Dorsal Horn Cells Connected to the Lissauer Tract and Their Relation to the Dorsal Root Potential in the Rat

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Lidierth, Malcolm and Patrick D. Wall. Dorsal horn cells connected to the Lissauer tract and their relation to the dorsal root potential in the rat. J. Neurophysiol. 80: 667–679, 1998. We have examined the role of dorsal horn cells that respond to Lissauer tract stimulation in regulating primary afferent depolarization (PAD). PAD was monitored by recording the dorsal root potential (DRP) in the roots of the lumbar cord. Recordings were made of the discharges of Lissauer tract-responsive cells, and their discharges were correlated with the DRPs occurring spontaneously and those evoked by stimulation. Electrical microstimulation of the Lissauer tract (<10 μA; 200 μs) was used to activate the tract selectively and evoke a characteristic long-latency DRP. Cells that were excited by Lissauer tract stimulation were found in the superficial laminae of the dorsal horn. They exhibited low rates of ongoing discharge and responded to Lissauer tract stimulation typically with a burst of impulses with a latency to onset of 5.6 ± 2.7 ms (mean ± SD) and to termination of 13.6 ± 4.1 ms (n = 105). Lissauer tract-responsive cells in L5 were shown to receive convergent inputs from cutaneous and muscle afferents as they responded to stimulation of the sural nerve (100%, n = 19) and the nerve to gastrocnemius (95%, n = 19). The latency of the response to sural nerve stimulation was 3.7 ± 1.5 ms and to gastrocnemius nerve stimulation, 8.3 ± 3.6 ms. Stimulation through a microelectrode at a depth of 1.5 mm in the sensorimotor cortex (100 μA, 200 μs) evoked a response in 17 of 31 Lissauer tract-responsive cells (55%) with a latency to onset of 21.9 ± 2.8 ms (n = 17). Stimulation of the sural nerve, nerve to gastrocnemius or sensorimotor cortex was shown to depress the response of Lissauer tract-responsive cells to a subsequent Lissauer tract stimulus. The ongoing discharges of Lissauer tract-responsive cells were correlated to the spontaneous DRP using spike-triggered averaging. Of 123 cells analyzed in this way, 117 (95%) were shown to be correlated to the DRP. In addition, the peaks of spontaneous negative DRPs in spinally transected animals were detected in software. Perievent time histograms triggered from these peaks showed the discharge of Lissauer tract-responsive cells to be correlated to the spontaneous DRPs in 57 of 62 cells (92%) recorded. We conclude that these data provide compelling evidence that the Lissauer tract, and the dorsal horn cells that it excites, mediate the PAD evoked from multiple neural pathways.

INTRODUCTION

Barron and Matthews (1938) showed that there was a prolonged depolarization of the central end of a dorsal root after the arrival of an afferent volley over a neighboring dorsal root. It was shown later that this dorsal root potential (DRP) was produced by depolarization of the terminals of primary afferents (Wall 1958) and was associated with presynaptic inhibition produced either by blockade of impulse transmission (Howland et al. 1955) or by a decrease of transmitter release (Eccles 1964).

It has been difficult to identify the spinal neurons responsible for inducing the prolonged negative DRP for the simple reason that dorsal root or peripheral nerve stimulation excites many cells in the spinal cord, and it is highly unlikely that they all contribute to DRP generation. The major search for cells responsible for the DRP has concentrated on the DRP provoked by stimulation of large diameter muscle afferents but this evokes a very weak potential (Wall 1958). This work began with Eccles and co-workers (Eccles 1963) and has been extended with beautiful single-unit correlations (Jankowska and Riddell 1995; Rudomin et al. 1987). However, very few candidate cells have been identified, and these lie in lamina III and deeper. In the past, we concentrated on the very large DRP evoked by a single stimulus to large cutaneous afferents (Wall and Devor 1981). A source-sink analysis of the location of activity during this DRP showed activity concentrated in laminae I and II (Wall 1962). Furthermore, the spread of the disturbance from one segment to another was shown to involve the Lissauer tract (Wall 1962). For these reasons, we decided to search the superficial laminae as well as the deeper parts of the dorsal horn for cells the firing of which was correlated with the DRP.

We needed to correlate the firing of cells with the DRP in circumstances that did not necessarily involve stimulating afferent fibers or descending systems as these obviously produce widespread postsynaptic activity in cells that may or may not be involved in the DRP-generating mechanism. We chose two examples. The first was the ongoing generation of DRPs that occurs spontaneously in the absence of stimulation. These DRPs occur in spinal preparations (Lidierth and Wall 1996) and therefore do not depend on descending pathways for their generation. They also continue when all dorsal roots are sectioned and, therefore, do not depend on afferent input to the cord (Lidierth and Wall 1996). The second example was the DRP produced by low-strength electrical stimulation of the lateral Lissauer tract. This DRP has been described elsewhere both in cats (Cervero et al. 1979; Wall and Yaksh 1978) and rats (Wall and Lidierth 1997). A stimulus of <5 μA, 200 μs applied through a microelectrode to the Lissauer tract evokes a large negative DRP in neighboring dorsal roots with a latency to onset of 15 ms, to peak at 30 ms, and with overall duration in excess of 80 ms. Such small stimuli produce no activity in either myelinated or
unmyelinated afferent fibers or in ventral roots and are effective in evoking DRPs in intact or spinal animals (Wall and Lidierth 1997).

Elsewhere we presented preliminary evidence that a group of cells exists in the upper laminae of the dorsal horn that can be excited by the Lissauer tract and also by the sural nerve, nerve to gastrocnemius, and sensorimotor cortex (Wall and Lidierth 1997). We now provide a detailed description of these cells and we show that both the stimulated and spontaneous activity of these cells is closely correlated with the generation of a DRP. Together these data make these cells strong candidates to mediate primary afferent depolarization (PAD).

**METHODS**

Experiments were carried out on 23 male Sprague-Dawley rats (weighing 150–300 g) anesthetized with intraperitoneal urethan (1.25 g/kg). The experimental methods recently have been described in full elsewhere (Wall and Lidierth 1997). Briefly, the animal was held in a frame secured to the L1 spinous process and the pelvis. A laminectomy exposed the cord from L1 to the cauda equina, and the cord was covered with warm paraffin oil. A carotid artery and the trachea were cannulated, and the rectal temperature, expired carbon dioxide, electrocardiogram, and oil pool temperature were monitored and observed to be within normal limits. Animals were observed for ≥1 h to ensure deep and steady anesthesia and then were paralyzed with gallamine (20 mg ia) and artificially respired keeping the expired CO2 at 3–4%.

For DRP recording, the selected root was placed on a pair of chloridized silver hook electrodes. The filters were set at 0.1-Hz low-cut and, minimally, 150-Hz high-cut. Platinum-plated tungsten-in-glass microelectrodes were used to record the action potentials of superficial dorsal horn neurons and these were electronically discriminated (see Fig. 1A).

**Stimulus protocols**

The stimulus protocols followed those of Wall and Lidierth (1997). Stimuli to the Lissauer tract were delivered through glass-coated tungsten microelectrodes. 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. However, when the interaction between Lissauer tract evoked DRPs and those evoked by other stimuli was examined the appropriate dorsal root was chosen. 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.

The sural nerve was dissected free, cut, and mounted on stimulating silver hooks. The stimulus (≤10 µA, 200 µs, 1 Hz) was a single shock sufficient to produce a maximal DRP on the L6 dorsal root. 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 and stimulated with a train of three stimuli separated by 2

![Figure 1](https://example.com/figure1.png)
ms and of 8-μA strength and 200-μs duration. Trains were delivered once per second. For cortical stimulation, a craniotomy was made over the relevant area. 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 with the trains being delivered once per second.

**Histology**

The depth of penetration and distance from the dorsal root entry zone were noted for each recording point. The distance between the dorsal root entry zones on each side of the cord was noted and used to correct for shrinkage of the tissue during histological processing. At the end of the experiment, the animal was killed by anesthetic overdose, and a fine sharpened tungsten wire was inserted into one of the electrode tracks and left in situ while the cord was fixed by flooding with 10% formalin in saline. The cord was removed and postfixed in 10% formalin before a 1-mm-thick section containing the electrode tracks was cut by hand using a razor blade. The section then was dehydrated in a series of alcohols and cleared with methyl salicylate. The cleared section was drawn in a camera lucida, and the recording grid was superimposed on the drawing aligned with the surface, the root entry zone, and the marker wire (cf. Wall 1967).

**Data analysis**

Data were digitized on-line using SPIKE2 or Spike2 for Windows software (Cambridge Electronic Design, England). For the DRP, the digitization rate was minimally 1 kilosample/s with data prefiltered at 150 Hz high cut (−3 dB). DRPs subsequently were filtered digitally with a minimally 100-Hz high cut. Poststimulus time histograms and spike-triggered averages of the DRPs were computed with Spike2 for Windows.

The peaks in spike triggered averages of the DRP were measured automatically in software. The baseline mean level of the DRP and its standard deviation were measured over an 80-ms-long time window beginning 160 ms before the spike trigger. If a peak in the DRP exceeding the mean by ≥1 SD was detected within ±80 ms of the spike trigger, the average was presumed to show a positive correlation. The time to the onset and to peak of the averaged DRP then were measured with the onset being defined as the first negative-going inflection point before the peak.

To correlate the ongoing discharge of dorsal horn neurons with the spontaneous negative DRPs, the peaks of these DRPs were located using the Spike2 for Windows MemImport function. This function searches for a local maximum. When one is found, a subsequent minimum is searched for, and if the voltage difference between these exceeds a given threshold, the time of the local maximum is recorded as a peak (see Fig. 8A). These times then were used to trigger an average of the DRP and a perievent time histogram of the recorded dorsal horn neuron. The onset of the rising phase of the negative DRP in the averages was detected as the first negative-going inflection point preceding the peak. Peri-event time histograms were constructed with 1-ms binwidths. For further analysis, the histograms were smoothed using a 15-point Gaussian window. The baseline mean and standard deviation of the bins in a 125-ms window beginning 250 ms before the trigger were calculated. Peaks within ±125 ms of the trigger were deemed significant if they exceeded the baseline mean by ≥2 SD, and their onset was defined as the first bin to exceed the baseline mean by one standard deviation.

**RESULTS**

The stimulating electrode was placed on the lateral Lissauer tract between roots L2 and L3. The electrode tip initially was placed 100-μm lateral to the dorsal root entry zone, and its position was adjusted to produce an optimal long-latency DRP. Such long-latency DRPs were shown earlier to result only from stimulation of the Lissauer tract and not from stimulation of primary afferents lying dorsal to the stimulus or of projection fibers lying ventral to it (Wall and Lidierth 1997). The long-latency Lissauer tract evoked DRP typically begins 15 ms after the stimulus, peaks at 30 ms, and has a duration exceeding 80 ms. Its amplitude is about half that of the DRP produced by maximal A-fiber stimulation of the neighboring L3 dorsal root.

**Cells responding to the Lissauer tract stimulus**

The cord was searched for cells responding to the Lissauer tract stimulus in a regular grid of vertically penetrating electrode tracks (Fig. 1A). The search began at the root entry zone and proceeded to a depth of 1 mm in 10-μm steps. Parallel search tracks then were made 100-μm apart medial and lateral to the root entry zone until no further responsive cells could be located. The search grid extended across the cord at a rostrocaudal distance of no more than 1 mm from the stimulating electrode.

Responding cells were plentiful in the superficial layers. Any search track entering at the root entry zone would isolate at least two to three units with spike amplitudes of >100 μV in the first 200 μm of penetration. Examples of single sweep recordings of four such cells are shown in Fig. 1B. Poststimulus time histograms of the responses of two single cells to 30 stimuli and their relation to the evoked DRP are shown in Fig. 2. Responding cells had low rates of ongoing activity (0.2–2 Hz) in the absence of stimulation. Most such cells had receptive fields in the expected dermatome of L3-L4, and responded to touch and pressure on the rostral part of the upper leg. We did not carry out detailed analyses of the receptive fields. The cells responded to stimulation of the Lissauer tract with a burst of impulses beginning at 2–18 ms (5.6 ± 2.7 ms, mean ± SD; n = 105) and ending at 8–30 ms (13.6 ± 4.1; n = 105).

All the initial spikes in the burst had a small variation in latency (~1 ms) between successive stimuli. We observed no cells with a time-invariant latency of a type to be expected if the response was produced by the arrival of an antidromic action potential following stimulation of the Lissauer tract.

**Locations of cells responding to Lissauer tract stimulation**

We carried out a complete and detailed search grid in four animals in the L3 segment and isolated 52 responding units. For each recorded unit, the position at which the maximum spike height occurred was determined and the mediolateral position relative to the root entry zone was recorded. The position for each cell is shown in Fig. 1C on a composite transverse section of the L3 cord constructed by averaging the sections obtained from the four animals. The units are concentrated in the lateral two-thirds of superficial dorsal horn with some outliers in dorsal and dorsolateral white matter and in deep grey.

The Lissauer tract-responsive units were interspersed with units that failed to respond to Lissauer tract stimulation. Given the low strength of microstimulation used here, we
can not say with confidence that these cells received no synaptic input from the Lissauer tract and shall therefore deal with them no further.

Convergence from three other sources onto cells that respond to Lissauer tract stimulation

To examine convergence of input from Lissauer tract stimulation and stimulation of the sural nerve and nerve to gastrocnemius we moved the unit recording area to the L₄ or L₅ segment and recorded the DRP from a neighboring dorsal root. The Lissauer tract was then stimulated between L₃ and L₅ segments or, when blood vessels obscured the tract at this level, between L₃ and L₄. The stimulus parameters, evoked DRPs and cell responses to Lissauer tract stimulation were similar to those reported in the previous sections.

SURAL NERVE. The sural nerve was stimulated with single pulses of sufficient intensity to produce a maximal negative DRP (≤10 μA, 200 μs, 1 Hz). Single units were recorded in the dorsal laminae of L₅ and responded in the characteristic way to stimulation of the nearby Lissauer tract. Stimuli then were alternated between the sural nerve and Lissauer tract. All of 19 recorded cells that responded to the Lissauer tract stimulus responded also to stimulation of the sural nerve (Fig. 3A). The latency of the response to the sural nerve was 3.7 ± 1.5 ms (range 2–9 ms, n = 19), and duration of the burst was 11.8 ± 3.9 ms (range 5–19 ms, n = 19).

NERVE TO GASTROCNEMIUS. A single stimulus to the gastrocnemius nerve produces only a weak DRP, and we therefore used a train of three stimuli separated by 2 ms at strength of 8 μA, 200 μs, 1 Hz. This was sufficient to produce a maximal negative DRP. Of 19 cells that responded to the Lissauer tract, 18 responded also to stimulation of the gastrocnemius nerve. The latency of the response was 8.3 ± 3.6 ms (range 4–16 ms, n = 18), and the duration of the burst was 24.9 ± 8.5 ms (range 11–39 ms, n = 18). An example is given in Fig. 3B.

SENSORIMOTOR CORTEX. As shown previously (Wall and Lidierth 1997), stimuli through a microelectrode 1.5-mm below the surface of the cortex at an optimal point in the hindleg area of the somatosensory cortex produce a negative DRP in lumbar roots. For each animal, a search grid was made of the contralateral cortex to locate a stimulus point at which five stimuli separated by 2.5 ms with strength of 100 μA, 200 μs produced a substantial negative DRP on the recorded root. Of 31 cells that responded to stimulation of the Lissauer tract, 17 (55%) responded also to stimulation of the cortex. The latency of the response was 21.9 ± 2.8 ms (range 19–30 ms, n = 17). An example is shown in Fig. 3C.

Interaction between stimuli

When electrical stimuli are delivered to the sural nerve, nerve to gastrocnemius, or sensory cortex, the DRP evoked by a subsequent Lissauer tract stimulus delivered within 40 ms is greatly depressed (Wall and Lidierth 1997). This depression often is followed by a rebound in which Lissauer tract stimuli delivered 80–100 ms after the conditioning stimulus evoke a DRP that is larger than in the control. We examined the effects of conditioning stimuli to these structures on the discharges evoked in superficial dorsal horn neurons in response to subsequent Lissauer tract stimulation. A similar depression of the Lissauer tract-evoked neuronal discharge was observed for each of the sural nerve, nerve to gastrocnemius, and cerebral cortex, and specimen records for the nerve to gastrocnemius are illustrated in Fig. 4. In Fig. 4A, a stimulus was delivered alone to the Lissauer tract, whereas in Fig. 4, B–F, the stimulus was preceded by a train of stimuli to the gastrocnemius nerve. The nerve stimulus itself evoked a response as seen in Fig. 4, B–D (although in Fig. 4B, it overlaps with the Lissauer tract evoked response). There is an evident depression of the Lissauer tract-evoked discharge with conditioning stimulation between 20 and 60 ms before the Lissauer tract stimulus and in Fig. 4, E and F, evidence of a facilitated response. The effect of conditioning stimulation of the nerve to gastrocnemius on this single Lissauer tract-responsive neuron to a subsequent Lissauer tract stimulus thus mimicked that on the DRP in both its direction and its time course.

Correlation of ongoing DRPs with ongoing activity of cells activated by the Lissauer tract

Up to this point we have shown that provoked activity of these cells is associated with the DRP evoked by stimulation.
LISSAUER TRACT CELLS

Figure 3. Responses of 3 Lissauer tract-responsive neurons to stimulation of the sural nerve (A), the nerve to gastrocnemius (B), and the sensorimotor cortex (C) are shown as post-stimulus time histograms. Insets: responses of each cell to microstimulation of the Lissauer tract. All traces were formed from 30 successive stimuli.

We simultaneously recorded the ongoing spike activity of superficial dorsal horn cells that previously had been shown to respond to Lissauer tract stimulation and the DRP on a neighboring dorsal root. In the first instance, the spontaneous DRP was correlated to the ongoing discharge of the neuron using spike-triggered averaging. For this purpose, the DRP was digitally low-pass filtered with a cut-off of 100 Hz. Figure 5 illustrates some typical results from a spinoally transected rat (Fig. 5, A and B) and a rat with an intact spinal cord (Fig. 5, C and D). The solid vertical line superimposed on each average represents the time of the trigger spike. In each illustrated average, the spike is associated with a subsequent negative (upward) peak in the DRP. The dotted...
A total of 62 Lissauer tract-responsive cells were analyzed in this way from animals with a spinal transection at T_{11}/T_{12}. The ongoing discharge of 60 (97%) of the cells were found to be positively correlated to the DRP using the criteria given above. A further 61 cells were recorded in animals with intact spinal cords, and 57 (93%) of these exhibited a positive correlation to the DRP. The averages were robust and reproducible and required relatively few trigger spikes to achieve stability (~200–300).

For most cells, the onset of the negative DRP in the spike-triggered average preceded the spike trigger. The frequency distribution of the time to onset relative to the spike is illustrated in Fig. 6. In spinally transected animals, the onset of the DRP preceded the spike by 14.6 ± 2.6 (SE) ms (n = 62; range 93 to –8 ms), whereas in spinally intact animals, the figure was 14.5 ± 4.6 ms (n = 57; range 126–46). These means are not significantly different. Nonetheless, the frequency distributions illustrated in Fig. 6 are significantly different (Kolmogorov-Smirnov test, P < 0.001).

The synchronicity of the spontaneous DRPs across the entire lumbar cord, which has been reported elsewhere (Lidierth and Wall 1996), suggests that dorsal horn neurons regulating the DRP might also exhibit a high degree of synchrony both to each other and to the DRP. To examine this further, the times of the peaks of spontaneous negative DRPs were detected and cross-correlations be-
FIG. 5. Spike-triggered averages of the ongoing DRP. A and B: Lissauer tract-responsive neuron was recorded in a rat with the spinal cord transected at T11/T12. C and D: spinal cord was intact. Vertical solid lines in A–D show the position of the spike-trigger used to form the averages. Baseline mean ± 1 SD were measured as described in METHODS and are indicated by the horizontal dotted lines. Times of onset and peak of each averaged DRP are indicated in each case by the vertical dotted lines. For each trace, n indicates the number of spikes used as triggers.

FIG. 6. Frequency distribution histograms showing the time to onset of the spike triggered averaged DRP for all cells. A: data from rats with intact spinal cords. B: data from rats in which the spinal cord was transected at T11/T12.
tween these events and the ongoing discharges of the recorded single units were calculated as described in Methods. Figure 7 illustrates the process for one cell (which also was used for Fig. 5B) recorded in a spinally transected rat. In Fig. 7A, the middle trace shows the spontaneously oscillating DRP and arrows above mark the peaks of spontaneously occurring negative DRPs with an amplitude exceeding 5 μV. The spike train below shows the time of occurrence of spikes in the isolated single cell. The times of the peaks of the spontaneous negative DRPs were used to trigger an average of the DRP (Fig. 7B) and a perievent time histogram of the ongoing discharge in the isolated cell (Fig. 7C). As expected, the averaged DRP showed a prominent central negative peak and, owing to the oscillatory character of the DRP, this was flanked by further peaks separated by ~100 ms. Examination of the perievent time histogram in Fig. 7C shows that the recorded cell also exhibited a prominent peak and that this preceded the spontaneous negative DRP. Further inspection reveals that additional increases in the ongoing discharge of the cell preceded the smaller negative peaks in the DRP that occurred at ±100 ms in Fig. 7B. This is illustrated further in Fig. 8, which shows example correlograms for four cells and the DRP averages. Each set of traces is from a different animal all with spinal cords transected at T11/T12.

All 62 Lissauer tract-responsive cells that were recorded in spinal preparations were analyzed in this way. The time to onset of the negative DRP was taken as the point of inflection preceding a standard peak negativity of 10-μV minimum amplitude. Histograms were smoothed with a Gaussian window. A peak in the smoothed perievent time histogram was judged significant if it exceeded the baseline mean by ≥2 SD. The onset of the increased spike discharge was taken as the first point at which the baseline was exceeded by 1 SD (for details see Methods).

For 57 (92%) of the cells, the perievent time histogram exhibited a significant peak. The cells began to fire at a significantly higher rate than their baseline, on average by 4.6 ± 2.1 (SE) ms before the onset of the negative-going phase of the DRP. The firing rates of the cells then increased and reached their maxima on average 10.9 ± 1.8

**FIG. 7.** Temporal correlation of the discharge of a Lissauer tract-responsive neuron and the spontaneous oscillation of the DRP in a spinally transected rat. A: DRP together with the timing of its peak negativities as detected in software (see Methods). Relatively infrequent ongoing spikes in a simultaneously recorded Lissauer tract-responsive neuron were electronically discriminated as shown below. B: detected peak negativities have been used to trigger a waveform average of the DRP. Average shows, as expected, a prominent central negative peak and several smaller peaks occurring at multiples of approximately ±100 ms from the trigger point. C: detected peaks of the DRP have been used to trigger a perievent time histogram of the simultaneously recorded neuronal discharge. This also shows a prominent central peak but this is phase advanced relative to the DRP. Similarly phase advanced increases in neuronal activity were associated with the secondary negativities in the averaged DRP.
FIG. 8. Further examples of the averaged DRP and the peri-event time histograms triggered from the spontaneous negative DRPs as in Fig. 7. Each of the 4 specimen traces in A–D was taken from a different animal. Oscillatory character of the DRP is clear in each average. Each peri-event time histogram exhibits a prominent peak that phase-leads the negative DRP. In addition, secondary increases in neuronal discharge are associated with, and phase-lead, the secondary peaks in the DRP.

ms into the rising phase of the spontaneous negative DRP. The distribution of the times of onset and peak of the increased firing rates is shown in Fig. 9 for all cells.

DISCUSSION

We describe here a type of superficial dorsal horn cell that responds with a brief burst to single stimuli to the Lissauer tract. The Lissauer tract contains, other than primary afferents, fine fibers of which 13% are myelinated (Chung and Coggeshall 1982) and many of which are propriospinal fibers originating from the substantia gelatinosa and projecting back into the substantia (Szentagothai 1964). We observed no cells responding with the fixed latency characteristic of antidromic invasion. We therefore assume that we were recording neurons that had been transsynaptically excited by impulses arriving over propriospinal fibers in the Lissauer tract. The absence of antidromic spikes is not unduly surprising; we stimulated only a small fraction of the Lissauer tract, first because the stimulus was spatially restricted and second because low strength stimuli were used and are likely to have activated mainly the larger diameter fibers within the Lissauer tract. The likelihood of encountering
an antidromically activated neuron was therefore much less than that of locating a neuron synaptically activated by the diverging Lissauer tract fibers.

Origins of the responses

It is highly likely that some of the recorded units were dendrites or axons (Wall 1965) as some spikes were encountered on the surface of the cord where no cell bodies are found. Given the distribution of recorded units (Fig. 1C), it is probable that they originated mainly from units with cell bodies in the superficial dorsal horn.

Sural nerve. Large myelinated afferents in the sural nerve are able to produce a large negative DRP (Schmidt 1971; Wall and Devor 1981) and, as we show here, they are also capable of stimulating the units responding to the Lissauer tract. Transport studies with β-subunit of cholera toxin (Mannion et al. 1996) show myelinated cutaneous afferents terminating deep to the border of lamina II within lamina III. Those cells in more superficial laminae that were shown here to respond to sural nerve stimulation were presumably excited indirectly by such afferents. Such indirect activation was observed earlier among cells of the marginal zone (McMahon and Wall 1989).

Nerve to gastrocnemius. It has been shown repeatedly that large myelinated fibers originating from muscle may, on repetitive stimulation, produce a DRP (reviewed in Jankowska 1992; Rudomin et al. 1993). We show here that the majority of Lissauer tract-responding units also respond to stimulation of the nerve to gastrocnemius at a strength sufficient to produce a DRP. Although the bulk of these afferents terminate in deeper laminae, it is also clear that some terminate in lamina I (LaMotte 1977; Mense and Craig 1988).

Cortex. Stimulation of the cortex was shown to produce a DRP by workers in the Eccles school (Andersen et al. 1962), and its effects have been reported by many others (reviewed in Wall and Lidierth 1997). We showed in the rat that the DRP was produced by the motor component of the sensorimotor cortex and that the effect reached the lumbar cord by way of the dorsal column corticospinal tract (Nielsen et al. 1995). Seventeen of the 31 cells (55%) that we examined for convergent input from the Lissauer tract and sensorimotor cortex responded to both stimuli. The majority of pyramidal axons terminate in deeper laminae (Casale and Light 1991). However, transport studies reveal a substantial projection to laminae I, II, and III (Casale et al. 1988; Cheema et al. 1984).

Relationship to the DRP

Wall (1962) provided evidence for the involvement of the superficial dorsal horn in the generation of DRPs and for the involvement of the Lissauer tract in the intersegmental propagation of that disturbance. In the present study, we have examined the hypothesis that the Lissauer tract, and the superficial dorsal horn neurons to which it connects, mediate the DRPs evoked from multiple neural sources. We have established that Lissauer tract-responsive neurons respond also to input from the periphery and do so in a temporal pattern that is consistent with them contributing to the generation of the peripherally evoked DRP. Both cutaneous afferents in the sural nerve and muscle afferents in the nerve to gastrocnemius were shown to converge onto these neurons. In addition, we have examined the effect of cortical stimulation and similarly found that corticospinal input converged onto these cells and, again, that the cells discharged in advance of the onset of the cortically evoked DRP.

We have shown elsewhere for the rat, that these four inputs to the DRP-generating mechanism interact (Wall and Lidierth 1997). The DRP evoked by Lissauer tract stimulation was reduced for 60–80 ms after conditioning stimulation to sural or gastrocnemius nerves or cerebral cortex, and this inhibitory period often was followed by a period of rebound facilitation. The effects of such conditioning stimuli on the Lissauer tract-evoked discharge of dorsal horn neurons are shown here to follow an identical pattern, in both sign and timing.

We have taken thus far an essentially classical approach to establishing the Lissauer tract-responsive neurons as candidates for participating in the generation of the DRP. These observations establish the neurons as candidates and no more. Each of these inputs has widespread actions in the cord and the presence of convergence alone can not be regarded as a sound basis on which to assign the neurons a DRP-generating role. However, the observation that the lumbar spinal cord contains intrinsic mechanisms that also generate DRPs (Lidierth and Wall 1996) has afforded an important further opportunity to establish the role of these neurons.

In the anesthetized rat with an intact spinal cord, the DRP generating mechanism is tonically active and large spontaneous negative DRPs occur irregularly. Following Rudomin et al. (1987) and Jankowska and Riddell (1995), we have used spike-triggered averaging methods to examine whether the ongoing discharge of these neurons is correlated to the spontaneous fluctuations in the DRP. The vast majority (93%) of recorded neurons were associated with a significant spike-trigger averaged DRP. The temporal relationship between the spike and the onset of the averaged negative DRP was variable but for most neurons the onset of the most prominent negative DRP preceded the spike trigger, and it peaked after the spike trigger in all but four cells. However, instead of displaying a single identifiable negativity, the DRP averaged had a complex form. This undoubtedly complicates interpretation of these records. In Fig. 5, C and D, the most prominent central negativities are flanked by earlier and later positive-going phases. Although the latency to onset has been measured to the first negative-going inflection on the rising phase of the DRP, this point rarely occurs on a steady baseline potential. Thus while the inflection may well be a point at which a negative-going drive to the DRP begins, it could equally well represent a point at which a positive-going (or less-negative) drive is removed. Owing to this, spike-triggered averages such as those in Fig. 5 cannot provide precise information about the latency of the effect of a single neuron on the DRP.

When the spinal cord is transected at the 11th or 12th thoracic level, the DRP is released to oscillate at ~10 Hz. The temporal relationship between the spike and the onset of the negative-going phases of these complexes was little different to that in animals with intact cords and was subject to the same uncertainties regarding its interpretation.
Rudomin et al. (1987) also reported that positive phases preceded the negative DRPs “quite frequently” in spike-triggered averages from neurons in the intermediate grey. They concluded that these arose because of common excitatory inputs to afferents and interneurons. Synchronization of firing of interneurons is most likely in spinally transected animals where large oscillations in the DRP occur simultaneously on all dorsal roots of the lumbar spinal cord. This synchrony makes interpretation of the spike-triggered averages difficult and makes it impossible to define the temporal relationship between the neuronal discharges and their effects on the DRP. We therefore took a second approach to this correlation; we used the oscillations produced by this synchrony to provide a trigger with which we could perform the reverse correlation by averaging the neuronal discharges in relation to the peaks of the spontaneous negative DRPs. This DRP-triggered histogram method selects only DRPs above a specified amplitude and therefore reveals correlations between a neuron’s discharge and large-amplitude DRPs. Large DRPs are most likely to occur when a population of many neurons is recruited near-synchronously. Therefore DRP-triggered histograms may reveal a neuron’s membership of such a population and may do so even when relatively few among a train of recorded spikes are related in time to the trigger event.

We averaged the neuronal discharges in relation to the peaks of the spontaneous negative DRPs for 62 Lissauer tract-responsive neurons recorded in spinally transected animals. In these preparations, the peaks could be detected reliably because of the large size of spontaneous negative DRPs. With this type of analysis, the temporal correlation between the neuronal discharges and the DRP becomes clear. The shapes of the perievent time histograms in Fig. 8 closely follow the complex shapes of the averaged DRPs but are phase-advanced relative to them. As shown in Fig. 9, the majority of neurons exhibited an increase in activity 5–20 ms before the onset of the negative-going DRP and peaked during its rising phase. This is consistent with these cells contributing to the generation of the characteristically delayed DRP evoked by electrical microstimulation of the Lissauer tract and with them being recruited to contribute to the generation of the spontaneous oscillations of the DRP seen in the absence of stimulation.

**Comparison with other studies**

PAD-generating neurons have been described in other studies. In the cat, they have been described in deeper parts of the dorsal horn than in the present study and in the intermediate gray (Jankowska and Riddell 1995; Rudomin et al. 1987). However, these earlier authors recorded selectively from the intermediate grey (Rudomin et al. 1987) or from neurons with input from group II muscle afferents (Jankowska and Riddell 1995). The present data from the rat are in no way inconsistent with these earlier observations as we sought deliberately to locate neurons with input from the Lissauer tract and these, as shown above, are more superficially located.

Jankowska and Riddell (1995) searched specifically for neurons with input from group II muscle afferents and used spike-triggered averaging of the DRP to demonstrate a DRP-generating role for these cells. These authors achieved stable baselines in the spike-triggered averages and could tentatively but convincingly classify the cells as first- or last-order interneurons from the latency to onset of the averaged negative DRPs. The absence of preceding positivities in the averages may indicate that these neurons display less synchrony than those described here. It is interesting, however, that Jankowska and Riddell (1995) did find that DRPs evoked by microstimulation in the region of these interneurons were preceded by positive-going changes in the DRP.
It is likely that the cells described here and those described by Jankowska and Riddell (1995) are components of separate DRP-regulating circuits, and it is therefore all the more striking that the two groups of cells share several common properties. For both groups, relatively few spikes were required to produce a robust spike-triggered average, which indicates that their effects on primary afferents must be potent. Also, both groups receive convergent input from several classes of primary afferent and both receive descending input from the sensorimotor cortex (Jankowska and Riddell 1998).

Like Rudomin et al. (1987), we found the negative DRPs in the spike-triggered averages often to be preceded by a relatively positive phase. These authors ascribed this to common input impinging on both the afferents and the isolated interneuron and pointed out that synchronicity would be a requirement for the cells effectively to gate the actions of many afferent fibers simultaneously. These authors recorded selectively from the intermediate gray. It remains to be determined whether the common inputs they discussed provide input that is common also to the more superficially located neurons that we describe here.

In summary, we have examined the hypothesis that superficial dorsal horn neurons receiving excitatory synaptic input from the Lissauer tract mediate the PAD evoked from four neural inputs. Each of these inputs evokes a DRP, and all are shown here to evoke discharges in the Lissauer tract-responsive neurons that precede the onset of the DRPs. Furthermore, we have shown that these inputs interact in an identical manner in their effects on both the DRP and the neuronal discharges. Critically, we have shown that the ongoing discharges of these neurons are temporally correlated to the DRPs that occur spontaneously in the absence of applied stimuli. Putting these facts together, we propose that these cells are components of a mechanism for generating DRPs. We can not assign individual cells a specific location in the network responsible for generating DRPs because of the uncertainties of measuring the latency of their effects in spike-triggered averages. It could be that many of these cells are reporting the activity of other cells in the network that produces the DRP. Nevertheless, the fact that Lissauer tract stimulation evokes a DRP, and that neurons responding to that stimulus are restricted to the superficial dorsal horn, makes it reasonable to assume that last-order interneurons in the DRP-generating circuit were included in the present sample.

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