Journal of Physiology (1993), **463**, *pp.* 475–489
With 6 figures
Printed in Great Britain

PYRAMIDAL AND CORTICOSPINAL SYNAPTIC EFFECTS OVER RETICULOSPINAL NEURONES IN THE CAT

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(*Received 27 April 1992*)

SUMMARY

1. The spontaneous activity of 103 precruciate neurones (fifty-eight activated antidromically from the pyramidal tract but not from the corticospinal tract, PTNs; forty-five activated from both sites, CSNs) was used to trigger the average of the intracellularly recorded synaptic noise in 294 reticulospinal neurones (RSNs). These RSNs were recorded in the nucleus reticularis gigantocellularis of the contralateral medial bulbar reticular formation (NRGc) in chloralose-anaesthetized cats.

2. Twelve pyramidal tract neurones (six CSNs) were tested with a single RSN, twenty-six (10 CSNs) with two RSNs each, thirty (13 CSNs) with three RSNs each, and thirty-five (16 CSNs) with four RSNs each. Postsynaptic potentials were observed in the averages generated by twenty PTNs and fifteen CSNs.

3. The only synaptic effect produced by both PTNs and CSNs upon RSNs in our sample was excitatory, and in none of the tested cases ($n = 15$) were any changes found in the amplitude, shape, or duration of the excitatory postsynaptic potentials (EPSPs) after injection of depolarizing or hyperpolarizing currents. This suggests that the synapses are probably located at the distal dendrites.

4. Recording of the presynaptic spike allowed separation of the conduction time and synaptic delay from the total latency. According to our data there appear to be two different types of excitation of corticofugal neurones over RSNs: a monosynaptic effect produced by both PTNs and CSNs, and a disynaptic effect produced by PTNs but not by CSNs. The disynaptic EPSPs had statistically significant slower rise times and longer widths than the monosynaptic EPSPs.

INTRODUCTION

Previous electrophysiological studies have investigated the synaptic effects produced by corticofugal fibres from the motor cortex on reticulospinal neurones (RSNs) (Magni & Willis, 1964; Peterson, Anderson & Filion, 1974; Pilyavsky & Gokin, 1978; He & Wu, 1985; Iwamoto, Sasaki & Suzuki, 1990). In these studies, electrical stimulation of the sensorimotor cortex and/or the cerebral peduncle was invariably used. It has been suggested that pyramidal tract (PT) and corticospinal (CS) collateral branches may be involved since postsynaptic potentials (PSPs) are observed in RSNs following pericruciate and cerebral peduncle stimulation (He & Wu, 1985). Anatomical (Keizer & Kuypers, 1984) and electrophysiological

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(Alstermark, Pinter & Sasaki, 1983*a*, *b*) data also point to a rapid disynaptic cortico-reticulospinal pathway. To our knowledge, no quantitative and systematic analysis has been previously undertaken of the possible differential postsynaptic effects produced by PT and CS branches on RSNs. Therefore, we decided to study the PSPs produced by individual PT and CS fibres in RSNs by using single-fibre spike-triggered averaging (STA) techniques. This probably allows a more objective approach than electrical stimulation because for STA we identified single corticofugal fibres which served to trigger the average of synaptic noise in antidromically identified reticulospinal neurones. The STA of synaptic potentials is a very sensitive method for detecting synaptic interactions. Since Mendell & Henneman (1971) first used STA to study unitary excitatory postsynaptic potentials (EPSPs) evoked by single spindle Ia fibres on motoneurone pools, the method has proved to be a very useful tool. It has subsequently been used, among many applications, to reveal previously unknown monosynaptic connections (Kirkwood & Sears, 1974), to detect disynaptic EPSPs (Watt, Stauffer, Taylor, Reinking & Stuart, 1976), to demonstrate Ia reciprocal inhibition (Jankowska & Roberts, 1972*b*), and to average analog signals from rectified electromyographic recordings (Fetz, Cheney & German, 1976) (for review, see Kirkwood & Sears, 1980; Fetz, Toyama & Smith, 1991).

METHODS

General

A total of forty cats (2·5–4·0 kg) were anaesthetized (α-chloralose, 60 mg kg^{-1}, I.P.), paralysed (Pavulon, 1 mg kg^{-1} h^{-1}, I.V.), and artificially respired (ventilation adjusted to maintain end-tidal CO_2 between 4 and 4·5 %). Additional doses of anaesthesia were administered (I.V.) when necessary. The depth of anaesthesia was assessed by monitoring the heart rate and by observing the state of the pupil. Changes in heart rate and dilated pupils, or pupils reacting rapidly to the used electrical stimuli were considered to reflect inadequate anaesthesia, and supplementary doses of 30–40 mg kg^{-1} of α-chloralose were injected every 4–6 h, as required. Rectal temperature was maintained at 37·5 ± 0·5 °C by an abdominal heating pad under servocontrol. Tracheal and venous cannulae were inserted and the animal was positioned in a stereotaxic frame. A craneotomy was performed over the motor cortex, and the pyramids were approached ventrally at mid-olivary level. A rostral C2–caudal C4 laminectomy was carried out, and the entire cerebellum was suctioned. After reflection of the dura mater, all the exposed tissues were covered with warmed mineral oil to avoid desiccation (see Fig. 1 for the general experimental arrangement).

Stimulation

In order to identify pyramidal tract and corticospinal neurones (PTNs and CSNs), concentric bipolar stimulating electrodes (0·5 mm intertip space, 40–50 μm diameter and insulated except at the tip) were placed in the pyramids (under visual guidance and ipsilaterally to the recording in motor cortex) and in the dorsolateral contralateral funiculus (DLF, at C3 level). In some experiments, a third stimulating electrode was placed stereotaxically (at A4) in the ipsilateral cerebral peduncle (CP). Stimulation through the PT electrode allowed optimal placement of the movable electrodes in CP and in DLF. Another stimulating electrode was positioned in the cord ipsilateral and caudal to the DLF electrode and at the level of the ventromedial funiculus (VMF, 0·5–1·5 mm lateral to the mid-line, 5–6 mm deep). This served to activate antidromically the reticulospinal axons coursing through the ventral funiculi (no distinctions were made either between ventromedial and ventrolateral or between ipsilateral and contralateral reticulospinal axons). A fine tungsten stimulating/recording electrode was also introduced together with the recording micropipettes in the nucleus reticularis gigantocellularis (NRGc) in order to search for PT and CS collaterals and for averaging the extracellular records of field potentials produced by the collaterals. Strong cathodal shocks of 0·05 ms duration and with intensities up to 1·5 mA were applied to all the stimulating electrodes, relative to a Ag–AgCl reference anode implanted subcutaneously in the lower back. None of the cells antidromically activated from the DLF were

ever excited by VMF stimulation, and none of the PTNs or CSNs were excited from the brainstem electrode by current spread to the PT (e.g. Figs 4*A*, 5*B* and 6*B*: the antidromic latency to brainstem stimulation was always longer than that to PT stimulation, thus precluding antidromic activation due to current spread from NRGc to the pyramidal tract).

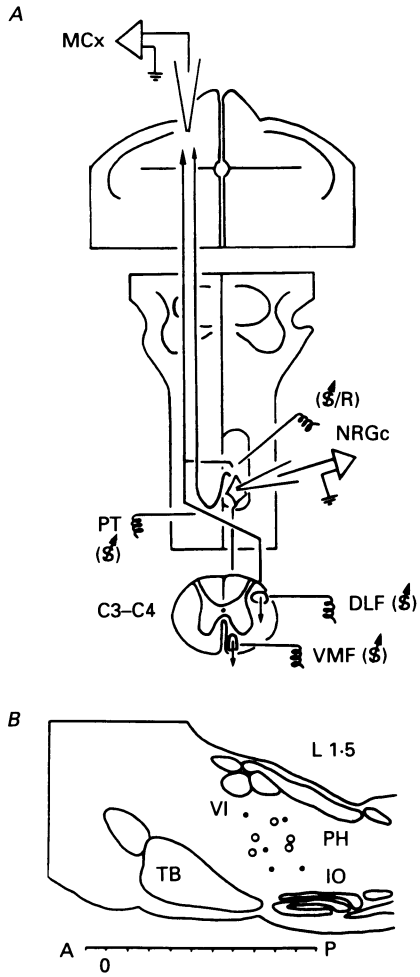

Fig. 1. *A*, experimental arrangement. *B*, diagram illustrating some of the sites where corticospinal (○) and pyramidal (●) effects on reticulospinal neurones were recorded. Abbreviations: MCx, intermediate precruciate motor cortex; NRGc, medial reticular bulbar formation (nucleus reticularis gigantocellularis); PT, pyramidal tract; DLF, dorsolateral funiculus; VMF, ventromedial funiculus; S, stimulation; R, recording; VI, abducens nucleus; PH, prepositus hypoglossal nucleus; TB, trapezoid body; IO, inferior Olive; L 1·5, 1·5 mm lateral to the mid-line.

Recording

Glass capillary micropipettes were drawn to 1–3 μm o.d. and filled with 2·5 M NaCl (impedance at 1 kHz in saline, 4–10 MΩ) for extracellular recording (occasionally AC–intracellular) within the precruciate motor cortex. Micropipettes of 0·5–2 μm o.d. were filled with 3 M sodium citrate (10–20 MΩ, measured *in vivo* at 1 kHz) for intracellular recording into the nucleus reticularis gigantocellularis (NRGc) contralaterally to the record in motor cortex (0·5–1·5 mm lateral to the mid-line; 2·5–5 mm deep, and at anteroposterior co-ordinates P6–P10). The micropipettes used to

record in the brainstem were made from thinner-walled glass than the cortical electrodes. A thinner wall yields a pipette with larger lumen and hence lower resistance, which allows easier injection of current. Pyramidal, corticospinal and reticulospinal neurones were classified as antidromically activated in accordance with standard criteria (e.g. Canedo & Lamas, 1989). The NRGc signals were fed to a DC high-input impedance amplifier equipped with a bridge circuit for intracellularly injecting current through the recording electrode. The recordings in motor cortex were AC coupled. The outputs from the amplifiers were monitored on oscilloscopes and stored on an FM magnetic tape (bandwidth DC–13·5 kHz) for further analysis.

Once a spontaneously active PT or CS neurone was isolated from the recording (only spontaneously active neurones were selected), the search for RSNs began. If the corticofugal cell was lost, another was found and intracellular recording in NRGc resumed. When a RSN was encountered, it was selected only if its resting potential was $\geqslant 50$ mV, and the STA was performed whether or not the RSN had spontaneous discharge. The validity of the STA method for averaging the synaptic PSPs produced by a single fibre depends on the absence of synchronized bombardment. This was the reason for selecting spontaneous units and for not using peripheral or chemical stimuli to fire the cells. Furthermore, peripheral (electrical shocks applied to the foot pads) as well as direct chemical (glutamate) stimuli evoked such a synchronization within precruciate cortex that discrimination of a single spike was very difficult. The trigger spikes were continuously monitored to avoid contamination of the average from spikes originating in other units in the recording with similar shapes and waveforms, as some of the simultaneously active neurones may send their axons to different levels of the corticospinal tract (Canedo & Towe, 1985). Two windows were cascaded for spike discrimination, one above and the other below baseline whenever various spikes had similar waveforms. The acceptance pulses of the second window served to trigger an averager whose input was the synaptic noise recorded in RSNs. In some cases it was possible to average the extracellular field potentials of the collaterals by the electrode previously used for antidromic activation (AA) (Fig. 6*E*2). The recording of the impulse in the presynaptic axon or its terminals (presynaptic spike) allowed separation of the conduction time and synaptic delay from the total latency, the latter being measured as the time from the foot of the triggering spike to the foot of the EPSP. The *a priori* assumption was made that the averaged presynaptic activity originated from the branch that made synaptic contact with the RSN. In those fibres in which the presynaptic spike could not be registered, the conduction time was calculated by subtracting 0·2 ms (assumed to be the utilization time for NRGc antidromic activation) from the antidromic latency to NRGc. In fact, the utilization times which were measured as the difference between AA and STA latency estimates in eighteen cases (Table 1) varied between 0·0 and 0·4 ms (mean $\pm$ S.D., $0{\cdot}22 \pm 0{\cdot}11$ ms).

Although in the deeply anaesthetized, paralysed animal, the sensory inputs are diminished and feedback produced by movement is absent, the possibility exists of synchrony between the cells generating the trigger spikes and other elements. Therefore, the signals were delayed (trigger spike with respect to itself) and, when possible, various spikes from the multiunit record in motor cortex were used as triggers. Negative results from synaptically unrelated cells and the absence of synchronized averaged potentials preceding that of the trigger spikes were taken as evidence that synaptic effects were not produced by third elements. The study was restricted to a maximum time interval of 10·24 ms following the trigger in motor cortex (1024 points per sweep, 10 μs per point, using a single channel of a Dagan-4800 signal averager, Dagan Corp, Minneapolis, MN, USA).

Histology

At the end of each experiment, the microelectrode was placed at the deepest point of recording in that experiment. The animal was then perfused with normal saline followed by 10% formalin, and the pontobulbar region was removed and post-fixed. Transverse 100 μm frozen sections were cut, mounted serially, stained, and the locations of recording sites were determined. The recordings were restricted to nucleus reticularis gigantocellularis (Fig. 1).

RESULTS

In order to be sure that the recordings were from the soma of the neurone, only antidromic responses to VMF stimulation showing clear breaks into initial segment–somadendritic components were selected (e.g. Fig. 2*A*). In extracellular

recordings, antidromic activation of reticulospinal cells often produced superimposed, synchronous potentials (e.g. Fig. 2*B*). These potentials originated from different units as demonstrated by the collisions shown in Fig. 2*C* and *D*. Figure 2*B* illustrates the characterization of a cluster of two reticulospinal units (traces *a* and

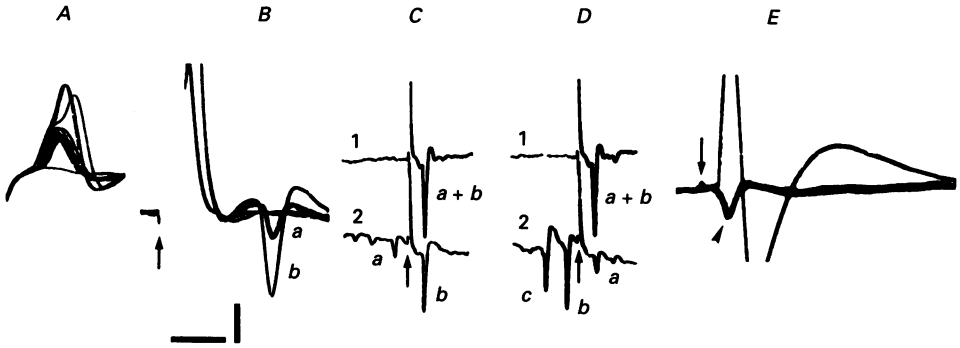

Fig. 2. *A*, identification of a reticulospinal cell (the stimulus artifact served to trigger the oscilloscope) at high frequency stimulation (200 Hz). As the stimulation proceeded, the spike broke into its initial segment–somadendritic components and then disappeared. *B–D*, ventromedial funiculus stimulation, at increasing intensities in the cervical cord, evoked the antidromic activation of two superimposed spikes in NRGc (traces *a* and *b*). Suprathreshold stimulation of the tract fired both units (*C*1 and *D*1), and when the spontaneous discharge *a* or *b* preceded the stimulus at the adequate interval the collision occurred, leaving only spike *b* (*C*2) or spike *a* (*D*2). Refractory periods were 0·7 ms for spike *a* and 0·8 ms for spike *b*. In the multiunitary recording of *D*2 a third non-reticulospinal spike (*c*) was also present. *E*, AC-intracellular recording of a reticulospinal neurone. Subthreshold antidromic stimuli (artifact marked by an arrow) for the impaled cell evoked a negative field potential (arrowhead), presenting the same latency as did the single unit. Stimulus artifacts marked by arrows. Voltage bar: *A*, 30 mV; *E*, 10 mV. For extracellular recordings in *B–D*, voltage bar: 50 μV. Time bar: *A*, 1 ms; *B* and *E*, 2 ms; *C* and *D*, 11 ms. *C* and *D* single sweeps. Positivity upwards for these and all subsequent records.

b). Stimulation increasing the strength from subthreshold for unit *a* to suprathreshold for unit *b*, demonstrated clear superposition of both potentials. Both units were spontaneously active and collision tests could be performed (Fig. 2). This synchrony in antidromic activation was also evident in some intracellular records. Figure 2*E* shows superimposed traces of a reticulospinal neurone intracellularly recorded (the recording was AC coupled). Juxtathreshold stimulation in the VMF demonstrated that at subthreshold intensities a clear negative field potential was evident intracellularly which had exactly the same latency as the impaled unit. This field potential probably reflected other units in the neighbourhood of the recorded neurone, which were responding antidromically at lower intensities. Clusters of superimposed, antidromic responses were also recorded in precruciate cortex as previously described (Canedo & Towe, 1985).

The data obtained are summarized in Table 1. The technical difficulties in finding connections between corticofugal and RSNs restricted our sample. A total of 294 reticulospinal neurones (Fig. 3) were intracellularly recorded and maintained long enough to accomplish averages generated by at least 512 trigger events. A total of

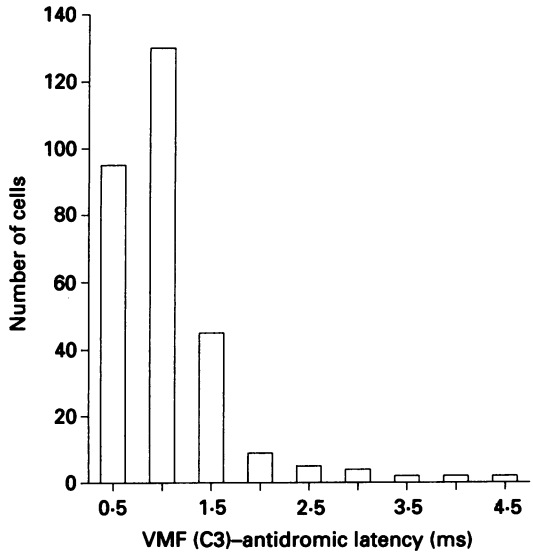

Fig. 3. Histogram illustrating the antidromic latency distribution to VMF stimulation of the reticulospinal neurones intracellularly recorded and tested for corticofugal connections ($n = 294$). Bins are 0·5 ms width and centred on the stated latencies.

TABLE 1. Synaptic effects produced by PT and CS neurones upon RSNs

	Antidromic latency (ms)			NRGc (STA latency) (ms)	EPSP latency (ms)	EPSP (TTP) (ms)	EPSP durat. (ms)	Synaptic delay (ms)
	PT	DLF	NRGc					
PTNs (non-CS)								
Fast ($n = 17$)	1·5±0·4	—	1·9±0·5	1·7±0·4 ($n = 6$)	2·6±0·9	0·9±0·5	4·5±1·2	0·9±0·5
Slow ($n = 3$)	4·6±2·3	—	6·4±2·4	6·2±2·3 ($n = 3$)	7·0±2·9	0·8±0·5	5·6±2·0	0·8±0·5
Total ($n = 20$)	1·9±1·4	—	2·5±1·9	2·3±2·4 ($n = 9$)	3·2±2·0	0·8±0·5	4·7±1·3	0·9±0·5
CSNs								
Fast ($n = 13$)	1·4±0·3	3·3±0·7	2·0±0·6	1·8±0·5 ($n = 7$)	2·3±0·6	0·5±0·1	4·07±0·57	0·5±0·1
Slow ($n = 2$)	2·8 2·5	5·9 4·8	3·4 3·4	3·3 3·3	3·8 3·9	0·2 0·4	4 4	0·5 0·6
Total ($n = 15$)	1·6±0·5	3·5±1·0	2·2±0·7	2·0±0·7 ($n = 9$)	2·5±0·7	0·4±0·1	4·0±0·5	0·5±0·1

Values are presented as means±S.D., except for the two slow CSNs for which individual values are included. The presynaptic spikes could be recorded on only eighteen of the thirty-five cases in which connections were positive. The real conduction times from motor cortex to NRGc (STA latencies) are therefore restricted to these eighteen neurones. Abbreviations: PT, pyramidal tract, bulbar location; DLF, dorsolateral funiculus, cervical location; NRGc, nucleus reticularis gigantocellularis; STA, spike-triggered averaging; TTP, time to peak; durat., duration.

103 spontaneously active precruciate neurones (fifty-eight activated from the pyramidal tract but not from the corticospinal tract, PTNs; forty-five activated from both sites, CSNs) were tested with these RSNs. Averaged postsynaptic

potentials were found in thirty-five cases: twenty PTNs and fifteen CSNs could be correlated with the averaged synaptic noise in RSNs. Although both classes of corticofugal neurones were used as counterparts with more than one RSN, we did not find postsynaptic potentials from a single fibre to more than one RSN. Twelve

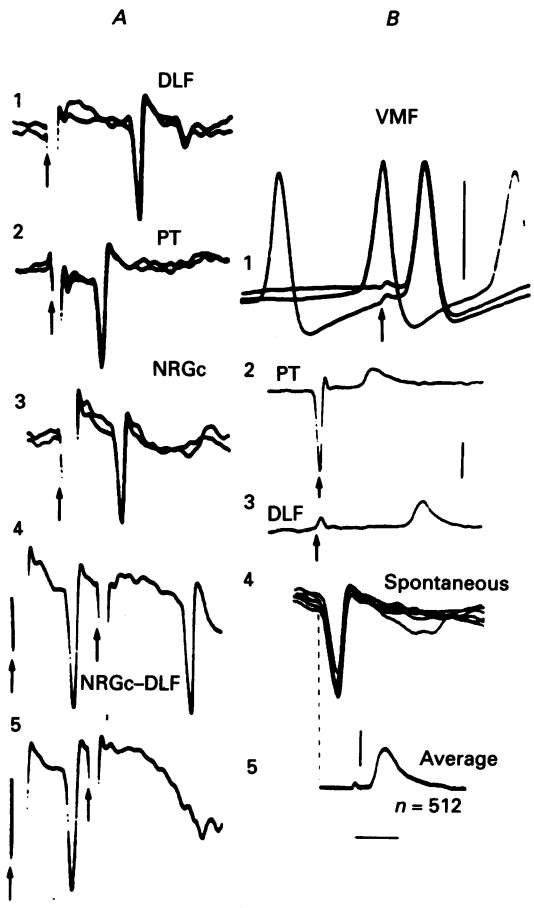

Fig. 4. *A*, antidromic identification of a CSN (*A*1 and 2) branching into the contralateral NRGc (*A*3). Collision between antidromic spikes at NRGc and at DLF is illustrated in *A*5. *B*, antidromic identification of an RSN by stimulation of VMF (*B*1). The superpositions in *B*1 show collision between spontaneous and antidromic spikes in one of the sweeps. Electrical stimulation at the PT (*B*2) and at the DLF (*B*3) evoked EPSPs in the RSN with a difference in latency of 1·5 ms. Superpositions of the spontaneous spikes produced by the CSN and which served to trigger the average of synaptic noise in the RSN (*B*5, 512 triggers) are shown in *B*4. Time bars, 2 ms; bar under panel *A* applies to *A*1–5 and *B*1–3; bar under panel *B* applies to *B*4 and 5. Voltage bars: *B*1, 50 mV; *B*2 and 3, 4 mV; *B*5, 50 μV. For extracellular recordings in *A*1–5 voltage bar in *B*5 represents 25 μV. The arrows point to the stimulus artifacts.

corticofugal neurones (six CSNs) were tested with a single RSN, twenty-six (ten CSNs) with two RSNs each, thirty (thirteen CSNs) with three RSNs each, and thirty-five (sixteen CSNs) with four RSNs each.

The only synaptic effect of corticofugal neurones upon RSNs we have encountered was excitatory, and in none of the ten monosynaptic and five disynaptic EPSPs which were tested did we find any change in the amplitude, shape or duration of the averaged EPSPs after injecting depolarizing or hyperpolarizing currents through the recording electrode (up to 1·5 nA).

The probability of finding an RSN correlated with a corticofugal fibre was reasonably high; this was possible because the tip of the stimulating electrode for searching for collaterals in NRGc was close to the recording micropipette (200–400 μm). Because of the necessity of having a stable recording, our sampling bias toward large RSNs is clear. Mean distance from VMF stimulation site to NRGc recording site was 60 mm. Antidromic conduction velocities for RSNs ranged from 12·7 to 150 m s^{-1} with a mean of 60 m s^{-1}.

Examples of the EPSPs encountered are illustrated in Figs 4, 5 and 6. Figure 4*A* shows the antidromic identification of a CSN (*A*1 and 2) sending a branch to the contralateral NRGc (*A*3). Collision between the spikes evoked at NRGc and at DLF is illustrated in *A*5 (collision interval = 2·8 ms). The neurone had spontaneous activity and it could be correlated with the averaged synaptic noise of the RSN antidromically identified by stimulation of VMF (Fig. 4*B*1). The superimposed traces of Fig. 4*B*1 show that whenever a spontaneous spike appeared preceding the stimulus, and at the adequate interval, the collision occurred. Traces 2 and 3 of Fig. 4*B* show that stimulation in the PT and DLF evoked EPSPs in this RSN. Traces 4 and 5 illustrate the STA technique used to study precruciate–reticular connections. After 512 triggers, an EPSP is apparent in the average record of the intracellular recording in NRGc (Fig. 4*B*5), and the arrival of the impulse in the presynaptic axon or its terminals can also be seen as a small positive wave. The appearance of the presynaptic spike allowed separation of the conduction time and synaptic delay from the total latency (time from the foot of the trigger spike to the foot of the EPSP).

The synaptic delay was calculated from the positive peak of the presynaptic spike when it was intracellularly recorded, or from its negative peak when it was recorded by the electrode previously used for antidromic activation (considered to be the instant at which the signal passes nearby the recording electrode) to the foot of the EPSP (Fig. 6*E*2). Thus, the synaptic delay of the EPSP shown in Fig. 4*B*5) is about 0·7 ms, and is therefore considered as a monosynaptic excitation.

Figure 5 illustrates an example of a PTN, not activated by corticospinal tract stimulation, which we postulate produced a disynaptic EPSP on an RSN. This interpretation is based on the following argument. The antidromic latencies to PT and to NRGc stimulation were 1·6 and 2 ms, respectively. The collision interval (PT–NRGc) was 0·9 ms, and the refractory period for NRGc stimulation was 0·5 ms. The latency from the trigger spike to the foot of the EPSP was about 3·3 ms (traces 4 and 5 of Fig. 5*B*). If a synaptic delay of 0·7 ms is assumed, then the conduction time from the motor cortex to NRGc will be 2·6 ms. If we also assume utilization time of 0·2 ms, then the real conduction time would be 1·8 ms, which added to a single synaptic delay of 0·7 ms gives a total of 2·5 ms for the expected latency from the trigger spike to the beginning of the rising phase of the EPSP. However, the difference between the measured and the expected times was of 0·8 ms (3·3–2·5), which could indicate either additional conduction time through the brainstem or the

existence of a disynaptic contact. Similar results were observed with seven other PTNs which could not be activated from the corticospinal tract, but in no other cases studied when using CSNs as triggers. Further arguments favouring the idea of disynaptic contacts produced by these eight neurones on RSNs are the slower rise

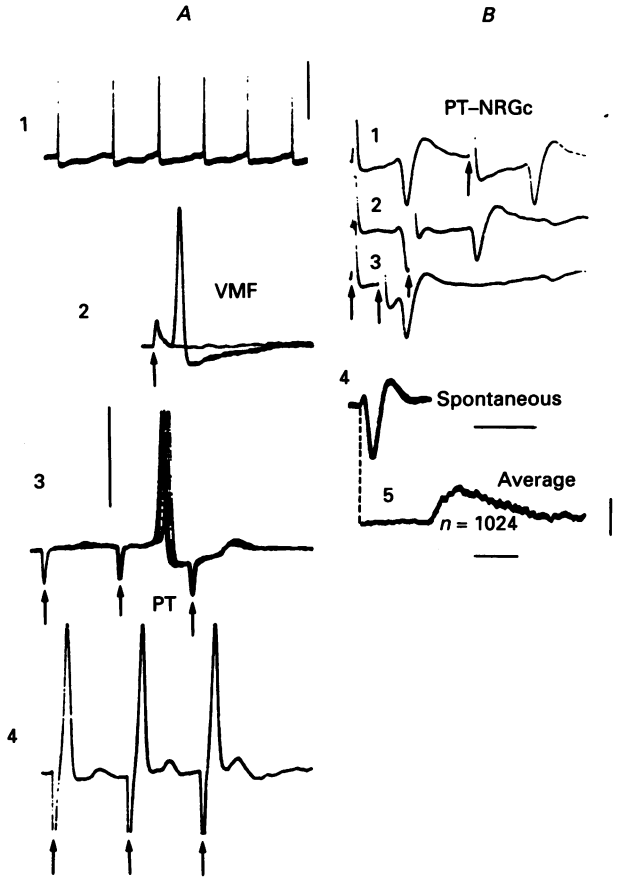

Fig. 5. *A*, antidromic identification of an RSN by stimulating VMF (*A*2). Its spontaneous discharge is illustrated in *A*1. Stimulation at the PT with trains of three pulses each (4·8 ms interpulse interval) evoked EPSPs in the RSN (*A*3 and 4). Note that increased intensities of PT stimulation produced a decrease in latency of the EPSPs as the stimulation proceeded (*A*4). *B*, a pyramidal non-corticospinal neurone branching into NRGc was isolated and antidromically identified. Collision between antidromic responses evoked at PT and NRGc is shown in *B*3. The spontaneous spikes of the PTN (*B*4) served to trigger the synaptic noise of the RSN (*B*5). Time bar under *B*4 applies to *A*1–4 and *B*1–4: *A*1, 40 ms; *A*2–4, 4 ms; *B*1–4, 2 ms; Time bar under *B*5: 2 ms. Voltage bars: *A*1–4, 50 mV; *B*5, 25 μV. For extracellular recordings in *B*1–4, voltage bar: 50 μV. Stimulus artifacts are marked by arrows.

times ($1{\cdot}45 \pm 0{\cdot}25$ ms, $n = 8$ *versus* $0{\cdot}61 \pm 0{\cdot}78$ ms, $n = 27$) and longer widths ($5{\cdot}93 \pm 0{\cdot}94$ ms, $n = 8$ *versus* $3{\cdot}98 \pm 0{\cdot}68$ ms, $n = 27$) shown by the averaged EPSPs they produced when compared with the monosynaptic EPSPs (e.g. Figs 4 and 6). The

U test indicated that both the rise times and widths of the disynaptic EPSPs were significantly greater than those of monosynaptic EPSPs ($P < 0{\cdot}01$). Furthermore, the differences between the expected monosynaptic delays (around 0·7 ms) and the measured values ranged from 0·6 to 0·9 ms (mean $\pm$ S.D., $0{\cdot}75 \pm 0{\cdot}12$ ms). Finally, the amplitude of the postulated disynaptic EPSPs averaged 15 μV (range, 7–25 μV) whereas the amplitude of the EPSPs presumed to be monosynaptically produced averaged 35 μV (range, 15–70 μV). Note also that PT stimulation at strong intensities produced shortening of the EPSP latency and a direct spike (Fig. 5A). This antidromic activation is probably due to spread of current to the reticulospinal axon as it passes medial and close to the inferior olive as shown by Grantyn, Ong-Meang Jacques & Berthoz (1987) for some reticulospinal axons. Mean antidromic latencies to PT stimulation, at bulbar level, were 2·4 ms (range, 1–7·4 ms) for the eight PTNs producing the postulated disynaptic EPSPs, 1·6 ms (range, 0·6–3·3 ms) for the twelve PTNs producing monosynaptic EPSPs in RSNs, and 1·2 ms (range, 0·6–2·8 ms) for the fifteen CSNs producing monosynaptic EPSPs on RSNs. Mean antidromic latencies to NRGc stimulation were 3 ms (range, 1·3–9 ms), 2·3 ms (range, 1–6·1 ms) and 2 ms (range, 0·7–3 ms) for PTNs producing disynaptic and monosynaptic EPSPs, and for CS collaterals evoking monosynaptic EPSPs in RSNs, respectively. Because the presynaptic spike could not be recorded in any of the eight cases in which disynaptic EPSPs were detected, true conduction times (STA latencies) cannot be compared with STA latencies observed in the fibres producing monosynaptic EPSPs. Thus, it appears that the PTNs producing disynaptic EPSPs are, on average, slower conducting than the PTNs and CSNs responsible for direct synaptic contacts, in agreement with data obtained using electrical stimulation (Pilyavsky & Gokin, 1978). None of the RSNs with antidromic latencies of 1·8 ms or longer (Fig. 3, $n = 24/294 = 8{\cdot}1\%$) to VMF stimulation could be demonstrated to receive synaptic contact from the corticofugal fibres tested. Furthermore, no correlation was found between the conduction velocities of the RSNs and the latencies of the EPSPs, except that all of the EPSPs were recorded in RSNs having antidromic latencies of 1·7 ms or less.

A particularly illustrative example of monosynaptic connection of a PTN on a RSN is shown in Fig. 6. Both recordings in motor cortex and in NRGc were intracellular (this was the only case in which pairs of intracellular recordings could be correlated). The motor cortex cell fired very irregularly and in bursts of four to eight spikes (Fig. 6A). Antidromic latencies to stimulation of cerebral peduncle, to PT and to NRGc were 0·5, 0·8 and 1·2 ms, respectively (Fig. 6B). The RSN recorded simultaneously had an antidromic latency of 1·7 ms (Fig. 6D). Figure 6E shows the averaged spontaneous cortical spike ($E1$) which served to trigger the recording of field potentials registered by the electrode in NRGc previously used for antidromic identification ($E2$, 5000 triggers). The conduction time from the motor cortex to NRGc was about 1·2 ms, and the synaptic delay was close to 0·5 ms, which indicates that the effect is clearly monosynaptic.

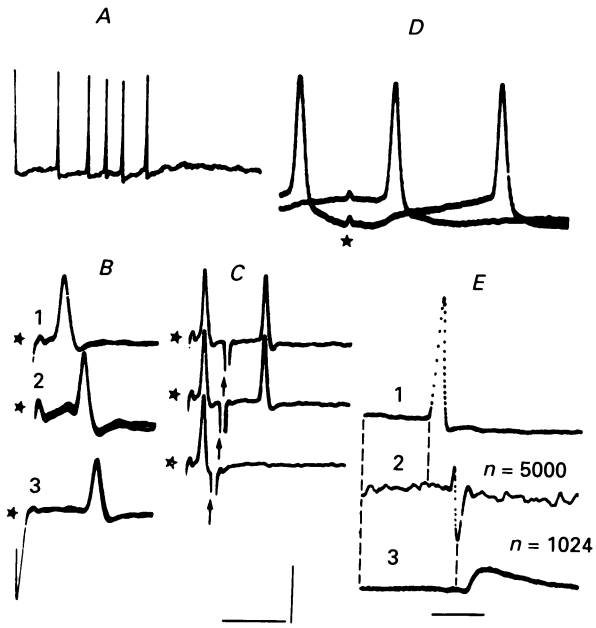

Fig. 6. Intracellular recording of a pyramidal non-corticospinal neurone (*A*) branching into NRGc. Antidromic responses to cerebral peduncle (CP), to PT, and to NRGc stimulation sites are successively shown in *B*1–3. Collision between antidromic spikes elicited at CP and at NRGc is illustrated in *C*. An RSN was simultaneously recorded within NRGc (*D*). In one of the sweeps of *D*, collision between a spontaneous and an antidromic spike occurred. The averaged records in *E* were delayed 2·5 ms (trigger spike in *E*1 with respect to itself) for easy comparison and measurement of all three signals. The presynaptic spike of the corticofugal collateral was averaged through the electrode previously used for antidromic identification (*E*2), and it served to estimate the synaptic delay for the EPSP produced on the RSN (*E*3). Time bar under *C* applies to *A*–*D*: *A*, 20 ms; *B*, 1 ms; *C*, 2 ms; *D*, 3 ms. Time bar under *E*3 applies to *E*1–3: 2 ms. Voltage bar applies to all panels: *A* and *D*, 50 mV; *B* and *C*, 35 mV; *E*1, 40 mV; *E*2, 10 μV; *E*3, 30 μV. Stimulus artifacts marked by arrows and stars.

DISCUSSION

Previous studies have demonstrated the existence of clusters of cortical neurones identified by antidromic stimulation of the corticospinal (Humphrey & Corrie, 1978; Canedo & Towe, 1985) and rubrospinal (Canedo & Lamas, 1989) tracts. The presence of similar clusters in the reticulospinal system (e.g. Fig. 2) indicates that this might be a general phenomenon, at least in the motor system. This probably reflects the synchronization necessary for a group of neighbouring neurones to produce summated effects on their target cells. The proximity of a group or cluster of corticofugal neurones will often mean that they are linked together by common afferent input and/or recurrent synaptic effects, which will tend to synchronize their activities.

Previous electrophysiological studies have shown that many reticular neurones

receive short-latency excitation from the pericruciate cortex (Magni & Willis, 1964; Peterson *et al.* 1974; Pilyavsky & Gokin, 1978; He & Wu, 1985; Iwamoto *et al.* 1990). It has also been proposed that 22·6% of the EPSPs observed in RSNs following stimulation of the pericruciate cortex and internal capsule were di- or polysynaptic (Pilyavsky & Gokin, 1978). A detailed anatomical report by Keizer & Kuypers (1984) demonstrated the existence of both PT fibres and CS collaterals into NRGc, a result which was recently corroborated in our laboratory using an electrophysiological approach (J. A. Lamas & A. Canedo, unpublished observation). Thus, direct and indirect control of corticofugal cells over RSNs previously demonstrated by electrical stimulation has now been confirmed for single PT and CS neurones by the present spike-triggered averaging study.

The main conclusions for the neurones sampled in this study are that both PT and CS collaterals make monosynaptic contact with RSNs, and that disynaptic EPSPs are produced by PT but not by CS collaterals. Furthermore, both monosynaptic and disynaptic EPSPs are unaffected by polarizing currents, indicating that the synapses could be located distant from the soma. The proposed disynaptic nature of some of the EPSPs is favoured by their slower rise times, long duration and by their smaller amplitude when compared with monosynaptic EPSPs. To detect disynaptic EPSPs using STA, the intercalated interneurone must be active, and the variation in its firing time will introduce changes in the latency of the EPSP and thus slow the rise time of the average. A disynaptic link is apparently weaker than a monosynaptic one, but the addition of effects produced by an unknown number of interneurones may multiply the effect of a single disynaptic interaction. Also, the delays showed by these EPSPs are consistent with a disynaptic linkage. However, slowing of conduction in presynaptic axonal branches and/or terminals (Jankowska & Roberts, 1972*a*; Shinoda, Yamaguchi & Futami, 1986) might be partly responsible for the estimated delays. The effect of a single fibre seems to be quite small, and the convergence of many fibres would be required to produce a significant excitation on RSNs. Such a convergence from various afferent sources does occur (Peterson, Franck, Pitts & Daunton, 1976), although evidence of convergence of motor cortex fibres onto individual RSNs is based on electrical stimulation. Compound EPSPs elicited by strong stimulation of the PT in our sample of twenty-seven RSNs receiving monosynaptic connections from corticofugal fibres averaged 2·3 mV (range, 0·9–4 mV). Since the individual EPSPs in the same RSNs averaged 35 μV, it seems that about sixty-five cortical fibres would converge, on average, on these RSNs. However, since the amplitude of unitary EPSPs were estimated from averaged records, they do not necessarily indicate the true amplitude of postsynaptic unitary potentials, and the number of cortical cells which produced the compound EPSPs might be overestimated.

Whether or not the corticofugal fibres projecting into NRGc serve to regulate the background activity of RSNs remains to be determined, but their role could well be to facilitate the excitation of RSNs by other afferents (vestibular, tectal, cerebellar, etc.) in order to produce a co-ordinated motor behaviour. The intermediate precruciate cortico-reticulospinal connections appear to be segregated into three categories: 'pure' non-PT corticobulbar cells (J. A. Lamas & A. Canedo, unpublished observation) whose synaptic effects upon RSNs remained to be elucidated, PT

collaterals producing mono- and disynaptic EPSPs, and CS collaterals producing exclusively monosynaptic EPSPs. Due to the small sample and to the necessity of having a stable intracellular recording of RSNs, our results are probably biased towards large reticulospinal neurones and therefore the possibility that CS collaterals could also produce di- or polysynaptic potentials on RSNs cannot be excluded.

Pyramidal and CS neurones with collaterals to NRGc are concentrated in those parts of the sensorimotor cortex where back, neck and shoulder muscles are represented (Keizer & Kuypers, 1984; this study). The role of the reticulospinal system has been particularly stressed in relation to the control of axial and proximal muscles (see Peterson, 1979). However, co-contraction of numerous muscles occurs in all kinds of movement. Movement of distal musculature and postural adaptations are not independent. Each movement of any limb must be accompanied by an adjustment in posture. The results of Massion and collaborators (for references, see Massion, 1979) in cats, point to a significant role of the pyramidal tract in synergies regulating movement and posture. Furthermore, Berthoz & Grantyn (1986) have shown that some RSNs are involved in complex motor synergies such as orienting movements of the eye, the head and the ears. Alstermark *et al.* (1983*a*) demonstrated that after transection of the pyramid at the obex, stimulation rostral to the lesion still evoked disynaptic EPSPs on contralateral neck motoneurones. This effect was probably mediated by bulbar reticulospinal neurones since stimulation of the pyramid rostral to a transection at the level of the trapezoid body did not produce synaptic effects, as described by the above authors.

Corticofugal neurones branch extensively at both spinal and supraspinal levels (Tsukahara, Fuller & Brooks, 1968; Endo, Araki & Yagi, 1973; Shinoda, Arnold & Asanuma, 1976; Humphrey & Corrie, 1978; Canedo & Towe, 1986; Shinoda *et al.* 1986). Reticulospinal cells in the nucleus reticularis pontis caudalis also branch profusely in the pontobulbar reticular formation (Grantyn *et al.* (1987) as well as at different levels of the cord (Peterson, Maunz, Pitts & Mackel, 1975). Both RSNs (Peterson *et al.* 1975) and CSNs (Shinoda *et al.* 1986) project to different segments in the spinal cord. The corticobulbar connections, including the CS collaterals, could help to maintain the background activity of RSNs at the same time as both CSNs and RSNs control the excitability of spinal neurones. It is well known that fast PTNs are associated with rapid arm movements and display phasic properties. Many of them fire in bursts shortly before a movement (for references see Wiesendanger, 1981); if some of those are fast corticospinal cells with collaterals into NRGc then their function would be to increase the background depolarization of RSNs and thus augment the efficiency of incoming signals before and during a particular movement. This background activity could be further enhanced by the slow PTNs contacting RSNs. The slow PTNs are well known to produce tonic, sustained discharges during the movement. Thus, movement synergies and the associated postural adjustments could be controlled by the same neurones regulating the movement. The plasticity of these interactions would, however, be regulated according to the incoming information (tectal, vestibular, proprioceptive, cerebellar). In fact, changes in the functional connections between reticulospinal cells and motoneurones have recently been inferred from experiments in unanaesthetized cats when at rest and walking (Drew, 1991).

This work was supported by a grant from the Direccion General de Investigacion Cientifica y Tecnica (PB89–0552). José A. Lamas is a fellow of the Formacion Personal Investigador program (Spain).

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