ACTIVITY IN SINGLE CUTANEOUS AFFERENTS: SPINAL PATHWAYS AND CORTICAL EVOKED POTENTIALS

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INTRODUCTION

The production of cortical evoked potentials by large afferent volleys is a well documented phenomenon[6,7,12,22,24]. Considerable evidence has been amassed that the maximal evoked response results from a small afferent volley in a single nerve (see *e.g.* ref. 18). In fact, Amassian[2] has calculated that activity in as few as 12 dorsal column fibers can produce the maximum cortical evoked response. Furthermore synaptic transmission in the afferent channels to the brain requires very little summation[14,16,18].

Effects elicited in the central nervous system by very small peripheral inputs have only recently begun to be studied[9,15,20,31,32]. This is surprising in the light of the work cited above and behavioral experiments which show that extremely small stimuli can be most effective in eliciting simple[20] as well as complex behaviors[28].

Weak stimuli applied to small areas of the skin can produce evoked cortical responses[1,19]; however, even these small stimuli probably evoke a number of action potentials in a number of fibers. In contrast, McIntyre *et al.*[15] have shown that a single action potential elicited in a single afferent fiber associated with a Pacinian corpuscle can produce an evoked potential in SII cortex of the cat. Similar results might be expected for other kinds of somatic afferents; however, the inconvenience of obtaining such strict control of the afferent activity has perhaps discouraged investigators from tackling the problem. Such control is now possible with new techniques developed in our laboratory (see *e.g.* ref. 4). These techniques and information on the functional organization of the type I cutaneous afferent system have encouraged us to undertake a similar study for these particular somatic afferents[10,13,29,31]. In addition, a study of the location of pathways which carry activity of type I afferents to the cerebral cortex was prompted by the apparent absence of activity from type I afferent fibers in the major projection pathways to the cerebral cortex — the spino-

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cervical tract[3,33] and the dorsal column system[26] — and the apparent contradiction of these data with behavioral studies[30].

METHODS

Adult cats were anesthetized with halothane and maintained under chloralose (50–70 mg/kg) after discontinuation of halothane. The fur of the left hindlimb was closely clipped and the leg was depilated. Animals were placed in the stereotaxic instrument and their legs were held rigidly in an easily accessible position by a clamp applied to a screw in the tibia. The cats were paralyzed by intravenous injection of gallamine triethiodide (Davis and Geck, Flaxedil, 20 mg/h) and artificially ventilated (30–50 ml at 15–20/min). Rectal temperature was monitored and maintained at 37 $\pm$ 1°C. In some experiments, sodium pentobarbital (Diamond Labs., Diabutal, 35 mg/kg) was the anesthetic agent and all other procedures were identical. Supplementary doses of anesthetic agents were administered as required. No differences in cortical responses which could be attributed to the anesthetic agent were observed.

The right pericruciate cortex was exposed in 17 cats and covered with warm mineral oil. Swelling of the brain after exposure was prevented by injection of decamethasone sodium phosphate (Merck, Sharp and Dohme, Decadron, 2 mg, i.m.) or dexamethasone (Schering, Azium, 2 mg each i.v. and i.m.). In some experiments a double pneumothorax was produced to reduce pulsations of the cortex and small movements of the skin of the leg. Evoked potentials were recorded from a 3 $\times$ 4 array of 12 silver wires held 1.5 mm apart in a lucite plate but free to float up and down with cortical movements. The tips of the wires were polished to prevent injury to the cortical surface and vasculature. Signals were amplified by Grass AC amplifiers with time constant of 0.45 sec, then digitized at 2.0 kc/sec, averaged and plotted with standard deviations by an IBM 1800 computer.

The afferent activity was monitored from one branch of the posterior femoral cutaneous nerve (NCFP) which was exposed and bathed in warm mineral oil. Recordings, from a flattened silver-wire electrode, were made with respect to an indifferent in nearby tissues. The amplitude of the stimuli (short mechanical pulses) was adjusted to near threshold for a single action potential from each pulse. Individual spike potentials evoked by controlled cutaneous stimuli were observed in single oscilloscope sweeps synchronized with the stimuli and discriminated from ongoing activity on the basis of spike amplitude and/or known characteristic discharges of the cutaneous afferent fibers. This method proved to be most precise in determining such input. Because the stimuli were near threshold, action potentials were produced by only 60–90% of the stimuli.

In a few experiments a dorsal rootlet, which contained activity from a convenient area of skin, was exposed by laminectomy of the S_1 and L_7 spinal segments. The amount of afferent activity was observed by averaging activity in the dorsal rootlet, a procedure described by Brown and Tapper[4]. The integrity of the pathways to the cortex was checked by monitoring gross cortical potentials evoked by stimulation

of the NCFP or the isolated dorsal rootlet. No damage to the pathways was indicated in any of the experiments for which data are presented.

The active cortical sites were located by observing initially positive responses (temporal muscle indifferent) to NCFP or cutaneous electrical stimulation or to large area (1.6 sq. cm) mechanical stimulation. Once the sites were located an indifferent electrode was made by connecting together those cortical electrodes which showed no average evoked response to these stimuli or small stimuli applied either on tactile pads or on adjacent skin. Interference from large spontaneous slow waves of wide extent was effectively eliminated by use of this 'cortical indifferent' in most cases. Stimuli presented once per 3 sec for 100 trials gave the same amplitude average evoked potential (AEP) as stimuli presented once per 10 sec, however, when the repetition rate was increased to 1/sec, a reduction in amplitude was usually observed. Because of this attenuation, the repetition rate was maintained at 1/3 sec for all control records.

After the responses were observed with high amplitude stimuli, we used only precisely controlled minute mechanical skin displacements. Short duration (4 msec) mechanical pulses were delivered to the skin using a stimulator with a 200 μm diameter probe attached[29]. The position of the probe was observed with a dissecting microscope. At rest position, the probe just touched the surface of the skin. The well-known conditioning–testing procedure was employed to measure the aftereffects of potentials evoked in the cerebral cortex of 6 cats. One hundred responses to the testing stimulus were averaged with conditioning–testing intervals from 10 to 500 msec in each animal.

In 4 experiments the L_5 and T_8 spinal segments were also exposed by laminectomy and lesions were made in the dorsal (T_8) and the dorsolateral (L_5) columns. The dura mater was incised and control averages were computed just prior to the creation of the lesion using a pair of No. 5 jeweler's forceps and very fine scissors. Care was taken to avoid surface vessels and hemorrhage was always minimal.

In each animal the dorsal column was lesioned first and then successive lesions of the dorsolateral column were made until the cortical response disappeared. The location and extent of transection of the ascending pathways was determined by histological examinations of 25 μm sections stained with hematoxylin and eosin following formalin fixation.

RESULTS

Responses to gross stimuli

Responses to mechanical displacements of short duration were observed at cortical loci found by electrical stimulation of the NCFP or skin, or large mechanical displacements of the skin. Such responses were observed only at locations where a large, initially positive potential was elicited by gross stimulation. Loci which showed early negativity or no response to gross stimuli also showed no response to confined small mechanical stimuli. Late positivities were often observed in response to gross stimuli but seldom for short duration pulses. These late positivities were also observed

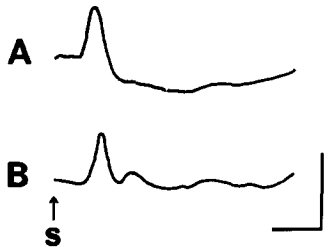

Fig. 1. Cortical responses to activation of specific receptors. Cortical AEPs following a single action potential/stimulus in a type I afferent (A) and in an afferent associated with a receptor located next to the tactile pad (B). Stimuli applied once every 3 sec. Cortical indifferent electrode. Time marked is 20 msec. Vertical bar represents 50 μV. The stimulus (S) occurred at the beginning of the trace, which contains 200 sample points.

at loci for which there was no early positivity. At these positions gross or small mechanical stimuli produced either no early response or an early negativity.

Effects of small mechanical pulses

In general, a cortical AEP was obtained with small mechanical pulses applied to the skin. Either one or two action potentials elicited in the NCFP by this stimulus were required to produce a monophasic positive averaged evoked potential with a latency of 12–20 msec. The duration of the response ranged from 25 to 40 msec and the amplitude measured 5–40 μV. These potentials were all recorded from the post-cruciate cortex; no such potentials were ever recorded from the precruciate cortex.

Stimuli of short duration and amplitude appropriate for production of a single action potential, or at most two, in the type I afferent produced a cortical response in most cases (Fig. 1A). That the action potential occurred in the appropriate afferent fiber was confirmed in all cases by application of the same stimulus to the skin immediately adjacent (*i.e.*, within a few hundred micra) to the tactile pad (off-pad). In many instances a cortical AEP was obtained following a single action potential evoked by the off-pad stimulus (Fig. 1B); however, this action potential, observed in the NCFP, could be distinguished by latency and/or amplitude from that produced by tactile pad stimulation.

The number of spike potentials necessary to produce cortical potentials with on-pad and off-pad stimuli was equally variable. In some off-pad loci one spike was sufficient; in others two spikes were required. A different response both in the nerve and in the cortex may be elicited by one spike from the proximal, distal, pre-axial and post-axial sides of the same tactile pad. It is clear from this observation that activation of Pacinian or similar receptors could not have produced the AEP evoked by stimulation of tactile pads. In addition, stimuli applied at some off-pad locations could evoke no spike potential in the nerve and no response in the cortex. This observation serves as a control against the possibility that unobserved activity was evoked over nerves with overlapping dermatomes. In no case could an AEP be seen when the stim-

ulating probe was lifted off the skin, thus the sound produced by the speaker cone of the mechanical stimulator did not cause any of the responses described here.

The latency from stimulus onset to the initial deflection of the cortical AEP was measured in 8 cats for stimuli on and off (adjacent to) tactile pads. The latencies in the two cases never differed by more than $\pm$ 2 msec, perhaps due to differences in peripheral conduction times. It was reliably observed that increasing the number of afferent action potentials elicited by tactile pad stimulation decreased the latency of the evoked cortical response while increasing its amplitude. Similar observations were not made for off-pad stimulation.

Areal extent of AEPs

Single action potentials produced positive AEPs at no more than 3 adjacent electrodes. Since the electrodes were placed on 1.5 mm centers, a maximum of 13 sq. mm of cortical surface contained the potential change recorded. The amplitude of response was greater at one electrode than at the others in every case, implying that the actual area of cortex activated by the stimulus was much less than this value.

Under some circumstances, evoked potentials could be seen in single oscilloscope sweeps following a single spike on the afferent fiber. It is likely that the electrode was lying directly on the focus of the response in these cases and when no such

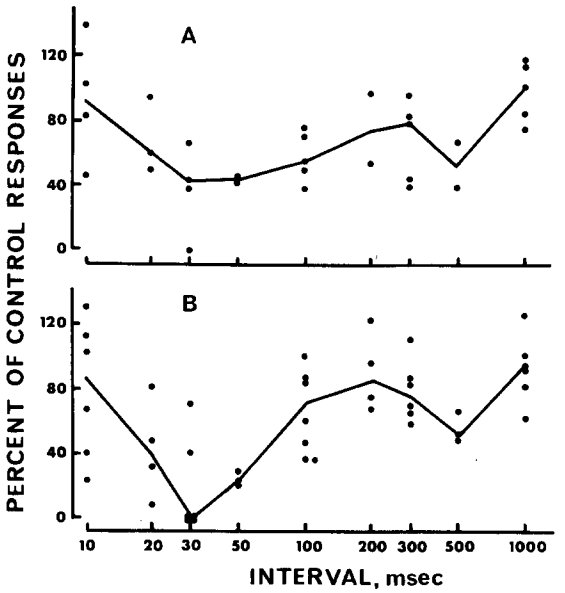

Fig. 2. Aftereffects of cortical AEPs. Amplitude of response to testing stimulus plotted as percent of control responses to single stimuli *vs.* the interval between conditioning and testing stimuli. In A are plots of responses to stimuli applied to tactile pads and in B responses to stimuli applied adjacent to the same tactile pads. All stimuli, double and single, presented once every 3 sec. At a given interval, every point represents the presentation of that interval to a different animal. Solid line joins median values at each interval.

response was visible the electrode was slightly off-focus (to use the terminology of Towe[34]). Thus the focus of the response is very small and responses which require averaging to be visible are merely due to potential spread from the focus.

The active locus was usually found by extensive exploration of the cerebral cortex. Once such a locus was found no further exploration was attempted. Thus it is possible that there were secondary active sites.

Interactions

Fig. 2A, B are plots of the amplitude of the response evoked by the testing stimulus of a conditioning–testing pair against the interval between the pair. In obtaining the data for Fig. 2A both the conditioning and testing stimuli were applied to a tactile pad within the receptive field of NCFP. The amplitudes are plotted as

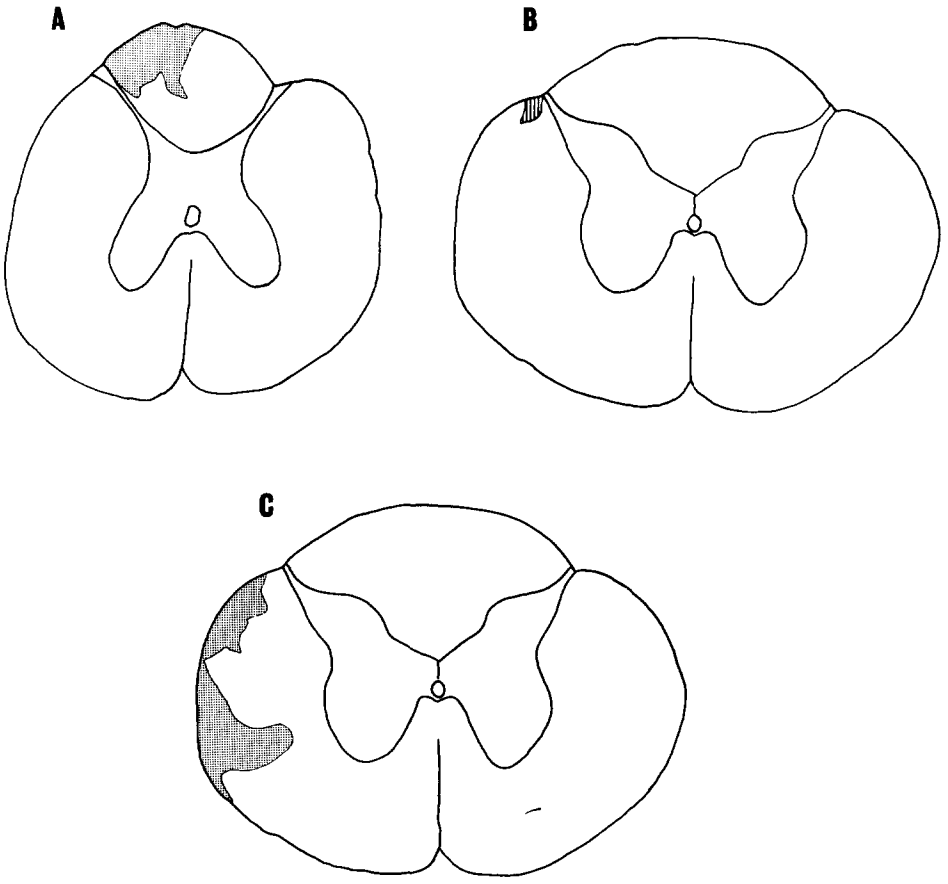

Fig. 3. Lesions of dorsal (DC) and dorsolateral (DLC) columns. A, Maximum extent of lesions made in DC at T_8, all ineffective. B, Area of DLC at L_5 common to all effective lesions. C, The sum of areas of all ineffective DLC lesions.

percent of the amplitudes of AEPs produced by the same stimulus delivered at a rate of one per 3 sec. The curve represents a composite of data from 6 animals and hence reflects responses from slightly different cortical locations. In Fig. 2B the same sort of plot is used for pairs of stimuli applied at a single skin location immediately adjacent to a tactile pad. It is clear that in both Fig. 2A and B there is an attenuation of response amplitude with a primary minimum at 30 msec. Perhaps there is a secondary minimum at about 500 msec but the sample size at this time period is too small to be certain. The early attenuation is similar to that described by McIntyre *et al.*[15] for Pacinian corpuscle evoked responses. At the 10 msec interval (Fig. 2A, B), there was a facilitation of the response in about 50% of the cases and an attenuation in the other 50%, however, the latter was less severe than at 20 or 30 msec. Although there was a good deal of variability in response at a given interval across animals, the variability for a single animal was less (not illustrated).

Lesions

Lesions made in the dorsal column at the eighth thoracic segment (T_8) of the spinal cord never decreased or abolished the evoked response following stimulation of a tactile pad. The maximum extent of the dorsal column lesions is shown in Fig. 3A.

On the other hand, some lesions in the dorsolateral column at the fifth lumbar segment (L_5) were observed to decrease or completely eliminate the response evoked by stimulation of tactile pads. Lesions which did disrupt the evoked potential always included the most dorsomedial part of the lateral column, the region usually assigned to the spinocervical tract (Fig. 3B). Lateral lesions which had no effect on the cortical potentials are illustrated in Fig. 3C.

DISCUSSION

Paralleling the results of McIntyre *et al.*[15], the present investigation demonstrates that single impulses initiated from cutaneous receptors have significant effects in the cerebral cortex. Single impulses in type I afferents and in other afferents with receptors near the tactile pad evoke positive cortical potentials and alter the excitability of the cortex for at least 1000 msec. The activity arrives at the cortex by way of fibers of the dorsolateral column and affects less than 13 sq. mm of the postcruciate cortex.

The efficiency of synaptic transmission in ascending tracts has been emphasized by many investigations (*e.g.* refs. 14, 18) and convincingly demonstrated by the experiments of McIntyre *et al.*[15]. It is striking that a wide variety of peripheral receptors, *i.e.*, Pacinian corpuscles, tactile pad receptors and others, have similar potency in the production of cortical AEPs.

The wave form and localization of AEPs described are consistent with repetitive firing of a few cortical elements. It is possible that amplification, *i.e.*, conversion of a single impulse into a burst of impulses, occurs at the spinal cord level at the first synaptic interruption. Amplifying effects at spinal synapses have recently been shown

for activity of type I afferents in cells of the lower lumbar and sacral dorsal spinal gray matter[32]. Hongo and Koike[9] observed a considerable temporal summation at spinal synapses following a single action potential in a cutaneous afferent fiber. Matthews[20] demonstrated in the frog that a single impulse in a single cutaneous afferent produced a dorsal root potential (DRP) visible in single oscilloscope sweeps. The occurrence of a DRP implies transmission through an internuncial chain to the primary afferent terminals. All amplification need not have occurred at the spinal level[21,23,27].

The depression of cortical response to a single action potential following a single conditioning potential is similar to that described by McIntyre *et al.*[15] from Pacinian corpuscle activation.

The cortical region from which unitary evoked potentials were isolated is the area described by Woolsey[35] as the pre-axial and lateral part of the post-axial leg region. Some difficulty was encountered in isolating tactile pad evoked potentials. Paul and Goodman[25] showed a separation of representation of slowly and rapidly adapting mechanoreceptors in the monkey's cortex in which the slowly adapting representation was predominantly in Brodmann's area 3 and the rapidly adapting in area 1. Such a separation, if it occurred in the cat, would explain this result.

This experiment clearly shows that the activity of type I afferents is carried in the dorsolateral column, corroborating the lesion results of Tapper's[30] behavioral study. The tractotomy experiments of Levitt and Levitt[11] apparently offer a contrast to this finding since they show no absence of touch-activated cortical units following dorsolateral column lesions, however, other receptors also give 'touch responses', *e.g.*, the intradermal receptors of type II afferent fibers[5].

Our effective lesions all involved the region of the spinocervical tract (SCT). Although Taub[33] and Brown and Franz[3] located no SCT axons which responded to stimulation of tactile pads, Mann *et al.*[17] found type I activity in cutaneous dorsal spinocerebellar tract (DSCT) axons and in cells of the L_6 and S_1 dorsal gray matter which send axons into the dorsolateral funiculus but not into the DSCT. Ha and Liu[8] showed that DSCT axons send collaterals into the LCN which accompany the collaterals from axons of cells in the dorsal gray of the lower lumbar and sacral spinal cord. Attempts to activate LCN neurons from the cerebellar cortex have, however, been unsuccessful. Some of these LCN inputs are not part of the SCT under the criteria employed by Brown and Franz[3] but might be considered part of the spino-cervico-thalamic systems.

SUMMARY

Averaged cortical evoked potentials were obtained from the postcruciate cortex of anesthetized cats in response to single action potentials over various cutaneous afferent channels. Type I afferents were particularly emphasized because of their well-defined receptive field and stimulus–response characteristics. Cortical potentials were evoked by one or two action potentials in single type I afferents or in other afferents which are associated with receptor structures near tactile pads. Such poten-

tials occurred after a latency of 12–20 msec, had a duration of 25–40 msec, had a maximum positivity of 5–40 μV, and engaged less than 13 sq. mm of cortex. In addition the second of two impulses found the cortical mechanisms depressed for up to 1 sec. These effects re-emphasize the security of somatic pathways to the cortex and provide evidence of fairly restricted projections for some afferent systems. Possible spinal pathways to the cortex are discussed.

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