INTRASPINAL LATENCY, CUTANEOUS FIBER COMPOSITION, AND AFFERENT CONTROL OF THE DORSAL ROOT REFLEX IN CAT

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INTRODUCTION

The dorsal root reflex (DRR) is a special manifestation of primary afferent interaction which can be used to further examine and extend our understanding of cutaneous fiber interactions derived from studies of the dorsal root potential (DRP) and intracellular recording of primary afferent depolarization (PAD). The presynaptic interactions of mammalian cutaneous afferents, for example, are thought by some[8,9] to be mediated by a group of spinal interneurons, thus accounting, in part, for the observed 3–4 msec intraspinal delay of the DRP[1,16] and DRR[7,27,28]. This hypothesis is supported by intra-axonal and intra- and extracellular recordings showing appropriately timed interneuronal discharges and primary afferent depolarization[8,9]. However, others have suggested, on the basis of morphological[23] and physiological data[2,29] that more direct interaction among afferent fibers might underlie some components of the DRR and DRP. We wished to obtain an independent estimate of the intraspinal delay for the DRR. Since the DRR is composed of propagated action potentials, it is possible to collide orthodromic and antidromic activity within the spinal cord and provide another estimate of the delay interposed between primary afferent interactions.

The DRR can also be used to reveal types of interacting fibers. Recent estimates of the average conduction velocities of fibers composing the cutaneous DRR have ranged from 14.2 m/sec in the phalanger[18] to 50 m/sec in the cat[3]. Toennies[27], on the basis of collision experiments in the cat, concluded that both large (A-alpha) and small diameter (A-delta) myelinated fibers were included in the reflex discharge. As regards the afferent limb of this reflex, there is general agreement that a full DRR can be elicited by stimulation of large myelinated fibers. Jänig and Zimmermann[12] have reported occasional long-latency single fiber DRRs following the addition of C fibers to an A fiber volley, but we wished to determine the efficacy of input volleys strictly limited to either A-delta or C fibers.

Additional details of the organization of the DRR should be revealed by studying the action of the different spinal afferents influencing DRR excitability. Pro-

longed depression of the DRR, for example, has been observed after DRR activation by single volleys as well as following tetanus of spinal afferents[9,13]. The latter phenomenon has been attributed to the post-tetanic hyperpolarization of the stimulated terminals[8,9,13]. On the other hand, an interneuronally mediated presynaptic inhibitory system has been considered responsible for the depressed excitability of the dorsal root potential (DRP) evoked by a single cutaneous volley[5]. We therefore wished to determine the effective inputs for modulation of DRR excitability and whether these afferents also excite the DRR mechanism.

In this report, we describe experiments in which collision and anodal polarization techniques have been used to determine the minimum intraspinal interaction delay of the DRR and the conduction velocity of fibers eliciting and composing the DRR in cat cutaneous afferents. We have also investigated the effect of single volleys, repetitive electrical, and natural somatic stimuli on the excitability of the DRR as recorded and elicited from both stimulated and unstimulated nerves and roots. The conclusions derived from these experiments are diagrammatically summarized in a model.

METHODS

Eighteen cats were anesthetized with pentobarbital (35–40 mg/kg); additional anesthetic was administered intravenously as needed to depress reflex responses to strong somatic stimulation. In 6 cats, surgical exposure of the spinal cord was avoided, only the cutaneous nerves (the sural and the cutaneous branch of the superficial peroneal) being exposed for stimulation and recording in a warm mineral oil or silicone (Dow Corning 200 fluid) bath. The remaining cats all underwent unilateral ventral rhizotomy (L_{4-5} to S_{3-5}) under a dissection microscope. The exposed spinal cord was kept under an oil bath warmed by radiant heat. Dorsal root filaments were identified and mounted on electrodes for stimulation and recording. Rectal temperature was kept between 35 and 38.5 °C by means of a heating pad. An infra-red CO_2 analyzer was used to monitor the expired CO_2 in some of these preparations.

Stimulating and recording electrodes were made of chlorided silver wire; for experiments employing anodal polarization block, the nerve rested on Ag–AgCl troughs in a manner previously described[4]. Glass or wooden probes were used for natural somatic stimulation. Electrical stimuli and polarizing currents were delivered via isolated constant current stimulators and the responses were recorded on photographic film or paper; additional observations and measurements were recorded with the use of a storage oscilloscope and camera. Amplifier filters were set to attenuate (— 3 dB) frequencies above 10,000 c/sec and below 300 c/sec or 0.2 c/sec.

In order to quantify changes in DRR excitability, the intensity of electrical stimulation was reduced so that the efferent reflex discharge consisted of a cluster of spikes from several nerve fibers. With the amplifier filters set to attenuate input frequencies below 300 c/sec an amplitude discriminator and counter[26] could be used to register the total number of spike discharges exceeding a fixed amplitude. This allows quantification of the response although it provides no information about the behavior

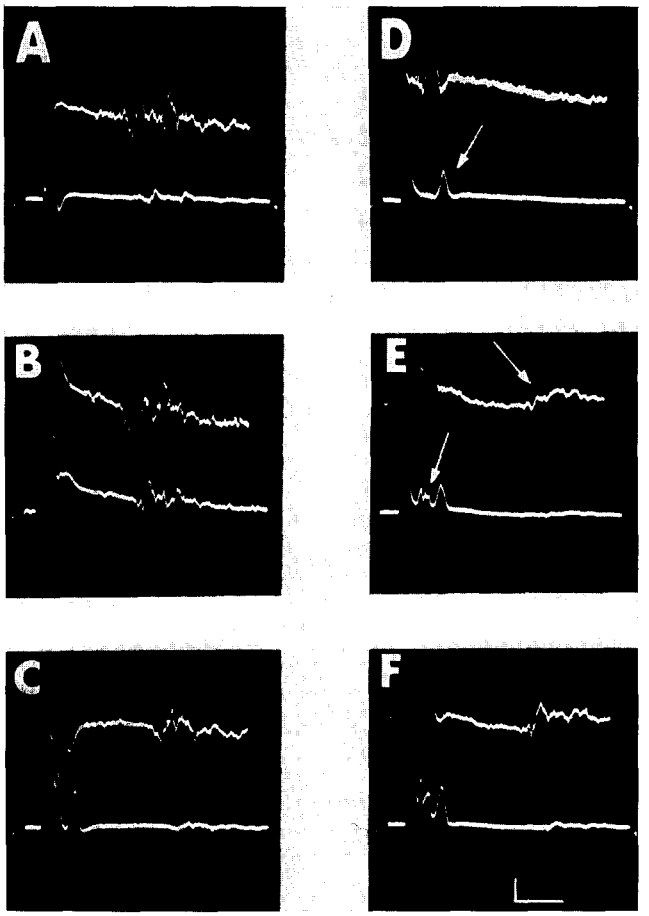

Fig. 1. Role of small fibers in eliciting DRR. Both traces show ingoing volley and returning DRR. Stimulation (0.2 msec duration pulse) and recording (proximal bipolar) from superficial peroneal nerve. Lower trace at reduced amplification and shifted slightly to right. Calibration lines in F are 5 msec and 100 μV upper, 350 μV lower, except 200 μV, lower in B. A, DRR elicited by pure submaximal A-alpha wave. B, DRR elicited by A-alpha and delta volley. C–F, Decline (C), elimination and recovery (E–F) of DRR with anodal polarization block of alpha fibers. Note the delta fibers are still conducting (arrow in D) but no DRR is present. In E–F a few A-alpha fibers (lower arrow in E) elicit a DRR (upper arrow in E) during recovery from polarization. The polarizing electrode (anode proximal) was between the distal stimulating electrode and the recording electrode.

of any single fiber. In constructing excitability curves, samples of poststimulus DRR activity were recorded at regular intervals following repeated trials of the conditioning stimulus.

RESULTS

General observations

Our observations on the DRR could be made without cooling or surgical exposure of the spinal cord. A DRR was seen in all preparations with rectal temperature

of 35–38.5 °C and with an expired CO_2 of 3–5%, but was not recorded at higher temperatures or lowered levels of expired CO_2. No DRR could be recorded from afferents with relatively high levels of ongoing activity, usually associated with recent surgical exposure or section of the nerve or rootlet. After such activity had subsided, however, the DRR could be observed.

Size of cutaneous fibers participating in the DRR

Afferent limb of the DRR. The effective afferent limb of the DRR is largely limited to fibers within the A-alpha beta (hereafter called 'A-alpha') group. No reflex antidromic discharge could be attributed to stimulation of the smaller diameter A-delta or C fibers. The DRR is fully developed before A-delta fibers are added to the orthodromic volley. However, this does not exclude the possibility that the A-alpha volley in some way suppresses or obscures the influence of smaller diameter fibers. Accordingly, anodal polarization was used in 8 experiments to block conduction in the A-alpha portion of the orthodromic volley so that an isolated A-delta volley could be delivered. Both sural and cutaneous superficial peroneal nerves were tested. In order that the recorded antidromic discharge not pass through the polarized region, the DRR was recorded proximal to the polarizing and stimulating electrodes (Fig. 1) or from the cut end of a separate, unstimulated nerve. Under both conditions, an A-delta volley alone failed to evoke any antidromic discharges which could be detected even at high gain on the recording amplifier. As anodal polarization is removed, the DRR resumes when A-alpha activity appears in the orthodromic volley (Fig. 1E–F).

In 6 experiments, the effectiveness of unmyelinated fiber volleys was tested by adding C fibers to the orthodromic volley and by delivering a C fiber volley following anodal polarization block of conduction in myelinated afferents. No antidromic response to C fiber stimulation could be detected at high amplifier gains. The possibility remains that such activity could not be detected because of dispersion in both the afferent and efferent volleys.

Efferent limb of the DRR. Toennies'[27] experiments provided evidence that both A-alpha and -delta cutaneous fibers participate in the antidromic DRR discharge in the cat. Megirian[18], however, found that the average conduction velocity of the antidromic discharge in the phalanger was 14.2 m/sec, and Brooks and Koizumi[2] concluded that fibers conducting above 30 m/sec comprise both the afferent and efferent limbs of the DRR in cat. Collision of an orthodromic test volley with the DRR (Fig. 2) indicates that the initial part of the cat DRR is composed principally of A-alpha and the latter part of A-delta fiber activity. The results, then, indicate a non-reciprocal interaction between A-alpha and A-delta fibers, the latter apparently playing a passive role, carrying but not initiating the DRR.

Intraspinal latency of the DRR

In order to obtain additional information about the intraspinal connections in the

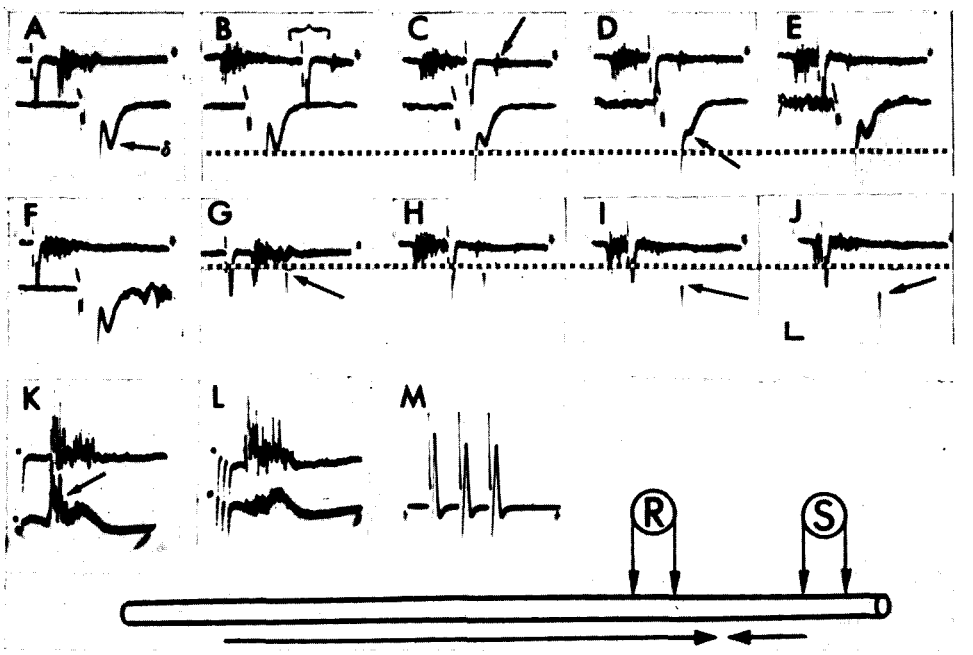

Fig. 2. Fiber composition of the DRR. A–J, All records from sural nerve. The bottom trace is × 5 expansion of that portion (see bracket in B) of the upper trace containing the compound action potential elicited by sural stimulation at S in the diagram. A, Control DRR to sural stimulation at S; delta input volley indicated. B–F, Sural DRR elicited by an A-alpha volley on the superficial peroneal nerve at varying intervals prior to stimulation of the sural at S. In D, for example, the descending DRR swept through the ascending sural volley and reduced the delta wave as recorded at R. The sural input volley elicited at S produces only a small DRR (arrow in C) because the DRR mechanism is refractory following the prior pure alpha volley on the superficial peroneal nerve. (This is additional evidence that A-delta fibers are unimportant in producing a DDR since only the sural volley included a maximal A-delta component.) G–J, The arrows indicate reduction in the A-alpha wave following collision of the DRR with some of the A-alpha fibers in the sural input volley. K and L, Stimulation of and recording from superficial peroneal nerve (lower trace), simultaneous recording from sural (upper trace). Three A-alpha volleys on the peroneal (M, 500/sec) block by collision (L) the A-alpha component of the peroneal DRR (arrow in K). Calibration lines in J: A–J, upper, 5 msec and 20 μV; lower, 1 msec and 20 μV except 100 μV G–J. K and L, 5 msec and 50 μV. M, 1 msec and 2 mV.

DRR pathway, a collision technique was used to estimate the minimum time delay between afferent and efferent impulses at the point of efferent discharge initiation within the spinal cord. In this method, the afferent volley evoking the DRR is followed by a second volley timed to collide with the reflex discharge within the spinal cord. Collision occurs if the sum of the inter-volley interval, plus the refractory period of the blocking impulse exceeds the intraspinal reflex latency; the shortest volley interval at which collision block fails provides an estimate of minimum intraspinal DRR latency. The results of the 10 collision experiments are best presented following a brief consideration of the major factors influencing their interpretations.

(1) The efficacy of the blocking volley clearly depends on volley size and com-

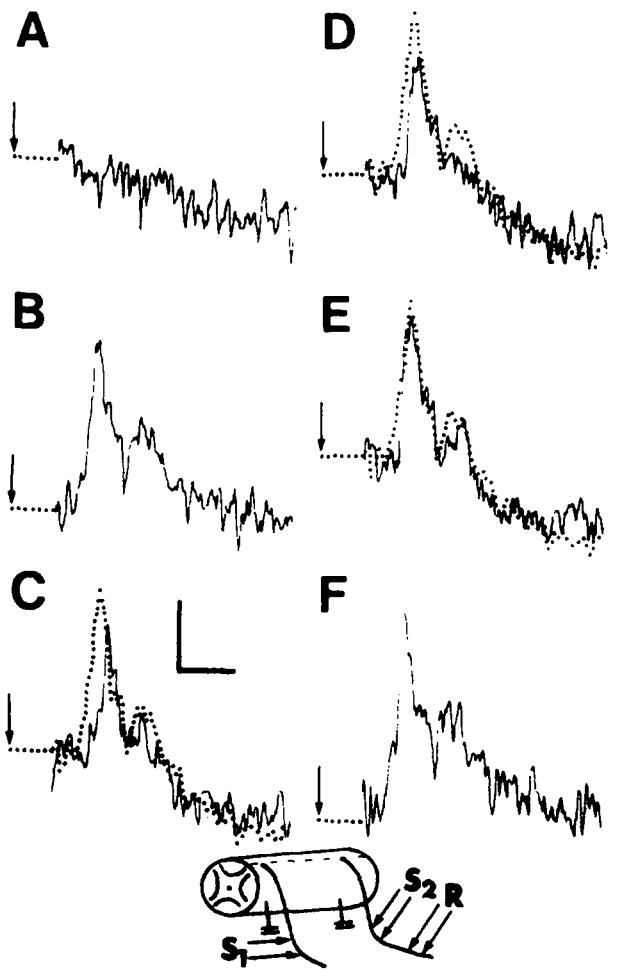

Fig. 3. Escape from DRR block at short inter-volley intervals. Volley eliciting DRR, delivered via one L_7 rootlet (S_1 in insert diagram), is followed by a blocking volley (S_2) in an adjacent L_7 rootlet used for monopolar DRR recording. B–F, Thirty averaged sweeps of DRR responses to S_1 delivered 1 msec prior to arrows. Calibration: 100 μV and 2 msec. B and F, Control DRRs to S_1 alone before (B) and after (F) the blocking experiments in C–E. Note (A) that the blocking volley (S_2) alone does not elicit a DRR but does attenuate the reflex due to S_1 when delivered 2.0 msec (C) and 1.0 msec (D) later. Nearly all DRR components escape this block when the inter-volley interval is 0.5 msec (E). Dotted lines in C–E show, for comparison, average unblocked DRR as the algebraic sum of B and F.

position. In these experiments, A-delta fibers were not included in the blocking volley so that there is no collision with the A-delta part of the reflex.

(2) Failure of complete collision block of A-alpha fibers may reflect the existence of multiple intraspinal circuits or may be due to the repetitive firing of single fibers in the efferent response to the initial or blocking volley[30]. Thus, as shown in Fig. 2K and M, two blocking volleys were required to effectively block the A-alpha DRR component. However, because accurate latency measurements required the use of a single blocking volley, detection of block is based on a consistent reduction in ampli-

tude of the earliest DRR components rather than on complete elimination of the reflex discharge.

(3) Since interaction occurs between fibers of different diameter, the conduction velocity differences could lead to errors in the estimate of intraspinal DRR latency. Conduction velocity differences have been minimized, in these experiments, by stimulating and recording from dorsal root filaments.

(4) The intraspinal DRR latency, here defined as the delay interposed between orthodromic and antidromic activity, includes both conduction and junctional (*e.g.*, synaptic) delays between interacting fibers. An additional delay may occur between the onset of post-junctional effects and the initiation of the reflex spike. Furthermore, persistence of post-junctional effects, as in the case of prolonged depolarization maintained by transmitter action, may be sufficient to initiate a reflex spike soon after the passage of the blocking impulse, thus allowing an early escape from block during a relatively refractory period. Both of these post-junctional factors, the delay in spike generation and persistence of depolarizing action, could lead to an overestimate of DRR latency as based on the minimal inter-volley interval producing collision block.

Fig. 3 shows the experimental arrangement and sample records of an experiment in which the DRR was evoked by stimulation of an adjacent rootlet while the blocking volley was produced by stimulation of the rootlet used for recording. In 4 of these experiments, monopolar recording was used and the blocking volley was below threshold for evoking a DRR so that only the response to adjacent rootlet stimulation was recorded. The blocking effect is less obvious than when a larger blocking volley is employed, but is clearly revealed with the use of a response averaging device (Computer of Average Transients, model 400). The results (Fig. 3) of each of these experiments show that collision occurs with inter-volley intervals of 1.0 msec, but there is little or no blocking at 0.5 msec intervals. With the larger blocking volleys used in 6 experiments there was evidence of collision at volley intervals of only 0.4 msec, but this effect was obscured by the DRR elicited by the blocking volley itself. For all 10 experiments, the estimated minimal inter-volley interval ranged from 1.0 to 0.4 msec with an average of 0.5 msec.

Occasionally, it is possible to record an early reflex response in one or a few fibers as reported by Van Harreveld and Niechaj[29]. These short-latency reflexes follow much higher rates of stimulation (up to 100/sec) than the longer-latency major reflex responses which are typically greatly attenuated at stimulus rates of 5/sec. Attempts to estimate the intraspinal latency of the short-latency discharges were unsuccessful because the orthodromic blocking volley obscured the responses.

Afferent control of DRR excitability

In order to simplify presentation and discussion, it will be useful to introduce and define some terminology. If depression of DRR excitability could be caused by changes restricted to the afferents used to produce the depression (*i.e.* the conditioned fibers), the effect will be designated as *restricted*. The post-tetanic hyperpolarization of afferent terminals[9] is an example of such an effect. DRR excitability changes might

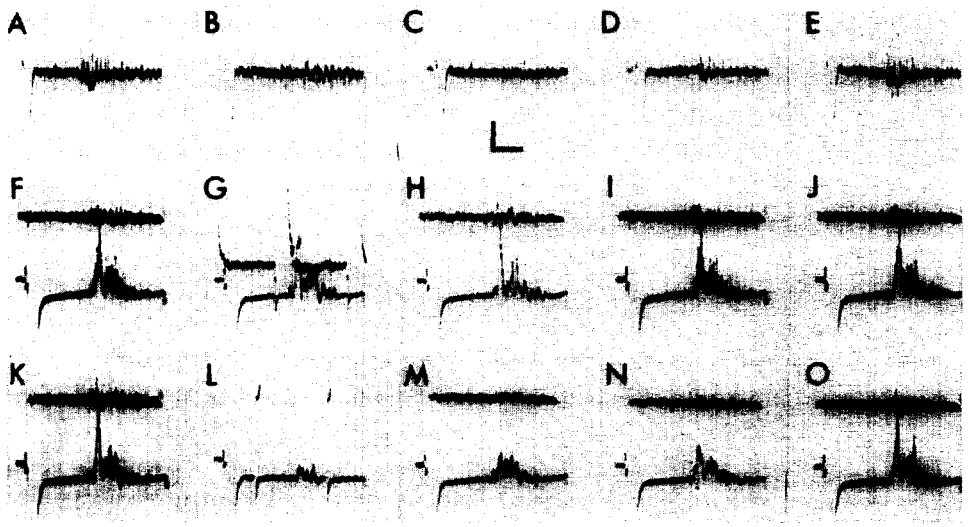

Fig. 4. DRR depression following natural somatic or repetitive electrical stimulation. A–E, Intact sural nerve. DRR to single orthodromic volley (A) is depressed during (B) 10 sec of continuous natural somatic stimulation to sural receptive field and for 10 (C) and 30 (D) sec thereafter. DRR excitability has recovered after 60 sec (E). Calibration: 50 μV, 4 msec. F–J, Stimulation of sural nerve elicits control DRR (F) on sural (lower traces) and cutaneous branch of superficial peroneal nerve (upper traces). Repetitive superficial peroneal stimulation (100/sec for 10 sec) at strengths below DRR threshold (G) does not depress the DRR on either nerve. Sample records shown 4 (H), 30 (I) and 60 (J) sec after conditioning tetany. Calibration: 50 μV, 2 msec (upper traces); 100 μV, 2 msec (lower traces). K–O. Same conditions and recording conventions as in F–J except that superficial peroneal tetany, now delivered above DRR threshold, depresses the DRR on both nerves. Sample records shown 4 (M), 30 (N) and 60 (O) sec after conditioning tetany.

then be observed only when the conditioned fibers elicited and/or carried the reflex discharge. However, if conditioning of primary afferents depresses a DRR that does not use the conditioned fibers as either input or output pathways, the depressive mechanism cannot be restricted to the conditioned terminals; this will be designated a *general* effect, indicating that the DRR depression has been distributed to unconditioned afferents by some mediating system.

There are two ways in which depression of the DRR is produced by primary afferent stimulation: (i) by excitation of the DRR itself *(post-excitatory depression)*, and (ii) following several seconds of orthodromic activity elicited either by physiological cutaneous stimulation or repetitive electrical stimuli *(post-tetanic depression)*. The results show that the activation and distribution of the depressive effect depends upon the afferent system employed.

Post-excitatory depression. A single orthodromic volley which evokes a DRR is followed by a period of prolonged depression of the reflex system in peripheral nerves and dorsal roots. The DRR of the sural nerve, for example, is depressed, after a 4–5 msec delay, for approximately 500 msec following the initial sural volley. The depression is not seen if the initial volley fails to evoke the DRR. A similar but shorter and less pronounced depression is also present in afferent systems which do not carry the initial orthodromic volley.

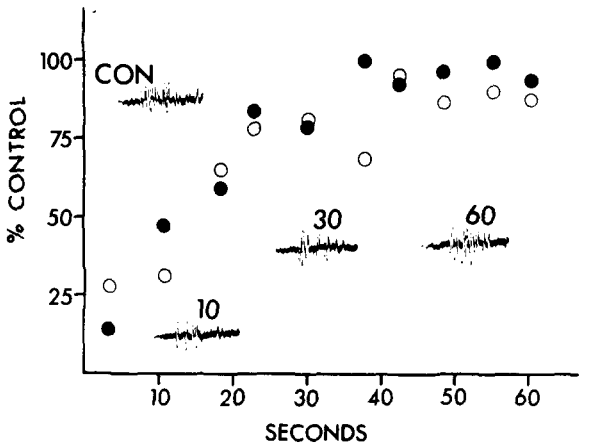

Fig. 5. Time course of post-tetanic DRR depression. Sural testing stimulation (1/3 sec) just sufficient to elicit multiple unitary DRR spikes (inserted records) which were counted at various times (seconds indicated in inserts) following 10 sec of continual natural (filled circles) or repetitive electrical (open circles) sural conditioning. Each point shows DRR spike count as a percentage of the control value and is based on 5 samples at each conditioning-testing interval, there being at least 5 min between samples. Some spikes retouched.

Post-excitatory depression is also observed when dorsal root filaments are used as afferent pathways. A single, suprathreshold stimulus of one L_7 rootlet strongly depresses the DRR elicited by and recorded from an adjacent rootlet. The post-excitatory depression observed after nerve or rootlet stimulation is therefore a general effect, not restricted to the conditioned fibers used to produce it. In all cases, the depression appears after a 4–5 msec delay and is seen only following conditioning stimuli which evoke DRRs in the afferent systems under examination.

Depression following physiological or tetanic stimulation. Our incidental observations, suggesting that a prolonged asynchronous barrage depresses the DRR mechanism, were tested by recording from the intact sural nerve while initiating an orthodromic discharge by gently rubbing the fur and skin over the sural receptive area for about 10 sec. The resulting post-conditioning excitability curve (Fig. 5) and the sample records (Fig. 4A–E) show the marked and prolonged DRR depression induced by the asynchronous afferent barrage. It is significant that the magnitude of the reflex depression induced by natural stimulation is as great as that following tetanic electrical stimulation of the same nerve (Fig. 5).

When cutaneous nerves are used, the general effects of tetany are clearly revealed by the DRR. Fig. 4K–O, for example, show that after tetanic conditioning of the cutaneous superficial peroneal nerve there is a depression both of the DRR recorded from that nerve and of the reflex evoked by and recorded from the sural. A general effect is also present during tetanic conditioning (Fig. 4L, lower). Thus, the two methods of producing DRR depression, post-excitatory and post-tetanic, both have general effects so far as cutaneous peripheral nerves are concerned.

If a L_7 rootlet is tetanized and then stimulated, its own DRR is greatly depressed and the DRR on an adjacent rootlet (Fig. 6A and B) is briefly depressed. However,

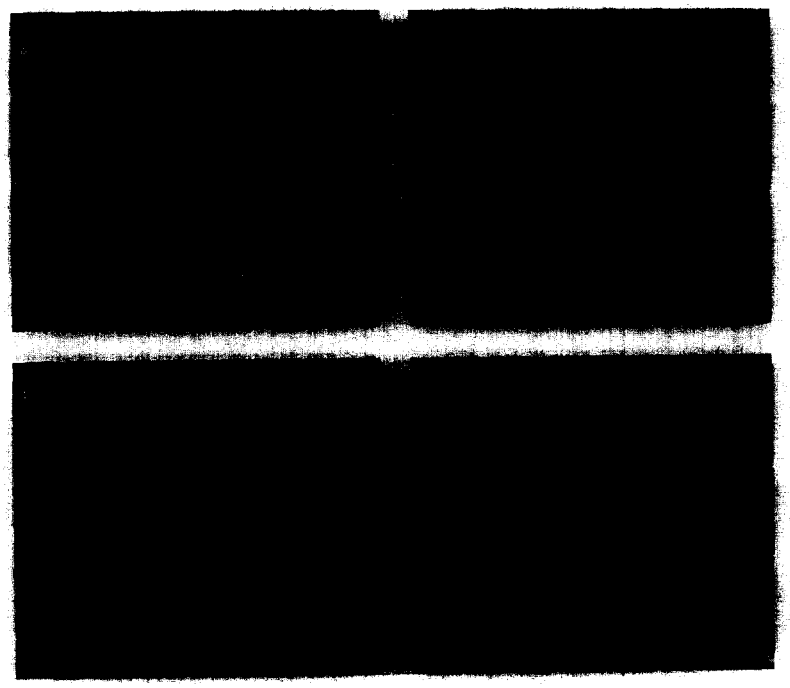

Fig. 6. Restricted post-tetanic DRR depression in L_7 dorsal rootlets. Arrangement of stimulating and recording electrodes as shown in Fig. 3A: DRR elicited by stimulation of an adjacent rootlet (S_1, lower trace), the rootlet used for recording (S_2, middle trace), and with S_1 preceding S_2 by 3.5 msec (upper trace). Note facilitation (reduced latency) of the S_2 response. B, DRR elicited by S_1 stimulation approximately 2 (lower), 19 (middle) and 60 (upper) sec following 30 sec of 100/sec full A-alpha tetanus of the S_1 rootlet. Note DRR depression in lower record. C, Same experiment as in B except that DRR excitability now tested by S_2 stimulation, showing no effect of the tetanus to S_1. Same experiment as in B and C, but with S_2 testing stimulus given 3.5 msec after S_1. Note that S_1 tetanus does not modify the facilitatory effect of prior S_1 stimulation. Calibration same as in Fig. 3. Three sweeps each trace.

the DRR elicited by and recorded from an adjacent L_7 rootlet is unaffected (Fig. 6C). The DRR depression may be too weak to affect the facilitatory interaction between the two rootlets (Fig. 6D), but even weak general effects could not be seen. Thus, the general effects of post-tetanic DRR depression appear limited to peripheral nerve interactions. In dorsal roots, post-tetanic effects are apparently restricted to the afferent fibers or to a DRR mechanism interposed between the afferent and efferent limbs of the reflex.

These DRR depressions cannot be explained as the summation of post-excitatory effects, for reflex discharges were not continually elicited throughout the natural or electrical conditioning stimuli. All but the initial volley in the conditioning tetanus, for example, falls within the period of post-excitatory depression of the reflex system. Nevertheless, to cause the depression the conditioning stimulus must excite a population of fibers normally capable of activating the DRR, because a single A-alpha volley too weak to evoke a DRR also fails to induce the DRR depression when delivered repetitively (Figs. 4F–J, 7A–D). When the stimulus intensity is increased so

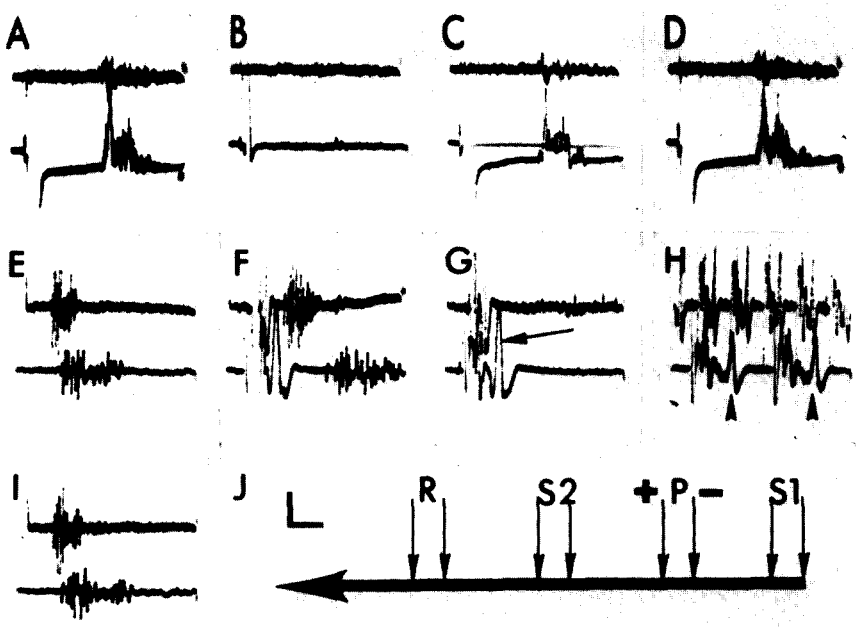

Fig. 7. Repetitive stimulation of volleys not eliciting a DRR. A–D, Single test stimulus to sural nerve evokes DRR in sural (lower traces) and in cutaneous superficial peroneal (upper traces) nerves. Tetanus of sural (100/sec for 10 sec) at intensities below DRR threshold (B) does not depress the DRRs as tested 4 (C) and 20 (D) sec later. Calibration (see J): 50 μV, 4 msec (upper traces); 100 μV, 4 msec (lower traces). E–J, Arrangement of sural recording (R), stimulating (S_1, S_2) and polarizing (P) electrodes shown in J, arrow indicating orthodromic conduction. Upper and lower traces show sural DRR to sural stimulation, the calibrations (see J) being 100 μV and 4 msec for lower traces; slightly higher (uncalibrated gain) and 10 msec for upper traces. E, Control DRR elicited by test stimulus at S_2. F, DRR elicited by a volley of A-alpha and A-delta fibers at S_1. G, Same as F except that anodal polarization has partially blocked the A-alpha volley, showing that the A-delta component alone (arrow) elicits no DRR. H, Continued anodal polarization allows 10 sec of 100/sec tetany of A-delta fibers (arrows) during nearly complete A-alpha block. I, Control response as in E, elicited 2 sec after the A-delta tetany shown in H. Note (E, I; A, C) that both early (A-alpha) and late (A-delta) DRR components are present following tetany of A-alpha or A-delta fibers which do not elicit DRRs.

that reflex discharge is produced by single volleys, prolonged post-tetanic depression is observed on both conditioned and unconditioned nerves (Fig. 4K–O).

Similarly, A-delta volleys alone fail to evoke either the DRR or its depression. This is shown in Fig. 7 where isolated A-delta volleys were delivered as anodal polarization blocked the A-alpha volley. DRR excitability was tested by means of recording and stimulating electrodes proximal to the polarized region, thus avoiding possible interference with conduction owing to residual effects at the polarizing electrodes (Fig. 7J). Single isolated A-delta volleys do not evoke reflex discharge (Fig. 7G) and, in accord with the observations on ineffective A-alpha volleys (Fig. 7A–D) a conditioning tetanus of isolated A-delta volleys fails to induce any sign of depression of either the early or late phase of the DRR (Fig. 7E–I). The same results are obtained when isolated C fiber volleys are used. Post-tetanic and post-excitatory DRR depression, then, are similar in that both require conditioning volleys capable of DRR

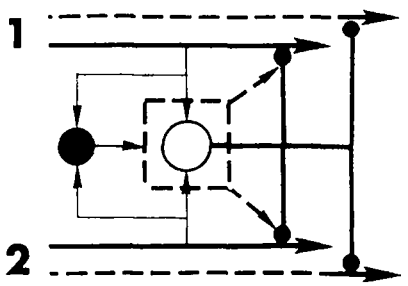

Fig. 8. Summary diagram of DRR organization. The A-alpha afferents (solid arrows) of cutaneous nerves 1 and 2 each excite an interneuronal population (open circle) which depolarizes the terminals of A-alpha and A-delta afferents (dashed arrows), eliciting a DRR in both nerves. A-delta fibers do not significantly influence the DRR system. A-alpha cutaneous fibers also excite a separate interneuronal population (filled circle) which depresses the excitability of the DRR system of both nerves, producing general post-excitatory and post-tetanic depression. The mechanism of this depression is unspecified, but could include pre- and postsynaptic effects (broken rectangle and arrows).

excitation. They differ in that the general effects of post-tetanic depression were observed only when peripheral nerve inputs were interacted.

To summarize, in this analysis of afferent control of the DRR, we have utilized either two nerves (sural and cutaneous superficial peroneal) or two dorsal rootlets (L_7). For each pair we have systematically examined all possible ipsilateral permutations of conditioning, testing and recording arrangements, in which the conditioning stimulus was either a single tetanic electrical stimulation or, in the case of the peripheral nerves only, natural stimulation. A model which summarizes the results and conclusions has been formulated and is presented in the discussion.

DISCUSSION

Fibers participating in the cutaneous DRR

The results confirm the observations of others[17,27] that cutaneous A-alpha volleys alone are sufficient to evoke a full cutaneous DRR. Volleys of smaller diameter afferents, delivered alone or in addition to A-alpha discharge appear to be relatively ineffective, as the smaller PAD attributed to C fiber activation[12] would suggest. It is unlikely that a reflex discharge to an A-delta volley was overlooked because the A-delta portion of the DRR is clearly detectable with bipolar recording. Reflexes evoked by C fibers could have escaped detection if the efferent activity were largely limited to these slowly conducting fibers, but there is sufficient synchrony in an orthodromic C fiber volley to evoke detectable dorsal root potentials in both the anesthetized[12] and unanesthetized cat[10,20,32]. These observations indicate that if the DRR is interneuronally mediated, the discharges of those dorsal horn cells predominantly excited by isolated A-delta or C fiber volleys are relatively ineffective in DRR activation. Differences in the presynaptic action of large and small fiber volleys have also been observed in studies of the DRP[11,12,20,31].

The results of the collision experiments confirm Toennies'[27] observation that the cat cutaneous DRR includes both A-alpha and A-delta efferent activity. Our experiments do not reveal the presynaptic effects of A-delta fibers suggested by Selzer and Spencer[24] for visceral–cutaneous interactions. This difference may appear because the DRR does not provide information about weaker presynaptic effects, or because there are differences between visceral and cutaneous presynaptic organization; for example, the results of Selzer and Spencer[24] also indicate that large diameter cutaneous afferents do not depolarize visceral A-delta fibers.

DRR latency: relation to neural mechanism

The results of the collision experiments reveal that, with the possible exception of the very early DRR seen in a few fibers[29], some process interposes a delay at the point of primary afferent interaction which initiates a DRR. The minimum value of this delay is important in considering the mechanism generating the DRR.

Since primary afferent depolarization (PAD) is the first sign of interaction between fibers, estimates of minimum interaction delays based on the intraspinal measurement of PAD latencies[8] provide the most satisfactory basis for comparison with our results. Since PAD latencies of less than 2.0 msec have been recorded[8], the escape from blocking observed from some fibers with 1.0 msec inter-volley intervals (Fig. 3C and D) suggests that, for these fibers, the refractory period of the blocking volley is 1.0 msec or less at the point of fiber interaction. If this is also the value of the refractory period for fibers which just escape from collision block when 0.5 msec volley intervals are used (Fig. 3D and E), then some interaction delays may be as short as 1.5 msec. This estimate is similar to that obtained by PAD latency measurements[8]. It is likely that 1.5 msec is near the minimum value for this delay because otherwise one would expect the final escape from collision block at inter-volley intervals well below 0.5 msec or perhaps only when the blocking volley led the eliciting volley by a time nearly equal to the refractory period of the fibers. Overestimates of interaction delay might occur if post-junctional effects persisted throughout the refractory period of the blocking volley, thus delaying the antidromic response. However, the results show (Fig. 3) that the partially blocked DRR is attenuated, not simply delayed or increased in duration.

If an interneuron mediates this interaction via chemically mediated synapses, approximately 1.0–0.6 msec of this delay is attributable to two synaptic delays and an additional period of time must be allowed for postsynaptic effects to lead to generation of the reflex spike. In some cases, then, there may not be sufficient time for serial activation of more than one interneuron as has been proposed for PAD or the DRP[5].

Direct interaction among fibers is suggested by the close apposition of primary afferents within the microbundle organization of the dorsal horn[23]. Van Harreveld and Niechaj[29] and Rudomin and Munoz-Martinez[22] have also shown that some components of the DRR are independent of interneuronal activity. However, light and electron microscopic study of dorsal horn connections has not revealed evidence for direct axonal contacts between dorsal root afferents[21]. Although the structural

elements mediating the DRR remain to be identified, interneuronal mediation would seem to be the most likely mechanism for the generation of DRRs by contralateral stimulation[27]. This mechanism is also compatible with the minimum intraspinal interaction delays of ipsilateral DRRs as estimated by the collision technique.

Afferent control of the DRR

The results suggest that the mechanism of post-excitatory depression is linked to the activation of the DRR itself. This comparatively brief depression apparently affects all afferent systems which carry the DRR and is not limited to the afferents which carry the orthodromic volley. The results are similar for the DRP[6]. In contrast, the more prolonged post-tetanic depression is not associated with reflex discharge, for it is produced by orthodromic asynchronous discharge or repetitive volleys during which a DRR is not continually evoked. It is unlikely that post-tetanic DRR depression is due to an effect restricted to either the efferent or afferent limbs of the DRR. Tetanus of A-delta or subthreshold A-alpha fibers does not depress the DRR carried by these fibers (Figs. 4F–J and 7); moreover, restricted phenomena cannot account for the results obtained by prolonged natural or repetitive electrical stimulation of cutaneous nerves since the DRR of unconditioned nerves is clearly depressed (Fig. 4K–O). These general effects require a mediating system.

Summary diagram of DRR organization

The model shown in Fig. 8 summarizes the major findings and conclusions. In accord with the observations on DRR latency and distribution, it is assumed that the DRR is mediated by an interneuronal mechanism like that responsible for the DRP and PAD[28]. In the model (Fig. 8), the A-alpha fibers of two cutaneous nerves each activate the DRR system, producing antidromic discharge of both A-alpha and A-delta afferents in both nerves. The smaller diameter cutaneous fibers have little or no effect on the DRR system.

Activity in A-alpha cutaneous fibers is also responsible for post-tetanic and post-excitatory DRR depression. As indicated in the diagram, it is postulated that this is mediated by a separate interneuronal system which receives A-alpha cutaneous input and depresses, by mechanisms not specified, the DRR system of unstimulated afferents. The recent experiments of Somjen[25] suggest the possibility that glial elements could mediate general DRR depression. The sustained potential of dorsal spinal gray, attributed primarily to glial depolarization, is increased by repetitive stimuli which depresses the DRP and increases the ventral root reflex; this phenomenon lasts for several seconds, and is most effectively produced by cutaneous nerve stimuli[25]. However, in contrast to the results presented here, the smaller diameter cutaneous fibers were the most effective inputs and single orthodromic volleys were ineffective[25]. Mediation of DRR depression by direct connections between terminals is incompatible with the delay in onset of the depression. A change in the composition

of the extracellular fluid is unlikely to cause the depression in view of the effectiveness of single volleys and the differential effectiveness of large cutaneous fibers.

Interneurons mediating the DRR depression should be excited by cutaneous afferents and discharge at about the time of DRR generation, perhaps firing repetitively in order to account, in part, for the prolonged DRR depression which is observed. Interneurons with these properties have been identified in the cat dorsal horn and their function has been related to interneuronally mediated primary afferent depolarization[5]. It seems equally possible that some of these cells may mediate post-excitatory and cutaneous post-tetanic DRR depression.

The summary model does not include the restricted DRR depression effects found with tetanization of dorsal root fibers. The differential effects of rootlet and cutaneous nerve tetany may reflect the differences between cutaneous nerve and dorsal root afferents with respect to afferent fiber composition and inter-segmental interactions. The restriction of DRR depression to the tetanized dorsal root afferents may be due to the post-tetanic hyperpolarization[15] which is known to potentiate the DRP[14] and PAD[8,9,13] recorded from tetanized fibers. Concurrent DRR depression would result if the PAD potentiation did not compensate for the hyperpolarization of the afferent terminals. Post-tetanic hyperpolarization cannot, however, explain the general DRR depression attributed to cutaneous A-alpha fiber tetany.

Since barbiturates strongly attenuate the development of a positive DRP[14], the organization of presynaptic interactions revealed by these experiments is probably an experimental simplification of that present in the awake animal. Nonetheless, our results suggest that A-alpha cutaneous afferents would be the predominant fibers attenuating synaptic transmission from both large and small diameter cutaneous fibers, an arrangement consistent with that proposed for a 'gate control' theory of pain[19]. The DRR depression produced by these large cutaneous afferents, however, also suggests that prolonged cutaneous A-alpha activity would depress presynaptic depolarization. On the other hand, our experiments indicate that fine fiber activity would have little effect on the excitability of presynaptic depolarizing mechanisms. The amount of large fiber activity, then, would determine both the magnitude of presynaptic depolarization and the excitability of the mechanism which generates it.

SUMMARY

The intraspinal delay, fiber composition, and excitability of the cutaneous dorsal root reflex (DRR) was studied in pentobarbital-anesthetized cats.

A maximal cutaneous DRR is elicited by a volley of cutaneous fibers with conduction velocities above the A-delta range. The addition of A-delta and C fibers does not increase DRR amplitude or duration; nor does an isolated A-delta or C fiber volley, delivered during anodal polarization block of the larger fibers, elicit a DRR. Collision experiments reveal that the initial phase of the cutaneous DRR recorded from nerves is composed of activity in large myelinated fibers; the later phase is due to active A-delta fibers. Thus, the observations reveal that A-delta fibers carry, but do not elicit, the DRR.

A collision technique shows that the minimum delay for the inter-fiber interaction producing the DRR may be as short as 1.5 msec, a value compatible with DRR mediation by one interneuron.

Prolonged DRR depression follows mechanical stimulation of the skin and single shock or repetitive electrical stimulation of the A-alpha cutaneous afferents capable of DRR excitation; neither large (A-alpha) fiber volleys below DRR threshold nor isolated A-delta volleys depress the DRR. In the case of cutaneous nerves, this depression affects unconditioned nerves, but the effects of dorsal rootlet tetany are restricted to DRRs elicited by or recorded from the conditioned rootlet.

The results are summarized in a model in which A-alpha cutaneous afferent fibers activate separate interneuronal systems mediating DRR excitation and depression.

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REFERENCES

1 BARRON, D. H., AND MATTHEWS, B. H. C., The interpretation of potential changes in the spinal cord, *J. Physiol. (Lond.)*, 92 (1938) 276–321.
2 BROOKS, C. M., AND KOIZUMI, K., Origin of the dorsal root reflex, *J. Neurophysiol.*, 19 (1956) 61–74.
3 CALMA, I., AND QUAYLE, A. A., Repetitive antidromic discharges in fast cutaneous nerve fibers, *Brain Research*, 11 (1968) 268–272.
4 CASEY, K. L., AND BLICK, M., Observations on anodal polarization of cutaneous nerve, *Brain Research*, 13 (1969) 155–167.
5 ECCLES, J. C., KOSTYUK, P. G., AND SCHMIDT, R. F., Central pathways responsible for depolarization of primary afferent fibers, *J. Physiol. (Lond.)*, 161 (1962) 237–257.
6 ECCLES, J. C., KOSTYUK, P. G., AND SCHMIDT, R. F., Presynaptic inhibition of the central actions of flexor reflex afferents, *J. Physiol. (Lond.)*, 161 (1962) 258–281.
7 ECCLES, J. C., KOZAK, W., AND MAGNI, F., Dorsal root reflexes of muscle group I afferent fibres, *J. Physiol. (Lond.)*, 159 (1961) 128–146.
8 ECCLES, J. C., AND KRNJEVIĆ, K., Potential changes recorded inside primary afferent fibers within the spinal cord, *J. Physiol. (Lond.)*, 149 (1959) 250–273.
9 ECCLES, J. C., AND KRNJEVIĆ, K., Presynaptic changes associated with post-tetanic potentiation in the spinal cord, *J. Physiol. (Lond.)*, 149 (1959) 274–287.
10 FRANZ, D. N., AND IGGO, A., Dorsal root potentials and ventral root reflexes evoked by non-myelinated fibers, *Science*, 162 (1968) 1140–1142.
11 HODGE, C. J., Potential changes inside central afferent terminals secondary to stimulation of large and small diameter peripheral nerve fibers, *J. Neurophysiol.*, 35 (1972) 30–43.
12 JÄNIG, W., AND ZIMMERMANN, M., Presynaptic depolarization of myelinated afferent fibres evoked by stimulation of cutaneous C fibres, *J. Physiol. (Lond.)*, 214 (1971) 29–50.
13 KOKETSU, K., Intracellular potential changes of primary afferent nerve fibers in spinal cords of cats, *J. Neurophysiol.*, 19 (1956) 375–392.
14 LLOYD, D. P. C., Electrotonus in dorsal nerve roots, *Cold Spring Harb. Symp. quant. Biol.*, 17 (1952) 203–219.
15 LLOYD, D. P. C., Post-tetanic potentiation of response in monosynaptic reflex pathways of the spinal cord, *J. gen. Physiol.*, 33 (1964) 147–170.

16 LLOYD, D. P. C., AND MCINTYRE, A. K., On the origins of dorsal root potentials, *J. gen. Physiol.*, 32 (1949) 409–443.
17 MEGIRIAN, D., Centrifugal discharges in cutaneous nerve fibers evoked by cutaneous afferent volleys in the acutely spinal phalanger, *Trichosurus vulpecula*, *Arch. ital. Biol.*, 106 (1968) 343–352.
18 MEGIRIAN, D., Centrifugal cutaneous nerve discharges in the decerebrate phalanger, *Trichosurus vulpecula*, *Arch. ital. Biol.*, 108 (1970) 388–399.
19 MELZACK, R., AND WALL, P. D., Pain mechanisms: a new theory, *Science*, 150 (1965) 971–979.
20 MENDELL, L. M., AND WALL, P. D., Presynaptic hyperpolarization: a role for fine afferent fibres, *J. Physiol. (Lond.)*, 172 (1964) 274–294.
21 RALSTON, H. J., Dorsal root projections to dorsal horn neurons in the cat spinal cord, *J. comp. Neurol.*, 132 (1968) 303–330.
22 RUDOMIN, P., AND MUNOZ-MARTINEZ, J., A tetrodotoxin-resistant primary afferent depolarization, *Exp. Neurol.*, 25 (1969) 106–115.
23 SCHEIBEL, M. E., AND SCHEIBEL, A. B., Terminal patterns in cat spinal cord. III. Primary afferent collaterals, *Brain Research*, 13 (1969) 417–443.
24 SELZER, M., AND SPENCER, W. A., Interactions between visceral and cutaneous afferents in the spinal cord: reciprocal primary afferent fiber depolarization, *Brain Research*, 14 (1969) 349–366.
25 SOMJEN, G. G., Evoked sustained focal potentials and membrane potentials of neurons and of unresponsive cells of the spinal cord, *J. Neurophysiol.*, 33 (1970) 562–582.
26 SPEARS, R., SMITH, G., AND CASEY, K. L., A pulse height discriminator and post-stimulus histogram system using integrated circuits, *Physiol. Behav.*, 5 (1970) 1327–1329.
27 TOENNIES, J. F., Reflex discharge from the spinal cord over the dorsal roots, *J. Neurophysiol.*, 1 (1938) 378–390.
28 TREGEAR, R. T., The relation of antidromic impulses in the dorsal root fibres to the dorsal root potential in the frog, *J. Physiol. (Lond.)*, 142 (1958) 343–359.
29 VAN HARREVELD, A., AND NIECHAJ, A., A possibly monosynaptic component of the dorsal root potential, *Brain Research*, 19 (1970) 105–116.
30 WALL, P. D., Repetitive discharge of neurons, *J. Neurophysiol.*, 22 (1959) 305–320.
31 YOUNG, R. F., AND KING, R. B., Excitability changes in trigeminal primary afferent fibers in response to noxious and nonnoxious stimuli, *J. Neurophysiol.*, 35 (1972) 87–95.
32 ZIMMERMANN, M., Dorsal root potentials after C-fiber stimulation, *Science*, 160 (1968) 896–898.