on the L₁ root. However, LT stimulation did inhibit the DRP evoked by a subsequent Lissauer tract stimulus. Recordings were made from superficial dorsal horn neurons. Coverage of input from LT sural, and gastrocnemius nerves and cortex was observed. Spike-triggered averaging was used to examine the relationship between the ongoing discharge of superficial dorsal horn neurons and the spontaneous DRP. The discharge of 81% of LT responsive cells was correlated with the DRP.

INTRODUCTION

Barron and Matthews (1938) showed that there was a prolonged depolarization of the central end of a dorsal root if an afferent volley arrived over a neighboring dorsal root. This was seen to be an important phenomenon because it showed that nearby afferent axons interacted with each other in the spinal cord and because a brief event in axons set off very prolonged changes in their neighbors. The dorsal root potential (DRP) was divided into six components (Lloyd 1952) with DRP V being a prolonged negative wave. Wall (1958) showed that this negative DRP was associated with depolarization of the afferent terminals (primary afferent depolarization, PAD). This in turn was associated with presynaptic inhibition attributed to blockade of impulse transmission by Howland et al. (1955) or to a decreased release of transmitter by Eccles (1964). The DRP is associated with negative-positive dorsal cord potential (DCP) recorded from an electrode on the dorsal surface of the cord relative to a nearby reference electrode (Willis and Coggeshall 1999). We report here on these potentials in the rat where the shapes but not the latencies are the same as those seen in the cat.

After the earlier work where the afferent volley was generated by stimulation of the dorsal root, the Eccles school, in particular, turned to stimulating individual peripheral nerves (reviewed in Schmidt 1971). Here, for convenience, we stimulated either the sural nerve or the nerve to gastrocnemius. A single shock to the A fibers in the mainly cutaneous nerve generates a large DRP in the neighboring dorsal roots. A single shock to the purely muscle nerve to the gastrocnemius generates only a weak DRP (Wall 1958), and we, like many others, used a brief repetitive volley to generate a clear DRP (Jankowska 1992). The mechanisms producing these two DRPs are presumed to be interrelated because they mutually inhibit each other and both partly depend on γ-aminobutyric acid (GABA) (Willis and Coggeshall 1991).

A further type of DRP studied was provoked by a single shock to the Lissauer tract. Such potentials have been recorded previously in the cat (Cervero et al. 1978; Wall and Yaksh 1978). In the rat, the Lissauer tract is an easily accessible broad band of fibers on the surface of the cord (Fig. 1). It contains small fibers of which 13% are myelinated (Chung and Coggeshall 1982), and there are propriospinal fibers originating from the substantia gelatinosa and projecting back into the substantia (Szentagothai 1964). It also contains branches of unmyelinated primary afferent fibers because a number of fibers disappear if nearby dorsal roots are cut (Chung and Coggeshall 1982; Chung et al. 1979) and some contain calcitonin gene-related peptide, which is regarded as a primary afferent marker (McNeill et al. 1988; Traub et al. 1990). However, in confirmation of an earlier study (Wall and Yaksh 1978), the potential described here...
will be shown not to involve stimulation of primary afferents. There was a particular reason to compare the Lissauer tract-evoked DRP with those evoked by primary afferent stimulation because there is a suspicion that the substantia gelatinosa may be involved in generating DRPs (Wall 1962).

Prolonged negative DRPs also may be generated in lumbar cord from various sites in the brain. The sensorimotor cortex has been shown to generate DRPs (Abdelmoumene et al. 1970; Andersen et al. 1962, 1964; Carpenter et al. 1963), and presynaptic afferent inhibition has been shown to occur in man after magnetic stimulation of the motor cortex (Iles 1996; Nielsen and Petersen 1994). Orbital cortex in cat, but not monkey, produces DRPs (Abdelmoumene et al. 1970), whereas in the cat under chloralose anesthesia, visual flash (Besson and Rivot 1973) and auditory click (Besson and Rivot 1972) stimuli generate lumbar DRPs. DRPs may be generated also by stimulating brain stem structures including the medullary reticular formation, locus coeruleus, raphé nuclei, and nearby reticular formation (Lundberg and Vyklicky 1966; Quevedo et al. 1995; Riddell et al. 1993). Here we examine the cortically evoked DRPs in the rat and compare them with those evoked from four other inputs: namely dorsal roots, sural and gastrocnemius nerves, and Lissauer tract.

The existence of DRPs has been well known for 50 yr, and the motivation for this paper is by no means simply to confirm the existence in the rat of phenomena clearly present in other species. The correlation of DRPs with the gating of afferent impulses has been studied extensively for 30 yr, but the unsolved problem remains that we do not understand with certainty the nature of the interneurons responsible for generating the DRPs (Jankowska 1992). The difficulty arises clearly from the fact that the inputs used to generate the DRPs obviously fire many types of cell, and it is not easily possible to identify which generate DRPs and which are interneurons in simultaneously active circuits with other functions. Here we compare DRPs generated by the five very different inputs and show their interactions to compare the response of interneurons during evoked DRPs or tonic DRPs (Wall 1995) or spontaneous variations of the DRP (Lidierth and Wall 1996). This paper supplements the previous work of many others in three ways: it compares DRPs produced from five sources, it examines their interactions, and it defines more precisely their sources in the Lissauer tract and cortex. The reason for wanting a precise comparison of latencies, shapes, and interactions of the DRPs from the five sources is that this data should be reflected in the firing properties of the interneurons responsible for generating the DRPs and so aid their identification. To justify this aim, we present here samples of the interneuron responses that show just such convergence of the five inputs and whose spontaneous firing is time-locked to spontaneous DRPs. We have obtained such recordings from large numbers of interneurons and will report details in papers now in preparation. Here, we give examples of dorsal horn interneurons activated by Lissauer tract stimulation that receive a convergent input from the other four sources and whose spontaneous activity is shown by spike-triggered averaging to be correlated to the spontaneous DRP. This paper therefore is intended to set the background on which the activity of interneurons can be compared with some of the observed properties of the DRPs.

METHODS

All experiments were carried out on male Sprague-Dawley rats weighing 150–300 g anesthetized with intraperitoneal urethan (1.25 g/kg). The general experimental method has been described elsewhere (Fig. 1) (see also Wall 1994; Wall and Bennett 1994). Briefly, the animal was held in a frame secured to the L1 spinous process and the pelvis with an extensive laminectomy from L1 to the cauda equina. The exposed cord was covered with warm paraf-
fin oil. One carotid and the trachea were cannulated, and the rectal temperature, expired carbon dioxide, ECG, and oil pool temperature monitored and observed to be within normal limits. On those occasions where particular mechanical stability was required, the animal was observed for ≥1 h to be anesthetized deeply and steadily and then was paralyzed with gallamine (20 mg ia) and artificially respired, keeping the expired CO₂ at 3–4%.

Stimulation

DORSAL ROOT. Dorsal roots were dissected free and cut at their exit from the dura. They were mounted on two silver hooks separated by 5 mm. The anode was on the cut end of the dorsal root. A single stimulus (±10 μA, 200 μs, 1 Hz) was sufficient to produce a maximal DRP.

SURAL NERVE. The sural nerve was dissected free in the popliteal fossa, cut in the periphery, and mounted on stimulating silver hooks separated by 5 mm. The stimulus (±10 μA, 200 μs, 1 Hz) was a single shock sufficient to produce a maximal DRP on the L6 dorsal root.

GASTROCNEMIUS NERVE. The lateral and medial nerves to gastrocnemius were dissected separately in the popliteal fossa, cut, and freed of connective tissue up to the sciatic nerve. They were mounted together on silver hooks separated by 3 mm. The stimulus strength was raised, while recording differentially on a filament of L₃ cut centrally, until an initial compound action potential of peak amplitude was recorded. The stimulus was raised to 8 μA, 200 μs; sufficient to produce a maximal myelinated fiber compound action potential with a conduction velocity of 20–60 m/s. No attempt was made to examine the effects of smaller stimuli. The DRP then was evoked by a train of three such stimuli separated by 2 ms.

LISSAUER TRACT. Stimuli were delivered through Merrill-Ainsworth (1972)-type glass-coated tungsten microelectrodes with 25-μm exposed tips. The electrode was placed in a micromanipulator on the surface of the Lissauer tract usually between L₂ and L₃, where the Lissauer tract is most easily accessible (Fig. 1). However, when the interaction between Lissauer tract evoked DRPs and those evoked by other stimuli were examined, the appropriate dorsal root was chosen (see below). Stimulation strength was ±5 μA, 200-μs single shocks at 1 Hz with the microelectrode tip negative with respect to a large electrode in nearby muscle.

CORTEX. A craniotomy was made over the relevant area, the dura was reflected, and the cortex covered with oil. A tungsten stimulating microelectrode was lowered into the cortex with a micromanipulator. Stimuli were trains of five pulses separated by 2.5 ms, each pulse of ±100 μA, 200 μs again with tip negative. Cortically evoked DRPs were recorded on dorsal root L₂–L₄.

Recording

DORSAL ROOT. For dorsal root potential recording, the selected root was placed on a pair of chloridized silver hook electrodes, one on the cut end of the root and the other 1 mm from the cord. Because these are prolonged potentials, the filters were set at 0.1 Hz low-cut. For action potential recording on the root, the proximal hook was moved along the root to a measured distance from the cord or fine filaments were dissected from the root. For these compound action potential or unit recordings, the filter setting was 100 Hz to 15 kHz. To decrease noise, eight recordings were averaged.

DORSAL CORD POTENTIAL. One electrode was placed on the dorsal midline of the cord in the same segment from which the dorsal root potential was recorded. The other recording electrode was placed on muscle immediately lateral to the cord electrode. Filters were set as for the DRP.

SINGLE UNIT RECORDING. Platinum-plated tungsten-in-glass microelectrodes were used to record the action potentials of superficial dorsal horn neurons via an AC coupled high-input impedance amplifier. The methods were those used previously in this laboratory (Wall 1994; Wall and Bennett 1994).

MEASUREMENT OF EXCITABILITY OF TERMINAL ARBORIZATIONS. These experiments used the method developed by Wall (1958). The aim of this experiment was to measure the excitability of terminal arborizations of myelinated afferents at various times after the generation of a volley either from a dorsal root or from the Lissauer tract. To stimulate the terminals of L₂ afferents, a glass-covered tungsten Merrill-Ainsworth microelectrode with a 25-μm exposed tip was lowered vertically into the dorsal horn of L₂. A stimulus through this microelectrode provoked an antidromic compound action potential recorded on the L₂ dorsal root placed on a pair of hooks. The location of the microelectrode and the strength of the stimulus (<5 μA; 200 μs, 1 Hz) were adjusted until a small stable compound action potential was recorded on the root and was well below the maximum. The latency, shape, and conduction velocity of this compound action potential were in the large A-fiber range. If the primary afferent axons became more excitable because they were depolarized (PAD), more axons were stimulated by the fixed intensity stimulus and the height of the compound action potential increased. The excitability of the L₂ afferent terminals was measured in this way at various times after the standard stimulus had been delivered either to the L₂ dorsal root or to the Lissauer tract between L₂ and L₃.

Neonatal capsaicin

To remove most of the unmyelinated C fibers from the afferent fibers and therefore from the Lissauer tract, capsaicin was administered to neonates (Jansco et al. 1977; Nagy et al. 1980; Wall et al. 1982). Two-day-old rats were anesthetized with halothane and given an intraperitoneal injection of 50 mg/kg capsaicin. The solution was 1.5% capsaicin dissolved in 10% ethyl alcohol and 10% Tween 80 in 0.9% saline. On recovery, the animals were returned to their mother. The procedure was repeated on neonatal day 4. These animals then grew up in the normal way for 10 wk, by which time, the males weighed 300 g and were used in acute experiments.

RESULTS

Dorsal root-evoked DRP and DCP

The dorsal root potential provoked by stimulating a neighboring dorsal root has been published previously (Wall 1994). It consists of a brief triphasic wave (DRPs I, II, and III of Lloyd 1952) associated with the arrival of the afferent volley. It is followed by the short DRP IV, during which the proximal electrode is positive. By 5 ms, the prolonged negative dorsal root potential (DRP V) begins (see Table 1). Simultaneous recording of the dorsal cord potential shows a brief negative potential followed by a more prolonged positive shift.

Sural nerve-evoked DRP and DCP

A single shock to the sural nerve generates a prolonged negative dorsal root potential on the L₆ dorsal root (Fig. 2A). It is similar in shape and duration to that generated in cat (Eccles 1964). The relatively long latency to onset (typically 11 ms, Table 1) includes the conduction time of the afferent volley and the period of the brief positive DRP.
TABLE 1. Latencies to onset, peak, and offset

<table>
<thead>
<tr>
<th>Source</th>
<th>Dorsal Root Potential, ms</th>
<th>Dorsal Cord Potential, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Onset</td>
<td>Peak</td>
</tr>
<tr>
<td>Dorsal root</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Sural nerve</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>Gastrocnemius nerve</td>
<td>14</td>
<td>39</td>
</tr>
<tr>
<td>Lissauer tract</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>Cortex</td>
<td>32</td>
<td>75</td>
</tr>
</tbody>
</table>

The latencies (ms) to onset, to peak, and to offset of the components of the dorsal root and dorsal cord potentials examined here and evoked by stimulation of a neighboring dorsal root, sural nerve, gastrocnemius nerve, Lissauer tract, or cerebral cortex.

(DRP IV of Lloyd 1952). The latency is also longer than that of the dorsal root-evoked DRP because the arriving afferent volley is less synchronized.

**Nerve to gastrocnemius**

A single shock to the nerve to gastrocnemius generates only a small and variable DRP as in the cat (Wall 1958). We therefore stimulated the nerve with a train of three shocks at 500 Hz as have others in the cat (Eccles 1964) to produce a clear DRP on the L₆ root (Fig. 2B). With these multiple stimuli, the onset of the negative DRP is delayed until 14 ms but the dorsal cord potential recorded simultaneously from the surface of the L₆ segment shows that impulses had arrived by 2 ms (Fig. 2B; Table 1).

**Lissauer tract**

When a stimulating microelectrode was placed on the surface of the Lissauer tract between roots L₂ and L₃ and 100 µm lateral to the dorsal root entry zone, a single shock of <5 µA provoked a delayed dorsal root potential (Figs. 2C and 4B). It began at 15.0 ± 1.9 ms (mean ± SD, n = 20) without any preceding positive wave. Its amplitude was about half that produced by stimulating the L₃ dorsal root.

With the stimulating electrode directly on the surface of the Lissauer tract, the threshold current for evoking a DRP was 1.5–2.0 µA. If the stimulus point was moved close to the root entry point, the same stimulus provoked the typical rapid DRP beginning at 4–5 ms. This DRP was preceded by a spike conducted on the recorded dorsal root, showing that afferents had been stimulated. If the stimulus was moved 200 µm lateral to the root entry zone, the 15-ms latency DRP was provoked as from 100 µm lateral. At 300 µm lateral, the threshold rose to 4 µA, and the onset latency was delayed to 20 ms. Further lateral movement onto the surface of the dorsolateral funiculus led to a further rise of threshold and a decrease in amplitude of the response. If the stimulating electrode was made to penetrate the cord perpendicular to the surface of the Lissauer tract from a point 100 µm lateral to the root entry zone, the threshold to provoke the delayed DRP rose at a depth of 20 µm. When the stimulating electrode reached a depth of 100 µm, it provoke a rapid compound action potential on the recording root presumably because it was within range of the penetrating myelinated afferent terminal arborizations of fibers originating from L₂.

In three animals, a search was made of the entire dorsal horn in a vertical and mediolateral grid at 100-µm intervals to find any areas from which the characteristic Lissauer tract potential (i.e., 15 ms onset latency with no antidromic compound action potential on the dorsal root) could be generated. Except for the Lissauer tract area, all stimulus locations that generated a dorsal root potential after stimuli ≤10 µA, 200 µs produced the rapid onset dorsal root potential (5–8 ms) and a conducted spike on the dorsal roots.
FIG. 3. Time course of changes in excitability of primary afferent terminals after stimulation of Lissauer tract (A) or a neighboring dorsal root (B). Graphs show amplitudes of submaximal antidromic volleys recorded on L2 dorsal root and evoked by microstimulation of primary afferent terminals in superficial dorsal horn at L2 at various intervals after a stimulus to Lissauer tract or L3 dorsal root. Amplitudes are expressed as a percentage of those seen in absence of conditioning stimulation.

In all the subsequent reported experiments, the stimulating microelectrode was placed on the surface of the Lissauer tract 100–200 μm lateral to the root entry zone with a stimulus <5 μA while the dorsal root potential was monitored continually and averaged to observe the late onset DRP and to ensure the absence of an earlier, primary afferent evoked component.

**Primary afferent depolarization provoked by Lissauer tract stimulation**

Wall (1958) had shown that the excitability of the terminal arbors of primary afferents, as measured by the height of an antidromic compound action potential provoked in dorsal horn and recorded on dorsal roots, increased with the same time course as the prolonged negative dorsal root potential that followed stimulation of the neighboring dorsal root. In the present experiments, the same change was examined in primary afferents after stimulation either of a neighboring dorsal root or after stimulation of the Lissauer tract. A stimulating microelectrode was lowered vertically 200 μm into the dorsal horn of L2 halfway between the midline and the root entry zone. The stimulus was adjusted (<5 μA) to provide a small stable antidromic compound action potential recorded on the cut L2 dorsal root. The average height of eight compound action potentials produced at 1 Hz was measured. Then either the dorsal root of L3 or the Lissauer tract between L2 and L3 was stimulated in the standard way. The excitability of the afferent terminals as measured by the height of the compound action potential then was measured at the time intervals shown in Fig. 3. It will be seen that the dorsal root stimulation was followed by an increased excitability of the afferents that had peaked by 15 ms. When the Lissauer tract was stimulated before the primary afferent terminals within the cord, there was also a rise of excitability, but the rise was slower than that observed after dorsal root stimulation. Because excitability was measured with 5-ms steps of interval between the conditioning and test stimuli, the peaks and latencies are accurate only to ±5 ms. It is evident that the increased excitability, which has been shown by other methods to be associated with primary afferent depolarization, is produced as expected by dorsal root stimulation and also, after a delay, by Lissauer tract stimulation. These comparisons were made in three animals.

**Did the Lissauer tract stimulation fire primary afferents?**

Because, as described in the introduction, the Lissauer tract contains fine primary afferent fibers, one might expect the stimulus applied to the Lissauer tract to stimulate axons of both known components in the Lissauer tract, namely the propriospinal fibers from the substantia gelatinosa cells and the primary afferents. If primary afferents were being excited by the Lissauer tract stimulus, then signs of an antidromic volley should be apparent on the nearby root. It will be remembered that in all of the experiments described above, the dorsal root potential was being monitored on the L2 dorsal root while the Lissauer tract was being stimulated between the L2 and L3 roots. The early phase of these recordings was examined repeatedly at high amplification with averaging of at least eight responses for signs of such antidromic volleys, and none were observed provided that the Lissauer tract stimulus was held <5 μA and the evoked DRP was of the delayed variety. To improve recording of conducted impulses on the L2 dorsal root, the proximal recording electrode was moved 1 cm distal to the cord. Further, to improve resolution, fine filaments were dissected free from the root and mounted on bipolar recording electrodes.
in five animals. Again, no signs were recorded of antidromic action potentials. If the Lissauer tract stimulus was raised >10 μA, clear signs of fast myelinated action potentials were observed that could have been due to stimulus spread to nearby dorsal roots or dorsal columns. Of course, such action potentials were associated with a rapid onset DRP. If the position of the microelectrode was deliberately moved off the Lissauer tract onto the root entry zone, clear recordings were obtained in both rapidly conducting myelinated fibers and in unmyelinated afferents conducting impulses at ≤1 m/s.

**Effect of neonatal capsaicin**

Because the Lissauer tract includes branches of unmyelinated fibers, it was of interest to stimulate the Lissauer tract in animals treated with capsaicin soon after birth because this removes most afferent C fibers (see Methods). Two animals were examined at the age of 10 wk. The Lissauer tract and the dorsal root L3 was stimulated in the standard way while recording the dorsal root potential on the L2 dorsal root. The shape of the two dorsal root potentials and the stimulus needed to provoke them could not be distinguished from the many recordings made in untreated animals. To check the effectiveness of the capsaicin in removing the C fibers, the sciatic nerve was stimulated maximally (5 mA; 200 ms; 1 Hz) while recording on the cut sural nerve (Wall et al. 1982). No signs were detected of the slowly traveling compound action potential characteristic of C fibers that can always be detected in intact animals. In a further control check that the capsaicin treatment had been effective, one hindfoot of a gently held unanesthetized animal was dipped into 49 ± 2°C water. In previous experiments (Gibson et al. 1982; Wall and Fitzgerald 1981), it had been shown in blinded experiments on 30 animals that the normal animal withdrawal time had a mean of 5 s, and the treated animals 9 s with a $P = 0.005$ significance for the difference. The two animals used here had withdrawal times of 9, 12, 11, 11, 10 s and 10, 13, 11, 11, 10 s in five successive trials.

**Bilateral effects of Lissaeur tract stimulation**

Barron and Matthews (1938) showed that dorsal root stimulation evoked DRPs bilaterally. Figure 4 shows that stimulation of the Lissaeur tract also evokes bilateral DRPs. The contralateral DRP exhibits an ~5 ms longer latency to both onset and peak. For comparison, Fig. 5 shows the ipsilateral and contralateral DRPs on the L2 dorsal roots after stimulation of the ipsilateral L2 dorsal root. The ipsilateral dorsal root was stimulated at 10 μA, 200 μs, 1 Hz, which was sufficient to generate a maximal DRP.

**Cortex**

In 16 animals, the cortex was stimulated in the region of the foot representation in the sensorimotor cortex while recording on a contralateral dorsal root (L2 or L4). The stimulating microelectrode, with its tip negative, was lowered into the cortex on 0.5 mm grid coordinates centered on the bregma at the midline. At no location was it possible to evoke a DRP by a single shock (<1 mA, 200 μs, 1 Hz), but small potentials could be measured after three shocks at 400 Hz. For reliable recording, a train of five shocks at 400 Hz was used routinely, and the threshold measured. Typical examples of the evoked DRP and the dorsal cord potential recorded on a neighboring segment are shown in Fig. 5A. The lowest threshold was found for each animal at a depth of 1.5 mm below the surface of the cortex with a stimulus strength of ~100 μA. As the electrode was lowered into the cortex, the threshold dropped as the 1.5 mm deep point was approached and rose again as the electrode was lowered further. This finding was taken as evidence for the intracortical locus of the area provoking the DRP. Further evidence is provided by the existence of a precise map of effective points in the cortex when the stimulus was moved horizontally (Fig. 6). These findings together suggest an intracortical origin rather than spread of the stimulus to subcortical structures.

The locations of the optimal points in the 16 animals with respect to the midline and bregma are shown in Fig. 6 where they are superimposed on a standard figure modified from Neafsey (1990). The points were distributed throughout the leg areas of both classical motor agranular cortex (MI) and classical sensory cortex (SI). However, microstimulation studies have shown that, in the leg area of the rat sensorimotor cortex, sensory and motor areas are superimposed (Neafsey 1990). In the arm area, this overlap of sensory and motor areas is incomplete (Donoghue and Wise 1982). To take advantage of this separation to assess whether stimulation of sensory or of motor areas of cortex was most effective at evoking DRPs, recordings were made from a cervical dorsal root, and the optimum area of cortex for provoking a DRP was determined in the same way as for the leg area. The optimum location is shown for six animals marked with stars in Fig. 6 for the contralateral arm area. In every case, the optimum site was in the motor area or close to the border between motor and sensory areas where motor responses are elicited easily by microstimulation (Donoghue and Wise 1982). Responses were not elicited with stimuli of <100 μA from the more lateral regions, which are more purely sensory.

Stimulation of the ipsilateral cortex in both the arm and leg areas also produced a DRP in the relevant root and the optimal point of stimulation was at the mirror location on the two sides. This bilateral effect is not surprising because stimulation on one side produced DRPs on both ipsilateral and contralateral roots (Fig. 4).

The route from the cortex to the lumbar DRP was examined in four animals. The lower thoracic cord at T12 was exposed and the dura reflected. The tips of sharpened jewelers’ forceps were placed on the dorsal columns extending from the left to the right root entry zones. A superficial crush across the entire width of the dorsal columns was made without effect on the cortically evoked DRP. The tips were marked, and the complete transverse lesion of the dorsal columns was extended gradually to a depth of 0.7 mm, which was similarly without effect. However, when the lesion was extended to a depth of 1.1 mm, there was a complete abolition of the cortically evoked DRP. Evidently the fibers responsible for triggering the DRP were running in the ventral third of the dorsal columns, i.e., the region in which the corticospinal tract is known to run in the rat (Casale et al. 1988).

**Interaction between dorsal root potentials of different origins**

Interactions between dorsal root potentials originating from different peripheral sources have been studied by others.
(reviewed in Schmidt 1971). Similarly, interactions between DRPs of cortical origin and of peripheral origin have been examined (Besson and Rivot 1973). We therefore concentrated here on the mutual interaction between the DRP of Lissauer tract origin and those of other origins, which has not been recorded by others.

DRPs reach maximum amplitude when only a fraction of the input is stimulated (Wall and Devor 1981). There is therefore a danger when studying interactions that one or both of the interacting inputs may be saturating the DRP-producing mechanism. To avoid this problem, we studied interactions when each input was producing only half-maximal DRPs so that both facilitation and inhibition could be observed. Specimen records are shown in Fig. 7 from three experiments where stimuli were delivered to the sural nerve, gastrocnemius nerve, or sensorimotor cortex (Fig. 7, A, E, and I) and to the Lissauer tract (Fig. 7, B, F, and J). Figure 7, C, G, and K, shows the potentials evoked when stimuli to the peripheral nerves or cortex were used to condition the response to a subsequent test stimulus to the Lissauer tract. Computer subtraction from these of the responses evoked by the conditioning stimulus alone provides the calculated Lissauer tract-evoked components illustrated in Fig. 7, D, H, and L. The Lissauer tract-evoked DRP clearly was reduced by preceding stimulation of the gastrocnemius or sural nerves and, in this case, abolished by preceding stimulation of the sensorimotor cortex.

Traces similar to those in Fig. 7 were produced for a range

![Fig. 4](image1.png)  
**FIG. 4.** Left: DRP was recorded on ipsilateral and contralateral L₂ dorsal roots after stimulation of ipsilateral L₃ dorsal root. Right: DRP recorded on both ipsilateral and contralateral L₂ dorsal roots while stimulating Lissauer tract in gap between L₂ and L₃.

![Fig. 5](image2.png)  
**FIG. 5.** Superimposed traces of L₄ dorsal root potential (*top*) and L₃/L₄ dorsal cord potential (*bottom*) recorded simultaneously and evoked by stimulation of cerebral sensorimotor cortex (*A*) with a train of 5 stimuli separated by 2.5 ms and commencing at time 0 and Lissauer tract (*B*) with a single shock at time 0.

![Fig. 6](image3.png)  
**FIG. 6.** Optimum locations for evoking DRPs by intracortical microstimulation in lumbar dorsal roots of 16 rats and cervical roots of 6 rats. Points are superimposed on a map of sensorimotor cortex modified from Neafsey (1990) showing arm and leg areas of primary sensory cortex. Effective points lie in motor cortex (M1) and in medial part of classical sensory cortex (S1), which is known to have a strong motor function in rat (Donoghue and Wise 1982).
FIG. 7. Effects of conditioning stimuli (▲) to sural nerve (A–D), gastrocnemius nerve (E–H), or cerebral cortex (I–L) on response evoked by a subsequent Lissauer tract stimulus (▲). Averaged responses to conditioning and test stimuli when delivered alone are shown as well as those when stimuli were presented together at intervals shown. Traces in D, H, and L were obtained by subtraction of responses to conditioning stimuli alone from those evoked by conditioned test stimuli. In A–C, arrows x, y, and z show amplitudes that were measured to generate plots such as those in Fig. 8.

of intervals between conditioning and test stimuli and the amplitudes of the responses (x–z in Fig. 7, A–C) were measured. Voltages were measured at the latency of the peak of the response to the test stimulus given alone, and the amplitude of the conditioned response (z) was expressed as a percentage of the sum of the responses to conditioning and test stimuli delivered alone (x + y). These are plotted in Fig. 8. As the latencies of the DRPs evoked from different sources are different (Table 1), the intervals indicated in Fig. 8 are the intervals between the peaks of the DRPs evoked by conditioning and test stimuli when delivered alone.

The DRP produced by the Lissauer tract stimulus clearly was inhibited for a period of 40 ms by preceding input from the sural nerve, gastrocnemius nerve, or cortex. However, when the reverse situation was examined, the DRP produced from the sural or gastrocnemius nerves or from the cortex was not affected by a preceding stimulus to the Lissauer tract. Nonetheless, when paired stimuli to the Lissauer tract were presented, the DRP evoked by the second of the pair was reduced in amplitude (Fig. 9).

These data present two apparent anomalies: Lissauer tract stimulation inhibits the DRPs evoked by subsequent Lissauer tract stimulation but not those evoked by primary afferent or cortical stimulation and the Lissauer tract depolarizes primary afferents and, presumably therefore, induces presynaptic inhibition of them but, from the plots of Fig. 8, appears not to inhibit the central action of those afferents in generating DRPs. We shall return to this in the DISCUSSION but for the moment will ask how postsynaptic, rather than presynaptic, effects may contribute to the nonreciprocal nature of the interactions between primary afferent inputs and the Lissauer tract evoked DRP. To this end, recordings were made from superficial dorsal horn neurons whose actions may contribute to the generation of DRPs.

**Dorsal horn interneurons**

The dorsal horn was searched with platinum plated tungsten microelectrodes while the Lissaeur tract was stimulated (<5 μA, 200 μs, 1 Hz) between L3 and L4 and the characteristically delayed evoked DRP was recorded on L3. Large numbers of responding cells were recorded in an area within 1 mm of the stimulating electrode and within 1 mm of the cord surface. This area included, but was not restricted to, laminae I–III. The cells responded with a repetitive burst beginning at an average of 4 ms (range 2–10 ms, n = 32) and ending at an average of 15 ms. Units were isolated easily so that at least two units with a spike height >100 μV commonly could be examined on each penetration. In separate experiments, we then examined Lissauer tract responsive cells for signs of convergence from one of the other inputs of interest. All of 20 units responding to sural nerve stimulation at A-fiber strength also responded to Lissauer tract stimulation. An additional 20 cells responded to stimulation of the nerve to gastrocnemius as well as to Lissauer tract stimulation. Eleven of 18 (69%) cells responding to stimulation of the sensorimotor cortex responded also to Lissauer tract. It is evident that substantial convergence of
the five inputs occurs on these cells. This convergence and the interactions of the inputs will be the subject of papers now being written.

Further experiments were undertaken to examine the relationship of the Lissauer tract responsive neurons to the DRP; the spontaneous DRP was spike-trigger averaged from the ongoing discharges of the cells. A total of 142 Lissauer tract responsive cells were recorded in the superficial dorsal horn of L3. Of these 115 (81%) were found to be correlated with the spontaneous DRP recorded on the L2 dorsal root. Example averages are shown in Fig. 10. The Lissauer tract responding cells include a subset of those dorsal horn interneurons that are related to PAD. The spontaneous activity of the cells and of the DRPs was inherent to the cord as they continued when the spinal cord was transected at L1 and when all dorsal roots were cut. A substantial group of cells that are excited by the convergent inputs that we have examined as sources of the DRP exist in the superficial dorsal horn. Furthermore, the spontaneous activity of these cells is time-locked with the appearance of spontaneous DRPs.

DISCUSSION

It is our intention here to bring together certain features of dorsal root potentials that one would expect to be reflected in the firing properties of interneurons responsible for generating those dorsal root potentials. We have not attempted an exhaustive study of the differences of the DRPs generated by stimulation of the five sites. For example, the five potentials may share different populations of afferent fibers or different parts of their terminal arborizations. We have concentrated on those aspects that show convergence and interaction. Similarly, it is not our intention at this stage to describe in detail a class of interneurons that we prove to be

![FIG. 8. Interaction between stimuli to Lissauer tract and to either nerve to gastrocnemius (A), sural nerve (B), or cerebral cortex (C). Amplitudes of evoked negative DRPs were measured and expressed as a percentage of control responses evoked without conditioning stimulation. Dashed line, effect of conditioning stimuli to nerves or cortex on DRP evoked by subsequent stimulation of Lissauer tract. Intervals on abscissa are intervals between peaks of DRPs evoked by conditioning and test stimuli delivered alone (see text). Solid line, effects of conditioning stimulation of Lissauer tract on responses evoked by stimulation of gastrocnemius or sural nerves and cortex.](http://jn.physiology.org/lookup/fig/8)

![FIG. 9. Plots similar to those in Fig. 8 but showing amplitude of Lissauer tract-evoked DRP after preceding stimulation of Lissauer tract in a single experiment. DRP was recorded on L2 root while stimulating at L2/L3 border. This plot shows a single example of conditioning effect of a Lissauer tract stimulus on DRP evoked by a subsequent test stimulus that was identical to conditioning stimulus.](http://jn.physiology.org/lookup/fig/9)

![FIG. 10. Average of spontaneous DRP recorded on L3 root when triggering from ongoing discharge of 2 neurons (A and B) in dorsal horn of L3. Both cells were synaptically excited by Lissauer tract stimulation as shown by poststimulus time histograms in insets. Lissauer tract-evoked DRP is superimposed on histograms. Scale bars in A apply also to B.](http://jn.physiology.org/lookup/fig/10)
the source of the dorsal root potential. We are well aware that the generator mechanism is likely to be an interconnected chain of cells. Furthermore, there is likely to be a detector mechanism, which reports the state of primary afferent depolarization, as well as a mechanism, which generates the depolarization. We limit our report here to the interneurons that are candidate cells for being involved in the DRP mechanism because they respond to all five inputs and because their spontaneous firing is time-locked to the spontaneous DRP. These two properties simply make the cells candidates; to pass the test, they will have to be subject to further examination of their response patterns during convergence and interaction of inputs that are followed by DRPs.

**Origin of the cortically evoked DRP**

This paper also shows that the potential of cortical origin is provoked preferentially from the motor cortex rather than sensory cortex. These cortically provoked potentials depended on the integrity of the spinal white matter containing the corticospinal tract. The cortex as a source of negative DRPs has been known since the work of Andersen et al. (1962, 1964) and has been studied in great detail along with other descending pathways (Quevedo et al. 1995; Rudomin et al. 1993). Previous work had not differentiated precisely which area of the sensorimotor cortex was responsible. Here, by using intracortical microelectrodes and by limiting the stimulus strength, it was clear that the source was indeed of cortical origin. Furthermore, the sensory and motor leg areas overlap so extensively in cat and rat that it is not possible to differentiate which area is responsible for generating the DRP. However, the rat motor arm area is located medial to the sensory area, and it was apparent here that stimulation of medial areas was more effective at evoking DRPs in the cervical dorsal roots. There are many direct and indirect routes from cortex to cord. It has been natural that most work has concentrated on pyramidal tract and its ventral terminations particularly on motor neurons. However, there are in fact widespread terminations of the pyramidal tract in both ventral and in dorsal horns including the most superficial laminae in monkey, cat, and rat (Casale et al. 1988; Cheema et al. 1984). These fibers could provide the anatomic substrate for the observation here that section of the pyramidal tract in the ventral part of the dorsal columns eliminated the cortically evoked DRP.

**Origin of the Lissauer tract-evoked DRP**

The characteristic long-latency DRP arising without stimulation of afferents could only be evoked in the rat by stimulation in the immediate vicinity of the Lissauer tract. This differs from the crucial observation of Rudomin et al. (1993) who provoked DRPs without any observed activation of afferents by microelectrode stimulation in the region of laminae III and IV in the cat. This experiment, and the work of others, before them led to the proposal that DRPs were generated by interneurons in that region (reviewed in Jankowska 1992; see also Jankowska and Riddell 1995). We therefore deliberately tried to repeat these experiments in the rat but failed. This may simply be because of the relative sizes of the rat and cat spinal cord so that our stimulus currents invariably spread to activate primary afferents. The observation here that the discharge of cells as deep as lamina III, and a few cells even deeper, are correlated to the DRP is entirely consistent with data from the cat (Jankowska and Riddell 1995; Rudomin et al. 1993).

The long latency of the Lissauer tract-evoked DRP naturally suggested that we might be stimulating unmyelinated afferent fibers that are known to exist in the Lissauer tract (Chung and Coggeshall 1982; Chung et al. 1979). We therefore repeatedly examined the nearby whole dorsal root (and filaments from that root) and failed to detect signs of a conducted antidromic volley in the afferents. The delayed DRP without activation of afferents was observed routinely with stimuli as low as 2 μA. If the stimulus was increased to ≥10 μA, activation of A fibers and a DRP with a latency of <5 ms was observed. Similarly, with increased stimulus strength or with movement of the stimulating electrode to the root entry zone, both A and C compound action potentials were recorded on the nearby root. The observation that the Lissauer tract-evoked action potential was indistinguishable from the normal in rats treated as neonates with capsaicin, a procedure known to eliminate most afferent C fibers (reviewed in Wall and Fitzgerald 1981), adds to the evidence that the delayed potential was not produced by stimulation of unmyelinated afferents.

In the cat, Cervero et al. (1978) made unilateral lesions of the dorsal columns and dorsolateral funiculus sparing the Lissauer tract and showed that these lesions reduced the DRP evoked on stimulation of a neighboring dorsal root. Subsequent lesions of the ipsilateral lateral Lissauer tract had no further effect on the amplitude of the evoked DRP. Given the observation here that the Lissauer tract-evoked DRP, like the dorsal root-evoked DRP (Barron and Matthews 1938), occurs bilaterally, the observations of Cervero et al. (1978) now may be explained readily. First, the Lissauer tract is the lateral extension of a fiber tract, which extends across the surface of the dorsal horn immediately ventral to the dorsal columns (Wall and Yaksh 1978). Dorsal column lesions may be expected to disrupt the more medial parts of this tract and so may be expected to reduce its actions and therefore the size of the evoked DRP. Subsequent to a unilateral dorsal column lesion, Lissauer tract-evoked responses will be mediated by the fibers remaining on the side of the lesion but also by the entire, uninterrupted, tract contralaterally. This may explain why Cervero et al. (1978) found that completing the lesion ipsilaterally had little further effect on intersegmentally evoked DRPs. It may be noted here, that Cervero et al. (1978) reported an increase in latency of the intersegmental DRP after ipsilateral dorsal column lesions; an observation that is in keeping with these responses being mediated via the contralateral Lissauer tract.

In the work of Cervero et al. (1979) where the Lissauer tract was stimulated, conduction velocity was in the range of small myelinated axons (4.6–18.3 m/s). The most likely source of the potential is stimulation of myelinated fibers known to run in the Lissauer tract (Chung and Coggeshall 1982).

**Nonreciprocity of interactions**

The five inputs studied here share certain features apart from provoking a prolonged negative DRP. All five are
partly dependent on a GABA mechanism because the DRPs are reduced by the antagonists picrotoxin or bicuculline; as shown for peripheral inputs (Eccles et al. 1963; Rudomin et al. 1993), cortex (Benoist et al. 1972), and Lissauer tract (Thompson and Wall 1996). All five inputs interact with each other as has been shown for peripheral inputs (reviewed in Schmidt 1971) and for cortical inputs (Besson and Rivot 1972; Rudomin et al. 1993) and for the Lissauer tract here. However, unlike the other four inputs, the effects of the Lissauer tract on the DRPs were found here not to be reciprocal; the Lissauer tract-evoked potential was inhibited by preceding stimuli to the other inputs but Lissauer tract stimulation did not inhibit the DRPs evoked from the sural nerve, gastrocnemius nerve, or cerebral cortex. As noted above, this presents an apparent anomaly as Lissauer tract stimulation evokes presynaptic inhibition of primary afferents but does not appear to inhibit their ability to evoke DRPs. As noted in results, the stimuli to each input were adjusted to evoke a half-maximal DRP; stronger stimulation of the Lissauer tract therefore might have evoked inhibition of the other inputs if it had been used. Nevertheless, the present data show that such inhibition, if it exists, is much weaker than that evoked by stimulation of the sural or gastrocnemius nerves or cortex.

Interneurons

For two reasons, we chose to concentrate on cells responding when the Lissauer tract was stimulated and the characteristic long-latency DRP was generated. First, previous work had suggested that the substantia gelatinosa and Lissauer tract were involved in DRP generation (Wall 1962). More importantly, the effect of the Lissauer tract stimulus is limited to the superficial dorsal horn, whereas the other four inputs examined here produce widespread excitation, including activation of motoneurons.

There are large numbers of such cells excited by convergent inputs from dorsal root, sural nerve, nerve to gastrocnemius cortex and, in addition, one class is excited by the Lissauer tract. Furthermore, spike-triggered averaging of the DRP from the spontaneous activity of the cells shows that the firing of these cells is precisely time-locked to the appearance of a spontaneous DRP (Lidierth and Wall 1996).

The existence of these cells is very clear, but it is not at all clear how the five inputs gain access to the cells, how these two classes of cells interact and how they relate to the membrane potential of primary afferents. It will now be necessary to examine the response of these cells during the simultaneous convergence of various inputs that we now know interact in a nonreciprocal fashion. Analysis of the time-course of these responses and of simultaneously recorded members of these and other types of interneurons may allow us to propose a circuit diagram of how these cells stand in the DRP generating mechanism.

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