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$GABA_B$-related activity involved in synaptic processing of somatosensory information in S1 cortex of the anaesthetized cat

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1 The possible role of $GABA_B$ receptor mechanisms in information processing in primary somatosensory (S1) cortex was assessed by use of extracellular recording combined with microiontophoretic methods from 161 neurones in anaesthetized, paralysed cats.

2 Baclofen-induced suppressions of cell responses were reversible and stereoselective, the (+)-isomer being inactive and the (−)-isomer having two to three times the apparent potency of γ-aminobutyric acid (GABA). The responses measured were threshold to natural stimulation of receptive fields (RFs), responsiveness to thalamic electrical stimulation, change in RF size and magnitude of firing elicited by iontophoretic glutamate.

3 The action of GABA always was mimicked by muscimol or 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) but not always by (−)-baclofen; in certain cases (−)-baclofen enhanced neuronal responses while the opposite occurred with GABA or with the other $GABA_A$ agonists. The elevation of response thresholds by (−)-baclofen was relatively stronger in peripheral than in central subregions of cutaneous RFs, by contrast with the action of muscimol which was relatively non-selective as to the area in which it was effective.

4 Glutamate-induced and thalamically-evoked cortical responses as well as spontaneous activity were differentially sensitive to the suppressant effects of muscimol and (−)-baclofen.

5 Bicuculline methiodide reversibly blocked THIP- and muscimol-induced suppressions of tactile- (air puffer)-induced S1 responses but spared those produced by (−)-baclofen. Phaclofen and δ-amino-n-valeric acid were essentially inactive as blockers of (−)-baclofen-induced effects and in fact often acted as (−)-baclofen-like agonists, phaclofen being considerably weaker than δ-amino-n-valeric acid in this respect.

6 The range of suppressant effects produced by GABA as well as by muscimol and THIP, considered in conjunction with the actions of bicuculline methiodide, suggest that the effects observed by ejected GABA are likely to be due principally to $GABA_A$ processes, those mediated by $GABA_B$ receptors largely being masked. However, $GABA_B$ mechanisms are extant and do appear to be active, probably presynaptically and probably at sites distal to the soma.

Introduction

Many properties of the physiological responses of neurones in the primary somatosensory (S1) cortex are under the normal synaptic control of γ-aminobutyric acid (GABA)-mediated processes. These include such variables as receptive-field (RF) size, threshold to activation by natural simulation of the cutaneous surface, responsiveness to electrically induced activation of the ventroposterior lateralis (VPL) nucleus of the thalamus and spontaneous discharge (Batuev *et al.*, 1982; Hicks & Dykes, 1983; Alloway & Burton, 1986). Apparently the modality specificity of somatosensory neurones, either in S1 or in S2 cortex (Dykes *et al.*, 1984; Alloway *et al.*, 1988; 1989), or in the thalamus (Hicks *et al.*, 1986) is not influenced by GABA-mediated inhibition. The actions of GABA in shaping the responses of S1 cortical neurones are modifiable by substances which interact with benzodiazepine recognition sites (Oka *et al.*, 1986; Oka & Hicks, 1990) and are independent of and qualitatively unaffected by the particular form of anaesthesia employed, being observed whether the animals are anaesthetized with barbiturate, ketamine or urethane (Hicks *et al.*, 1985) or halothane/nitrous oxide (Alloway *et al.*, 1988).

GABA may exert its actions in cerebral cortex through at least two distinguishable classes of receptor, originally termed A and B types by Bowery *et al.* (1980). There is evidence that $GABA_B$ receptors may be located in large part upon presynaptic terminals and perhaps also at sites relatively distant from the soma (Fox *et al.*, 1978; Potashner, 1978; Bowery *et al.*, 1980; Johnston *et al.*, 1980; Curtis *et al.*, 1981; Newberry & Nicoll, 1985; Krogsgaard-Larsen *et al.*, 1986). Since the only means employed to date in investigating the functional roles of GABA in S1 cortex have been through the use of substances active at $GABA_A$ receptors (e.g., bicuculline methiodide, BMI) or mixed agonists (i.e., GABA itself), it was considered important in furthering our understanding of the way inhibition in sensory pathways is produced centrally, to assess the possible function of $GABA_B$ receptors in S1 synaptic integration by use of an established test paradigm (Dykes *et al.*, 1984; Oka *et al.*, 1986) and with pharmacologically specific agents. A preliminary note containing an account of a portion of these results has appeared recently (Kaneko & Hicks, 1988).

Methods

Experiments were performed on adult cats or either sex, weighing between 1.7 and 5.7 kg. The animals were anaesthetized with pentobarbitone sodium (Somnotol; 35 mg kg^{-1}, i.p.) initially and paralyzed with gallamine triethiodide (Flaxedil; 10 mg kg^{-1}, i.v.). Anaesthesia was maintained throughout the course of the experiment at a stable level through continuous perfusion (6 ml h^{-1}) of pentobarbitone sodium at a dose usually equal to, but not exceeding 2.0 mg kg^{-1} h^{-1}, in a 50 ml mixture based on lactated Ringer solution which also contained gallamine triethiodide (84 mg), dextrose (500 mg) and dextran (2.5 g). The cortical electroencephalogram was continuously available for monitoring

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whenever desired, to assist in the rapid assessment of the state of the animal's anaesthesia and whenever indicated, additional doses of barbiturate were administered. In such cases, testing was suspended temporarily until sufficient time had been allowed for a stable plateau of anaesthesia to be re-established. Additional indices that were used to aid in judgements about the adequacy of the anaesthesia were the change in CO_2 production, spontaneous neuronal activity and the pupillary state of the eyes.

Respiration was maintained with tracheal cannulation and forced ventilation by a respirator pump with end-expiratory CO_2 levels kept at 3.5–4.0%. Body core temperatures were maintained within the physiological range (37.5 ± 0.5°C) by means of a heating pad, controlled thermostatically from a rectal probe. A venous cannula was inserted into a forelimb vein on the ipsilateral side to permit infusion of the mixture and supplementary injection of drugs. The contralateral forelimb including the forepaw was shaved thoroughly up to shoulder level. Cats were positioned in a head-holder frame and craniotomies performed with a hand-held drill to expose the sensorimotor cortex and to permit the insertion of one or a pair of bipolar stimulating electrodes into the VPL nucleus of the thalamus (co-ordinates: AP 1.00, ML −0.68, DV 0.45 to 0.15). The final location of this electrode(s) was checked by measurement of the greatest amplitude of the evoked field potential as recorded extracellularly from a site in area 3b of S1 cortex. Following reflection of the dura overlying S1, the cortex was covered with warmed agar (3%) made in Ringer solution.

Cell recordings were made extracellularly through carbon fibre-containing central barrels of multibarrel micropipette assemblies, the outer channels of which contained the following solutions of drugs in various combinations: γ-aminobutyric acid (GABA: 0.5 M, pH 3.5); β-(*p*-chlorophenyl)-GABA (baclofen; (±); (−)- or (+)-isomers) 20 mM, pH 4.5); muscimol (10 mM, pH 3.5); 4,5,6,7-tetrahydroisoxazolo[5,4-c] pyridin-3-ol (THIP; 20 mM, pH 3.5); β-(*p*-chlorophenyl)-3-aminopropylphosphonic acid (phaclofen; 20 mM, pH 4.5); δ-amino-n-valeric acid (δ-AVA; 0.1 M, pH 4.5); bicuculline methiodide (BMI; 5 mM, pH 3.3); L-glutamic acid (0.5 M, pH 8.0). All solutions of drugs below 100 mM were prepared in 0.9% saline to ameliorate iontophoretic expulsion (Hicks, 1984).

Upon isolation of a single unit, a standard series of tests was performed: activation of the VPL stimulating electrode allowed assessment of whether the response derived from activation of a thalamo-cortical projecting neurone, or was antidromic in nature. The rate of spontaneous discharge was recorded and the body surface was searched for a RF. The RF then was mapped carefully by hand, with small glass probes according to standard criteria for the extent of the area, threshold evaluation, velocity sensitivity and modality and submodality property (Dykes *et al.*, 1980; Sretavan & Dykes, 1983). These tests served to identify the general categories of afferent fibres likely to produce the excitatory responses observed (rapidly adapting, slowly adapting, low-velocity, rapidly adapting, joint, etc.). Cells were chosen for study which exhibited low threshold, rapidly adapting mechanoreceptive responses having RFs located on the forelimb surface.

For natural stimulation of the cutaneous surface, it was of paramount importance to elicit responses which could be reproduced in a consistent manner and so quantitatively controlled stimulation of the skin was produced by use of an air puffer system modified from that of Gardner & Costanzo (1980). This device allowed the independent placement of up to 6 air jet nozzles (puffers) activated under computer control and featured the ability to generate variable airpuff durations and inter-puff intervals. A seventh channel was connected to a pressure transducer permitting the monitoring of the absolute amount of pressure developed for any given puff. Peristimulus time histograms (psths) were constructed from the responses to 20 repetitive stimulus trains. Typically only one or two puffers were used for a test series at one neurone; in the case of 2 puffers, different RF subregions were selected so as to provide the ability of producing a spatially summing stimulus using concurrent puffer activation (Kaneko & Hicks, 1988; Oka & Hicks, 1990). Under this dual stimulation paradigm, the air pressure delivered to the RF from the 2, co-activated puffers, equalled that delivered through either of the single puffers alone.

Electrolytic lesions were placed within penetrations from which data of special interest were sampled through the passage of a short pulse of direct current through the recording electrode. These penetrations were recovered from the histological material in reconstructions of the electrode tracks. Following each experiment, the cat was anaesthetized very deeply with a barbiturate overdose and was perfused transcardially with heparinized, phosphate-buffered saline followed immediately by a 5% solution of formalin and a final solution containing a mixture of 0.1% glutaraldehyde and 10% formalin.

The brain was removed, blocked by hand and sectioned on a freezing microtome at 60 μm thickness. The sections were mounted on subbed slides, counterstained with cresyl violet and coverslipped for microscopic examination. Shrinkage was compensated for by scaling from the surface to the lesion at the end of the track. Cell positions within each electrode penetration were placed by proportional scaling of the depth as recorded from the microelectrode positioner by use of the appropriate correction for tissue shrinkage.

Results

The results reported here are based upon a sample of 161 neurones from S1 cortex, located between 300 μm and 1880 μm depth and corresponding in electrode track reconstructions to laminae II to VI (e.g., Figure 1). This figure illustrates 4 parasagittal sections through the somatosensory cortex. Each contains an electrode trajectory terminated by a lesion. Careful cytoarchitectonic analysis of each such section containing lesions taken from every experiment, allowed the assignment of area and layer to be made for each cell studied. A description of the histological criteria employed for this purpose appears in the legend to Figure 1.

The number of cells tested with each drug used in the present study is shown in Table 1, which also provides a summary of the drugs' effectiveness on those cells. From this table, it is evident that the rank order of potency for the 6 suppressants, from highest to lowest was: muscimol > (−)-baclofen > GABA > (±)-baclofen > THIP > (+)-baclofen. Indeed (+)-baclofen, even at doses well beyond those used for (−)-baclofen, rarely was effective as shown by our data that derive from a functionally intact, physiologically operating preparation; similar observations have been made elsewhere

Table 1 Effective currents of agonists in producing ≥20% reduction of cell firing

(±)-Baclofen	*(−)-Baclofen*	*GABA*	*Muscimol*	*THIP*	*(+)-Baclofen*
28.9 ± 2.3 (48/49)	12.6 ± 0.7 (95/102)	21.6 ± 3.9 (49/51)	7.3 ± 1.0 (37/37)	34.6 ± 3.9 (28/35)	50 (1/15)

The numbers represent the mean values ± s.e.mean of currents (nA) which were used to inhibit cells effectively.
The numbers in parentheses represent the number of cells inhibited/the number of cells tested for each drug.

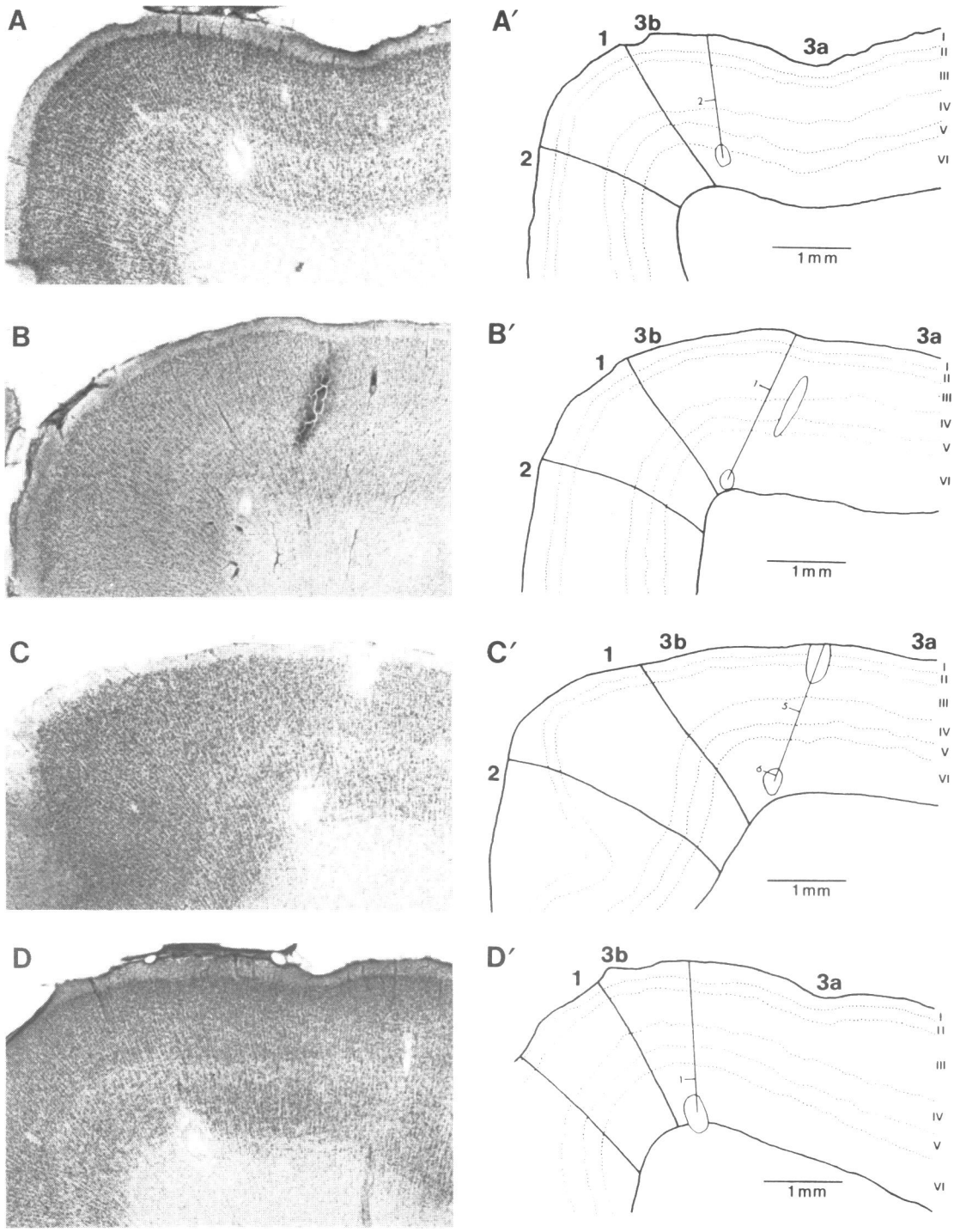

Figure 1 Photomicrographs of parasagittal sections from cresyl violet-stained somatosensory cortex, containing electrode penetrations ending in electrolytic lesions (compare with drawing at right showing lesion as a circled space at the end of the tracks), with corresponding reconstructions drawn by camera lucida to show laminar positions of some recorded cells (location shown beside cell number) and cytoarchitectonic boundaries. Area 3b is distinguished from area 1 by the observation that layer III cannot be subdivided, layer IV is wider in 3b than in 1 and appears relatively more cell-dense, containing many small, granule cells and fewer large pyramidal cells appear in layer V when compared with area 3a, for example (at right of each panel). Looking caudally (left) into area 1, the laminar organization becomes more distinctive, with a subdivided layer III that is less cell-dense, a narrower layer IV than in area 3b and only a few, clustered large layer V cells. Radial palisades are apparent here especially obvious in layer VI, which is thicker than in 3b. Area 3b is clearly differentiated from 3a by the density of layer IV cells and by the lack of darkly staining cells in layer V.

(Howe *et al.*, 1987) for cortical neurones recorded intracellularly *in vitro*. In our hands, (+)-baclofen seldom elicited a significant suppressant action (i.e., our criterion of a >20% reduction), either upon spontaneous firing or upon synaptically driven responses (cutaneously, via air puffer-evoked activity, or thalamically via VPL-evoked synaptic activation). THIP was considerably less potent than all the other agonists tested, including GABA itself which was of intermediate effectiveness.

In order to characterize better pharmacologically the suppressant actions induced by (−)-baclofen, the effects of this substance and of GABA, muscimol or THIP were compared

upon the excitations of cells elicited by puffer stimulation of their RFs before, during and after ejection of BMI. Quantitative comparisons have been made of such suppressions on 12 neurones, the results of which appear in Table 2, where the values in each column represent the means ± s.e.mean of the responses, expressed as a percentage of control (i.e., the lower the number, the greater the suppression).

Contrasting the effects of BMI upon (−)-baclofen-induced response suppressions with those induced by the other agonists, there was a marked difference in effect from control levels of suppression ($P < 0.01$, ANOVA) only with the latter group. That is, BMI produced statistically significant differences from control in the magnitude of the suppressions observed with GABA, muscimol or THIP, but not in the magnitude of the suppressions induced by (−)-baclofen, as is to be expected for $GABA_B$-related processes. Cells tested with BMI were exposed to ejecting currents ranging between 5 and 50 nA (mean ± s.e.mean, 15.1 ± 2.5 nA). Of 19 cells in total examined with BMI (12 of these were tested as well using the airpuffer), 15 were located in rapidly adapting background zones, 3 were in slowly adapting zones whilst one cell was found within a tap region (see Dykes *et al.*, 1984 for classification criteria). These cells were located between the depths of 527 and 1850 μm from the pial surface.

Enhancement (⩾20%) of spontaneous and/or driven activity during the ejection of BMI was observed in 17 of the 19 cells, the 2 cells remaining showing no discernible alteration of baseline response. When BMI was tested upon forms of responses evoked other than by puffer stimulation (i.e., spontaneous firing and electrical activation of VPL neurones projecting to cortical cells) that were suppressed by drugs, there was no effect upon (−)-baclofen-induced suppressions on 8 of 12 cells and a ⩽20% reduction on the remaining 4. By contrast, BMI was ineffective only in 2 of 12 cells tested with GABA, muscimol or THIP but brought about ⩾20% reductions of the suppressant effects in 10 of 12 cells. In 5 of this latter group of 10 cells, BMI showed complete antagonism of the effects of GABA, muscimol or THIP. An example of the selective action of BMI upon (−)-baclofen- and THIP-induced suppressions of air puffer-induced responses is provided in Figure 2. Note as well from this figure that baclofen and THIP also differed in their ability to diminish RF size.

To aid further in assessing the nature of the inhibitory processes activated by the various suppressants employed, the effects of the presumptive $GABA_B$ receptor antagonist, phaclofen, were examined. Twenty-seven cells were tested with this drug, 22 from rapidly adapting areas, 2 from slowly adapting and 3 from low-velocity, rapidly adapting regions, all the cells lying between 300 and 1840 μm depth. The range of ejecting currents of phaclofen was 20–200 nA, with a mean ± s.e.mean of 85.2 ± 8.0 nA. Phaclofen by itself had no discernible effect upon 17 of 25 cells but suppressed the activity of the remaining 8 cells. When tested upon the response suppressions induced by (−)-baclofen, the substance had no effect on just over half the cells tested (13 of 24), reduced the action of (−)-baclofen on 7 and enhanced the action of the agonist at the remaining 4. With reference specifically to the quantitatively determined evaluations using puffer-evoked responses — on 9 cells tested, phaclofen did not diminish or enhance significantly the puffer-induced response suppressions produced by the $GABA_B$ agonist, either at the centrally-located site of activation (mean control response reduced to 71.4 ± 11.8% with (−)-baclofen, compared with 57.2 ± 13.7% with (−)-baclofen in the presence of phaclofen), the peripherally located one (37.0 ± 9.6% compared with 25.9 ± 8.8%), or upon the spatially integrated form of RF stimulation (combined category: 73.2 ± 14.3% compared with 48.8 ± 12.4%; differences in all categories not statistically significant).

Two examples of this lack of effect may be seen in the responses illustrated by Figure 3. On only two cases of all

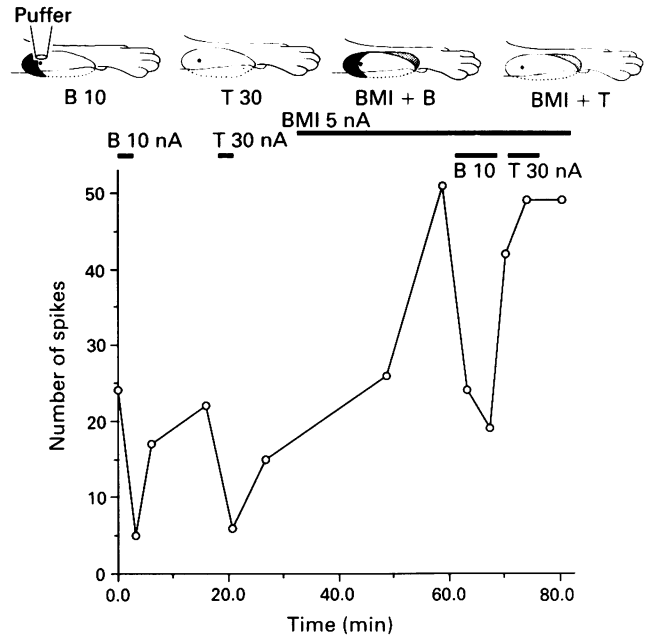

Figure 2 Selective antagonism by bicuculline methiodide (BMI) of 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP)-induced suppressant effects upon air puffer-evoked firing of an S1 cortical neurone. Each plotted point represents the magnitude of puffer-evoked response following 20 stimulus repetitions. Cell was rapidly adapting, evoked at 1.8 ms latency following electrical stimulation of the ventroposterior lateralis (VPL) nucleus of the thalamus and was situated at 916 μm depth in lower cortical layer III. Puffer-elicited responses were suppressed by (−)-baclofen (B), ejected at 10 nA and by THIP (T), ejected at 30 nA. BMI ejection at 5 nA enhanced puffer-evoked responses by 100% after about 25 min, but despite abolishing the THIP-elicited suppressions, exerted no antagonism of the (−)-baclofen effect. These interactions also are mirrored by the effects of the drugs as assessed concomitantly upon receptive field (RF) size and threshold, as displayed in the inset drawings overlying the graphed puffer data. A dot indicates the site of puffer-induced stimulation in the drawings. Outer circle is the border limit of the RF area; RF outlines beyond forelimb boundary (dotted lines) represent continuations for field on other side of forelimb. Black shaded-in area shows cutaneous region no longer responsive to tactile stimulation during drug condition (B 10; (−)-baclofen ejection at 10 nA; $t \sim 3$ min); stippled area shows cutaneous region becoming responsive *de novo* to tactile stimulation during drug condition (BMI; bicuculline ejection at 5 nA; $t \sim 24$ min). Note that effect of (−)-baclofen is not reversed by BMI. THIP was ineffective upon this cell in eliciting threshold or RF size change, as judged by the manual stimulation need to derive these data.

Table 2 Bicuculline methiodide (BMI) failed to antagonize suppressions induced by (−)-baclofen on air puffer-evoked firing

	(−)-*Baclofen*			*GABA, muscimol or THIP*		
	Central	*Peripheral*	*Combined*	*Central*	*Peripheral*	*Combined*
Without BMI	66.1 ± 17.6	20.7 ± 6.9	49.8 ± 14.0	40.5 ± 10.5	11.6 ± 5.0	37.7 ± 8.5
With BMI	73.4 ± 14.7	52.7 ± 11.3	53.0 ± 11.6	88.1 ± 16.6*	113.7 ± 46.5*	77.4 ± 18.2*

The numbers represent the means ± s.e.mean of the responses from 12 cells as a percentage of control (where control is the mean response prior to drug ejection).
An asterisk indicates that the value differs from that obtained without BMI in the same category by $P < 0.01$ (ANOVA).

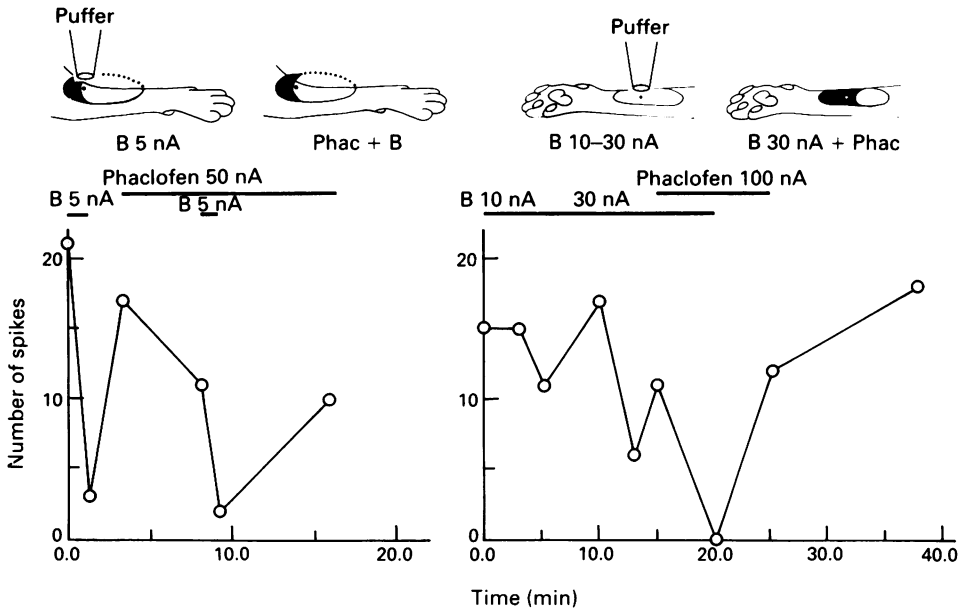

Figure 3 Lack of antagonistic effect exerted by phaclofen of (−)-baclofen-induced suppressant actions upon air puffer-evoked firing of an S1 cortical neurone. Conventions as in Figure 2. Left; phaclofen ejected at 50 nA does not block the (−)-baclofen-evoked response suppressions and actually exerts relatively weaker suppressant effects of its own. No reversal of (−)-baclofen-induced RF size diminutions or of threshold elevations was apparent. Right; (−)-baclofen dose-dependently suppressed air puffer-elicited firing of another S1 cortical neurone, whilst exerting no observable diminution of RF size or elevation of threshold. Addition of phaclofen (100 nA) for ~10 min concomitant with maintained administration of (−)-baclofen resulted in an enhanced suppression of, and an ensuing total abolition of, puffer-elicited activity and a marked reduction of RF size. Upon cessation of ejection of (−)-baclofen but continued administration of phaclofen, RF size returned to control level (not shown) whereas air puffer-elicited firing was maintained at a somewhat reduced level (~66% of control), until full recovery was obtained 12 min following the subsequent cessation of ejection of phaclofen.

tests made on 24 cells in which the effects of (−)-baclofen were also examined, did phaclofen exert actions similar to that expected of a $GABA_B$ receptor antagonist. One of these exceptional cases is illustrated in the oscillographic examples of Figure 4, showing the responses of a single cell to electrical stimulation of the VPL thalamus. In this figure, the shock artifact is indicated by the dot, the evoked field potential response by asterisks and the action potentials by arrows.

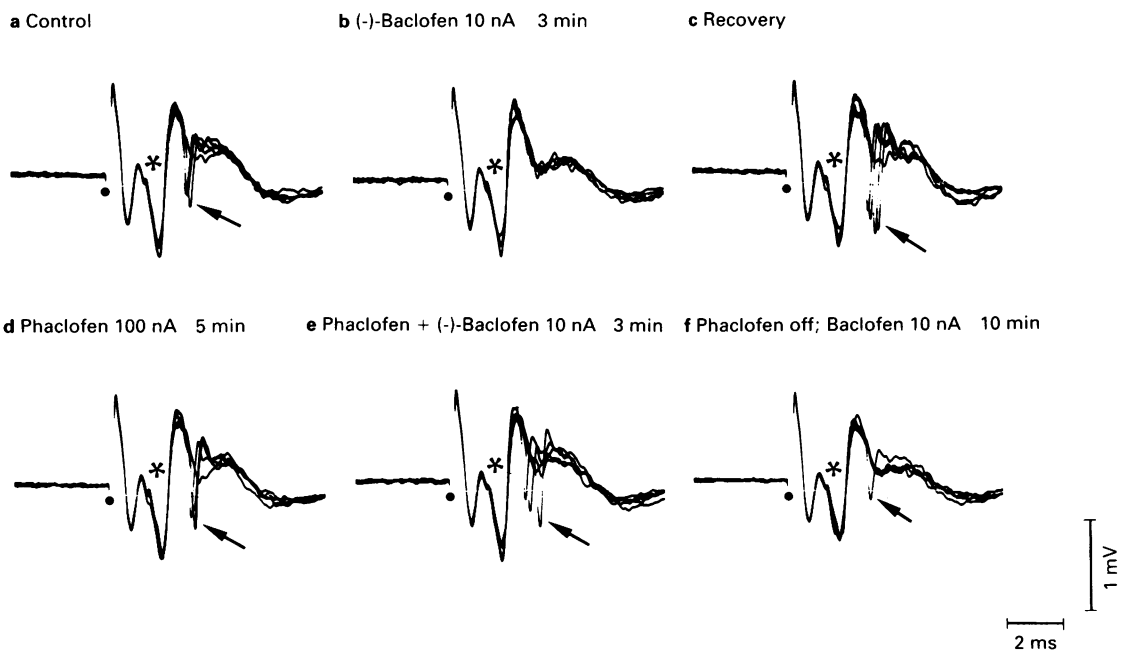

Figure 4 Exceptional case showing the effects of (−)-baclofen and phaclofen alone and the antagonism of the former by the latter when given in combination, upon the synaptic activation (electrical stimulation of VPL nucleus of the thalamus) of an S1 cortical neurone of RA submodality, located at a cortical depth of 1558 μm, corresponding to cortical layer VI. Each oscillographic record is the response observed following 5 sequential stimuli, photographed with sweeps superimposed. (●) Stimulus artifact; (*) evoked field potential; (→) orthodromically evoked response. (a) Control, 4 spikes evoked from 5 stimuli. (b) (−)-Baclofen 10 nA ejected for 3 min, 0 spikes evoked from 5 stimuli. (c) Recovery from effect of (−)-baclofen, 7 min after cessation of drug ejection, 4 spikes evoked from 5 stimuli. (d) Phaclofen, 100 nA ejected for 5 min, 4 spikes evoked from 5 stimuli. (e) Phaclofen ejection maintained as before, now after 8 min, concurrent ejection of (−)-baclofen 10 nA ejected for 3 min, 3 spikes evoked from 5 stimuli. (f) Phaclofen ejection terminated 7 min previously, (−)-baclofen ejection maintained as before, now 10 min total administration time, 1 spike evoked from 5 stimuli. Similar result seen on only one other cell, out of a total of 24 tests using VPL stimulation.

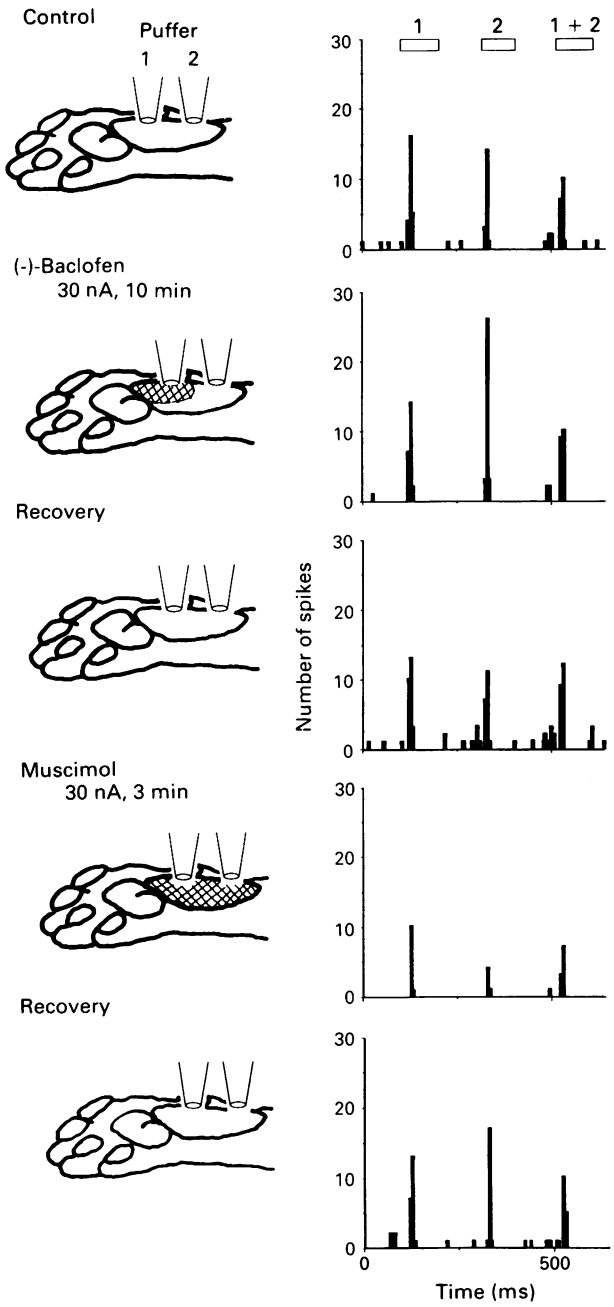

Figure 5 Alterations in cutaneous responsiveness induced by iontophoretically administered (−)-baclofen (30 nA, ejected for 20 min) and muscimol (30 nA, ejected for 3 min). Peristimulus time histograms at right are of 20 successively sampled series of air puffer-elicited RF stimulus trains, obtained before, during and after the ejections of the agonists indicated. Above are represented the times where puffer 1 (peripherally-located to the RF excitatory focus, or centre), puffer 2 (centrally located very near the RF 'hot spot') and the combined stimulus, puffer 1 + 2, were activated as indicated. Ordinate scale, no. of spikes elicited per 20 stimulus trains; abscissa scale, time in ms. Binwidth, 5 ms. Air puffer positions relative to the cell's RF and threshold evaluations shown schematically are depicted by the drawings at left as determined during each testing condition. Hatched areas represent sites exhibiting raised threshold to tactile stimulation. Note the 'mixed' effect elicited by (−)-baclofen in contrast to the non-selective suppression effected by muscimol.

There is a partial antagonism of the suppressant effects of (−)-baclofen upon VPL-elicited cell firing as shown in Figure 4e, compared with the control effect of (−)-baclofen alone appearing in Figure 4b. Note the reversibility of effect of phaclofen in Figure 4f, evident as a resumption of the (−)-baclofen-induced inhibitory effect.

Table 3 Numbers of cells showing mixed responses[1] to agonists assessed through effects upon air puffer evoked firing

(±)-*Baclofen*	(−)-*Baclofen*	*GABA*	*Muscimol*	*THIP*
3/22	11/37	0/27	0/12	0/8
	14/59		0/47	

[1] Mixed responses: ⩾20% reduction of cell firing in certain RF subregions while ⩾20% enhancement in others. Numerator represents number of cells showing mixed response; denominator represents number tested.

Another substance thought to act as an antagonist of $GABA_B$ receptors is δ-AVA and so its effects were examined on 4 neurones (between 300 and 1480 μm depth, at currents of 20–100 nA). In each case, δ-AVA showed suppressant effects of its own upon evoked responses yet had no apparent modifying action upon the (−)-baclofen-induced suppression of cell firing.

(−)-Baclofen occasionally produced enhancement of naturally elicited excitatory responses of S1 neurones. This enhancement occurred when the central excitatory mechanism was evoked, usually in the combined stimulation situation (Figure 5). In the case shown here, only the responses elicited by activation of the centrally located puffer (puffer 2) were enhanced by (−)-baclofen, ejected at 30 nA for 10 min (= a mixed response). This effect reversed upon cessation of drug ejection but subsequent administration of the $GABA_A$ agonist, muscimol, strongly suppressed all three responses: clearly a different pattern of activity from that observed with (−)-baclofen. During (−)-baclofen ejection, stimulation of the RF with hand-held probes revealed an elevated response threshold in the peripheral RF regions, but produced no evidence of any clear-cut change in the central area from the normal, low threshold status. However, ejection of muscimol (30 nA for 3 min) did change threshold from low to medium-high. Table 3 summarises the data obtained with GABA agonists and shows the relative proportions of mixed responses elicited by each. Note the marked dissimilarity between the $GABA_B$-selective agonists and the others, as well as the stereoselectivity exerted by baclofen.

(−)-Baclofen was equipotent with muscimol in suppressing puffer-induced responses evoked from peripheral regions of the RF (based on the mean of a sample of 12 cells where puffer-elicited excitations were challenged by both (−)-baclofen and muscimol). When the central region was stimulated either alone or in combination with the peripheral region, (−)-baclofen was much less effective than was muscimol (Table 4). This confirms and extends earlier observations made when GABA and (±)-baclofen were used on an additional sampling of 29 S1 cells (Kaneko & Hicks, 1988).

Note also from Table 4 that the effects of both (−)-baclofen and muscimol were indistinguishable in their actions upon spontaneous firing ($P > 0.05$). Not only were there differences between the effectiveness of (−)-baclofen and muscimol on excitation elicited from different RF subregions, but also their potency and time course differed when compared upon L-glutamate-evoked activity (Figure 6). (−)-Baclofen was weaker in suppressing excitatory responses than muscimol and required more time to reach a maximal effect. On 12 cells, and with plateau levels of ejected drug (i.e., > 10 min ejection) to minimize differences attributed to transport number, diffusion, etc, (−)-baclofen blocked L-glutamate-driven responses completely on just one cell. Muscimol, by contrast, blocked firing completely in 8 of the same 12 cells tested, using identical ejection currents. This difference is statistically significant ($P < 0.01$; Fisher's exact probability test) and its import is discussed at greater length below (cf. Howe *et al.*, 1987). On average, from the sample of cells tested with both muscimol and (−)-baclofen, the latter reduced L-glutamate-driven

Table 4 Effects of (−)-baclofen and muscimol on air puffer-evoked firing and spontaneous activity

	Air-puffer elicited responses			*Spontaneous*
	Central	*Peripheral*	*Combined*	*activity*
(−)-Baclofen	84.3 ± 12.9**	29.0 ± 7.2	75.6 ± 9.8**	19.9 ± 7.0
Muscimol	46.0 ± 8.0*	28.7 ± 7.3	40.7 ± 7.6	6.9 ± 2.7

The numbers represent the means ± s.e.mean of the responses from 12 cells as a percentage of control (where control is the mean response prior to drug ejection).
A single asterisk indicates that the value differs from that obtained with (−)-baclofen in the same category by $P < 0.05$ and two asterisks indicate that the value differs from that in category of peripherally evoked firing (for that drug) by $P < 0.01$ (ANOVA).

responses to 23.7 ± 5.2% of control (mean ± s.e.mean) whilst muscimol effected reductions to 3.1 ± 1.5% of control (significantly stronger suppression than that with (−)-baclofen by $P < 0.01$; ANOVA) at average ejection parameters of (−)-baclofen of 12.9 ± 2.3 nA for 4.4 ± 0.4 min and of muscimol of 7.6 ± 2.1 nA for 2.8 ± 0.7 min.

Figure 7 contrasts the effect of (−)-baclofen and muscimol on VPL-evoked responses. This example is typical of results obtained from a further 6 cells, where at identical currents of ejection and duration, one or the other substance was less effective in suppressing the synaptically evoked response than its comparator. In the illustrated example, 20 nA of drug

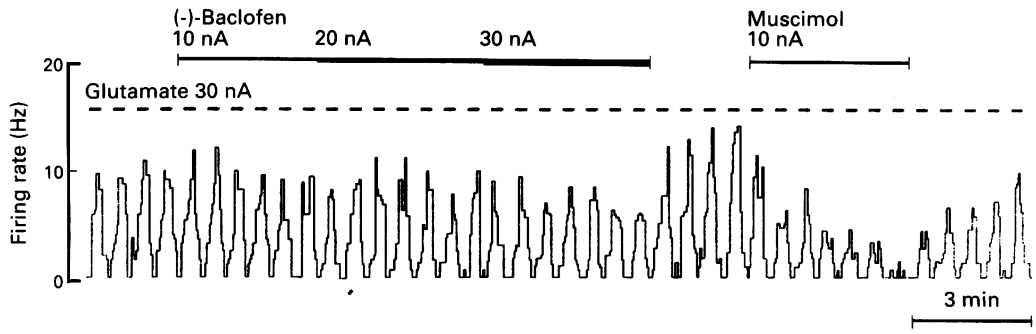

Figure 6 Comparison of various features characterizing the suppressant activities of (−)-baclofen and muscimol upon L-glutamate-elicited firing of an S1 cortical neurone activated periodically (30 s ejections and 30 s retention intervals) by the amino acid excitant, and displayed as a moving-chart recording of the output of a ratemeter. Ordinate scale: frequency of firing; abscissa scale: time. (−)-Baclofen was ejected for ~12 min at progressively-increasing doses, from 10–30 nA. Muscimol was tested only at the one dose of 10 nA. Note the different time courses to maximal effect at each dose, and the different potencies of the two suppressants.

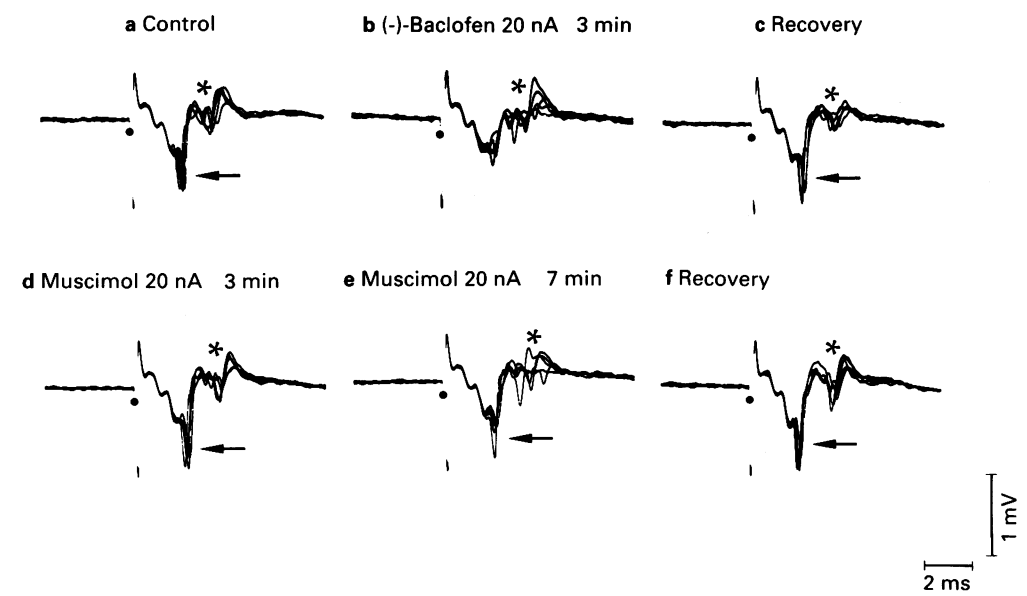

Figure 7 Comparison of the VPL-induced excitatory synaptic effects as influenced differentially by (−)-baclofen and muscimol. Conventions as for Figure 4 except 6 successively sampled sweeps are represented by each oscillograph; responses are from an S1 cortical neurone of RA submodality, located at a cortical depth of 1313 μm, corresponding to cortical layer V, having a high threshold RF situated on a forepaw digit pad (glabrous RF). The cell was driven orthodromically at this stimulation intensity at a success rate of 15 ~ 17/20 shocks, at a latency of 1.6 ms. (a) Control, 6 spikes evoked from 6 stimuli. (b) (−)-Baclofen 20 nA ejected for 3 min, 0 spike evoked from 6 stimuli. (c) Recovery from effect of (−)-baclofen, 4 min after cessation of drug ejection, 5 spikes evoked from 6 stimuli. (d) Muscimol, 20 nA ejected for 3 min, 6 spikes evoked from 6 stimuli. (e) Muscimol at same ejecting current but prolonged to 7 min, 1 spike evoked from 6 stimuli. (f) Recovery from muscimol, 10 min following cessation of ejection of all drugs, 6 spikes evoked from 6 stimuli.

ejected for 3 min was sufficient for (−)-baclofen but not muscimol to effect a synaptic block. The latter required a prolonged ejection time of 7 min before its effect approximated that of (−)-baclofen. (−)-Baclofen and muscimol abolished VPL-elicited firing on 4 and 3 cases of 6 tests, respectively, at mean currents (±s.e.mean) of 9.7 ± 2.4 nA (for 4.3 ± 0.9 min) and 8.2 ± 2.6 nA (for 4.3 ± 0.8 min), respectively. The mean extent of this antagonism for the two substances was 6.7 ± 4.2% of control for (−)-baclofen and 13.3 ± 6.7% of control for muscimol.

Discussion

This report confirms earlier findings and extends the work to encompass our previous suppositions (Kaneko & Hicks, 1988) about the likely nature of $GABA_B$ processes operating in S1 cortex of the anaesthetized cat. Prior to discussing our major results, some general methodological issues are best considered first. Whereas it is well known that barbiturates enhance the receptor binding of GABA (Olsen, 1982), in experiments where the variable of anaesthetic regimen was evaluated to permit comparisons of static and dynamic response properties (Hicks *et al.*, 1985) no differences were observed with respect to RF size, submodality property, threshold or velocity sensitivity. The only notable feature that varied with anaesthesia (barbiturate vs. urethane preparations) was in the rate of ongoing neuronal activity. Such lack of anaesthetic effect also was confirmed recently in a study of neurones from S2 cortex of cats, where barbiturate and halothane/N_2O anaesthetics were used (Alloway *et al.*, 1988). In this latter study, no differences were found in their measures of dynamic response properties, which included cell phase-locking with the periodic stimulus (their %E), threshold, submodality property or bicuculline antagonism of the effects of GABA. However, they did also note a reduced overall rate of spontaneous firing under conditions of barbiturate anaesthesia, as had been seen in S1 cortex previously (Hicks *et al.*, 1985).

In the present study, GABA was of a somewhat intermediate potency compared with its agonists in eliciting equivalent suppressions, THIP and (+) and (±) forms of baclofen being less potent whilst (−)-baclofen and muscimol exerted 2–3 times greater relative effectiveness. Potency inferences based upon comparing equi-effective ejecting currents of selective $GABA_A$ and $GABA_B$ receptor agonists are thought unlikely to be compromised significantly by artifacts associated with the microiontophoretic methodology used here for several reasons (Hicks, 1984; Stone, 1985). The data are very much in accordance with findings from *in vitro*, drug superfusion studies in which intracellular recording was used (Newberry & Nicoll, 1985; Kemp *et al.*, 1986) and there were quite marked differences in potency apparent amongst the isomeric forms of the same compound ((+); (−)- and (±)-baclofen). These potency differences are unlikely to be ascribed to: (1) differences in transport number, (2) of drug distribution within the tissue and away from the point source of ejection, or (3) of preferential uptake by glial elements of one compound compared with another, since isomerism comprises their sole distinguishing feature. Whereas these considerations may influence the data related to GABA vs. its agonists to some extent, the close similarity of potency values to *in vitro* data, plus the observation that muscimol and THIP, compounds very similar physico-chemically, differed greatly in their apparent potencies, allows us to place confidence in our qualitative statements about relative pharmacological effectiveness, within broad limits. Moreover, conducting a quantitative analysis of potency was not the aim of the present study.

Certainly the reduced effectiveness of (−)-baclofen compared with GABA or muscimol upon L-glutamate-driven responses is in good agreement with more quantitatively based, intracellular recording studies in which slice superfusion techniques were used. It is well recognised from such work that GABA or muscimol provokes a much higher conductance than does (−)-baclofen, and as the latter is less able to block L-glutamate-induced depolarizations, the attenuation of glutamate-induced activity must depend solely upon the difference in shunting (Howe *et al.*, 1987).

Several related observations support our earlier contention that $GABA_B$ receptors are involved in the synaptic processing of somatosensory information at cortical neurones. (−)-Baclofen is effective as a neuronal suppressant of cutaneously and thalamically evoked responses stereospecifically and at potencies comparable to those seen elsewhere in reduced preparations. Its effects are not blocked by BMI, yet mimic in certain respects, such as threshold elevation and RF size diminution, those observed with GABA. In particular, the $GABA_B$ agonist exerted area-selective response suppressions (from the RF peripheries) that were characteristic only of effects obtained with (−)-baclofen. The $GABA_A$ agonists and GABA did not elicit a similar pattern of activity, arguing for separate and distinct forms of inhibition by the different classes of compounds upon the same neurones, depending on the method of response elicitation employed.

The form of response suppression obtained with $GABA_B$ receptor stimulation is interesting to consider. As reported earlier and evaluated more extensively here, we have found that the effectiveness of (−)-baclofen is greatly diminished whenever natural stimulation involved the RF central excitatory mechanism. In contrast, if the cells were activated exclusively by stimulation of the RF periphery, without concurrent activation of the RF central zone, (−)-baclofen elicited profound suppressant activity. This observation suggests several clues as to the likely underlying mechanisms of action of the drug with respect to receptor location and distribution.

One possibility has been suggested by *in vitro* data from sensory cortex (Connors *et al.*, 1988), where baclofen stimulates receptor mechanisms that activate a small, prolonged K^+ conductance. This could alter dramatically the transfer characteristics of cells, causing them to increase their threshold for the generation of single spikes or repetitive discharges, but allowing a concomitant elevation of the sensitivity of their initial rates of firing to depolarizing stimuli. At higher membrane currents, an increased response of the cell to strong stimulation would be seen and this is exactly the type of observation made here *in vivo*, with central RF stimulation. Connors *et al.* (1988) have suggested that the $GABA_B$-related inhibitory postsynaptic potentials (i.p.s.ps) would tend to decrease background firing rates and diminish the cells' responses to weak excitatory inputs but leave unimpaired or even enhanced, their sensitivity to transient, intense synaptic inputs. Our observations with physiologically relevant forms of cell activation that activate the RF centre lend support to this view.

An additional possibility that is not exclusive of the above one, concerns the idea that there may be a selective distribution of $GABA_B$ receptors across the surface membrane area of the cells. Several of our observations suggest that $GABA_B$ receptors are distributed relatively distally, not only to the soma where our recordings are made, but also in relation to the distribution of $GABA_A$ receptors, that are activated in our system selectively, rapidly and potently by muscimol. We observed that the effect of (−)-baclofen differs from that of muscimol in that the former requires a relatively longer duration of ejection to effect response reductions that approach in magnitude those elicited by muscimol. Furthermore, (−)-baclofen was on average nearly half as potent as was muscimol (it is inadvisable to use GABA in such comparisons as it is taken up avidly by the neuropil and by glial elements; Balcar & Johnston, 1973; Hertz, 1979).

Consistent with these lines of reasoning is the further observation that as (−)-baclofen exerted potent suppression only when the central excitatory mechanism was not activated, the preferred site of termination of thalamic afferents for the most potent input (the RF 'hot spot', or central excitatory mechanism) would be near the somatic, soma-dendritic, or

peri-somatic synaptic-recipient zones. This is the favoured site of action in the cortex of inhibitory synaptic terminals (Jones & Powell, 1970; Valverde, 1976; Houser *et al.*, 1983; Hendry *et al.*, 1984; Somogyi *et al.*, 1984) which, judging from the potency and onset latency of the effects elicited with our tested drugs, employ receptors that are stimulated by muscimol. This same point has been addressed in an elegant *in vitro* study using a topological analysis of intracellular potentials evoked by GABA, the results of which support our receptor distribution hypothesis (Connors *et al.*, 1988; their Figure 3). It is hardly surprising therefore that GABA, which is necessarily restricted in iontophoretic studies to a relatively peri-somatic action due to the uptake processes mentioned above, and muscimol, the $GABA_A$ selective agonist, act in a non-selective manner in suppressing both peripheral, central and combined forms of natural RF stimulation. This is because such suppressant effects would be expected to be manifested where the RF central excitatory mechanism is expressed by its afferent input; i.e., near the soma/axon hillock region. $GABA_B$-related activity could be expressed only under those conditions where this highly secure, potent and centrally expressed RF excitation is absent, and when only the peripherally distributed inputs from RF surround regions are responsible for the generation of the naturally elicited excitatory responses.

Further support for such an hypothesis has appeared recently (Alloway *et al.*, 1989) in an *in vivo* study on S1 cortical neurones of cats, where the magnitudes of naturally elicited inhibitions were mapped linearly across RF axes in 4 directions. These investigators found that inhibitions were elicited throughout the topographical extent of the RF, including the RF centre and the surrounding region outside the normal excitatory RF boundary. The spatial profile of these inhibitory effects was consistent with a centre-surround formulation, more centrally located excitatory inputs being subjected to relatively more intense GABA-mediated inhibition. They concluded that this inhibition might be more important for limiting the size of RFs in the peripheral RF region, which contributes relatively more than does the central RF in making a tactile discrimination.

One other point arising from this study that merits discussion is the finding of occasional mixed effects of (−)-baclofen, and this may have some bearing upon the question posed above regarding its mechanism of action. Mixed responses here refer to cases where a $\geqslant 20\%$ reduction of cell firing was observed in certain RF subregions (invariably peripheral ones) whilst a $\geqslant 20\%$ enhancement was seen in others (in the RF 'hot spot'). Such mixed responses with the $GABA_B$ agonists were elicited on one quarter of the cells examined with the puffer, 30% being obtained with the (−)-baclofen isomer. (−)-Baclofen is known to exert presynaptic effects in many test systems in the CNS so far examined (Fox *et al.*, 1978; Potashner, 1978; Bowery *et al.*, 1980; Johnston *et al.*, 1980; Curtis *et al.*, 1981; Krogsgaard-Larsen *et al.*, 1986; but see Gallagher *et al.*, 1984; Howe *et al.*, 1987; Connors *et al.*, 1988; Deisz & Prince, 1989, for a description of postsynaptic baclofen responses). If $GABA_B$ receptors are distributed, even in part, on the presynaptic terminals of GABA-containing neurones that normally release their transmitter onto a postsynaptic target bearing $GABA_A$ receptors, then it is easy to envisage a facilitation (through an indirect, disinhibitory process) of the RF central excitatory mechanism, concomitant with a suppression exerted directly by (−)-baclofen at distal sites employing (possibly postsynaptic) $GABA_B$ receptors directly upon the recorded cell. Indeed, a highly similar sort of synaptic arrangement and type of effect as this has been described recently by Deisz & Prince (1989) using intracellular recording, where GABA released from presynaptic terminals was shown to exert a negative feedback on its release through activation of a $GABA_B$ receptor, in slices of guinea-pig neocortex. Moreover, as shown in this and another, related study (Connors *et al.*, 1988), the eventual effect of baclofen in cortex is very much dependent upon its site of administration and its dose, the drug causing an apparent increase of excitatory postsynaptic potentials by virtue of its preferential attenuation of inhibitory synaptic processes.

The most persuasive evidence for a functional role of $GABA_B$ receptors in S1 cortical processing would be data using selective antagonists of the receptors (Hicks, 1983). Unfortunately the two major candidate substances for $GABA_B$ antagonists, phaclofen and δ-AVA, were ineffective in blocking inhibition in any reliable fashion. Indeed, their profile of activity more often than not contained features which one would expect from agonists rather than antagonists. In other cortical systems (Baumfalk & Albus, 1988; Dutar & Nicoll, 1988), phaclofen has been described as selectively antagonizing (±)-baclofen-induced responses (on only 2 cells in the latter study), yet phaclofen itself was also observed to act directly on over half the responses elicited in the other study (visual cortical neurones *in vivo*; Baumfalk & Albus, 1988). In this latter study as well, complete antagonism of the suppression induced by baclofen was noted on 11 of 21 neurones tested, with an additional 4 of the 21 cells exhibiting no apparent competition. Clearly, variable results with phaclofen amongst different groups warrants a cautious approach in its use in making judgment about receptor populations involved in inhibitory systems.

In summary, we conclude that $GABA_B$ processes appear to be present in S1 cortex of cats. They have a particular functional role to play that specifically involves the control of size and threshold of RFs in their relatively peripheral regions, and this may very well relate to tactile discrimination sensitivity processes. Whether such receptors are situated presynaptically or postsynaptically, or both, whether they reflect disinhibitory synaptic information processing, and whether the view that their distribution distal to the soma is valid, are hypotheses that await further experimental evaluations.

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References

ALLOWAY, K.D. & BURTON, H. (1986). Bicuculline-induced alterations in neuronal responses to controlled tactile stimuli in the second somatosensory cortex of the cat: A microiontophoretic study. *Somatosens. Res.*, **3**, 197–211.

ALLOWAY, K.D., ROSENTHAL, P.R. & BURTON, H. (1989). Quantitative measurements of receptive field changes during antagonism of GABAergic transmission in primary somatosensory cortex of cats. *Exp. Brain Res.*, **78**, 514–532.

ALLOWAY, K.D., SINCLAIR, R.J. & BURTON, H. (1988). Responses of neurons in somatosensory cortical area II of cats to high-frequency vibratory simuli during iontophoresis of a GABA antagonist and glutamate. *Somatosens. Res.*, **6**, 109–141.

BALCAR, V.J. & JOHNSTON, G.A.R. (1973). High affinity uptake of transmitter: Studies on the uptake of L-aspartate, GABA, L-glutamate and glycine in the cat spinal cord. *J. Neurochem.*, **20**, 529–539.

BATUEV, A.S., ALEXANDROV, A.A. & SCHEYNIKOV, N.A. (1982). Picrotoxin action on the receptive fields of the cat somatomotor cortex neurons. *J. Neurosci. Res.*, **7**, 49–55.

BAUMFALK, U. & ALBUS, K. (1988). Phaclofen antagonizes baclofen-induced suppression of visually evoked response in the cat's striate cortex. *Brain Res.*, **463**, 398–402.

BOWERY, N.G., HILL, D.R., HUDSON, A.L., DOBLE, A., MIDDLEMISS, D.N., SHAW, J. & TURNBULL, M.J. (1980). (−)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA-receptor. *Nature*, **283**, 92–94.

CONNORS, B.W., MALENKA, R.C. & SILVA, L.R. (1988). Two inhibitory postsynaptic potentials, and $GABA_A$ and $GABA_B$ receptor-

mediated responses in neocortex of rat and cat. *J. Physiol.*, **406**, 443–468.

CURTIS, D.R., LODGE, D., BORNSTEIN, J.C. & PEET, M.J. (1981). Selective effects of (−)baclofen on spinal synaptic transmission in the cat. *Exp. Brain Res.*, **42**, 158–170.

DEISZ, R.A. & PRINCE, D.A. (1989). Frequency-dependent depression of inhibition in guinea pig neocortex *in vitro* by $GABA_B$ receptor feed-back on GABA release. *J. Physiol.*, **412**, 513–541.

DUTAR, P. & NICOLL, R.A. (1988). A physiological role for $GABA_B$ receptors in the central nervous system. *Nature*, **332**, 156–158.

DYKES, R.W., RASMUSSON, D.D. & HOELTZELL, P. (1980). Organization of primary somatosensory cortex in the cat. *J. Neurophysiol.*, **43**, 1527–1546.

DYKES, R.W., LANDRY, P., METHERATE, R. & HICKS, T.P. (1984). Functional role of GABA in cat primary somatosensory cortex: Shaping receptive fields of cortical neurons. *J. Neurophysiol.*, **52**, 1066–1093.

FOX, S., KRNJEVIĆ, K., MORRIS, M.E., PUIL, E. & WERMAN, R. (1978). Action of baclofen on mammalian synaptic transmission. *Neurosci.*, **3**, 495–515.

GALLAGHER, J.P., STEVENS, D.R. & SHINNICK-GALLAGHER, P. (1986). Actions of GABA and baclofen on neurons of the dorsolateral septal nucleus (DLSN) *in vitro*. *Neuropharmacology*, **23**, 825–826.

GARDNER, E.P. & COSTANZO, R.M. (1980). Spatial integration of multiple-point stimuli in primary somatosensory cortical receptive fields of alert monkeys. *J. Neurophysiol.*, **43**, 420–443.

HENDRY, S.H.C., JONES, E.G., DEFELIPE, J., BRANDON, C. & EMSON, P.C. (1984). Neuropeptide-containing neurons of the cerebral cortex also are GABAergic. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 6526–6530.

HERTZ, L. (1979). Functional interactions between neurons and astrocytes. I. Turnover and metabolism of putative amino acid transmitters. *Prog. Neurobiol.*, **13**, 277–323.

HICKS, T.P. (1983). Antagonism of synaptic transmission *in vivo*: Contributions of microiontophoresis. *Brain Behav. Evol.*, **22**, 1–12.

HICKS, T.P. (1984). The history and development of microiontophoresis in experimental neurobiology. *Prog. Neurobiol.*, **22**, 185–240.

HICKS, T.P. & DYKES, R.W. (1983). Receptive field size for certain neurons is determined by GABA-mediated intracortical inhibition. *Brain Res.*, **274**, 160–164.

HICKS, T.P., METHERATE, R., LANDRY, P. & DYKES, R.W. (1986). Bicuculline-induced alterations of response properties in functionally identified ventroposterior thalamic neurones. *Exp. Brain Res.*, **63**, 248–264.

HICKS, T.P., LANDRY, P., METHERATE, R. & DYKES, R.W. (1985). Functional properties of neurons mediated by GABA in cat somatosensory cortex under barbiturate and urethane anesthesia. In *Development, Organization and Processing in Somatosensory Pathways*. ed. Rowe, M. & Willis, W.D., pp. 265–276, New York: Alan R. Liss, Inc.

HOUSER, C.R., HENDRY, S.H.C., JONES, E.G. & VAUGHAN, J.E. (1983). Morphological diversity of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex. *J. Neurocytol.*, **12**, 617–638.

HOWE, J.R., SUTOR, B. & ZIEGLGÄNSBERGER, W. (1987). Baclofen reduces post-synaptic potentials of rat cortical neurones by an action other than its hyperpolarizing action. *J. Physiol.*, **384**, 539–569.

JOHNSTON, G.A.R., HAILSTONE, M.H. & FREEMAN, C.G. (1980). Baclofen: stereoselective inhibition of excitant amino acid release. *J. Pharm. Pharmacol.*, **32**, 230–231.

JONES, E.G. & POWELL, T.P.S. (1970). An electronmicroscopic study of the laminar pattern and mode of termination of the afferent fibre pathways to the somatic sensory cortex. *Phil. Trans. R. Soc.* B., **257**, 45–62.

KANEKO, T. & HICKS, T.P. (1988). Baclofen and γ-aminobutyric acid differentially suppress the cutaneous responsiveness of primary somatosensory cortical neurones. *Brain Res.*, **443**, 360–366.

KEMP, J.A., MARSHALL, G.R. & WOODRUFF, G.N. (1986). Quantitative evaluation of the potencies of GABA-receptor agonists and antagonists using the rat hippocampal slice preparation. *Br. J. Pharmacol.*, **87**, 676–684.

KROGSGAARD-LARSEN, P., FALCH, E., SCHOUSBOE, A. & CURTIS, D.R. (1986). GABA agonists and uptake inhibitors: stereoselectivities and anticonvulsant effects. In *Neurotransmitters, Seizures and Epilepsy* III. ed. Nisticò, G., Morselli, P.L., Lloyd, K.G., Fairello, R.G. & Engel, Jr, J. pp. 135–150. New York: Raven Press.

NEWBERRY, N.R. & NICOLL, R.A. (1985). Comparisons of the actions of baclofen with γ-aminobutyric acid on rat hippocampal pyramidal cells *in vitro*. *J. Physiol.*, **360**, 161–185.

OKA, J.-I. & HICKS, T.P. (1990). Benzodiazepines and synaptic processing in the spatial domain within the cat's primary somatosensory cortex. *Can. J. Physiol. Pharmacol.*, **68**, (in press).

OKA, J.-I., JANG, E.K. & HICKS, T.P. (1986). Benzodiazepine receptor involvement in the control of receptive field size and responsiveness in primary somatosensory cortex. *Brain Res.*, **376**, 194–198.

OLSEN, R.W. (1982). Drug interactions at the GABA receptor-ionophore complex. *Ann. Rev. Pharmacol. Toxicol.*, **22**, 245–277.

POTASHNER, S.J. (1978). Baclofen effects on amino acid release. *Can. J. Physiol. Pharmacol.*, **56**, 150–154.

SOMOGYI, P., FREUND, T.F. & KISVARDAY, Z.F. (1984). Different types of ^{3}H-GABA accumulating neurones in the visual cortex of the rat. Characterization by combined autoradiography and Golgi preparations. *Exp. Brain Res.*, **54**, 45–56.

STONE, T.W. (1985). *Microiontophoresis and Pressure Ejection*. p. 214. Chichester: John Wiley & Sons.

SRETAVAN, D. & DYKES, R.W. (1983). The organization of two cutaneous submodalities in the forearm region of area 3b of cat somatosensory cortex. *J. Comp. Neurol.*, **213**, 381–398.

VALVERDE, F. (1976). Aspects of cortical organization related to the geometry of neurons with intra-cortical axons. *J. Neurocytol.*, **5**, 509–529.

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