Dorsal Root Potentials and Ventral Root Reflexes Evoked by Nonmyelinated Fibers

Abstract. *Electrical stimulation of C-fibers in the cat superficial peroneal nerve, with the A-fibers either conducting or blocked by cold, evoked dorsal root potentials having the same polarity as those evoked by A-fibers. Ventral root reflexes evoked by A-fibers were facilitated by a pure C-volley in the same or another nerve, but dorsal root potentials evoked by A-fibers were reduced by isolated dorsal root potentials from C-fibers. In the absence of anesthetics, single C-volleys produced brisk and prolonged reflex discharges in ventral roots.*

The influence of nonmyelinated afferent nerve fibers (C-fibers) on spinal reflexes is poorly understood by comparison with that of the myelinated A-fibers. A theory that the terminals of afferent A-fibers are hyperpolarized by C-fiber activity is supported by experiments in which an isolated C-volley produced a small positive dorsal root potential (DRP) opposite in polarity to the large negative (depolarizing) DRP evoked by A-fibers and also facilitated reflex responses evoked by A-fibers without evoking a C-reflex (*1*). The absence of a C-reflex conflicts with other evidence that C-fibers do evoke prominent autonomic (*2*) and somatic (*3*) reflex responses, and the polarity of the DRP evoked by C-fibers has also been seriously challenged (*4*). Therefore, we have reinvestigated the effects of an isolated C-input to the spinal cord on both the DRP and ventral root discharges in anesthetized or unanesthetized spinal cats.

C-volleys in a peripheral nerve were isolated by blocking conduction in all A-fibers with a low-temperature thermode proximal to the stimulating electrodes. Recent studies (*5*, *6*) agree that conduction through single axons persists at significantly lower temperatures in C-fibers than in A-fibers but that no difference exists between A-fibers of different sizes. Complete isolation of C-fiber input to the spinal cord was readily obtained by careful monitoring and temperature control.

Ten adult cats were anesthetized with chloralose (80 mg per kilogram body weight, intravenously) or were prepared by removal of the forebrain and section of the spinal cord at C_1 under ether anesthesia. The latter were artificially respired and immobilized with gallamine triethiodide. The lumbar spinal cord was exposed and sectioned between L_3 and L_4. The DRP's were recorded from an L_6 or L_7 dorsal rootlet, and reflex responses were recorded from all or part of an L_7 or S_1 ventral root by conventional methods.

The ipsilateral common peroneal nerve and its superficial branch were exposed and carefully freed at three points. The afferent input to the spinal cord was provided by electrical stimulation of the superficial branch near the ankle. At the knee an intact, 12 mm segment of the common peroneal nerve containing the superficial branch was mounted on a grooved thermode and covered by a gel of 4 percent agar in saline to minimize thermal gradients across the nerve (*6*). A representative sample of the afferent input to the spinal cord was recorded from very fine nerve strands dissected from a fascicle of the sciatic bundle that was in continuity with the superficial branch (*7*). Exposed tissues were covered by a pool of paraffin oil. The thermode was cooled by a thermostatically controlled, circulating alcohol and water bath. Nerve temperature was monitored by a digital voltmeter from a calibrated thermistor bead in the groove of the thermode.

The afferent sample recorded proximally to the cooled nerve reliably indicated stimulus threshold and the stage at which only C-fibers were still conducting impulses to the spinal cord (Fig. 1, a–h, lower traces). Total block of A-fibers was further verified by the disappearance of their evoked DRP (A-DRP, Fig. 1, a–h, upper traces) or short-latency reflex responses in records from a ventral spinal root (Fig. 2, c–e). The much longer refractory periods of cooled axons and a necessity to keep the A-DRP as brief as possible made repetitive stimulation of the afferent nerve undesirable, and single pulses (0.25 msec in duration) at 8- to 10-second intervals were used for stimulation.

Normal deep body temperature was maintained by an electric blanket that was thermostatically controlled from a rectal probe. Arterial blood pressure was monitored in some experiments although direct microscopic observation of blood flow was also used as a more reliable indication of circulatory integrity. Confirmation of small DRP's evoked by C-fibers in unanesthetized animals was assisted by an averaging computer (Biomac 500).

Stimulation of C-fibers in the superficial peroneal nerve of anesthetized spinal cats consistently evoked negative DRP's (C-DRP's) having the same polarity as those evoked by A-fibers. The C-DRP was small or absent when preceded by the A-DRP but became larger as the A-DRP was reduced by cold block of the A-fibers. The large A-DRP in Fig. 1, a and b, became much

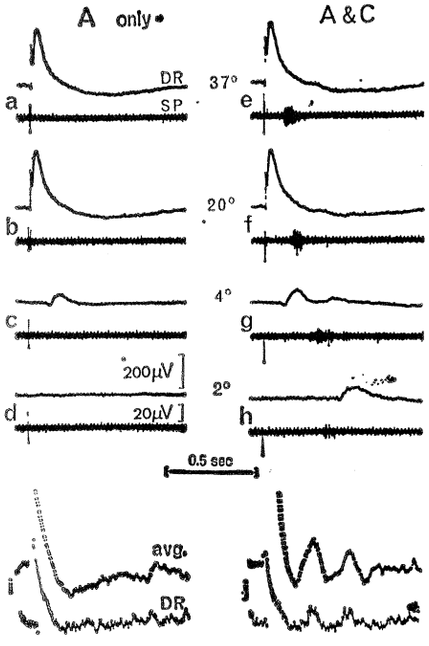

Fig. 1. (a–d) Dorsal root potentials evoked by A-fibers in a chloralose anesthetized spinal cat. (e–h) Dorsal root potentials evoked by A- and C-fibers in a chloralose anesthetized spinal cat. (i and j) Dorsal root potentials in an unanesthetized spinal cat. In a–h, the top traces are DRP's recorded from an L_7 dorsal rootlet and evoked by stimulation of the superficial peroneal nerve; conduction distance, 200 mm. Lower traces are action potentials monitored simultaneously from a multifiber filament of the nerve; conduction distance, 90 mm. The nerve was cooled by a conventional thermode midway between the stimulating and filament-recording electrodes until the A-fibers were blocked. Nerve temperatures (°C) refer to the two sets of traces on either side. In (i) and (j) the DRP's recorded from an L_7 dorsal rootlet (lower trace) were evoked by stimulation of the superficial peroneal nerve at below (i) or above (j) threshold for C-fibers. Upper traces are computer averages of 32 consecutive DRP's, amplified beyond the display capacity for the A-DRP. Nerve at normal temperature.

smaller in Fig. 1c and disappeared completely in Fig. 1d as the A-fibers were blocked. At the same temperature the addition of C-fibers to the afferent volley in Fig. 1, e–h, produced a C-DRP on the late phase of the A-DRP (Fig. 1, e and f). The C-DRP became larger and persisted after the A-fibers were blocked and the A-DRP had disappeared (Fig. 1, g and h).

As in Fig. 1, e–h, the C-DRP always correlated with the conduction velocity of the monitored C-volley, and the large increase in latency of the C-DRP equalled that of the C-volley which was considerably slowed across the cold thermode. The higher conduction velocity of A-fibers at all temperatures at which they can operate (*5, 6*) precludes their contribution to the C-DRP. The loss of a C-DRP when C-fibers were blocked at the thermode or failed to be excited by a subthreshold stimulus (Fig. 1, a–d) further supports its generation by impulses in C-fibers.

Stimulation of C-fibers in unanesthetized spinal cats also produced negative C-DRP's (Fig. 1j) which satisfied the criteria for their dependence on C-fibers including their absence below the C-threshold (Fig. 2i). These C-DRP's were generally smaller than those generated in cats anesthetized with chloralose, but they were less depressed by the preceding A-DRP which has a shorter duration in the unanesthetized animal (*8*).

Identification of the small C-DRP's in single traces was often less certain than in anesthetized cats because of spontaneous DRP's, but the absence (Fig. 1i) or presence (Fig. 1j) of an evoked C-DRP was confirmed by averaging (Fig. 1, i and j, upper records). The second smaller C-DRP in Fig. 1j corresponded in latency and threshold to the smaller C-fibers. This second C-DRP was selectively depressed by anesthetics.

The A-DRP's evoked by stimulation of A-fibers in the sural nerve (Fig. 2, a and b) were reduced when preceded by an isolated C-DRP within 600 msec. Likewise, C-DRP's were reduced or extinguished when preceded by an A-DRP evoked by the same or a different nerve.

Stimulation of C-fibers in unanesthetized spinal cats invariably evoked a late, asynchronous reflex discharge in L_7 or S_1 ventral roots (Fig. 2, c–e). Its duration varied in different preparations from 200 msec to several seconds, but its latency corresponded with that of the monitored C-volley and of the C-DRP. When the afferent A-fibers were blocked by cold, the unblocked C-fibers continued to evoke the reflex response but with a longer latency due to the slowing of their conduction velocity at the thermode (Fig. 2, d and e). The reflex diminished in size in proportion to the subsequent block of the C-fibers responsible for the reflex. C-reflexes were rapidly and reversibly extinguished by subanesthetic concentrations of ether, ethyl chloride, or sodium pentobarbital and could not be evoked in cats that were fully anesthetized.

Ventral root reflexes, evoked by stimulation of A-fibers in a dorsal root, were depressed by a preceding A- and C-volley from the superficial peroneal nerve (Fig. 2, f and g) but were enhanced by an isolated conditioning C-volley in both anesthetized (Fig. 2, h and i) and the unanesthetized cats. Within 400 msec after the arrival of a C-volley at the spinal cord these A-reflexes were increased by up to 60 percent.

Negative DRP's indicate depolarization of the presynaptic terminals of primary afferent nerve fibers which is the accepted mechanism for presynaptic inhibition by A-fibers (*8*). According to Mendell and Wall (*1*), C-fibers produce the opposite effect, that is, hyperpolarization of the presynaptic terminals of afferent A-fibers which generates a "positive DRP." The resulting presynaptic facilitation of afferent A-fiber activity by this mechanism has been incorporated into a general theory of pain perception (*9*). Our finding that C-fibers evoke only negative DRP's contrasts with that of Mendell and Wall (*1*) but confirms the recent report by Zimmermann (*4*) who blocked the A-fibers by a polarization technique. Furthermore, the negative DRP indicates that C-fibers, like A-fibers, depolarize the presynaptic terminals of primary afferent fibers but do so less effectively than the A-fibers. The interaction between A- and C-DRP's suggests that the same terminals of primary afferent fibers are depolarized by both A- and C-fibers.

The facilitation of A-reflexes by conditioning impulses in C-fibers may appear paradoxical since presynaptic depolarization implies presynaptic inhibition and a direct contribution of afferent C-fibers to spinal reflexes has been denied (*1*). However, the brisk reflex discharges evoked in unanesthetized cats by isolated C-volleys in our experiments indicate that C-fibers do

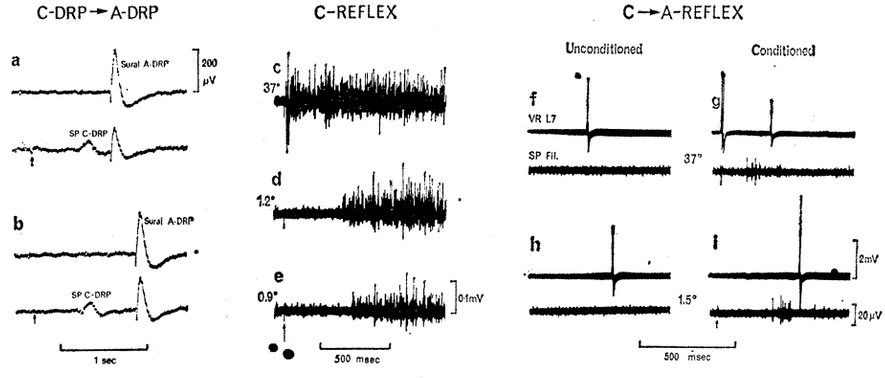

Fig. 2. (a and b) Depression of a dorsal root potential evoked by A-fibers (A-DRP) by a preceding dorsal root potential evoked by C-fibers (C-DRP) at two intervals in an anesthetized spinal cat. In each pair, the top trace shows the unconditioned A-DRP evoked by stimulation of A-fibers in the sural nerve and recorded from an L_6 dorsal rootlet. In the lower trace, the sural A-DRP was conditioned by a C-DRP evoked by a single volley in C-fibers of the superficial peroneal (SP) nerve, in which conduction in A-fibers was blocked at 1.4°C. Conduction distance, 210 mm. (c–e) Reflex response evoked by single shock stimulation of both A- and C-fibers in the SP nerve and recorded from L_7 ventral root in an unanesthetized spinal cat. At 1.2° and 0.9° C all the A-fibers were blocked. Conduction distance, 230 mm. (f–i) Facilitation of A-reflex by a single conditioning C-volley in the SP nerve of an anesthetized cat. Upper trace, L_7 reflex evoked by single shock stimulation of A-fibers in an L_7 dorsal rootlet. Lower trace, simultaneous recording from a small filament of the SP nerve. (f and h) Unconditioned reflex. In (g) the reflex was conditioned by single volleys in both A- and C-fibers of the SP nerve which also evoked an A-reflex. In (i) the reflex was conditioned by an SP C-volley alone, the A-fibers being blocked at 1.5°C. Interval lengthened in (h) and (i) to allow for additional conduction time in cooled nerve. Each record illustrates a mean response selected from 10 trials. Arrows in each set of records indicate the artifact from stimulation of the SP nerve.

make excitatory connections with motoneurons, a conclusion with considerable support (*3*). Therefore, facilitation of A-reflexes by C-fibers can be ascribed to spatial facilitation of a subliminal fringe common to both A- and C-fibers rather than to presynaptic hyperpolarization of primary afferent fibers.

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References and Notes

1. L. M. Mendell and P. D. Wall, *J. Physiol. London* **172**, 274 (1964).
2. D. Clark, J. Hughes, H. S. Gasser, *Amer. J. Physiol.* **114**, 69 (1935); D. N. Franz, M. H. Evans, E. R. Perl, *ibid.* **211**, 1292 (1966).
3. Y. Laporte and P. Bessou, *Compt. Rend. Soc. Biol.* **152**, 161 (1958); A. M. Alderson and C. B. B. Downman, *J. Physiol. London* **150**, 463 (1960); W. Koll *et al.*, *Arch. Ges. Physiol. Menschen Tiere* **272**, 270 (1961).
4. M. Zimmermann, *Science* **160**, 896 (1968).
5. A. S. Paintal, *J. Physiol. London* **180**, 1 (1965); *ibid.* **193**, 523 (1967).
6. D. N. Franz and A. Iggo, *ibid.*, in press.
7. A. Iggo, *ibid.* **142**, 110 (1958); M. R. Chambers and D. N. Franz, *ibid.* **195**, 11*P* (1968).
8. J. C. Eccles, *The Physiology of Synapses* (Springer, Heidelberg, 1964).
9. R. Melzack and P. D. Wall, *Science* **150**, 971 (1965).
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Radiobiological Damage: A New Class Identified in Barley Seeds Stored after Irradiation

Abstract. *A new class of radiobiological damage has been identified in irradiated barley seeds. Consisting of both physiological and genetic damage, it appears to be independent of oxygen and long-lived free radicals, and develops very slowly during storage after irradiation. Increasing the storage temperature accelerates its development.*

Three main classes of radiobiological damage have been recognized in bacterial spores, a dry (dormant, slowly metabolizing) system (*1*, *2*). These include Powers' class I (an "immediate" oxygen-independent portion), class II (an "immediate" oxygen-dependent portion in which oxygen must be present at the time of irradiation in order to be observed), and class III (an oxygen-dependent portion which lasts appreciable lengths of time and which is also termed the free-radical component).

We now report evidence for a new class of radiation damage identified in barley seeds, a dry system, through prolonged storage in a vacuum after irradiation. The damage appears to be initiated by a mechanism which is independent of oxygen and long-lived free radicals.

Seeds of hull-less barley *Hordeum vulgare* var. Himalaya (water content, 10 percent) were exposed in a vacuum to 40 and 25 krad of cobalt-60 gamma rays. Methods for evacuating and irradiating seeds have been described (*3*, *4*). After irradiation, all seeds were stored in a vacuum in sealed glass vials at 40°C for periods up to 6 weeks in the first experiment, and up to 12 weeks in the second experiment, before hydration in oxygenated (oxygen-bubbled) or oxygen-free (nitrogen-bubbled) water at 0°C for a period of 18 hours. After irradiation, the samples were stored at 40°C to accelerate the processes that occur during storage. The water content of the seeds in sealed, evacuated glass vials changes were little, if any, during storage (*3*) after irradiation.

At the end of the hydration period, the seeds were planted and the resultant seedlings were cultured and measured (*5*). The results, from three replications of 50 seedlings each, are reported as seedling injury (percentage of reduction in the mean heights of seedlings of the irradiated seeds compared to the mean heights of seedlings of the nonirradiated controls). Nonirradiated seeds were also stored at 40°C for periods up to 12 weeks, and seeds of each irradiated treatment were compared with the appropriate control.

Shoot tips were collected from some treatments of the second experiment and were cytologically examined for dicentric bridges and acentric fragments in the anaphase stage of the first mitotic division. Twenty cells from each of five seeds from two replications of each treatment (that is, a total of 200 cells for each treatment) were examined by the aceto-orcein smear technique (*3*). Three seeds from each of the three experimental replicates from each storage period were also analyzed for free-radical signals by electron paramagnetic resonance (EPR) (*3*).

Because oxygen was not present during irradiation, Powers' class II damage was not observed in these experiments. However, at least three classes of damage can be recognized from the results of the first experiment (Fig. 1). These include (i) an oxygen-independent portion of about 15 to 20 percent injury observed immediately after irradiation (class I damage); (ii) an oxygen-dependent portion of an additional 60

Table 1. Seedling injury, chromosome aberrations, and signal amplitude obtained from electron paramagnetic resonance (EPR) in barely seeds. Water content of the seeds was 10.1 percent. The seeds were exposed in a vacuum to 25 krad of ^{60}Co gamma rays and then stored for various periods in a vacuum at 40°C before hydration (hyd.) in oxygenated or oxygen-free water at 0°C for 18 hours; the free-radical signal was measured by EPR before and after hydration.

Storage time	Seedling injury reduction		Bridges per 100 cells		Fragments per 100 cells		EPR signal amplitude*
	O_2 hyd. (%)	N_2 hyd. (%)	O_2 hyd.	N_2 hyd.	O_2 hyd.	N_2 hyd.	
None	75.1	6.9	167	21	1060	108	104.3
9 minutes	45.3	6.9	82	38	586	130	77.0
1 hour	14.9	8.1					46.7
7 hours	10.5	6.3	31	31	85	115	27.3
2 days	7.7	6.4	31	39	147	200	10.7
10 days	12.1	10.1					8.7
3 weeks	20.3	18.2	53	54	219	254	7.3
5 weeks	22.2	21.8					6.7
8 weeks	22.6	26.5	48	56	239	254	6.0
12 weeks	38.1	37.6	99	93	351	304	4.7
Control (room temperature)	0.0	0.0	0.0	1.0	1.0	0.0	11.3
Control (12 weeks, 40°C)	0.0	0.0	0.5	0.0	1.5	1.0	4.0

* Arbitrary units.