Journal of Physiology (1995), **487.3**, *pp.*727–737

The pattern of excitatory inputs to the nucleus tractus solitarii evoked on stimulation in the hypothalamic defence area in the cat

L. Silva-Carvalho, M. S. Dawid-Milner and K. M. Spyer*

Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK

1. In anaesthetized, paralysed and artificially ventilated cats, recordings have been made in the nucleus tractus solitarii (NTS) to assess further the role of this nucleus in mediating the cardiorespiratory responses that are elicited on stimulation within the hypothalamic defence area (HDA).

2. The responses of NTS neurones to stimulation in the hypothalamus were assessed, as were their patterns of evoked response to electrical stimulation of the sinus (SN), aortic (AN), superior laryngeal (SLN) and vagus (VN) nerves.

3. Stimulation in the HDA affected the activity of 110 NTS neurones (85 studied in intracellular and 25 studied in extracellular recordings). The present study focused on those sixty-eight neurones that were excited by such stimulation (51 intracellular recordings and 17 extracellular recordings).

4. Of the sixty-eight neurones that were excited by HDA stimulation, seven revealed no changes in membrane potential or evoked discharge (2 neurones) but the stimulus facilitated the excitatory effects of stimulating either (or both) the SN and SLN. An additional group of neurones showed powerful excitatory responses to HDA stimulation (15 studied with extracellular and 35 with intracellular recording). Evoked EPSPs had onset latencies in the range of 1–30 ms. Of those thirty-five neurones displaying EPSPs, twenty-six were shown to receive convergent inputs on nerve stimulation. In nine neurones the early EPSP in response to HDA stimulation was followed by an IPSP.

5. In a further group of neurones HDA stimulation elicited a long-lasting IPSP, but this was not analysed further because its features have been described in detail in earlier studies from this laboratory.

6. The patterns of response of several neurones excited by stimulation in the HDA are consistent with them forming a group of NTS interneurones that mediate the hypothalamically evoked cardiovascular responses, including modulation of reflex function, which is a major feature of cardiorespiratory control. This possibility is discussed in the light of the present physiological observations and descriptions of an intrinsic NTS group of GABA-containing neurones that have been suggested to fulfil such a role.

Several studies over the last decade have indicated that the patterning of cardiorespiratory activity by the central nervous system (CNS) involves complex synaptic actions at several sites. These include the premotor brainstem neurones subserving either cardiovascular or respiratory function, the autonomic preganglionic neurones and respiratory motoneurones (Richter & Spyer, 1990), but additionally a modulation of the efficacy of cardiorespiratory reflexes may be involved (Spyer, 1990). The latter action has been suggested to require synaptic interactions at the level of the nucleus tractus solitarii (NTS). To gain an understanding of operation of these mechanisms it is necessary to study a relatively simple paradigm of cardiovascular (and respiratory) control. In this regard investigations on the patterning of cardiorespiratory responses to stimulation in the hypothalamic defence area (HDA) have proved valuable in identifying these physiological processes (Coote, Hilton & Zybrozyna,

* To whom correspondence should be addressed.

1973; Coote, Hilton & Perez-Gonzalez, 1979; and see Spyer, 1990, 1994 for review).

Stimulation within the HDA has been shown to elicit a characteristic pattern of cardiorespiratory response in the cat (Jordan, 1990 for review), including a central suppression of the baroreceptor reflex (Coote *et al.* 1979; Mifflin, Spyer & Withington-Wray 1988*b*; Spyer, 1990). This latter component of the response is produced by an inhibitory control of those NTS neurones that are excited by baroreceptor stimulation (Mifflin *et al.* 1988*b*; Jordan, Mifflin & Spyer, 1988) and is exerted by the activation of $GABA_A$ receptors localized on these neurones (Jordan *et al.* 1988). Evidence has been obtained indicating that an intrinsic group of NTS GABA-containing neurones may act as a target for descending input from the hypothalamus (Izzo, Sykes & Spyer, 1992). Interestingly, there is also evidence showing that the cardiovascular effects of activating the pontine parabrachial nucleus (Felder & Mifflin, 1988, 1994) and the uvula cortex of the cerebellum (Paton & Spyer, 1990) are produced, in part, by a similar control of the baroreceptor reflex function at the level of the NTS. The anatomical connections of GABA-containing terminals within the NTS are now well established (Maqbool, Batten & McWilliam, 1991; Izzo *et al.* 1992; Maley, 1994 for discussion), but the physiological characteristics of the postulated interneurones that mediate this control of baroreceptor function have only been inferred from indirect analyses (Mifflin, Spyer & Withington-Wray, 1988*a*, *b*; Jordan *et al.* 1988).

These putative interneurones have been suggested to be represented by the small number of NTS neurones that were shown to be excited by hypothalamic stimulation but were unaffected by reflex inputs (Mifflin *et al.* 1988*b*). This limited observation is the spur to the present detailed study that seeks to define the physiological properties of those NTS neurones that are the essential interneurones in these synaptic mechanisms. Their role is made even more significant by the fact that many of the NTS neurones that are known to receive an input from the arterial baroreceptors, and an inhibitory control from the hypothalamus, also respond to activation of vagal afferents, including those that relay in the superior laryngeal nerve (Biscoe & Sampson, 1970; Mifflin *et al.* 1988*a*, *b*). Hence, the activation of the HDA has the potential to modulate other reflexes (Lopes & Palmer, 1978) and this is considered in fuller detail in accompanying papers (Dawid-Milner, Silva-Carvalho, Goldsmith & Spyer, 1995; Silva-Carvalho, Dawid-Milner, Goldsmith & Spyer, 1995).

METHODS

Experiments were carried out in ten cats (body weight, 2·8–3·0 kg) anaesthetized with sodium pentobarbitone (Sagatal; 40 mg kg^{-1}, initial dose I.P., supplemented when necessary with 4–5 mg I.V., see later). A femoral artery was cannulated with a balloon-tipped catheter (Swan-Ganz; Baxter Healthcare Co., Thetford, UK), which was positioned in the descending aorta. The catheter lumen was used for continuous recording of arterial blood pressure. A catheter was inserted into a femoral vein for administration of drugs and supplementary anaesthetic. The trachea was cannulated caudal to the larynx and the animals breathed spontaneously a mixture of O_2-enriched room air.

Animals were paralysed with gallamine triethiodide (Flaxedil; 4 mg kg^{-1} I.V., supplemented with 1–3 mg kg^{-1} h^{-1} I.V.) on the completion of surgery. The level of anaesthesia was assessed by observing the presence or absence of a significant withdrawal reflex to pinching a paw before paralysis and by the absence of alterations in blood pressure, phrenic nerve activity and heart rate. Throughout the experiment, a stable level of these variables was used as an indication of the anaesthetic level and any change under resting conditions was countered by supplemental anaesthetic doses, as described above.

The right sinus nerve (SN), aortic nerve (AN), vagus nerve (VN) and superior laryngeal nerve (SLN) were isolated for electrical stimulation (1–2 pulses, 0·1 ms, 1–20 V given at 1 Hz). A Swan-Ganz catheter was advanced via the external carotid artery into the right carotid sinus. Baroreceptors were excited either by the inflation of this balloon (inflation volume, 0·05–0·1 ml saline) or by inflating a balloon-tipped catheter located in the descending aorta. The arterial chemoreceptors of the carotid body were activated by injection of small doses of either 0·1% sodium cyanide or CO_2-saturated saline via the central lumen of the catheter placed in the carotid sinus.

The right phrenic nerve was isolated and cut low in the neck and the central end was desheathed and placed on a bipolar silver electrode for recording central respiratory drive. A bilateral pneumothorax was established to prevent movements of the brainstem and a positive end-expiratory pressure (2–3 cmH_2O) was maintained. The ventilatory minute volume was adjusted to maintain end-tidal CO_2 in the range of 4–5%. Rectal temperature was maintained at 37–38 °C by a servo-controlled heating pad.

The animals were positioned in a stereotaxic frame and the hypothalamus was stimulated using a concentric bipolar stimulating electrode (SNE 100; Rhodes Medical Electrodes, Clark Electromedical, Pangbourne, UK). For initial localization of the HDA, 1 ms pulses, 50–200 μA at 100 Hz were given during 5 s to evoke the characteristic cardiorespiratory response of the defence reaction (increase in blood pressure, heart rate, amplitude and/or frequency of phrenic burst), together with retraction of the nictating membrane, mydriasis and piloerection. The defence area was delineated using the criteria of Mifflin *et al.* (1988*b*); stimulus sites were shown to block the cardiovascular responses to baroreceptor stimulation. During neuronal recording the stimulus was reduced to short trains of up to ten pulses (0·1 ms given at 1 Hz). This markedly reduced, or abolished, the changes in blood pressure evoked by the continuous stimulation but retained the changes of the phrenic nerve activity. The sites of stimulation were marked with electrolytic lesions at the end of each experiment by passing continuous 500 μA DC current through the electrode for 10–20 s and subsequently localized histologically.

The brainstem was exposed and stabilized. Intracellular recordings were taken from neurones in the vicinity of the NTS with glass-filament microelectrodes filled with 1–5 M Bapta in 3 M potassium acetate (see Richter, Champagnat, Jacquin & Benaka, 1993). DC impedances ranged between 30 and 120 MΩ. Intracellular potentials were recorded conventionally (Axoclamp 2A; current clamp mode). Neurones were considered to

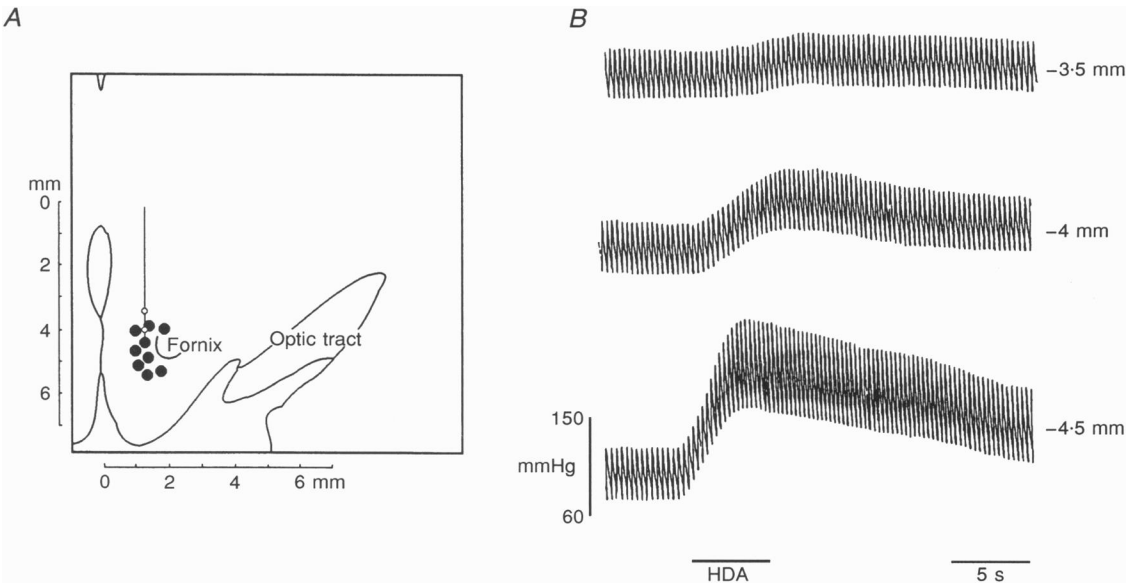

Figure 1. Pressor response to HDA stimulation

A, the location of the stimulation sites in the hypothalamic defence area (HDA; ●) that elicited the cardiorespiratory features of defence response in 9 of 10 cats. Also shown are 2 sites of stimulation (○) during a single electrode penetration through the hypothalamus that evoked blood pressure responses as shown in *B*. In *B*, the largest increase in blood pressure corresponds to the filled circle in line with the open circles.

be satisfactorily impaled if the membrane potential was greater than −45 mV. The position of recording sites was noted with respect to the measured position of the penetration with regard to the obex and the depth below the surface that was read from the digital read-out of the nanostepper. Penetrations were restricted to an area extending from 1 mm caudal to 2·5 mm rostral to the obex, and within 2 mm of the mid-line.

All data were stored on magnetic tape (Racal Store 7) and off-line analysis was undertaken using a computer A/D system with data capture and analysis software (CED 1401 plus, Spike 2, Sigavg, Cambridge Electronic Design, Cambridge, UK).

For statistical analysis we used Student's t test for paired observations. We consider values of t corresponding to $P < 0{\cdot}05$ to be significant.

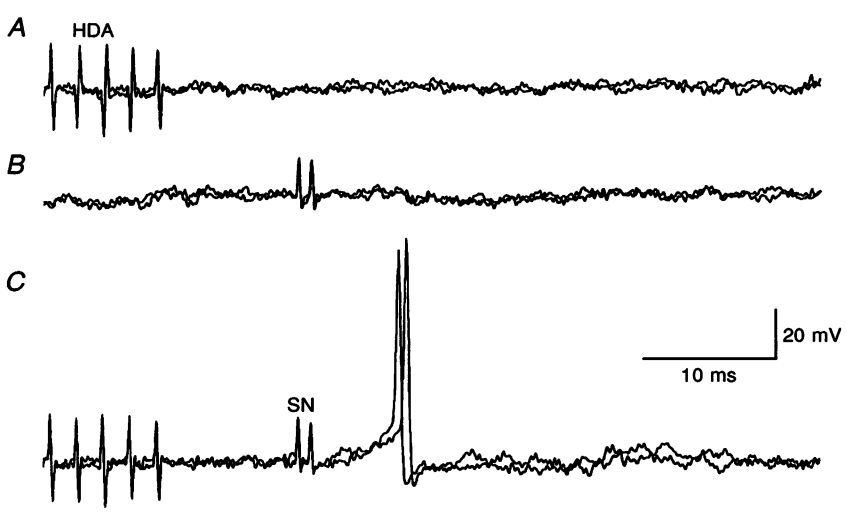

Figure 2. HDA stimulation-evoked facilitation of SN responses

Intracellular recording (2 superimposed sweeps; membrane potential, −55 mV). *A* shows the absence of any direct effect of the HDA (5 pulses, 500 Hz, 0·1 ms, 100 μA delivered at 1 Hz). In *B*, sinus nerve stimulation (SN; 2 pulses, 1 kHz, 0·1 ms, 5 V given at 1 Hz) failed to evoke any response. However, when this stimulus was preceded by a conditioning stimulus to the hypothalamus, as in *A*, an excitatory response was evoked (*C*).

RESULTS

Cardiovascular responses evoked from the hypothalamic defence area

The cardiovascular and respiratory components of the defence response were elicited by stimulation within the hypothalamus. This stimulus evoked a sustained increase in arterial blood pressure and heart rate, with an increase of frequency or amplitude of the phrenic burst and other autonomic features, including pupillary dilatation, retraction of the nictitating membrane and piloerection (see also Coote *et al.* 1979). Stimulation at these sites was also effective in suppressing the cardiovascular features of the baroreceptor reflex, as shown with the protocol identical to that used by Mifflin *et al.* (1988*b*). The histologically verified electrode positions are shown in Fig. 1*A*, together with examples of the blood pressure responses seen during a single electrode penetration through the hypothalamus (Fig. 1*B*).

Neuronal recordings within the nucleus tractus solitarii

The activity of a total of 110 units whose activity was affected by HDA stimulation has been studied; their positions, based on the co-ordinates of electrode penetration, were within the NTS. Of these, twenty-five cells were studied with extracellular recordings and eighty-five with intracellular recordings (membrane potentials > -45 mV). These neurones were also subjected to an analysis of their responses to stimulation (single or double, 0·1 ms pulses at 1 kHz, 1–15 V given at 1 Hz) of a range of peripheral afferent inputs (SN, VN, SLN and AN). The present study focused on those NTS neurones that received excitatory inputs on HDA stimulation (17 recorded extracellularly and 51 recorded intracellularly), although the overall pattern of response was within the pattern described by Mifflin *et al.* (1988*a*, *b*). An additional group of fifteen neurones (2 recorded extracellularly and 13 intracellularly) that were activated antidromically on HDA

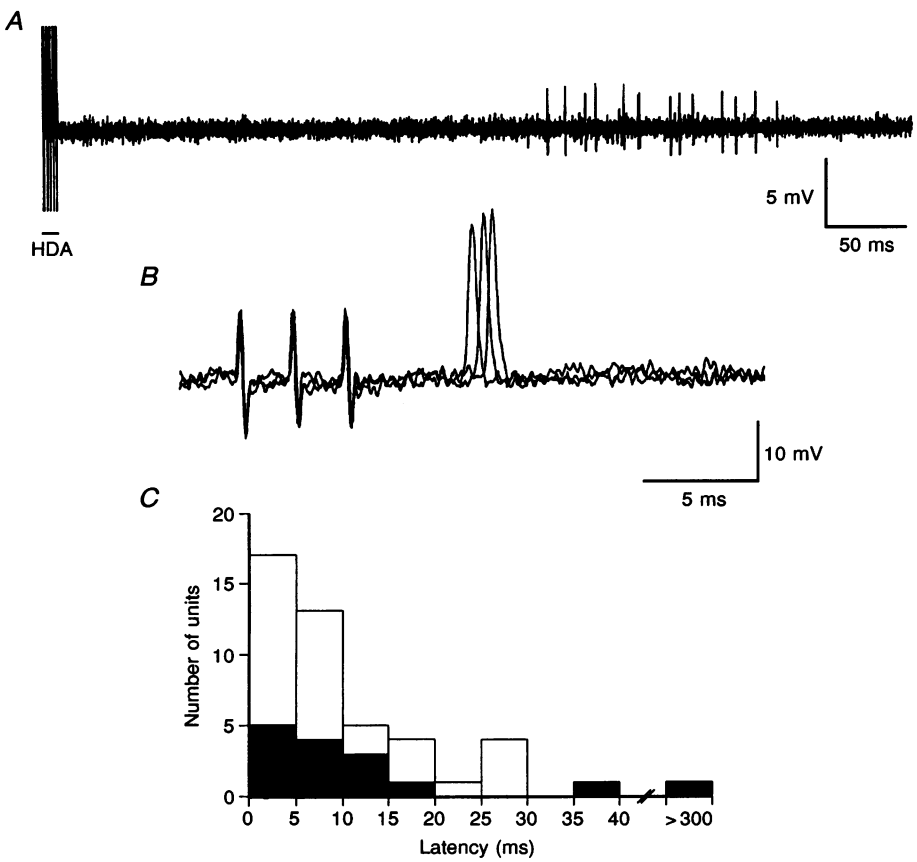

Figure 3. Characteristics of HDA stimulation-evoked excitatory responses

A shows an example of an extracellular recording of the activity of a cell that was activated by stimulation of the HDA (5 pulses, 500 Hz, 1 ms, 100 μA given at 1 Hz) responding with a latency of about 300 ms. *B* shows an intracellular recording of a unit (membrane potential, −45 mV) activated by HDA stimulation (3 pulses, 1 ms, 500 Hz, 100 μA, given at 1 Hz). In *A* and *B* each trace is a superimposition of 3 sweeps. *C* provides an illustration of the latency of evoked excitatory responses as a histogram. Open bars represent data from intracellular recordings (this includes latencies measured for EPSPs; EPSPs/IPSPs and action potentials from 44 neurones); filled bars represent data from extracellular recordings ($n = 15$).

stimulation were also observed (no further details will be provided).

No attempt has been made in this study to determine the biophysical characteristics of the NTS neurones investigated, since the major concern was to determine the pattern of synaptic inputs evoked by various stimuli. Further, no quantification of the evoked EPSP and IPSP is provided, since standardized stimulus protocols in terms of intensity of stimulation or timings in the respiratory or inflation cycle were not applied. However, EPSPs ranged between 0·5 and 10 mV and IPSPs between 0·5 and 9·0 mV.

Facilitatory responses. A group of nine NTS neurones revealed no changes in membrane potential (7 neurones) or evoked discharge (2 neurones in extracellular recordings) on HDA stimulation, but the stimulus facilitated the effects of stimulating either (or both) the SN and SLN (Fig. 2). Extracellularly, observations are limited in value, since subliminal effects of HDA stimulation were not investigated by exhaustive and detailed conditioning –test protocols with respect to either SN or SLN inputs at a wide variety of intensities of stimulation. However, a conditioning stimulus to HDA provoked an increase in the number of action potentials (from 1 to 3 spikes in one case and from 3 to 6 spikes in the other). Facilitation in intracellular recordings was observed in four units receiving SN inputs, in one receiving SLN inputs and in two units receiving both. The HDA conditioning stimulus provoked a decrease in the latency to onset of the responses to both SN and SLN stimulation that ranged between 1·3 and 3 ms. This was accompanied by an increase in the number of action potentials in four cells (from $2 \pm 0{\cdot}7$ to $3{\cdot}75 \pm 1{\cdot}7$, $P < 0{\cdot}05$). In three cells, SLN (1 cell) and SN (2 cells) stimulation evoked EPSPs alone (amplitude, 2·5–5·0 mV). In the presence of a conditioning stimulus, SLN or SN stimulation at the same intensity then evoked an action potential response (Fig. 2).

Excitatory effects on NTS neurones. Fifteen neurones studied extracellularly were shown to be excited synaptically on HDA stimulation; four of these showed no response to nerve stimulation. The latency for excitation ranged widely, from 1 to 300 ms (Figs 3*A*, 4*A* and 5*B*), although the majority of neurones (13 of 15) had a latency for excitation of less than 30 ms. In intracellular recordings, thirty-five NTS neurones were shown to receive an EPSP (onset latency, 1–30 ms) with, on occasions, accompanying action potential discharges (see Fig. 4*B*). Of these, nine neurones were unaffected by stimulation of the various peripheral nerves, whilst twenty-six showed convergent inputs from other sources. In those neurones receiving convergent inputs, a SN input was seen in twenty-three cases, SLN in sixteen, VN in seven and AN in four. Figure 3 illustrates the range in latencies of evoked spikes in extracellular recordings and EPSPs and/or action potentials in intracellular recordings. From the twenty-

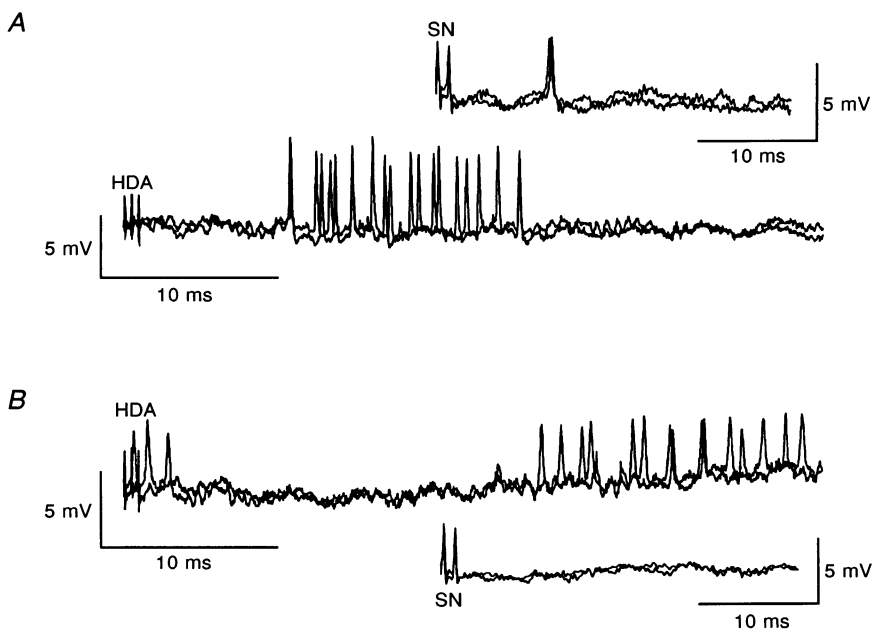

Figure 4. Potential neuronal interactions in NTS

A, an extracellular record taken from a cell that was unaffected by stimulation of the SN (2 pulses, 1 kHz, 0·1 ms, 8 V, given at 1 Hz) but responded to the stimulation of the HDA (3 pulses, 500 Hz, 1 ms, 50 μA given at 1 Hz) as shown in the lower traces. *B*, a recording from another neurone in the immediate vicinity of neurone A (separated by less than 50 μm) that was excited by stimulation of the SN (same parameters as in *A*; upper traces) in which HDA stimulation provoked a silencing of on-going activity (same parameters as in *A*; lower traces). In *A* and *B*, 2 traces are superimposed.

three cells receiving simultaneous inputs from the SN and HDA (Fig. 5*A* and *B*), four were tested for their response to baroreceptor activation and responded with an increase in activity (Fig. 5*C*). Another group of twelve neurones out of these twenty-four were excited on stimulation of the arterial chemoreceptor (not illustrated; but see Silva-Carvalho *et al.* 1995).

Neurones showing an EPSP–IPSP in response to HDA stimulation. In nine neurones HDA stimulation evoked an EPSP followed by an IPSP (see Figs 6 and 7). The latency of the EPSP was 3–10 ms, and the latency of the accompanying IPSPs was 10–30 ms. The maximum amplitude of the IPSP was seen after 60–80 ms. Four of these neurones were unaffected by baroreceptor activation, and one neurone was inhibited by sinus inflation. In five of these nine units convergent inputs (Fig. 6*B*) were seen on stimulation of peripheral inputs (SN in 5, VN in 1 and SLN in 2 neurones). Four neurones, however, showed no other inputs.

The patterns of summation between peripheral inputs and the effects of HDA stimulation were dependent on the timing of inputs. With HDA stimulation as a conditioning stimulus, test stimuli to peripheral nerves could be shown to elicit EPSP summation, or if the test stimulus was timed appropriately, the HDA-evoked IPSP (amplitude, 3·1–6·4 mV; latency, 20–40 ms) could act to shunt EPSPs evoked by other inputs. An example is provided in Fig. 6, where the timing of stimuli results in a diminution of a SN-evoked excitatory response (shown by a decrease in the number of action potentials evoked by SN stimulation from 2 to 1) at a particular interval together with a subsequent summation of the inhibitory actions of both HDA and SN stimulation (Fig. 6*D*). In cells that received no other inputs, IPSPs evoked by HDA stimulation (Fig. 7*C*) inhibited the on-going activity during the period corresponding to the evoked IPSP (Fig. 7*B*). In this particular cell the IPSP was reversed using a hyperpolarizing current of 2 nA, the reversal potential being approximately −75 mV (Fig. 7*B*).

HDA-evoked IPSPs. In a previous study (Mifflin *et al.* 1988*b*) particular emphasis was placed on the inhibitory actions elicited in the NTS on stimulation within the HDA. Since we have identified novel excitatory actions within the NTS as a consequence of HDA stimulation, it was

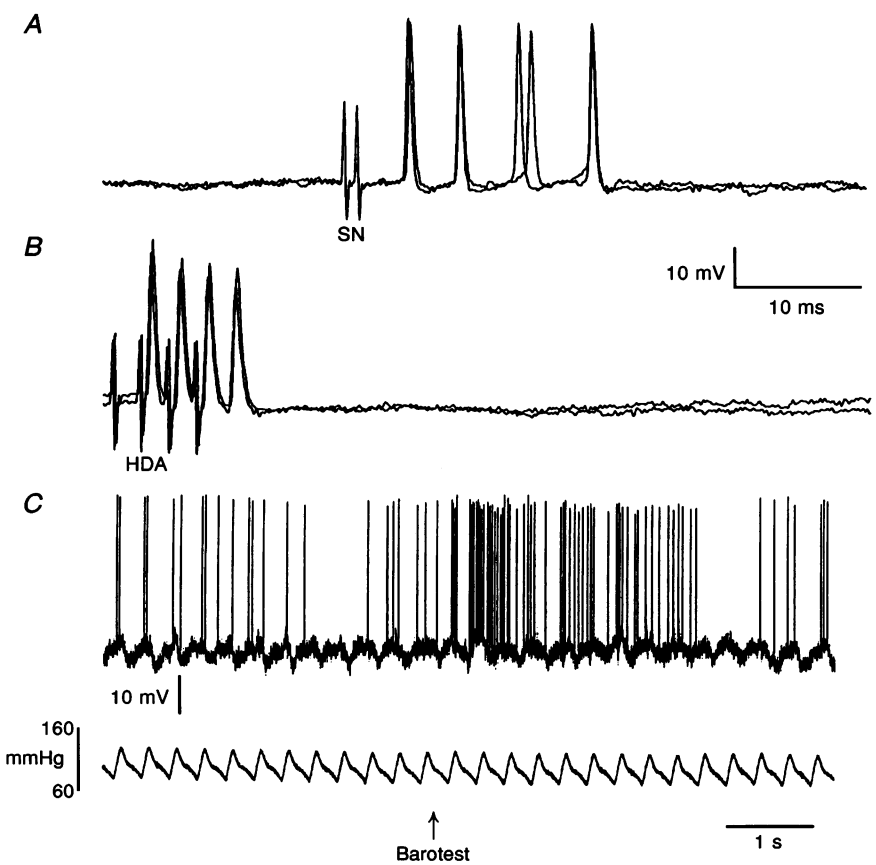

Figure 5. Excitatory convergence of baroreceptor and HDA effects

A, intracellular recording (membrane potential, −50 mV) from a cell that responded to SN stimulation (2 pulses, 0·1 ms, 6 V given at 1 Hz) with evoked action potentials. *B*, short-latency action potentials evoked by HDA stimulation (4 pulses, 0·1 ms, 500 Hz, 40 μA given at 1 Hz). In *A* and *B*, 2 traces are superimposed. *C*, the response of this neurone to the inflation of a balloon in the carotid sinus (Barotest). Upper trace, neuronal discharge; lower trace, arterial blood pressure.

necessary to establish that inhibitory actions of the type previously reported were elicited under the conditions of the present experiments. IPSPs were often evoked in NTS baroreceptor-sensitive neurones during hypothalamic stimulation (see Fig. 8*B*). Detailed analysis was undertaken in only five neurones. In these the latency to onset of the evoked IPSP ranged from 10 to 40 ms, and they had long durations (150–200 ms). Equivalent inhibitory effects were seen in extracellular recordings (Fig. 4*B*).

HDA stimulation decreased the magnitude of the excitatory responses elicited by either SN (from 4·16 ± 0·7 to 2·06 ± 0·6 mV, $n = 3$, $P < 0·05$) or SLN (from 4·07 ± 1·71 to 2 ± 1·5 mV, $n = 4$, $P < 0·05$) stimulation (see Fig. 8*C*, compare with Fig. 8*A*). Since this pattern of interaction has been so clearly described elsewhere (Mifflin *et al.* 1988*b*; Dawid-Milner, Silva-Carvalho, Goldsmith & Spyer, 1994, 1995), no further description will be provided.

It should be noted, however, that the neurone illustrated in Fig. 4*B* (neurone B) was recorded just a few micrometres (< 50 μm) below a neurone (neurone A) excited on HDA stimulation (Fig. 4*A*). The timing of excitation in neurone A, which was unaffected by SN stimulation, coincided with the period of inhibition seen in neurone B, which was excited by SN and baroreceptor stimulation. Although close physical proximity does not imply connectivity, it is attractive to suggest that neurone A represents an inhibitory neurone regulating the discharge of neurone B.

Occasionally, summation of evoked IPSPs ($n = 3$) was also seen (see Fig. 6*D* for the IPSP component of EPSP–IPSP). When the IPSPs evoked by individual stimulation of HDA and SN were timed to coincide, an enlarged IPSP was seen, which was the sum of the individual IPSPs. In one of these three neurones, the HDA-evoked IPSP was reversed using a current of 3 nA.

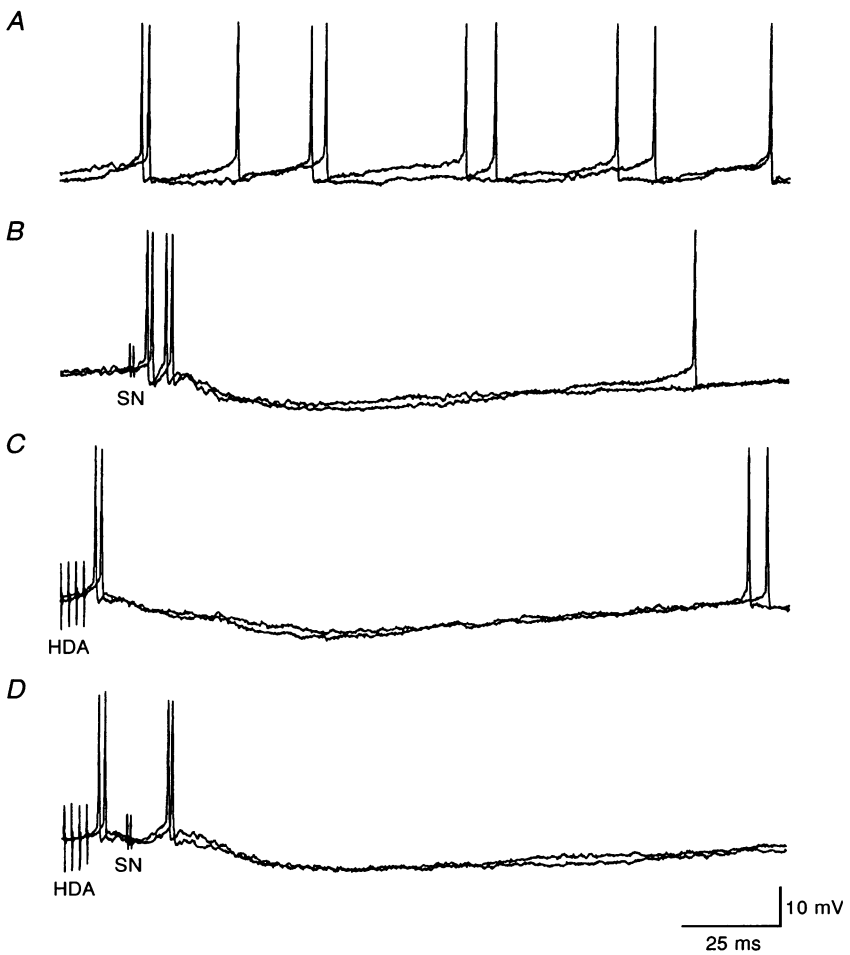

Figure 6. Interneuronal response to HDA stimulation

Intracellular recording of the activity of a cell (membrane potential, −60 mV). *A*, on-going activity of the cell. *B*, stimulation of SN (2 pulses, 1 kHz, 0·1 ms, 9 V given at 1 Hz) evoked an EPSP with bursts of spikes followed by an IPSP and silencing of on-going activity. *C*, stimulation of the HDA (5 pulses, 500 Hz, 1 ms, 150 μA given at 1 Hz) provoked an EPSP followed by an IPSP. In *D*, SN stimulation follows 20 ms after conditioning stimulus to HDA, resulting in a reduction in the number of spikes evoked by SN stimulation and silencing of on-going activity. All panels show 2 superimposed traces.

DISCUSSION

The present study has identified a significant population of NTS neurones that is excited on electrical stimulation in the HDA. Whilst the prevalence of excitatory input cannot be quantified from the results of this study, since efforts were made to restrict analysis of inhibitory inputs that have been detailed by our laboratory in an earlier study (Mifflin *et al.* 1988*b*), it is clear that excitation is more prevalent than was inferred in that original study. In that study only seven of forty-six (15%) neurones studied in intracellular recordings gave excitatory responses to HDA stimulation, whilst in the present study 68 of 110 neurones showed some level of excitation (62%) on hypothalamic stimulation. There are no systematic differences in protocols between this study and that of Mifflin *et al.* (1988*b*). These observations have considerable importance in interpretation of the role of the NTS in the regulation of cardio-respiratory reflex function.

There is a growing appreciation that the GABA-containing neurones of the NTS may have a major role in the regulation of the cardiovascular system (see Spyer, 1994 for discussion). This extends from the modulation of baroreceptor reflex function (see Mifflin *et al.* 1988*b*; Jordan *et al.* 1988; Spyer, 1990, 1994) to a role in the expression of the cardiovascular responses in exercise (McWilliam & Shepheard, 1988). GABA-mediated actions in the NTS are diverse, including actions at both $GABA_A$ and $GABA_B$ receptors, although the physiological role of the latter is unproven *in vivo* (Brooks, Glaum, Miller & Spyer, 1992). The physiological characteristics of an intrinsic GABA-containing neurone, in terms of the pattern of response to peripheral and central inputs, is as yet unresolved. The present data may go some way to filling this void, although we have as yet no definitive evidence that interneurones identified on the basis of their responses to hypothalamic stimulation are indeed GABA containing. Even so, there are at least some features of

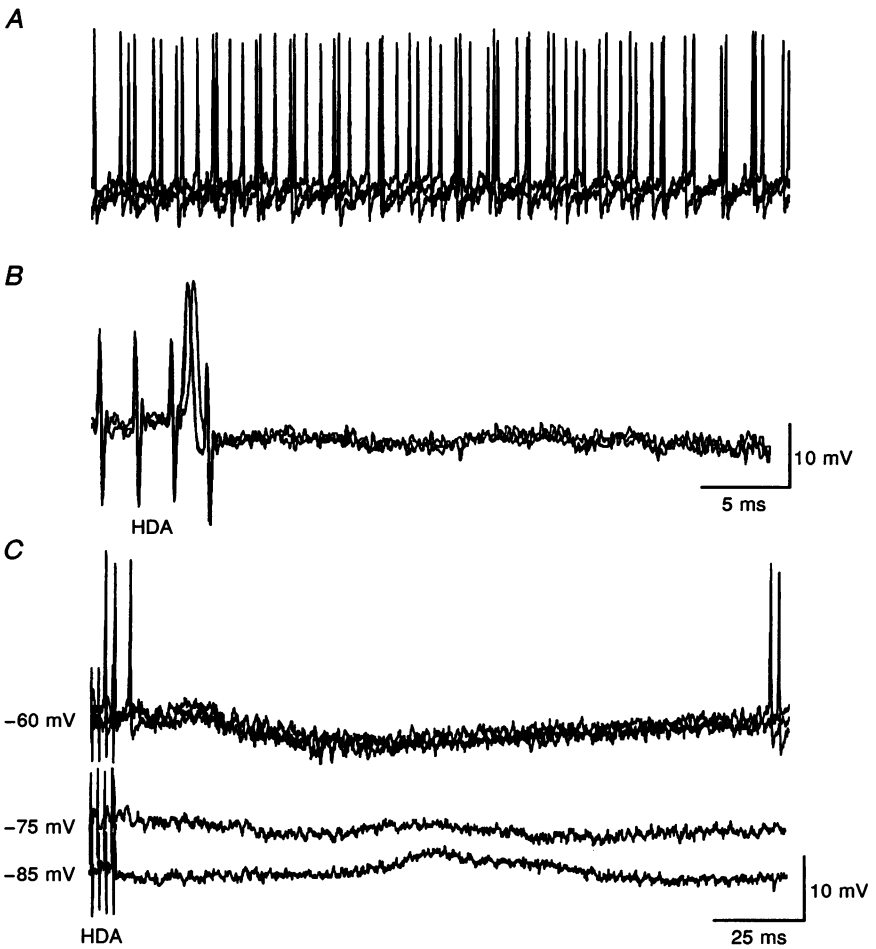

Figure 7. EPSP–IPSP response pattern to HDA stimulation

A, an intracellular record taken from a cell with on-going activity. *B* and upper trace of *C* show stimulation of the HDA (4 pulses, 500 Hz, 1 ms, 100 μA, given at 1 Hz) provoking an EPSP with action potential discharge followed by an IPSP that silenced on-going discharge. Lower traces in *C* show that hyperpolarizing current of 2 nA reversed the IPSP at a membrane potential of −75 mV (see single HDA sweeps at −75 and −85 mV). *A*, *B* and upper record in *C* show 3 superimposed traces.

their response patterns that make this a likely, or at least a plausible argument.

The neurones most likely to be GABA containing are those NTS neurones that are excited on HDA stimulation but are unaffected by stimulating peripheral afferent inputs, such as the SN, SLN and VN (see also Mifflin *et al.* 1988*b*). In the present study, thirteen of the sixty-eight (i.e. 19%) neurones showing only excitatory responses to HDA stimulation fell into this category. Four of this type were described in the earlier investigation (Mifflin *et al.* 1988*b*). In the context of function, it is interesting to note the temporal sequence of discharge evoked in one such potential GABA-containing interneurone (Fig. 4*A*) as it relates to the discharge evoked in a neighbouring cell to an identical hypothalamic stimulus (Fig. 4*B*). The period of evoked discharge in neurone A corresponds so closely to the period of inhibition in neurone B that a causal relationship is compelling. Whilst this is a potentially important observation, it is dangerous to rely on such a limited example in developing a causal relationship; the latency of excitatory effects and their durations cover the range of inhibitory actions that are described in this report and have been demonstrated earlier in NTS neurones on HDA stimulation (see Mifflin *et al.* 1988*b*). Indeed, some excitatory inputs had latencies as long as 300 ms, and even short-latency responses usually had long-duration effects, so that a causal relationship between excitation of one group of neurones and inhibition in another is quite firmly based on experimental observations. No evidence is yet available to preclude the fact that a proportion of these neurones may function as excitatory interneurones, feeding excitation onto GABA-containing neurones, or providing excitatory influences onto chemoreceptor-activated NTS neurones (see Silva-Carvalho *et al.* 1994).

The neurones showing excitatory responses were recorded over a more extensive area of the NTS than those areas described, on the basis of immunocytochemical studies, as containing GABA neurones (Izzo *et al.* 1992). This may

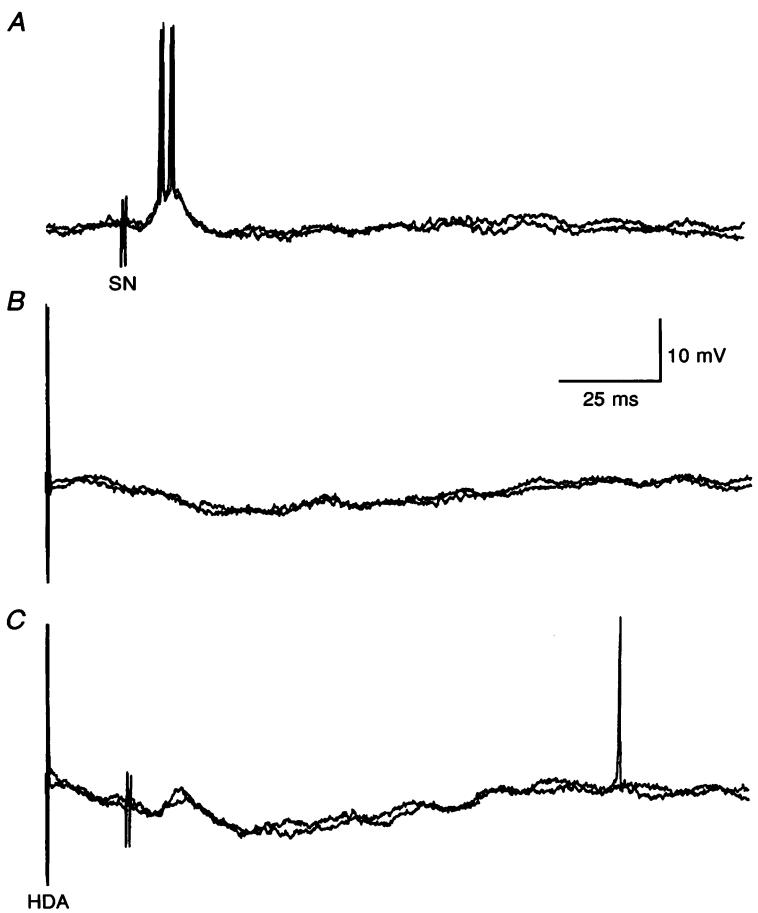

Figure 8. Inhibitory actions of HDA within NTS

A, intracellular recording (membrane potential, −62 mV) from a cell that responded to SN stimulation (2 pulses, 0·1 ms, 1 kHz, 10 V given at 1 Hz) with an EPSP and action potential discharge. *B*, an IPSP evoked by the stimulation of the HDA (5 pulses, 0·1 ms, 500 Hz, 50 μA given at 1 Hz) is shown; only the last HDA pulse is illustrated. *C*, when the SN stimulation was preceded by HDA stimulation (interval, 20 ms) the IPSP evoked by HDA stimulation shunts the response to SN stimulation (*C*). Each panel shows 2 superimposed traces.

reflect the limited identification of GABA-containing neurones that is possible when using an antibody directed against GABA itself; antibodies to the synthetic enzyme glutamic acid decarboxylase (GAD) (Maley & Elde, 1982) tend to label only GABA-containing terminals, having too low a level of antibody to localize adequately perikarya containing GABA (see Izzo *et al.* 1992 for discussion). This implies that immunocytochemical detection may underestimate the extent of GABA-containing neurones within the NTS, labelling only a limited subpopulation. It is thus imperative that, in future studies, intracellularly recorded neurones are identified and localized using either HRP or biocytin iontophoresis and then these neurones should be subjected to GABA immunochemistry so that final proof can be obtained.

In addition to the demonstration of excitatory synaptic inputs onto NTS neurones as a consequence of HDA stimulation, including inputs onto putative GABA-containing neurones, evidence has also accumulated for a more general facilitatory influence of hypothalamic stimulation. This effect is consistent with the neuronal organization of the NTS that has been proposed in a recent review (Spyer, 1994). In this model, many NTS neurones do not receive direct input from either the periphery (such as SN, SLN or VN) or central sites, but are contacted by other NTS neurones, which themselves receive patterned synaptic input from some, or all, of these sites. In the present study, several neurones were observed that had no discernible synaptic input from either the SN or HDA separately, even when the intensity of stimulation was raised. Yet when the two inputs were activated together an excitatory response was seen (Fig. 2). This suggests that the neurone was excited by an antecedent neurone, whose output was dependent on summation of input from these two sources. Given the latency of the facilitatory responses, this observation also argues for a summation of peripheral and central inputs at very early stages in the reflex pathway, an observation that is consistent with data accumulated in this and earlier studies (see Mifflin *et al.* 1988*b*; Silva-Carvalho, Dawid-Milner, Goldsmith & Spyer, 1993). These 'facilitatory' interactions were not analysed exhaustively in the present study, so their occurrence may well be more extensive than indicated in this report.

A novel observation in the present study was the recognition of neurones in the NTS that were excited by both SN and HDA inputs and also were shown to receive an excitatory input from the arterial baroreceptor. Such responses have been reported only in extracellular recordings in the presence of ionophoretically applied bicuculline, the $GABA_A$ receptor antagonist (Jordan *et al.* 1988). The early excitatory effect of HDA stimulation was absent in control conditions, indicating that the predominant hypothalamic influence was inhibitory (see also Mifflin *et al.* 1988*b*) and mediated by GABA. Several NTS neurones with defined baroreceptor inputs showed EPSP–IPSP sequences to HDA stimulation (see Fig. 6). This implies that hypothalamic stimulation can, under appropriate conditions, both facilitate and inhibit the baroreceptor reflex through actions in the NTS. This was suggested first by Jordan *et al.* (1988) to illustrate the heterogeneous characteristics of the HDA and regions concerned with affective behaviour in general; it can elicit defence reactions but also 'playing-dead' responses, depending on the site and intensity of stimulation. In this context it is worth stressing the vulnerability of electrical stimulation studies to this type of complexity, since such stimulation will excite perikarya in the vicinity of the electrode tip but also axons of passage. The HDA may well be a site of particular complexity in terms of axonal pathways as well as containing neurones that project into the descending pathways responsible for the autonomic features of affective behaviour (Jordan, 1990 for discussion). The most prevalent observations in this, and earlier studies, is that HDA stimulation inhibits the baroreceptor reflex (in the cat) and this can be reconciled with the neuronal response patterns seen in the majority of NTS neurones that are sensitive to baroreceptor input.

Whether the direct excitatory pathway is non-specific with respect to its innervation of both putative GABA-containing neurones and 'baroreceptor'-sensitive neurones and other neurones of the NTS remains to be investigated. It is clear that the majority of NTS 'baroreceptor'-sensitive neurones encountered in these studies do not show, under the conditions of these experiments (i.e. anaesthetized, artificially ventilated cats), evidence of the early excitatory input, implying that descending control of GABA-containing interneurones is the most pronounced effect of HDA stimulation, at least in the cat.

Biscoe, T. J. & Sampson, S. R. (1970). Responses of cells in the brainstem of the cat by stimulation of the carotid sinus, glossopharyngeal, aortic and superior laryngeal nerves. *Journal of Physiology* **209**, 359–373.

Brooks, P. A., Glaum, S. R., Miller, R. J. & Spyer, K. M. (1992). The actions of baclofen on neurones and synaptic transmission in the nucleus tractus solitarius of the rat *in vitro*. *Journal of Physiology* **457**, 115–129.

Coote, J. H., Hilton, S. M. & Perez-Gonzalez, J. F. (1979). Inhibition of the baroreceptor reflex on stimulation in the brainstem defence centre. *Journal of Physiology* **288**, 549–560.

Coote, J. H., Hilton, S. M. & Zbrozyna, A. W. (1973). The ponto-medullary area integrating the defence reaction in the cat and its influence on muscle blood flow. *Journal of Physiology* **229**, 549–560.

Dawid-Milner, M. S., Silva-Carvalho, L., Goldsmith, G. & Spyer, K. M. (1995). Hypothalamic modulation of laryngeal reflexes in the anaesthetized cat: role of the nucleus tractus solitarii. *Journal of Physiology* **487**, 739–749.

FELDER, R. B. & MIFFLIN, S. W. (1988). Modulation of carotid sinus afferent input to nucleus tractus solitarius by parabrachial nucleus stimulation. *Circulation Research* **63**, 35–43.

FELDER, R. B. & MIFFLIN, S. W. (1994). Baroreceptor and chemoreceptor afferent processing in the solitary tract nucleus. In *Nucleus of the Solitary Tract*, ed. BARRACO, I. R. S., pp. 169–185. CRC Press, London.

IZZO, P. M., SYKES, R. M. & SPYER, K. M. (1992). Aminobutyric acid immunoreactive structures in the nucleus tractus solitarius, a light and electron microscopic study. *Brain Research* **591**, 69–78.

JORDAN, D. (1990). Autonomic changes in affective behaviour. In *Central Regulation of Autonomic Function*, ed. LOEWY A. D. & SPYER, K. M., pp. 349–366. Oxford University Press, New York.

JORDAN, D., MIFFLIN, S. W. & SPYER, K. M. (1988). Hypothalamic inhibition of neurones in the nucleus tractus solitarius of the cat is GABA mediated. *Journal of Physiology* **399**, 389–404.

LOPES, O. U. & PALMER, J. F. (1978). Mechanisms of hypothalamic control of cardiac component of sinus nerve reflex. *Quarterly Journal of Experimental Physiology* **63**, 231–254.

MCWILLIAM, P. N. & SHEPHEARD, S. N. (1988). A GABA-mediated inhibition of neurones in the nucleus tractus solitarius of the cat that respond to electrical stimulation of the carotid sinus nerve. *Neuroscience Letters* **94**, 321–326.

MALEY, B. E. (1994). Immunohistochemical localisation of neurochemical synaptic circuits of the nucleus tractus solitarii at the light microscopic and ultrastructural levels. In *Nucleus of the Solitary Tract*, ed. BARRACO, I. R. S., pp. 63–73. CRC Press, London.

MALEY, B. & ELDE, R. (1982). Immunohistochemical localization of putative neurotransmitters within the feline nucleus tractus solitarius. *Neuroscience* **7**, 2469–2490.

MAQBOOL, A., BATTEN, T. F. C. & MCWILLIAM, P. N. (1991). Ultrastructural relationships between GABAergic terminals and cardiac vagal preganglionic motoneurones and vagal afferents in the cat: A combined HRP tracing and immunogold labelling study. *European Journal of Neuroscience* **3**, 501–513.

MIFFLIN, S. W., SPYER, K. M. & WITHINGTON-WRAY, D. J. (1988*a*). Baroreceptor input to the nucleus tractus solitarius in the cat: postsynaptic actions and the influence of respiration. *Journal of Physiology* **399**, 349–367.

MIFFLIN, S. W., SPYER, K. M. & WITHINGTON-WRAY, D. J. (1988*b*). Baroreceptor inputs to the nucleus tractus solitarius in the cat: modulation by the hypothalamus. *Journal of Physiology* **399**, 369–387.

PATON, J. F. R. & SPYER, K. M. (1990). Brainstem regions mediating the cardiovascular responses elicited from the posterior cerebellar cortex in the rabbit. *Journal of Physiology* **427**, 533–552.

RICHTER, D. W., CHAMPAGNAT, J., JACQUIN, T. & BENAKA, R. (1993). Calcium currents and calcium-dependent potassium currents in mammalian medullary respiratory neurones. *Journal of Physiology* **470**, 23–33.

RICHTER, D. W. & SPYER, K. M. (1990). Cardiorespiratory control. In *Central Regulation of Autonomic Function*, ed. LOEWY, A. D. & SPYER, K. M., pp. 189–207. Oxford University Press, New York.

SILVA-CARVALHO, L., DAWID-MILNER, M. S., GOLDSMITH, G. E. & SPYER, K. M. (1993). Hypothalamic-evoked effects in cat nucleus tractus solitarius facilitating chemoreceptor reflexes. *Experimental Physiology* **78**, 425–428.

SILVA-CARVALHO, L., DAWID-MILNER, M. S., GOLDSMITH, G. E. & SPYER, K. M. (1995). Hypothalamic modulation of the arterial chemoreceptor reflex in the anaesthetized cat: role of the nucleus tractus solitarii. *Journal of Physiology* **487**, 751–760.

SPYER, K. M. (1990). The central nervous organisation of reflex circulatory control. In *Central Regulation of Autonomic Functions*, ed. LOEWY, A. D. & SPYER, K. M., pp. 168–188. Oxford University Press, New York.

SPYER, K. M. (1994). Central nervous mechanisms contributing to cardiovascular control. *Journal of Physiology* **474**, 1–19.

Acknowledgements

The study was supported by a programme grant from the Wellcome Trust to K. M. Spyer. The authors are grateful for the excellent technical assistance of Ms A. Bradley and to Shirley-Anne Jurmann for the preparation of the manuscript.

Authors' present addresses

L. Silva-Carvalho: Instituto de Fisiologia, Faculdade de Medicina de Lisboa, Ava Egas Moniz, 1600 Lisboa, Portugal.

M. S. Dawid-Milner: Departmento de Fisologia, Facultad de Medicina, Universidad de Malaga, Malaga, Spain.

Received 29 September 1994; accepted 8 March, 1995.