On-going and reflex synaptic events in rat superior cervical ganglion cells

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1. Synaptic events evoked by brief noxious cutaneous stimuli were recorded in sympathetic neurones in the superior cervical ganglion of anaesthetized rats.

2. On-going excitatory synaptic potentials (ESPs) and/or action potentials (APs) were recorded in 69% of neurones at mean frequencies that varied from 0·01 to 6·3 Hz in different cells. From histograms of ESP amplitude during membrane hyperpolarization, it appears that most cells received one (52%), or two or more (36%), suprathreshold inputs and several subthreshold inputs with overlapping amplitudes.

3. Pinching the skin for 1–3 s evoked either a brief burst of synaptic events (lasting about 300 ms) preceding a few seconds of inhibition (burst–inhibitory (BI) neurones), or simply an excitation (excitatory (E) neurones), or no response (O neurones). In 60% of BI neurones, a second burst occurred after the end of the pinch.

4. BI neurones had a higher frequency of on-going synaptic activity (2·9 ± 0·5 Hz, $n = 15$) than E neurones (0·2 ± 0·1 Hz, $n = 5$) or O (0·2 ± 0·1 Hz, $n = 5$) neurones. Most neurones with two or more suprathreshold inputs were BI neurones. In 20% of neurones (all BI with high rates of synaptic activity), several other inputs had ESPs with amplitudes close to threshold.

5. Subthreshold and suprathreshold inputs responded in the same way in only 45% of neurones, but suprathreshold inputs were excited in 73% of BI and all E neurones. The order of recruitment of different inputs varied from trial to trial. If classification was based only on suprathreshold responses, there were 36% BI, 32% E and 32% O neurones.

6. In the majority of neurones, postganglionic discharge was initiated exclusively by suprathreshold inputs, even during reflex excitation.

7. Qualitatively similar, but smaller, responses were evoked by a puff of air on the abdomen in 71% of cells tested.

8. The data suggest that the natural discharge of SCG neurones is largely determined by the activity of one or two preganglionic inputs with high quantal contents. BI neurones may include vasoconstrictor neurones, whereas the other types include secretomotor, pilomotor and other neurones projecting to targets in the head.

The sympathetic nervous system consists of several different functional subsystems, each supplying a particular type of target tissue and thus being involved in the regulation of a specific function. In anaesthetized animals, as well as in conscious humans, sympathetic axons can be recognized as belonging to a particular subsystem on the basis of their characteristic reflex discharge patterns which are appropriate for the observed target tissue responses (Wallin & Fagius, 1988; Jänig & McLachlan, 1992). Because similar reflex patterns are found in subsets of both preganglionic and postganglionic neurones, it has often been assumed that sympathetic ganglia act primarily to relay signals that have been integrated at spinal levels. Thus, the centrally coded message is not distorted during transmission across ganglia (Jänig, 1995).

In sympathetic ganglia, there is considerable convergence and also divergence of preganglionic axons. For example, in the rat superior cervical ganglion (SCG), on average nine preganglionic axons have been estimated to make synaptic contact with each postganglionic neurone and each preganglionic axon to form synapses on about 240 ganglion cells (Purves & Lichtman, 1985; Purves, Rubin, Snider & Lichtman, 1986). These complex interconnections are not

easy to reconcile with the idea of function-specific pre- and postganglionic pathways that directly relay impulses. Transmission of the pattern of discharge may reflect co-ordinated summation of preganglionic synaptic responses (Skok & Ivanov, 1983). However, another way to preserve functional specificity during transmission through ganglia would be to limit action potential (AP) generation to only a few individual preganglionic inputs. In *in vitro* studies, the number of inputs to a ganglion cell has been studied by graded stimulation of the preganglionic nerve trunk or individual white rami. Postganglionic paravertebral neurones always seem to receive one (some two) preganglionic inputs that generate large responses which are suprathreshold for initiation of an AP ('strong' or 'dominant' inputs; Holman & Hirst, 1977; Skok & Ivanov, 1983; Hirst & McLachlan, 1984), whereas the majority of inputs are subthreshold (weak) and generate only excitatory synaptic potentials (ESPs). It is unresolved whether the discharge behaviour of postganglionic neurones is determined only by strong inputs or whether ESPs, after summation, also contribute to the natural activity of postganglionic neurones.

In the present study, we made intracellular recordings from neurones in the SCG of anaesthetized rats to test: (i) whether or not transmission of impulses involves summation of convergent weak inputs during on-going and/or reflex activity, (ii) whether all preganglionic inputs to a given neurone show the same response to a particular natural stimulus, and (iii) whether functionally distinct types of preganglionic axon can be distinguished from the synaptic activity in the rat SCG, as has been demonstrated in the lumbar sympathetic outflow (Häbler, Jänig, Krummel & Peters, 1994) and cat cervical sympathetic trunk (CST) (Boczek-Funcke, Dembowsky, Häbler, Jänig, McAllen & Michaelis, 1992). When single preganglionic inputs are activated in *in vitro* experiments, the amplitudes of the evoked ESPs vary over about 5 mV, because of variation in quantal content between responses (McLachlan, 1975). We assumed that peaks in the amplitude histograms of on-going ESPs, recorded with the membrane held at hyperpolarized membrane potential (V_m) so as to block AP initiation, would identify individual active preganglionic inputs. In addition, we attempted to elicit changes in synaptic activity by examining responses to noxious cutaneous stimuli. Such stimuli have proven useful to discriminate between sympathetic subsystems in previous studies on cats and rats (Jänig, 1985; Boczek-Funcke *et al.* 1992; Häbler *et al.* 1994).

METHODS

Female Wistar rats (12–13 weeks old; 180–245 g body weight) were anaesthetized with either urethane (1·0–1·3 g kg^{-1} I.P., 5 animals) or sodium pentobarbitone (45 mg kg^{-1} I.P., 8 animals). Surgical anaesthesia was maintained throughout the experiments by supplementary doses of anaesthetic (0·125 g kg^{-1} urethane, 0–15 mg kg^{-1} sodium pentobarbitone), given intravenously through a catheter in the right saphenous vein, sufficient to abolish fluctuations in mean arterial blood pressure (MABP) and heart rate under control conditions and to suppress blink to light touch of the cornea and all limb withdrawal reflexes, including during the experimental pinches. All experimental protocols were approved by the University of New South Wales Animal Care and Ethics Committee.

Each animal was placed supine and allowed to breathe spontaneously through a tracheal cannula. MABP was monitored via a catheter in the caudal artery. The urinary bladder was continuously drained via a thin polyethylene cannula passed through the urethra. Rectal temperature was maintained between 33 and 35 °C by a feedback-controlled heating blanket. Core temperature was intentionally kept low in an attempt to maintain high levels of cutaneous vasoconstrictor activity and facilitate the detection of inhibitory effects (Häbler *et al.* 1994).

The neck of the animal was opened via a ventral mid-line incision. After removal of the overlying muscles and salivary glands, the left aortic nerve and bilateral laryngeal nerves were sectioned in order to prevent them from being activated by the mechanical interventions in the neck and producing unwanted depressive effects on respiration and MABP (Bartlett, 1986). The left SCG and CST were isolated from the carotid artery, retaining adjacent connective tissue and blood vessels. The internal and external carotid nerves projecting from the SCG were each cut 1–2 mm from the ganglion. The SCG was then pinned onto a small rubber platform leaving 1·0–1·5 cm of the CST free so that movements of the animal would not disturb the ganglion. From the time when the SCG was freed from the carotid artery, the preparation was covered with oxygenated physiological salt solution (PSS; for composition see Davies, Ireland & McLachlan, 1996). In most experiments, just prior to intracellular recording, the PSS was replaced with warmed (35 °C) paraffin oil bubbled with 95% O_2–5% CO_2, but two preparations were superfused throughout the recording period with oxygenated PSS warmed to 35 °C (Ivanov & Purves, 1989).

Arterial pH, P_{O_2} (P_{a,O_2}) and P_{CO_2} (P_{a,CO_2}) were monitored in 0·2 ml samples of arterial blood from the caudal artery cannula prior to and during recording. The inhaled air was supplemented with O_2 to keep P_{a,O_2} at a minimum of 100 mmHg. Blood parameters (means ± S.E.M.) during the recordings reported here were: P_{a,O_2}, 126 ± 7 mmHg; P_{a,CO_2}, 51 ± 2 mmHg; and pH 7·3 ± 0·1. Blood volume in the animals was maintained by replacing withdrawn blood with saline.

Intracellular recordings from ganglion cells were made using microelectrodes filled with 0·5 M KCl (resistance 60–100 MΩ) using methods described in detail previously (Cassell, Clark & McLachlan, 1986; Davies *et al.* 1996). MABP, body temperature, V_m and current were displayed and stored on a MacLab/S system (ADI Pty Ltd, Castle Hill, NSW, Australia) using 1 kHz sampling frequency for voltage and current and 200 Hz for MABP and temperature. In addition, intracellular current and voltage recordings were simultaneously digitized at 1–5 kHz, stored on a PC and analysed as described previously for *in vitro* studies (Cassell *et al.* 1986; Cassell & McLachlan, 1987). Passive membrane properties were determined at intervals during the impalement from the voltage transient evoked in response to a small hyperpolarizing current step. Initially, hyperpolarizing DC was passed through the recording microelectrode to expedite sealing of the cell after impalement. Many of the records were taken with the membrane held close to −60 mV by hyperpolarizing current (0·01–0·5 nA) in order to aid recognition of the responses to individual preganglionic

inputs; this also reduced the probability that some of the larger ESPs would reach threshold (see Results and Discussion). Recordings of synaptic activity were usually made in bridge mode (to reduce high frequency noise) after checking that the electrode resistance was correctly balanced in single-electrode current-clamp mode. Membrane current could be recorded under single-electrode voltage-clamp conditions only when recordings were made under oil. Measurements of resting V_m were taken when the microelectrode was withdrawn from the cell. Rates of on-going synaptic activity of less than 0·1 Hz were always determined on records more than 6 min in duration. It should be noted that, as in *in vitro* studies, all neurones had been acutely axotomized, however, the changes in membrane properties that follow axotomy occur over several days (Purves, 1975; Sanchez-Vives & Gallego, 1993).

Noxious mechanical stimuli were delivered briefly (usually for 1–3 s) by firmly pinching the skin of the plantar surface or across the phalanges of the ipsilateral or contralateral hindpaw, or the skin of the abdomen, with toothed (Edson) forceps as described previously (Häbler *et al.* 1994). In some experiments, the internasal septum, the ipsilateral forepaw or the ipsilateral ear were pinched in the same way. The duration of the pinch was recorded manually by switching on and off a marker signal. The time of onset of the pinch was identified from the abrupt change in synaptic activity nearest to the marker and/or the onset of the change in MABP. The duration of the pinch was taken from the recorded markers. Recovery intervals of at least 1 min were allowed between pinches.

Once the SCG was covered with paraffin oil or perfused with PSS, the experiments continued for 2–6 h, after which the animal was killed with an I.V. overdose of sodium pentobarbitone. Synaptic activity in oil-covered preparations did not persist as long as that in PSS-perfused preparations. We noted in some of the oil-covered preparations that the activity in a cell suddenly changed over a few minutes from the typical pattern described by others (Skok & Ivanov, 1983; Ivanov & Purves, 1989), with maximum frequencies of synaptic events of less than 10 Hz, to a more or less continuous discharge of very small amplitude ESPs which could be recorded for periods of up to 1 h. After this change in activity, reflex responses were very rare and most subsequent impalements yielded only cells without synaptic activity. We interpret this barrage of small ESPs as 'degeneration release' of quanta of transmitter (for example see Nikolsky, Oranska & Vyskocil, 1996) due to depolarization of the preganglionic terminals by hypoxia. Recordings from cells showing this behaviour and those from all subsequently impaled cells in the same preparation were discarded.

The responses of individual SCG neurones to brief pinches were analysed by constructing peristimulus time histograms. Data were included in the quantitative analysis only if the following criteria were met: (i) MABP was at least 70 mmHg and stable; (ii) V_m was more negative than −40 mV and stable; (iii) baseline noise was no more than 2 mV so that synaptic events could be clearly distinguished; and (iv) APs which occurred either spontaneously or in response to a depolarizing current step were more than 60 mV in amplitude.

Frequency of synaptic events (subthreshold/weak and suprathreshold/strong) was determined from the number occurring in consecutive 5 s periods for 25 s before the pinch, in 1 s periods for 5 s from the start of the pinch and in 5 s periods from 5 to 25 s after the pinch. Changes in frequency over several trials were averaged and related to mean frequency in the period before the pinch. Maximum and minimum frequencies, and the time bins in which they occurred, were determined from the highest and lowest values following the pinch (Table 2). Excitation was also characterized by the number of synaptic events and the burst duration, whereas the duration of any subsequent inhibition was measured from the last synaptic event in the burst until the next synaptic event (Table 3, Fig. 8).

Data are presented as means ± standard error of the mean (S.E.M.) except where specified. Generally, mean data for each cell (i.e. averaged responses to several pinches) were averaged to provide the overall mean values for each group of neurones. Statistical significance ($P < 0·05$) was determined using appropriate non-parametric tests (Mann–Whitney U test, Kruskal–Wallis test or Wilcoxon signed-rank test for matched pairs; Statview®, Abacus Software). Differences between slopes of regression lines were tested using Student's t test on slope coefficients. To determine the significance of the changes in discharge following the pinch, Fisher–Pitman randomization tests were performed (Krauth, 1988), using small sample procedures. This test examined the likelihood that the responses in the first and second time bins after the onset of the pinch differed from the on-going activity levels determined in 10 × 1 s bins both before and after the pinch.

RESULTS

General observations

Upon impalement, SCG neurone resting V_m was often low and input resistance was 50 MΩ or more, but these normally increased over the next few minutes. Synaptic activity was detected in 69% of cells during impalements lasting longer than 1–2 min (103 cells in ganglia from 13 animals).

In the subpopulation in which nociceptive reflexes were studied, input resistance (R_{in}) was 139 ± 21 MΩ, input time constant (τ_{in}) 14 ± 2 ms and derived cell input capacitance 107 ± 8 pF ($n = 19$, see Fig. 1*A a*). Resting V_m ranged from −40 to −71 mV (mean, −50 ± 1 mV; $n = 23$). During long (250 ms) suprathreshold depolarizing current steps, all neurones discharged phasically (Fig. 1*A b*; Cassell *et al.* 1986; Davies *et al.* 1996). Steady-state current–voltage relations in the subthreshold V_m range were linear in the hyperpolarizing range to potentials more negative than −110 mV in thirteen of seventeen cells (76%; Fig. 1*A c*); in the remaining cells, slight inward rectification was present at potentials more negative than −85 to −90 mV (see Cassell *et al.* 1986; Wang & McKinnon, 1996). When the V_m was held at −58 ± 3 mV, APs were 89 ± 1 mV in amplitude and had half-widths of 1·71 ± 0·14 ms ($n = 7$) (Fig. 1*B a*). Threshold voltage was 23 ± 2 mV ($n = 7$) positive of this holding potential (i.e. −35 mV). In the absence of synaptic activity, after-hyperpolarizations (AHPs) were −9·2 ± 1·9 mV in amplitude, 294 ± 49 ms in duration and 62 ± 17 ms in half-width ($n = 5$) (Fig. 1*B b*). The outward tail current underlying the slow component of the AHP (recorded under voltage-clamp conditions at resting V_m, see Cassell *et al.* 1986) was 0·15 ± 0·03 nA peak amplitude and had a single exponential decay with a time constant (τ) of 107 ± 15 ms ($n = 6$, Fig. 1*B c*). A slower component of the tail current lasting more than 1·5 s was clearly present in

one of these neurones (not shown). These properties are similar to those recorded in the rat SCG neurones *in vitro* (Sanchez-Vives & Gallego, 1993; Davies *et al.* 1996).

The data reported here come from twenty-five cells in eleven animals in which nociceptor reflexes were tested and which satisfied the criteria detailed in Methods. These impalements were held for 39 ± 9 min (range, 5 min to 3 h). MABP during recording periods was slightly higher under anaesthesia with pentobarbitone (91·6 ± 2·6 mmHg, $n = 19$) than that under urethane (81·6 ± 3·5 mmHg, $n = 5$) ($P = 0{\cdot}08$, Mann–Whitney U test). Any differences in cell properties and the frequency and form of on-going synaptic activity were not statistically significant between neurones recorded under either anaesthetic or in either bathing solution, and reflex responses of all patterns (see below) were observed in similar proportions (Table 1). We therefore pooled the data regardless of anaesthetic or bathing conditions.

On-going synaptic activity

The majority of neurones showed on-going synaptic activity (88%) which almost always included APs (Figs 2*A* and 6*A*). The frequency of synaptic events over several minutes of recording was <0·1 Hz in 16% of cells, 0·2 to < 1 Hz in

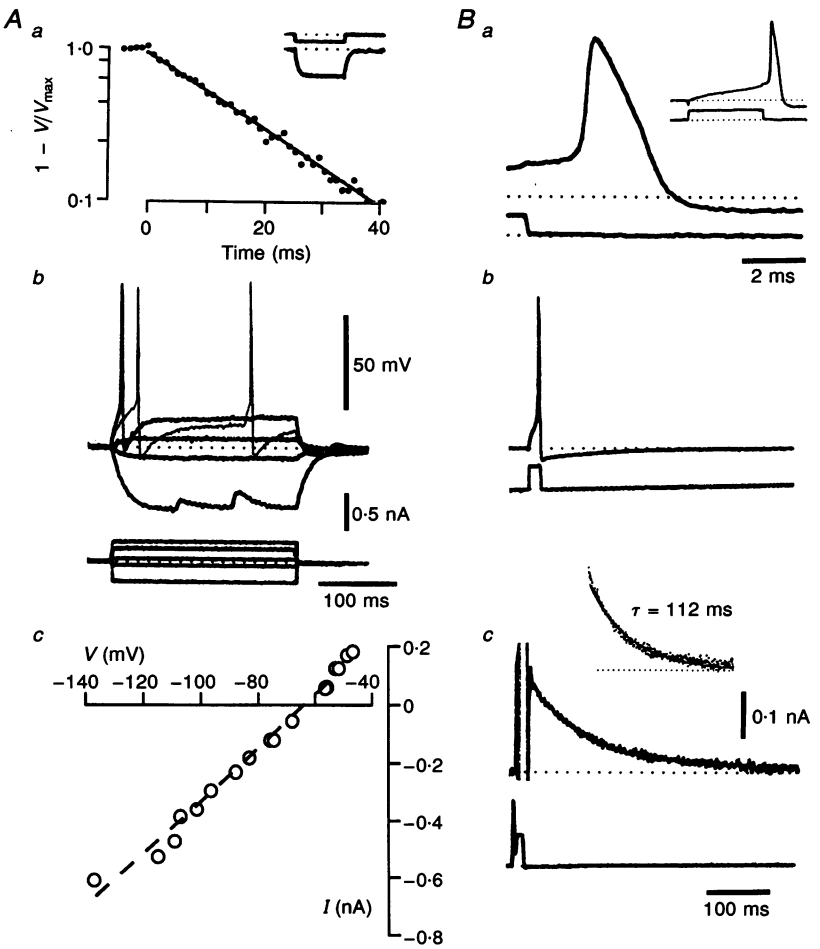

Figure 1. Properties of rat SCG neurones *in vivo*

A a, passive electrotonic response to a small hyperpolarizing current step (0·05 nA for 250 ms, inset) is a single exponential (plot). V is membrane potential change at various times after onset of current step, V_{max} is the steady-state level of membrane potential reached during the current step. From this analysis, R_{in} was 113 MΩ, τ_{in} 14·8 ms and cell capacitance 131 pF. *A b*, records of V_m changes (upper traces) during passage of 250 ms current steps (lower traces). Thicker depolarizing trace shows phasic discharge, thinner trace shows a second AP arising from a synaptic potential by summation. Note also ESPs during large hyperpolarization. *A c*, steady-state current–voltage relation in the subthreshold range of V_m, showing no inward rectification. *Ba*, AP recorded in another SCG neurone (upper trace) following a depolarizing current step (0·32 nA for 15 ms, lower trace, see inset). *Bb*, AP in the same cell as shown in *Ba* on a slower time base, showing the AHP. *Bc*, outward tail current (upper trace) recorded under voltage-clamp conditions following initiation of an active response by a voltage step from −60 to −40 mV (lower trace). This current was fitted by a single exponential with a time constant of 112 ms (see inset). Voltage calibration in *A b* applies throughout *B*. Voltage in inset in *A a* is shown at twice this gain, that in the inset in *Ba* is shown at half this gain. Current calibration in *A b* applies to lower traces in *Ba* and *b*. Time calibration in *Bc* applies also to *Bb*.

Table 1. Comparison of neurones recorded under different conditions

	Resting V_m (mV)	R_{in} (MΩ)	τ_{in} (ms)	On-going frequency: Suprathreshold (Hz)	On-going frequency: Subthreshold (Hz)	Excitation (Hz)	Inhibition (Hz)	Reflex type: BI (%)	Reflex type: E (%)	Reflex type: O (%)
A. Animals under different anaesthetics										
Urethane	−52 ± 1	203 ± 74	14·2 ± 4·8	0·6 ± 0·2	1·7 ± 0·8	+2·3 ± 0·7	−1·1 ± 0·4	66	17	17
n	5	5	5	6	6	6	6			
Pentobarbitone	−48 ± 2*	107 ± 11	13·5 ± 1·3	0·3 ± 0·1	1·4 ± 0·4	+1·4 ± 0·3	−0·9 ± 0·2	58	21	21
n	18	14	14	19	19	19	19			
B. Ganglia under different bathing conditions										
Paraffin oil	−50 ± 1·2	163 ± 38	14·2 ± 2·4	0·5 ± 0·1	1·1 ± 0·4	+1·6 ± 0·6	−0·8 ± 0·2	61	8	31
n	11	10	10	13	13	13	13			
PSS	−49 ± 3	113 ± 15	13·1 ± 1·9	0·2 ± 0·1	1·9 ± 0·6	+1·4 ± 0·2	−1·1 ± 0·4	58	33	8
n	12	9	9	12	12	12	12			

*$P \leq 0{\cdot}05$, Mann–Whitney U test.

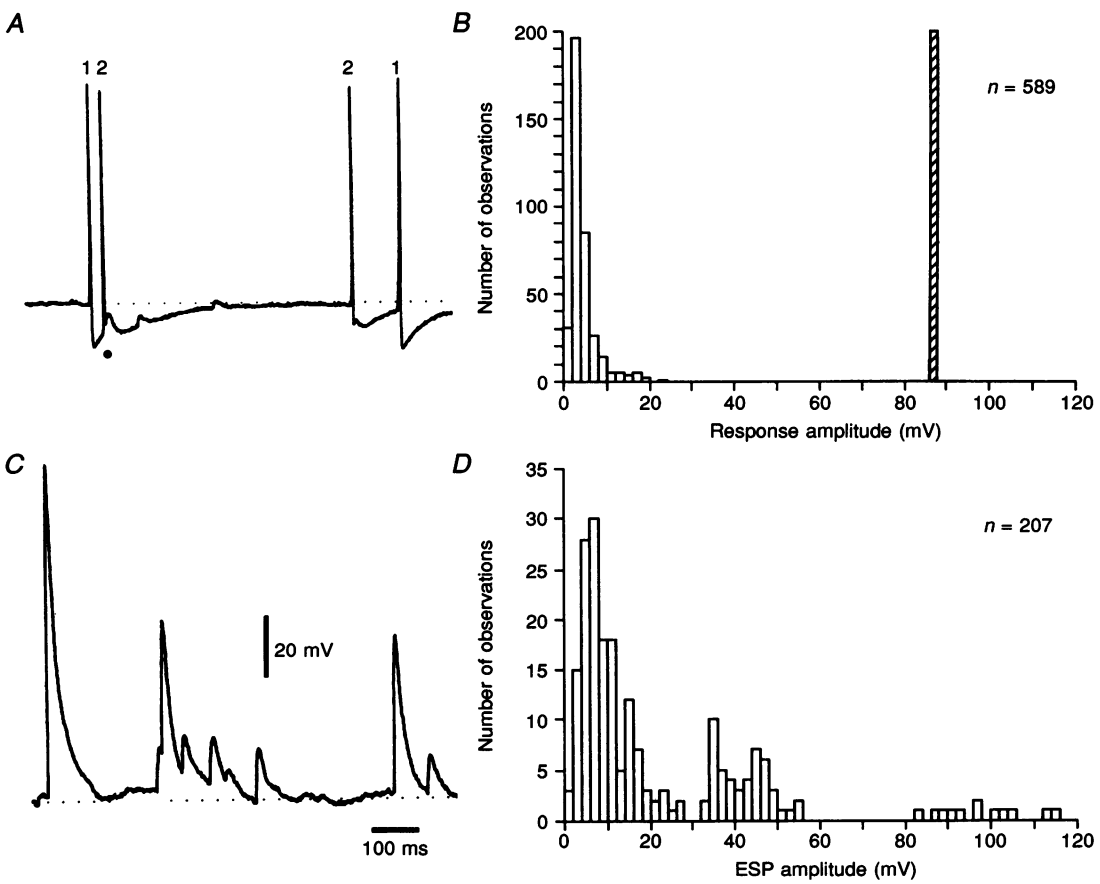

Figure 2. On-going activity in an SCG neurone

A and *C*, records of V_m at resting V_m (−50 mV) (*A*) and during hyperpolarization to −130 mV (*C*) to block AP generation. Calibrations in *C* apply also to *A*. At least two suprathreshold responses in *A* (1 and 2) can be distinguished by the configuration of their AHPs. The suprathreshold (first 2) event occurring during the AHP after the first AP (first 1) had a larger after-depolarization (●) than that indicated by the second 2 because of the greater driving potential. *B* and *D*, frequency histograms of ESP amplitudes at resting V_m (*A*) and during hyperpolarization to −130 mV (*B*). Hatched column in *B* indicates APs generated at resting V_m. Hyperpolarization revealed at least two populations of very large ESPs (> 25 mV).

32% of cells and ⩾1 Hz in 52% of cells. Synaptic activity varied markedly between cells in the same experiment and there was no relation between the frequency of synaptic events (either sub- or suprathreshold) and MABP.

The on-going frequency of synaptic events determined over a 15 s period prior to the stimulus was 1·8 ± 0·4 Hz (range, 0–6·3 Hz; $n = 25$). This frequency did not differ from the on-going frequency determined over periods of 1·0–4·5 min ($n = 5$). Only one neurone lacked any detectable on-going or reflex activity (not shown). Synaptic events were identified as suprathreshold (strong) or subthreshold (weak). APs usually arose with little inflection on the rising phase and were followed by AHPs lasting up to 400 ms (Fig. 2*A*).

Subthreshold ESPs recorded at −50 to −60 mV decayed exponentially with a time constant (16·3 ± 1·7 ms; $n = 7$, paired data) similar to or slightly longer than the passive membrane time constant determined from small electrotonic responses (13·9 ± 2·4 ms, $n = 7$, $P = 0·09$, Wilcoxon signed-rank test). The longer time course of the ESP results from the slow second component of synaptic current present in rat autonomic neurones (Callister & Walmsley, 1996). However, if the resting V_m was less negative than −45 mV, the falling phases of larger ESPs had undershoots indicating membrane rectification. At these more depolarized resting V_m values, threshold for the AP was only ~12 mV positive of resting V_m (Fig. 2*B*).

Subthreshold ESPs occurred more frequently (1·5 ± 0·4 Hz) than APs (0·4 ± 0·1 Hz). As recordings from most cells were made with the membrane hyperpolarized by ~10 mV, the rates of AP discharge at resting V_m was probably underestimated. For example, in one cell, in which a high frequency of synaptic events was recorded at both potentials, the frequency of APs at resting V_m (1·8 Hz) was more than twice that (0·8 Hz) recorded with the membrane 10 mV more negative. However, in some cells in which the frequency and amplitude of subthreshold synaptic events was low (<2 Hz), the frequency of APs at resting V_m was

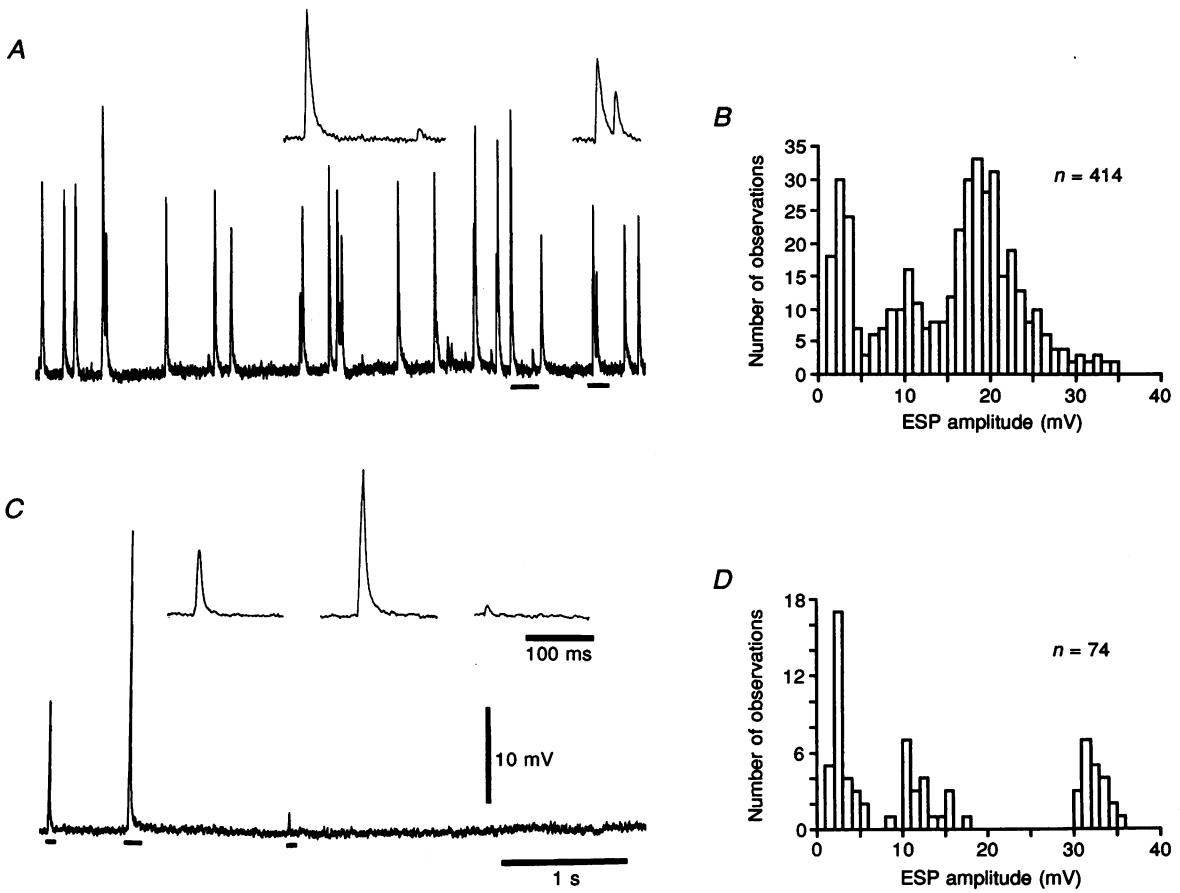

Figure 3. On-going activity and frequency histograms of ESP amplitude from two other SCG neurones

A and *C*, records of on-going activity with the membrane hyperpolarized to −80 mV (*A*) and −85 mV (*C*) to block AP generation. Insets at half the gain show some of the ESPs (at times indicated by bars below the records) on an expanded time base. *B* and *D*, frequency histograms of ESP amplitudes at −80 mV (*C*) and −85 mV (*D*). Calibrations in *C* apply also to *A*. At resting V_m, all events ⩾18 mV initiated APs. Cell in *A* had several inputs with amplitudes 15–35 mV that contributed to discharge at resting V_m, whereas cell in *C* had only one input that was suprathreshold.

the same as that when the cell was hyperpolarized by 10 mV because the ESPs underlying the APs were uniformly large (> 25 mV at −80 mV, see below).

When the membrane was hyperpolarized to −80 mV or more negative potentials, most APs were blocked and it was possible to determine the amplitude of the underlying ESPs (see Figs 2 and 3). In thirteen of fifteen cells (87%), small amplitude ESPs (peak amplitude, <8 mV) were the most prevalent. There was usually a trough in the histogram between 5 and 10 mV (in 83% of cases) and a second broader peak about 15–18 mV that gradually declined towards 30 mV (Fig. 3*B*). Several convergent inputs must have contributed to this higher amplitude peak as it was too wide to be accounted for by quantal variance of a single input (McLachlan, 1975). Assuming that the reversal potential for acetylcholine is close to 0 mV (Yawo, 1989), many of these responses would have evoked APs at resting V_m. In neurones with very low levels of on-going activity (Fig. 3*C*), it usually appeared that only a few inputs were active.

On the assumption that individual inputs can be identified by peaks in the ESP amplitude histogram, it was often possible to deduce the presence of individual strong inputs. In addition, particular suprathreshold inputs could sometimes be recognized by the configuration of their AHPs. In eight neurones (32%), some APs had a fast rising phase and were followed by an after-depolarization of relatively constant amplitude (see Figs 2*A*, 4*D* and 6*A b* and *d*). When such cells were hyperpolarized to more negative than −90 mV, the largest ESPs had modal amplitudes greater than 35 mV. In one of these cells, one group of APs was not blocked until the membrane was hyperpolarized to −130 mV, revealing ESPs with at least two modes of amplitude at ~45 and ~100 mV (Fig. 2*D*). In four other neurones, there was a second mode of ESP amplitudes that was just suprathreshold at resting V_m (e.g. Fig. 6*B*). In another four cells, two modes were just suprathreshold at resting V_m. Five cells received only one consistently suprathreshold input. Thus, suprathreshold inputs varied considerably in the size of the underlying ESP (i.e. quantal content), with only a proportion of them having a very high safety factor. Overall, thirteen neurones (52%) appeared to receive one strong input and nine neurones (36%) at least two. In the other cells, the largest ESPs occasionally reached threshold but the majority of ESPs recorded in all cells were too small and their frequency was too low to contribute significantly to neuronal discharge (e.g. Figs 2*A* and 5*A*).

Responses to pinching the skin in various body regions

Pinching the skin of the foot or abdomen for 1·9 ± 0·1 s produced small reflex changes in MABP which varied slightly in size and direction depending on the depth of anaesthesia. Overall, the initial change in MABP was +1 ± 1 mmHg over 5 ± 1 s followed by a fall of 4 ± 1 mmHg lasting 7 ± 2 s ($n = 22$).

In twenty of twenty-five neurones (80%), the pinches evoked either an increase or a decrease in the frequency of synaptic events. From the amplitude and configuration of these events, it was evident that the changes in synaptic activity almost always involved several of the active preganglionic inputs as well as recruiting silent ones. Excitatory and inhibitory responses were observed in different cells in a single animal and were not correlated with the existing level of MABP (correlation coefficient squared, $r^2 < 0·2$), so that the different responses are unlikely to have reflected differences in depth of anaesthesia or physiological state. Furthermore, the changes in synaptic activity occurred much faster than the changes in MABP and were almost always independent of their direction or magnitude, i.e. the changes in synaptic activity could not be attributed to baroreflex activity.

Repeated pinches to the same body region produced qualitatively similar responses in individual neurones. Furthermore, the responses to pinching the ipsilateral hindpaw were qualitatively similar to those elicited by pinching the contralateral hindpaw, the skin of the abdomen (Figs 4*B* and 5*A*) and, in the few instances they could be tested, pinching the ipsilateral forepaw or ear (see also Grosse & Jänig, 1976; Boczek-Funcke *et al.* 1992). The response to a somatotopically closer stimulus (i.e. to the forepaw) tended to be larger than that to more distant stimuli (see Fig. 7). Responses to pinching the nose were more marked and sometimes differed qualitatively and are described separately below.

In order to characterize the responses in different neurones, pinches were repeated and responses for both hindpaws and the abdomen were pooled and averaged (between 1 and 14 pinches were tested per neurone; mean, 4·7 ± 0·6). Excitation and inhibition were identified by increases and decreases, respectively, in the frequency of synaptic events relative to the frequency prior to the pinch. The frequency from 10 to 25 s after the pinch was not significantly different ($P > 0·18$, Wilcoxon signed-rank test) from that over an equivalent period before the pinch, indicating that the changes in synaptic activity initiated by a brief pinch generally lasted less than 10 s. In four cells in which several pinches were tested over each of two periods separated by 48 ± 21 min (range, 19–109 min), there was no apparent change in the character or magnitude of the reflex responses.

Identification of three patterns of response to pinch

The responses to pinch varied in ways that were largely but not exclusively correlated with the on-going frequency of synaptic activity (see below). Cells were classified into one of three groups: those showing 'burst–inhibitory' responses (BI), those showing only excitation (E); and those showing no response (O).

BI neurones. The most common pattern (15/25, 60% of cells). These neurones showed significantly more on-going synaptic activity, both of sub- and suprathreshold events (2·9 ± 0·5 Hz), than the other neurones ($P = 0·0002$,

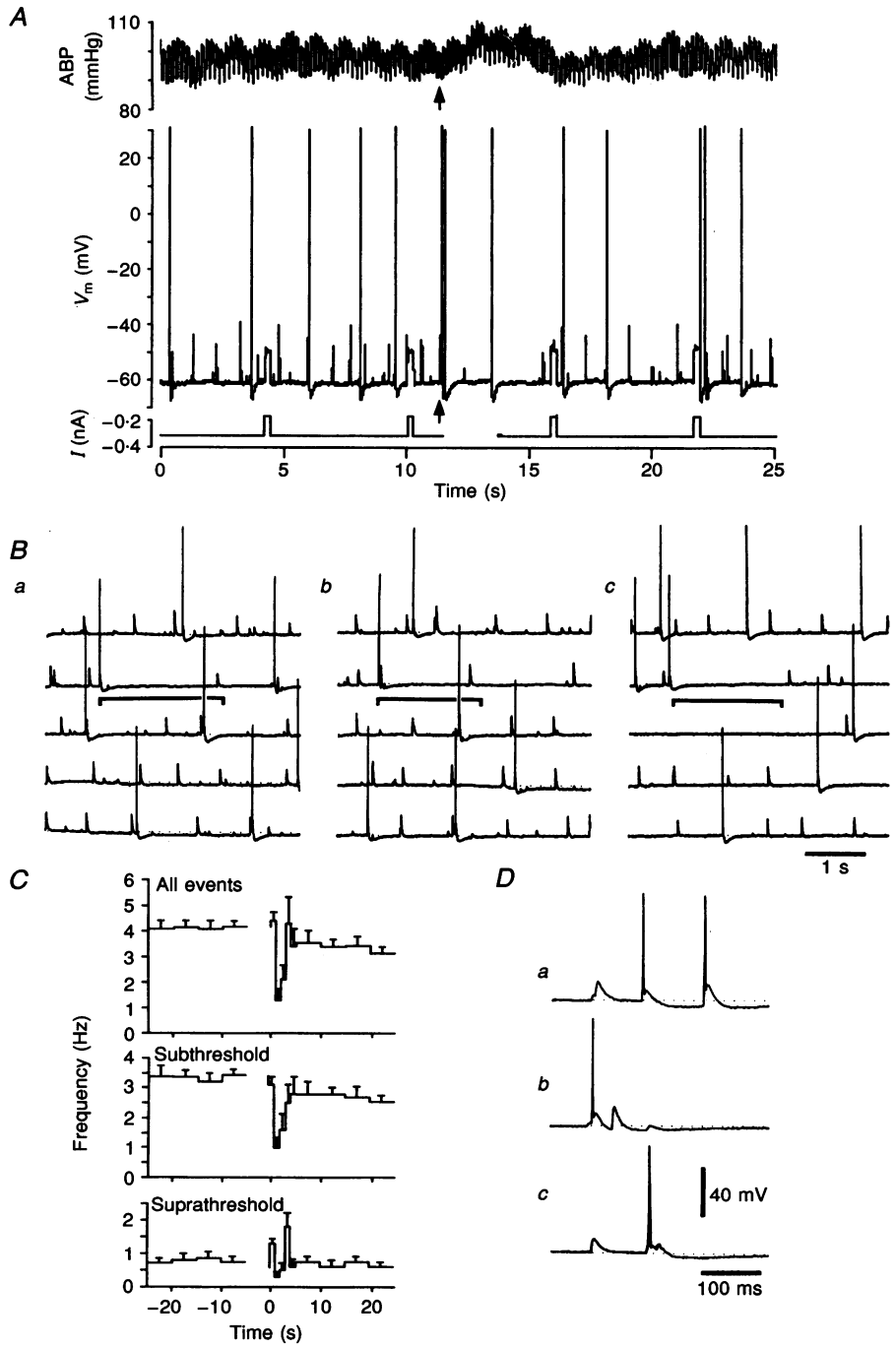

Figure 4. Responses to brief pinch in a BI neurone

A, simultaneous recordings of MABP, V_m and transmembrane current (*I*) before, during and after briefly pinching the ipsilateral hindpaw. The gap in the current trace is the marker indicating the duration of the pinch. The arrows indicate the onset of the burst and the rise in MABP. Note the second discharge close to the end of the pinch and the subsequent inhibition outlasting the stimulus. Short depolarizing current pulses were passed through the microelectrode to monitor passive membrane properties; these sometimes summed with ESPs to initiate an AP. *B*, responses in the same neurone as in *A* to other pinches of the ipsilateral hindpaw (*a*), the contralateral hindpaw (*b*) and the abdomen (*c*). Sets of five consecutive traces of V_m (from top downwards with 1·1 s gaps between each) with the duration of pinches indicated below the second trace. *C*, peristimulus histograms of frequency of all synaptic events (top panel), subthreshold events (middle panel) and APs (bottom panel) ($n = 10$ pinches). Zero time is the onset of the pinch and bars indicate S.E.M. *D*, characteristics of the excitatory burst in response to pinching the ipsilateral hindpaw (*a*), the contralateral hindpaw (*b*) and the abdomen (*c*) (shown on an expanded time base). The responses have been aligned to show the onset of the burst. The recruitment order of different synaptic events varies and summation of ESPs does not elicit an AP.

Kruskal–Wallis test). The pinch evoked a brief excitatory burst of synaptic events lasting up to 0·7 s which was followed by 0·5–2·8 s of inhibition (Figs 4 and 5; Table 3). The mean frequency of synaptic events rose in the first 1 s after the onset of the pinch (to 4·9 ± 0·7 Hz; range, 1·7–9·5 Hz) and fell to a minimum of 1·5 ± 0·4 Hz (range, 0–4·8 Hz) in the subsequent 1 s (Table 2). These represent significant changes in activity of +58% and −50%, respectively, relative to the on-going frequency before the pinch (both $P = 0{\cdot}048$; Fisher–Pitman randomization test; $n = 10$). In eight BI neurones, weak ESPs were either of small amplitude (<5 mV) and/or they occurred at low frequency (<1 Hz) whereas, in the other seven cells, many ESPs were at least 10 mV in amplitude. These included five neurones with on-going synaptic activity at more than 4 Hz (e.g. Figs 3*A* and 9*A*). In such cells, inhibition was more prominent.

In eight BI neurones (53%) the inhibition was interrupted by a second brief burst of synaptic events (reaching 4·3 ± 0·7 Hz, 38 ± 6% higher than pre-pinch frequency and 76 ± 11% of the initial burst). This usually occurred in the third 1 s, 1·3 ± 0·4 s after the end of the pinch, and often consisted of suprathreshold events.

The overall changes in frequency of sub- and suprathreshold inputs and the timing of these events are shown in Table 2, whereas Table 3 shows the characteristics of the excitatory bursts and ensuing period of synaptic silence. It is evident from Table 3 that the excitatory burst was much briefer than the stimulus, whereas the inhibition usually outlasted it.

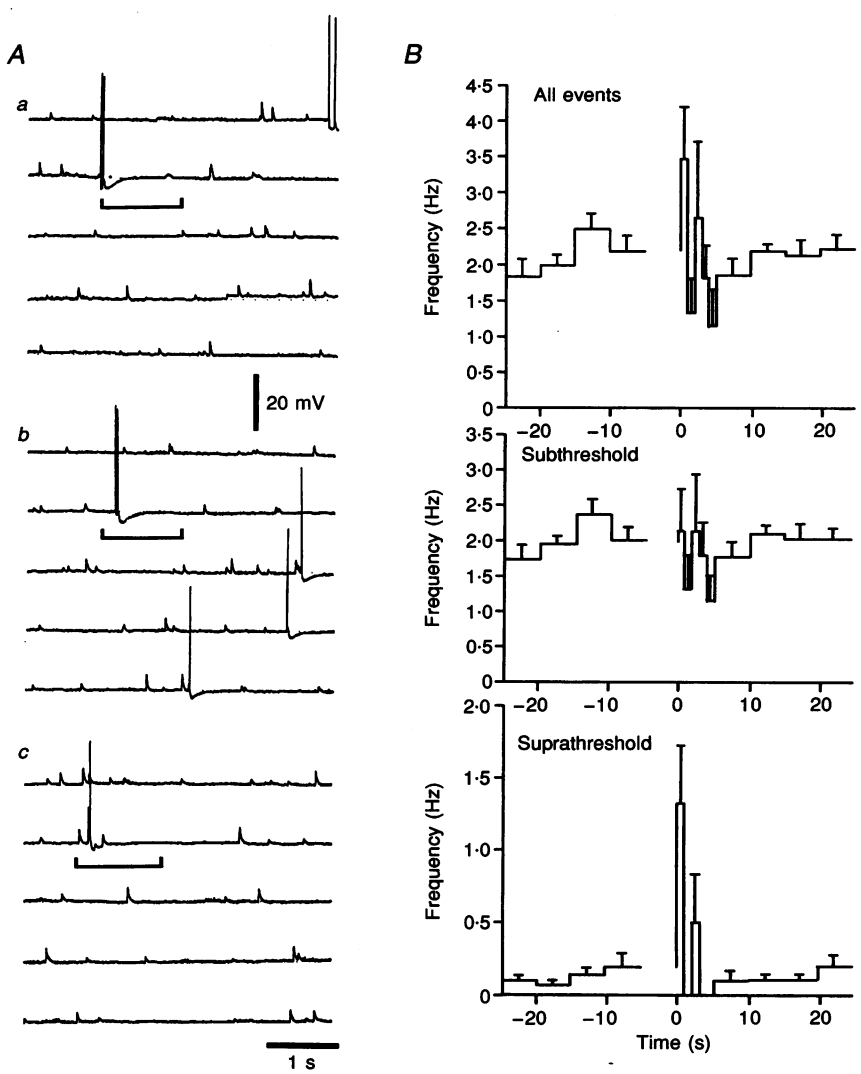

Figure 5. Responses to brief pinch in another BI neurone

A, response of another BI neurone to briefly pinching the ipsilateral hindpaw (*a*), the contralateral hindpaw (*b*) and the abdomen (*c*). Sets of five consecutive traces of V_m (from top downwards with 1·1 s gaps between each) with the duration of pinches indicated below the second trace. In this neurone, suprathreshold inputs that were rarely spontaneously active were always recruited during the initial burst response. *B*, peri-stimulus histograms of frequency of all synaptic events (top panel), of subthreshold events (middle panel) and of APs (bottom panel) ($n = 6$ pinches). Zero time is the onset of the pinch and bars indicate S.E.M.

Table 2. Characteristics and timing of sub- and suprathreshold responses to brief pinches of the skin in three groups of SCG neurones

Cell type	On-going frequency (Hz)	Maximum frequency (Hz)	Minimum frequency (Hz)	2nd peak frequency (Hz)
BI neurones ($n = 15$)				
Subthreshold	2·3 ± 0·5	4·1 ± 0·7* (at 0·1 ± 0·1 s)	1·3 ± 0·3‡ (at 1·4 ± 0·3 s)	2·8 ± 0·6† (at 2·9 ± 0·2 s)
Suprathreshold	0·5 ± 0·1	1·1 ± 0·2† (at 0·1 ± 0·1 s)	0·3 ± 0·1* (at 1·4 ± 0·2 s)	1·0 ± 0·2† (at 3·7 ± 0·6 s)
E neurones ($n = 5$)				
Subthreshold	0·2 ± 0·1	1·3 ± 0·2§ (at 0·4 ± 0·2 s)	0·0 ± 0·0 (at 2·8 ± 0·6 s)	0·2 ± 0·1 (at 7·0 ± 2·7 s)
Suprathreshold	0·04 ± 0·04	0·6 ± 0·1§ (at 0 ± 0 s)	0·0 ± 0·0 (at 1·8 ± 0·6 s)	0·2 ± 0·1 (at 4·0 ± 0·4 s)
O neurones ($n = 5$)				
Subthreshold	0·1 ± 0·1	0·5 ± 0·3 (at 1·3 ± 0·8 s)	0·0 ± 0·0 (at 2·5 ± 0·6 s)	0·2 ± 0·1 (at 4·5 ± 0·9 s)
Suprathreshold	0·1 ± 0·1	0·3 ± 0·2 (at 1·6 ± 0·6 s)	0·0 ± 0·0 (at 2·6 ± 0·6 s)	0·1 ± 0·1 (at 3·0 ± 0 s)

On-going frequency was determined over 15 s prior to the pinch; maximum, minimum and second peak frequencies were determined during 1 s time bins after the pinch. Times in parentheses are the mean values of the time bins in which the changes occurred (time 0 is the first 1 s after the beginning of the pinch so that, for example, time = 1·2 s means that most responses occurred during the second time bin). In most cells, responses were recorded near −60 mV V_m; thus the frequency of suprathreshold inputs may be underestimated, particularly in some BI neurones. Significant differences from on-going activity (Wilcoxon signed-rank test): *$P \leqslant 0{\cdot}001$; †$P \leqslant 0{\cdot}005$; ‡$P \leqslant 0{\cdot}01$; §$P \leqslant 0{\cdot}05$.

The reflex patterns in sub- and suprathreshold inputs were not always the same in a given cell. As the majority of both on-going and reflexly evoked synaptic events were small ESPs (<8 mV), the reflex pattern in these weak inputs, which showed burst–inhibition in eleven BI neurones, dominated the classification of neurones into different types. However, both sub- and suprathreshold inputs showed burst–inhibition in only five BI neurones (e.g. Fig. 5). In the other cases, suprathreshold inputs were predominantly inhibited ($n = 3$) or excited at the onset and after the pinch ($n = 2$, e.g. Fig. 9*A*). In the remaining neurones, the suprathreshold inputs showed the BI pattern but the subthreshold inputs showed only inhibition ($n = 3$, Fig. 4*C*) or only excitation ($n = 1$). In one BI neurone, there were no suprathreshold inputs.

Table 3. Characteristics of excitation in response to pinch

Cell type	Duration of pinch (s)	No. of synaptic events	Duration of burst (s)	Intraburst frequency (Hz)	Silence after burst (s)	Relative excitation
BI neurones ($n = 15$)						
All events	1·6 ± 0·1	5·2 ± 0·9	0·3 ± 0·1	20·2 ± 2·8	1·5 ± 0·2	13 ± 4
Subthreshold		4·0 ± 0·9		14·8 ± 2·3		
Suprathreshold		1·2 ± 0·2		5·7 ± 1·4		
E neurones ($n = 5$)						
All events	3·1 ± 0·4†	4·4 ± 1·0	2·0 ± 0·3*	2·2 ± 0·2*	5·2 ± 1·4†	6 ± 1
Subthreshold		3·3 ± 1·0		1·6 ± 0·2		
Suprathreshold		1·1 ± 0·4		0·6 ± 0·2‡		

Silence is the interval from the end of the burst to the next synaptic event. Relative excitation is the ratio of the intraburst frequency to the on-going frequency prior to the pinch; this excludes cells with no on-going activity. Significant differences between BI and E neurones (Mann–Whitney U test): *$P \leqslant 0{\cdot}001$; †$P \leqslant 0{\cdot}005$; ‡$P \leqslant 0{\cdot}01$.

Nine of the BI neurones had at least two suprathreshold inputs so that, on average, BI cells had significantly more strong inputs (1·6 ± 0·1) than the other cell groups ($P = 0·01$, Kruskal–Wallis test). All but one of the strong inputs to BI cells showed on-going activity and, in eleven of fifteen neurones (73%), strong inputs were activated at the onset of the pinch. However, within one cell, in some trials the burst primarily involved suprathreshold inputs, whereas in others it consisted of a barrage of only ESPs. Similarly, when different synaptic inputs were recruited during the burst, the order in which they occurred was not constant (Fig. 6). This was also the case for the events during the burst that followed the pinch.

The only property that distinguished the BI neurones was the level of resting V_m which was significantly less negative in BI (−47 ± 1 mV) than in E cells (−57 ± 4 mV, $P = 0·014$, Mann–Whitney U test).

E neurones. Pinching evoked only an increase in synaptic activity in five of twenty-five cells (20%). On-going activity (0·2 ± 0·1 Hz for all synaptic events) was significantly less than in BI neurones ($P < 0·001$, Mann–Whitney U test). The maximum frequency of synaptic events (1·7 ± 0·3 Hz; range, 0·7–2·3 Hz) occurred during the first 1–2 s after the onset of the pinch (Table 2, Fig. 7). This increase in frequency reached the on-going frequency on average eight times but the lowest firing frequency after this (0·1 ± 0·1 Hz)

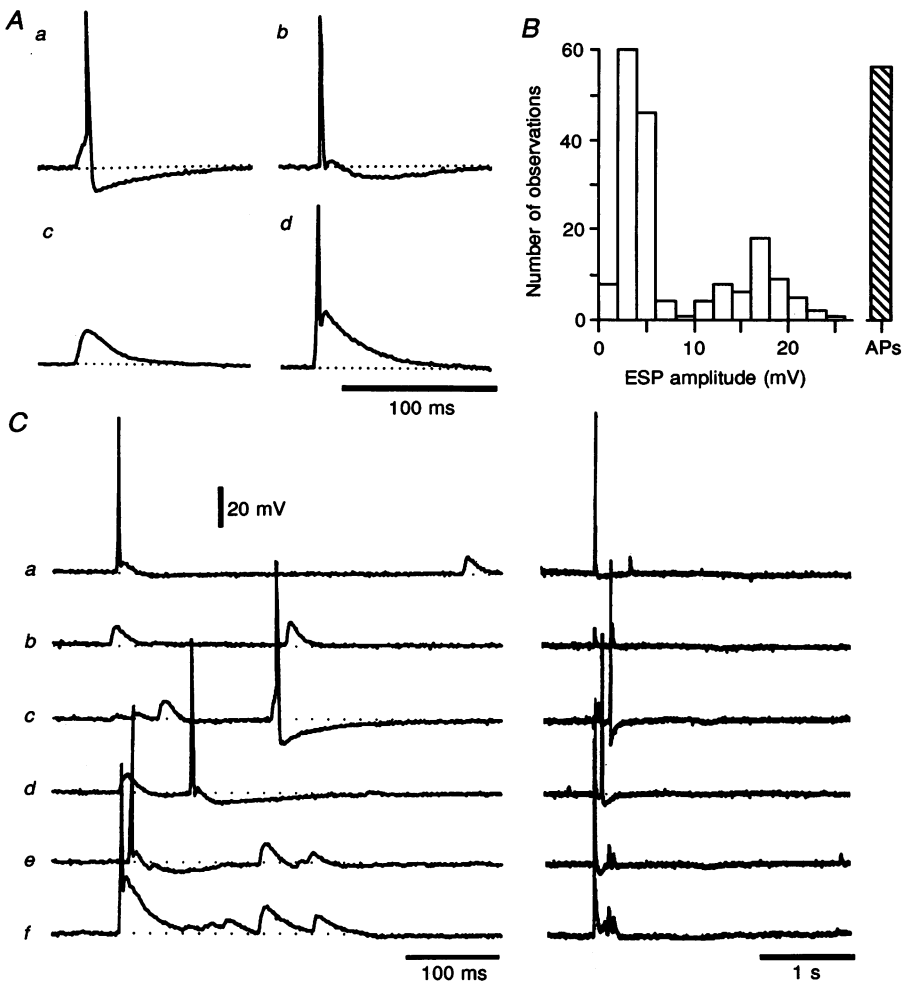

Figure 6. Variable recruitment of different synaptic inputs during burst in another BI neurone
A, during resting activity, two suprathreshold synaptic events could be identified by their distinct configurations. The event in *a* is just suprathreshold (threshold about −13 mV), whereas that in *b* shows a large after-depolarization (V_m held at −55 mV); when the membrane was hyperpolarized to −80 mV (*c*) there were many ESPs 13–22 mV in amplitude, and all APs had very large after-depolarizations (*d*). *B*, histogram of ESP amplitudes recorded at −80 mV. The hatched column indicates the number of large suprathreshold events (as in *A d*). *C*, ESPs and APs during the burst response to brief pinches of the ipsilateral hindpaw (*a–d*), the contralateral hindpaw (*e* and *f*). V_m in *a* and *b* was −60 mV, in *c–e* was −55 mV, and in *f* was −80 mV. The responses have been aligned to show the onset of the burst. The same records are shown to the right on a slower time base showing the absence of activity after the brief burst. Both sub- and suprathreshold events can be recognized during the burst but the sequence and timing of responses differ between pinches. Voltage calibration in *C* applies also to *A*.

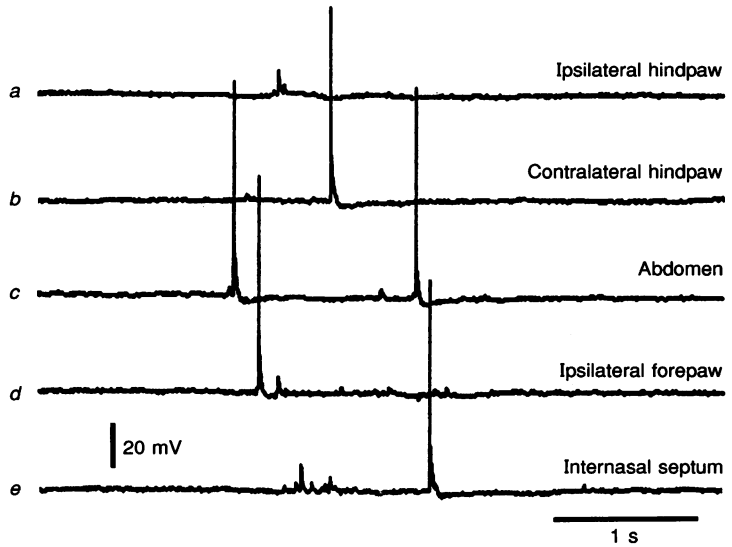

Figure 7. Variability of synaptic responses in an E neurone to pinching different body regions
Records of V_m showing ESPs and APs in response to brief pinches of the ipsilateral hindpaw (*a*), the contralateral hindpaw (*b*), the abdomen (*c*), the ipsilateral forepaw (*d*), and the internasal septum (*e*). There was no on-going activity in this cell and the pinches (~1 s in duration) occurred near the middle of each trace. At least three different amplitude responses can be distinguished. Note the variation in recruitment order of the different inputs and the greater number of synaptic events evoked from more proximal body regions.

was not significantly different from the on-going frequency before the pinch ($P = 0{\cdot}19$). The number of events occurring in the first 2 s after the pinch was significantly greater than during on-going activity ($P = 0{\cdot}015$, Fisher–Pitman randomization test, $n = 5$). The excitation lasted for a significantly longer proportion of the pinch ($0{\cdot}66 \pm 0{\cdot}05$) than did the burst in BI neurones ($0{\cdot}24 \pm 0{\cdot}05$, $n = 12$, $P < 0{\cdot}005$, Mann–Whitney U test). The peak intraburst frequency of all synaptic events and also of strong responses during the excitation of E neurones was significantly lower than in BI neurones (Table 3). Both sub- and suprathreshold inputs were involved in the excitation in all five E neurones but it was again apparent that the order in which different synaptic inputs were recruited was variable (Fig. 7).

E neurones received on average $1{\cdot}2 \pm 0{\cdot}2$ strong inputs. The suprathreshold input had on-going activity in only one of these neurones, but in all cases at least one was recruited by pinching.

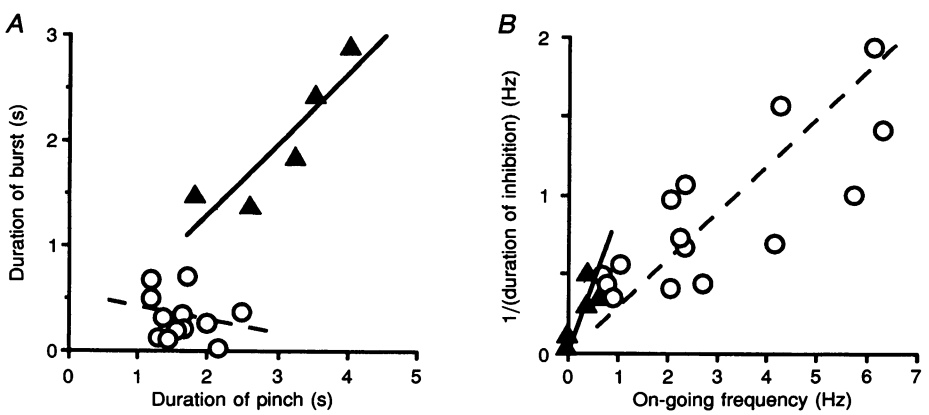

Figure 8. Timing of responses to pinch in different neurones
A, relation of the duration of excitation to the duration of the pinch. ○, BI neurones; ▲, E neurones. The regression line fitted through the data for BI neurones has a slope of 0·7 ($r^2 = 0{\cdot}79$) and that for E neurones has a slope of −0·1 ($r^2 = 0{\cdot}06$). *B*, relation of the reciprocal of the duration of the period of silence after the burst evoked by the pinch to the on-going frequency of synaptic events. Symbols as in *A*. The slope of the regression line fitted through the data for BI neurones (0·27, $r^2 = 0{\cdot}89$, dashed line) is significantly different from 1 ($P < 0{\cdot}001$), whereas that for E neurones (0·9, $r^2 = 0{\cdot}81$, continuous line) is not ($P > 0{\cdot}5$). This confirms that the silent period in BI neurones is due to active inhibition.

O neurones. In the remaining five of twenty-five neurones (20%), there was no detectable response to the pinch. The frequency of on-going activity ($0{\cdot}2 \pm 0{\cdot}1$ Hz) was low relative to that in BI neurones ($P < 0{\cdot}002$, Mann–Whitney U test), but similar to that in E neurones. It is therefore notable that MABP during recordings from O cells was significantly higher (100 ± 5 mmHg, $P = 0{\cdot}05$, Mann–Whitney U test) than in E neurones (82 ± 3 mmHg). In two of these cells, no on-going activity at all was observed for at least 5 min prior to the test pinches, although rare ESPs were recorded at other times and increased in frequency in response to other stimuli, such as pinching the nose (see below) or inflating the bladder. There were no on-going APs in three O neurones but two of them had strong inputs that were activated at other times during the recording.

Comparison between groups. The distinction between BI and E neurones was confirmed by two other analyses. First, the relation between the duration of the burst and the duration of the pinch in BI and E neurones is shown in Fig. 8*A*. This shows a direct relation between these parameters in E neurones that was clearly absent in BI neurones. Furthermore, the interval during which there was no synaptic activity (the duration of inhibition) after the burst was inversely related to the on-going frequency of synaptic events in the same cell. This relation can be most clearly seen when the reciprocal of the period of synaptic silence is plotted against the frequency of activity prior to the burst (Fig. 8*B*). The data for E neurones falls on a line with a slope close to 1 confirming the lack of inhibition. That is, the inhibitory interval following the excitation was similar to the mean interval between events prior to the stimulus. However, the slope was much less than 1 for BI neurones, indicating that the interval before the first synaptic event after the burst was prolonged. Data for four BI cells lay close to the line of unity but, in each of these cases, the brief interval resulted because a second burst interrupted the inhibition.

The groups have been classified on the basis of all synaptic events evoked by the pinch, both sub- and suprathreshold. If the suprathreshold events are taken to indicate the ganglionic 'output', then the neurones would have been classified as nine BI, eight E and eight neurones that were not responsive to the pinch. This may not be appropriate in all cases because the membrane was often held at a hyperpolarized level.

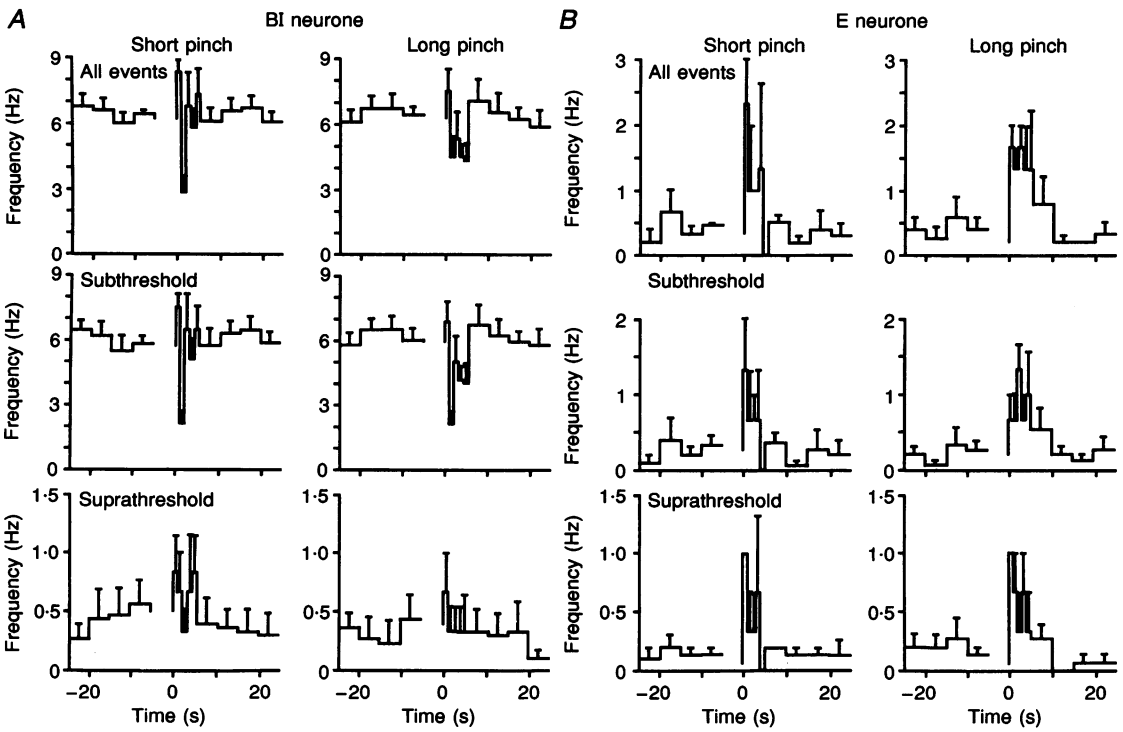

Figure 9. Prolonging the pinches prolonged the reflex responses

Peristimulus histograms for a BI neurone (*A*) and an E neurone (*B*). In the BI neurone, pooled responses to pinching the ipsilateral hindpaw ($n = 4$ short, 1 long), the contralateral hindpaw ($n = 2$ short, 2 long) and the abdomen ($n = 1$ short, 3 long) for $1{\cdot}6 \pm 0{\cdot}2$ s (short) and $4{\cdot}4 \pm 0{\cdot}4$ s (long); V_m held at -60 mV. In the E neurone, pooled responses to pinching the ipsilateral hindpaw ($n = 2$ short), the contralateral hindpaw ($n = 1$ short, 1 long) and the abdomen ($n = 3$ long) for $2{\cdot}1 \pm 0{\cdot}2$ s (short) and $6{\cdot}4 \pm 2{\cdot}3$ s (long); V_m at -70 mV. The pinches began at time 0. Note that the on-going frequency is similar prior to each set of pinches and that the responses are similar but both the inhibition and the excitation, respectively, are prolonged.

Effect of increasing the duration of the pinches

In three BI and three E neurones, responses to longer or repeated pinches lasting up to 10 s were tested. Changes in MABP were similar to or slightly more prolonged than those following a brief pinch. The synaptic responses were qualitatively similar to the reflexes evoked by brief pinch but were more long lasting in every case. Mean reflexes produced in one BI and one E neurone are compared with those to brief pinches in Fig. 9.

Responses to pinching the nose

When the internasal septum was pinched ($n = 14$), reflex responses elicited in SCG neurones were more pronounced than after pinching other regions of the body ($P = 0{\cdot}001$, Wilcoxon signed-rank test). There was also a more prolonged increase in MABP, which sometimes persisted for 15–30 s after the synaptic activity had recovered to pre-pinch levels. In six of eight BI neurones, the response was also a burst followed by a period of inhibition but both parts of the response were larger and more prolonged (Fig. 10*A*). In the other BI neurones, however, the response to pinching the nose was largely excitation. In BI neurones, the increase in frequency during the initial burst was $3{\cdot}6 \pm 0{\cdot}8$ times larger than the average response to pinching other regions ($P = 0{\cdot}012$, Wilcoxon signed-rank test), but the inhibition was of similar magnitude. Four E neurones tested were also excited by pinching the nose (Fig. 10*B*), as were two of the neurones which did not respond to pinching other regions. Three other (O) neurones did not respond at all to any of the pinches. Thus, stimulation of trigeminal nociceptors provoked stronger reflex responses than did the spinal afferents, and many of the responses (50%) were purely excitatory (see also Boczek-Funcke *et al.* 1992).

Responses to non-noxious stimuli

Responses to lightly touching the foot were tested in three neurones and in no case could any change in synaptic activity or MABP be distinguished.

Responses to a strong puff of air lasting 1–2 s onto the abdominal skin (sufficient to flatten the hairs) were examined in eleven BI, two E and one O neurones. The puffs evoked reflex responses lasting 1–2 s that were qualitatively similar to the responses to pinch in eight BI, one E and one O neurone (71%); in three of the other four cases there was no detectable response and one cell was briefly excited. In most cases, these responses differed from the natural variations in on-going activity only by their consistent timing. Of nine neurones in which multiple puffs ($3{\cdot}1 \pm 0{\cdot}6$) were presented, six gave BI responses, the excitation being somewhat smaller than the early response to pinching ($P = 0{\cdot}07$, $n = 6$,

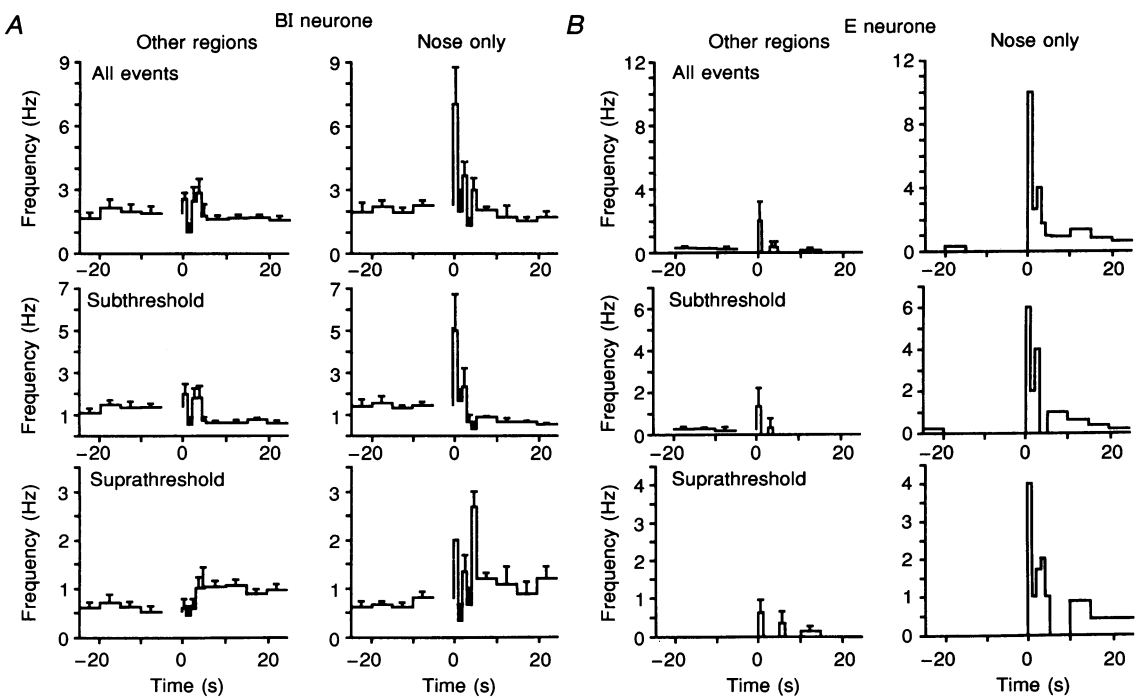

Figure 10. Pinching the internasal septum produced more pronounced responses than pinching other regions

Peristimulus histograms for a BI neurone (*A*) and an E neurone (*B*). In the BI neurone, pooled responses to pinching the ipsilateral hindpaw ($n = 4$), the contralateral hindpaw ($n = 2$) and the abdomen ($n = 1$) are compared with responses to pinching the nose ($n = 3$); V_m held at −55 mV. In the E neurone, single responses to pinching the ipsilateral hindpaw, the contralateral hindpaw and the abdomen are compared with the response to pinching the nose; V_m held at −60 mV. The pinches began at time 0. Note that the response to pinching the nose was largely excitatory in both neurones.

Wilcoxon signed-rank test). In the three other neurones, there was only brief inhibition. There were no changes in MABP associated with any of the responses to puffs of air.

DISCUSSION

This study was aimed at clarifying the role of convergent preganglionic inputs in determining the activity of postganglionic sympathetic neurones in the SCG of the anaesthetized rat. During on-going activity, activation of the neurones was dominated by one or two suprathreshold (strong) preganglionic inputs. In neurones with high levels of synaptic activity, there were also several inputs that generated ESPs with amplitudes close to threshold for the postsynaptic AP. However, in general, the probability that summation of subthreshold ESPs would initiate discharge of the postganglionic neurone was low. When reflex responses to brief cutaneous pinches were evoked, three types of response could be distinguished. The majority (60%) of neurones showed a brief burst of synaptic events followed by a more sustained inhibition that generally outlasted the pinch (BI neurones). In half of the remaining neurones, all of which had low levels of on-going activity, synaptic activity increased for most of the duration of the pinch (E neurones), whereas in 20% of cells there was no response (O neurones). Most of the preganglionic axons that converged on a particular ganglion cell (forming the pathway from the central nervous system to the peripheral target) responded in the same way to these natural stimuli. The simplest interpretation is that the different response patterns reflect the common behaviour of subgroups of functionally distinct neuronal pathways.

The on-going frequency of synaptic events (mean, 1·8 Hz) was the same as that reported previously for active neurones in the rat SCG (mean, 1·9 Hz; Ivanov & Purves, 1989), but the frequency of suprathreshold events was relatively low (0·4 Hz, cf. 0·9 Hz), presumably because we often recorded with the membrane hyperpolarized. The frequency of synaptic activity is about double that of extracellularly recorded spontaneously active postganglionic units in the rat hindlimb (0·3–3·6 Hz in identified muscle and skin vasoconstrictor axons, median ~1 Hz; Häbler *et al.* 1994). Active SCG neurones projecting to the salivary gland (which probably constitute the vasoconstrictor population) discharged at similar frequencies (0·15–1·48 Hz, mean 0·7 Hz; Bartsch, Häbler & Jänig, 1996), whereas 90% of salivary neurones (presumably secretomotor) were inactive (Ivanov, 1991). In the present study, the proportion of all impaled SCG neurones without synaptic activity (31%) was substantially lower than found previously (55%; Ivanov & Purves, 1989). The variation in the amount of on-going activity between neurones was marked.

It was clear that several of the convergent preganglionic inputs to each cell had on-going activity under the conditions of these experiments, but unfortunately the number of active inputs could not be estimated. In amplitude histograms of ESPs recorded with the membrane hyperpolarized to block AP generation, it was rarely possible to distinguish distinct modal groupings which varied as expected for quantal variation (McLachlan, 1975). The majority of the synaptic activity consisted of ESPs with amplitudes less than 8 mV. Either several inputs have similar small amplitude ESPs or the weak input(s) fire at higher frequencies than the larger inputs. Some ESPs less than 5 mV in amplitude must result from the spontaneous release of quanta (without presynaptic APs) (McLachlan, 1975). However, because small ESPs also dominated the reflex responses to cutaneous stimuli, it seems likely that the majority arose from activity in weak inputs. Even when weak ESPs summed during excitatory bursts, they were rarely sufficiently synchronous or of sufficient amplitude to reach threshold for AP initiation. Rather, the discharge of the neurones usually resulted from the activity of single strong inputs, i.e. those with large quantal contents that led to ESPs with amplitudes always well above threshold at resting V_m (Hirst & McLachlan, 1984). This contrasts with reports that, in the rabbit SCG, in addition to single strong inputs, several weak preganglionic inputs often summed to discharge the cell, apparently by synchronization of their activity within the spinal cord (Skok & Ivanov, 1983; Tatarchenko, Ivanov & Skok, 1990). Although the average number of inputs to each ganglion cell increases with animal size (Purves *et al.* 1986), the strength of the inputs in different species is unknown.

The amplitudes of the ESPs underlying the strong inputs varied considerably between cells; 88% of cells had strong inputs that were active. When two (or more) strong preganglionic inputs were active, one generally had a much larger underlying ESP (and therefore a higher 'safety factor') than the other(s). In a proportion of the neurones with high rates of on-going synaptic activity, several additional inputs had underlying ESPs with amplitudes that were around threshold and often evoked APs at resting V_m. However, such ESPs failed to reach threshold if they occurred during the AHP of a preceding AP (e.g. Fig. 4*D b*), whereas strong inputs were not blocked if that occurred (see Fig. 2). Thus, this subgroup of cells with high frequency activity showed more complex integrative behaviour such that the near-threshold ESPs were more effective (i.e. they initiated APs) when the frequency of synaptic activity was low than during excitatory bursts.

Given that the average number of convergent preganglionic inputs determined for rat SCG neurones *in vitro* is about nine (Purves *et al.* 1986), it appears from the on-going synaptic frequency that the rate of discharge of some preganglionic neurones is extremely low. This has been reported for preganglionic units recorded extracellularly in the spinal cord and identified antidromically from the CST in anaesthetized rats; 30–45% of these units were silent (Gilbey, Numao & Spyer, 1986; Lewis & Coote, 1995). In contrast, most active units in the CST discharge between 0·2 and 3·5 Hz (mean, ~1 Hz; T. Bartsch & J. Häbler,

unpublished observation). One possibility is that there is a subgroup of inputs to most cells that do not discharge (at least under anaesthesia) and another is that most preganglionic inputs in some particular pathways are silent, as might be expected given some of the functions of SCG cells (see below). It is unlikely that only few inputs converge on those cells with little on-going activity since multiple inputs could be recruited by pinching. Our recordings certainly suggest that many different inputs were active in cells with high rates of on-going activity. It was notable that no evidence of any high frequency discharge of individual preganglionic inputs, such as that observed in synchrony with inspiration in rat thoracic cord (Gilbey *et al.* 1986), could be detected during on-going or reflex activity.

The responses to a brief cutaneous pinch consisted of an excitation that was brief in BI neurones and more prolonged in E neurones. When the pinches were prolonged for several seconds, both the subsequent inhibition in BI neurones and the excitation in E neurones were prolonged, confirming the distinction between the response patterns. The variable sequence of recruitment of different inputs during reflex activation provides no support for the idea that there is coupling between preganglionic neurones, either via a common descending pathway (Skok & Ivanov, 1983) or as a result of electrical connections between the cells (Logan, Pickering, Gibson, Nolan & Spanswick, 1996). In addition, the burst of activity at the end of the inhibitory period in some BI neurones can most simply be explained if inhibition occurred at the membrane of the preganglionic neurone, rather than by withdrawal of a tonic excitatory barrage.

Suprathreshold and subthreshold inputs to BI neurones did not uniformly show burst–inhibition responses, suggesting that convergent preganglionic axons are not precisely linked in their reflex behaviour. This was observed previously in the ciliary ganglion (Johnson & Purves, 1983). Multiple weak inputs were usually involved in excitatory responses. However, the suprathreshold inputs that are the main determinant of postganglionic discharge participated in the burst in 73% of BI neurones and all inputs responded alike in E neurones. The data suggest that, overall, the behaviour of convergent inputs to a particular cell in response to pinch is relatively uniform.

The patterns of reflex discharge in sympathetic postganglionic neurones are associated with the responses of peripheral targets (Jänig & McLachlan, 1992). It is therefore of interest to compare the distribution of suprathreshold responses to pinch observed here (i.e. 36% BI, 32% E and 32% O neurones) with the possible functional groups of SCG neurones. Neurochemical and retrograde tracing studies of the SCG (Voyvodic, 1989; Gibbins, 1991) suggest that about 50% of the neurones project to blood vessels, some 25% are pilomotor and nearly 20% supply exocrine gland tissue. Smaller subpopulations supply the iris and the pineal gland. Secretomotor neurones supplying the rat salivary glands are silent (Ivanov, 1991; Bartsch *et al.* 1996) and might be either E or O neurones. Neurones projecting to the cat tail that were thought to be pilomotor had no on-going activity and were not affected by noxious stimuli (Grosse & Jänig, 1976); thus, pilomotor neurones may be part of the O population. In contrast, vasoconstrictor pathways to skin and muscle are tonically active at frequencies similar to those observed in some BI cells (Häbler *et al.* 1994).

Similar but very much smaller changes in synaptic activity were generated by puffs of air directed at the abdominal skin (see also Horeyseck & Jänig, 1974). Brief excitation followed by inhibition is the reflex pattern elicited in cutaneous postganglionic axons by single electrical stimuli that activate Aβ fibres alone or also recruit Aδ fibres or also C fibres (Jänig, Sato & Schmidt, 1972). Although most vasoconstrictor axons projecting to rat hindlimb muscles (MVC) (57%) were excited by longer pinches and most cutaneous vasoconstrictor (CVC) axons (53%) were inhibited (Häbler *et al.* 1994), the reciprocity of response patterns was less distinct than in the cat (Jänig, 1985). On a population basis, it seems unlikely that the BI and E patterns distinguish between the CVC and MVC populations projecting to the head, but it is possible that BI neurones include vasoconstrictor neurones. It is notable that, in some recordings of muscle vasoconstrictor activity in humans, brief inhibitions (sometimes preceded by a burst) were elicited by painful electrical stimuli to the body surface (Delius, Hagbarth, Hongell & Wallin, 1972). Cardiovascular neurones of the rostral ventrolateral medulla responded in the same way to pinch but not to noxious heat (Sun & Spyer, 1991) although the inhibition in this case was attributed to baroreflex effects. The 'presympathetic' neurones in the rostral ventrolateral medulla are the site of integration of many inputs from peripheral afferents, including the supraspinal component of somatosympathetic reflexes from nociceptors (Dampney, 1994). They include neurones involved in blood pressure regulation (via the MVC system) as well as antecedent neurones of the CVC system. If these neurones confer the BI pattern on both MVC and CVC pathways, after making divergent contacts with a number of preganglionic neurones, then SCG neurones with this pattern may all be vasoconstrictor neurones. The absence of two distinctive reflex patterns as observed in lumbar vasoconstrictor pathways may reflect the diversity of control of the vasculature in target tissues in the head.

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Acknowledgements

This work was supported by the National Health and Medical Research Council of Australia. H.-J. Häbler's participation was supported by the Humboldt Foundation. We are very grateful to Wilfrid Jänig and James Brock for their helpful comments on the manuscript.

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Received 13 November 1996; accepted 13 February 1997.