Different roles for $GABA_A$ and $GABA_B$ receptors in visual processing in the rat superior colliculus

K. E. Binns and T. E. Salt

Department of Visual Science, Institute of Ophthalmology, University College London, Bath Street, London EC1V 9EL, UK

1. The superficial grey layer of the superior colliculus (SGS) contains a high proportion of GABAergic inhibitory neurones. We have investigated the role of GABA receptors in synaptic transmission of aspects of visual activity in the SGS that may be driven by inhibitory mechanisms, such as surround inhibition and response habituation.

2. Multi-barrel glass iontophoretic pipettes were used to record single neuronal activity in the SGS of urethane-anaesthetized rats. Visual stimulation was provided by the display of moving bars and stationary spots of light on a monitor placed in the receptive field.

3. Both ejection of GABA and the $GABA_B$ agonist baclofen reduced responses to moving bars (interstimulus intervals $\geqslant 8$ s). The effects of GABA were reversed by the $GABA_A$ antagonist bicuculline, and the effects of baclofen were antagonized by the $GABA_B$ antagonist CGP 35348.

4. Surround inhibition was estimated by plotting the response to flashed spots of increasing diameter. In controls, expanding the spot diameter beyond the excitatory receptive field caused a decrease in the response. This inhibitory surround was reversibly reduced by bicuculline, but CGP 35348 had no effect.

5. Response habituation is the progressive reduction in the visual response during repetitive stimulus presentation. In controls, the visual response was reduced to $44 \pm 3\%$ of its initial level when a stimulus (moving bar) was presented 5 times with an interstimulus interval of 0·5 s. During CGP 35348 ejection, response habituation was reversibly reduced. Bicuculline had no effect on response habituation.

6. The effects of bicuculline on surround inhibition in the superior colliculus are consistent with similar studies in the lateral geniculate nucleus which indicate that $GABA_A$ receptors mediate this effect. The function of $GABA_B$ receptors in the visual system is less well researched. The reduction of response habituation with CGP 35348 demonstrates that, at least in the SGS, $GABA_B$ receptors have an important role in visual transmission which is distinct from that of $GABA_A$ receptors.

The superior colliculus (SC) integrates visual and other sensory information for the generation of goal-directed orientation movements towards novel sensory stimuli (Sparks & Nelson, 1987). To achieve accurate orientation responses sensory neurones in the superior colliculus must be able to determine what is a new stimulus in the environment and provide precise information about its location. The responses of visual neurones in the superficial grey layer (SGS) of the SC are known for their tendency to habituate; i.e. they respond strongly when an appropriate stimulus is first presented but poorly to subsequent presentations of the same stimulus (Drager & Hubel, 1975; Oyster & Takahashi, 1975; Chalupa & Rhoades, 1977). Hence, SGS neurones detect stimulus novelty. Some details about stimulus location are provided by the retinal-collicular projection, which observes a strict topographic order (Siminoff, Schwassmann & Kruger, 1966). In addition, visual receptive fields in the SC have inhibitory surrounds which enhance the precision of the representation (Rizzolatti, Carmarda, Grupp & Pisa, 1974; Berman & Cynader, 1975). The generation of inhibitory surrounds and response habituation are both likely to involve inhibitory mechanisms.

At least three types of inhibitory neurones are found in the SGS and several major inhibitory fibre tracts terminate within both the superficial and deep SC (Mize, 1992). Most of these inhibitory neurones and terminals accumulate GABA (Mize, 1992), and the levels of GABA found in the SGS are amongst the highest found in the CNS (Okada, 1974). Binding assays indicate that the number of $GABA_A$ and particularly $GABA_B$ receptors in the SGS is also high (Bowery, Hudson & Price, 1987). Despite the obvious likelihood that GABAergic inhibitory interactions shape

visual responses in the SGS these mechanisms have not been extensively studied.

We have investigated the functional significance of GABA receptors in visual activity in the rat SGS using natural stimulation and iontophoretic application of GABA agonists and antagonists.

METHODS

Surgery

Adult Lister Hooded (200–400 g) rats were prepared for recording under urethane (1·25 g kg^{-1}, I.P.) anaesthesia. Lignocaine was injected subcutaneously in all intended sites of surgical entry. During surgery the rats were held in a stereotaxic position with ear bars and a nose bar. A craniotomy was made to reveal the cortex overlying the right SC. A head holder was then mounted on the skull using screws and dental cement to allow the head to be maintained in a stereotaxic position after the ear and nose bars had been removed and to give greater access to the facial area. The electroencephalogram and electrocardiogram were routinely monitored for the duration of the experiment. Additional anaesthetic (0·02–0·04 g kg^{-1}, I.P.) was given when the heart rate increased and/or when the pinch withdrawal reflex returned. At the end of the experiments rats were overdosed with urethane. All procedures used had Home Office approval.

Recording and iontophoresis

As in our previous work (Binns & Salt, 1994) multi-barrel glass electrodes were lowered through the cortex into the SGS using a stepping micro drive. To avoid damaging cortical inputs an angled electrode approach was made. Usually a single track was made through the SGS and data were obtained from a series of recording sites 50–100 μm apart. A PSB (Pontamine Sky Blue, 2·5% in 0·5 M NaCl–0·5 M sodium acetate) dye-filled barrel was used to place dye spots in the electrode track for histological reconstruction of the recording sites.

Extracellular single neurone activity was recorded through the central barrel of the electrodes, which contained 4 M NaCl. Action potential spikes were gated with a waveform discriminator, and timed and recorded using a CED 1401 computer interface and VS software (Cambridge Electronic Design, Cambridge, UK), which generated post-stimulus time histograms (PSTHs). The remaining electrode barrels were used for iontophoretic drug application and were filled with a selection of GABA (γ-amino-n-butyric acid, 0·2 M in water, pH 3·5) baclofen (10 mM in 150 mM NaCl, pH 3·5), bicuculline methochloride (5 mM in 150 mM NaCl, pH 3), CGP 35348 (*P*-(3-aminopropyl)-*P*-di-ethoxymethyl-phosphinic acid; Olpe *et al.* 1990; 10 mM in water, pH 3) and NMDA (*N*-methyl-D-aspartate, 0·2 M in water, pH 8). GABA, baclofen, bicuculline and CGP 35348 were ionized as cations and held in the electrode barrels with small negative retaining currents. NMDA was ionized as an anion and retained with a positive current (5–25 nA). Positive ejection currents of GABA, baclofen, bicuculline and CGP 35348 were used and current balancing was provided through a barrel containing 1 M NaCl.

Visual stimulation

Approximately 2 mm from the cortical surface, the recording electrode encountered visual neurones with small spatial receptive fields (RFs). After locating the RF of a neurone a CRT (cathode ray tube; Tektronix) was placed in the RF, 15 cm from the left eye of the subject, in order to display a range of stimuli, including moving bars and stationary spots of various sizes, created by a Picasso visual stimulus generator under computer control.

Experimental protocol

As many of the following as possible were undertaken on an individual neurone.

Centring the CRT in the receptive field. The CRT was used to display a moving bar (5 × 5 deg, out 2 s, still 4 s), which made horizontal or vertical excursions across the screen at different elevations and azimuthal positions, respectively. Responses were displayed on-line and the position of the CRT was adjusted until the best response was obtained by a stimulus moving along either the vertical or horizontal meridian. This procedure was normally repeated several times during the time when an individual neurone was being studied to control for any eye movements made by the rat.

Assessing the extent of the antagonistic surround of a neurone. Stationary spots of various diameters (2–40 deg) were flashed (on 2 s, off 4 s) in random sequence (3 times each) and spot-diameter tuning curves were plotted on-line. This procedure lasted 330 s.

Assessing the parameters of response habituation. Travelling bars (5 × 10–20 deg, approximately the diameter of spot which elicited the best response) were displayed at various interstimulus intervals. In each trial the stimulus was repeated 5 times at an interval between 0·5 and 8 s. Each trial was shown 3 times in random order with an interval of 10 s at the end of each trial.

Pharmacological controls for GABA and bicuculline. To determine the iontophoretic ejection current (and duration), which antagonized the effects of GABA, computer-controlled cycles (duration 320 s) were constructed in which eight pairs of moving bars (5 × 10–20 deg) were presented. GABA was ejected for a short period (4–6 s) beginning just prior to the first of each pair. Thus the visual response to the first presentation of each pair was reduced. After adequate control cycles, bicuculline was ejected continuously throughout 2–3 cycles. The bicuculline ejection current necessary to block the effects of GABA was ascertained. Recovery data were then collected.

Pharmacological controls for baclofen and CGP 35348. The effects of the $GABA_B$ agonist baclofen on visual responses were assessed by constructing 320 s cycles during which a moving bar (parameters as determined above) was presented 20 times at 8 s intervals. After collecting control data, baclofen was ejected (50–100 nA, 50–60 s) during presentations 6–10/11 so as to reduce the visual responses. A similar technique was used to demonstrate the effects of $GABA_B$ receptor activation in cat S1 cortex (Kaneko & Hicks, 1990). CGP 35348 was then ejected to antagonize the effects of baclofen continually during subsequent cycles. After cessation of antagonist ejection, recovery data were collected.

Assessing the effects of baclofen on postsynaptic activation. Cycles were constructed in which NMDA was ejected for two brief periods (duration, 10–12 s) to evoke postsynaptic excitation. After controls, baclofen was ejected for 50–60 s, beginning 40–50 s before the second NMDA ejection.

Assessing the effects of bicuculline and CGP 35348 on response habituation. Further cycles were constructed during which stimuli that caused the response to habituate were presented. Normally, five repeated travelling bars (5 × 10–20 deg, interval 0·5 s) were used, and this stimulus was presented 8 times during the 320 s cycle. After control data had been collected bicuculline was ejected during a few cycles (current and duration as determined above). Recovery data were then collected and new

controls established before ejection of CGP 35348. Currents of 50–100 nA of CGP 35348 were usually ejected for two cycles and normally reduced response habituation. Final recovery data were collected after cessation of CGP 35348 ejection.

Assessing the effects of CGP 35348 and bicuculline on surround inhibition. Spot-diameter tuning curves were plotted (as described above) before, during and after ejection of CGP 35348 or bicuculline using the same ejection currents and durations determined above.

The completion of all the above protocols was rarely possible. Thus the first three were performed in each neurone. Then the effects of a single antagonist on both surround inhibition and habituation were studied, or the effects of both antagonists against either surround inhibition or habituation were studied. For each neurone, the appropriate pharmacological controls were always included and the current and duration of antagonist ejection were equivalent in the pharmacological controls and the studies of either surround inhibition or habituation.

Data analysis

Throughout the recording experiments data collection was observed on-line. Off-line, spot-diameter tuning curves were plotted using the summed on- and off-responses (with background subtraction). The spot diameter that evoked the maximum response, and spot diameters that produced a 25 and 50% reduction of the response from the maximum were calculated from the plots. The degree of response habituation was assessed by expressing the response evoked by the fifth presentation of a set of five repeated stimulus presentations as a percentage of the response evoked by the first using the formula:

$$\frac{100 \times \text{no. of spikes evoked by fifth presentation}}{\text{no. of spikes evoked by first presentation}}.$$

The effects of the agonists and antagonists on visual- and NMDA-evoked responses during control pharmacology were quantified by expressing the magnitude of the response during drug ejection as a percentage of the response during controls. Non-parametric

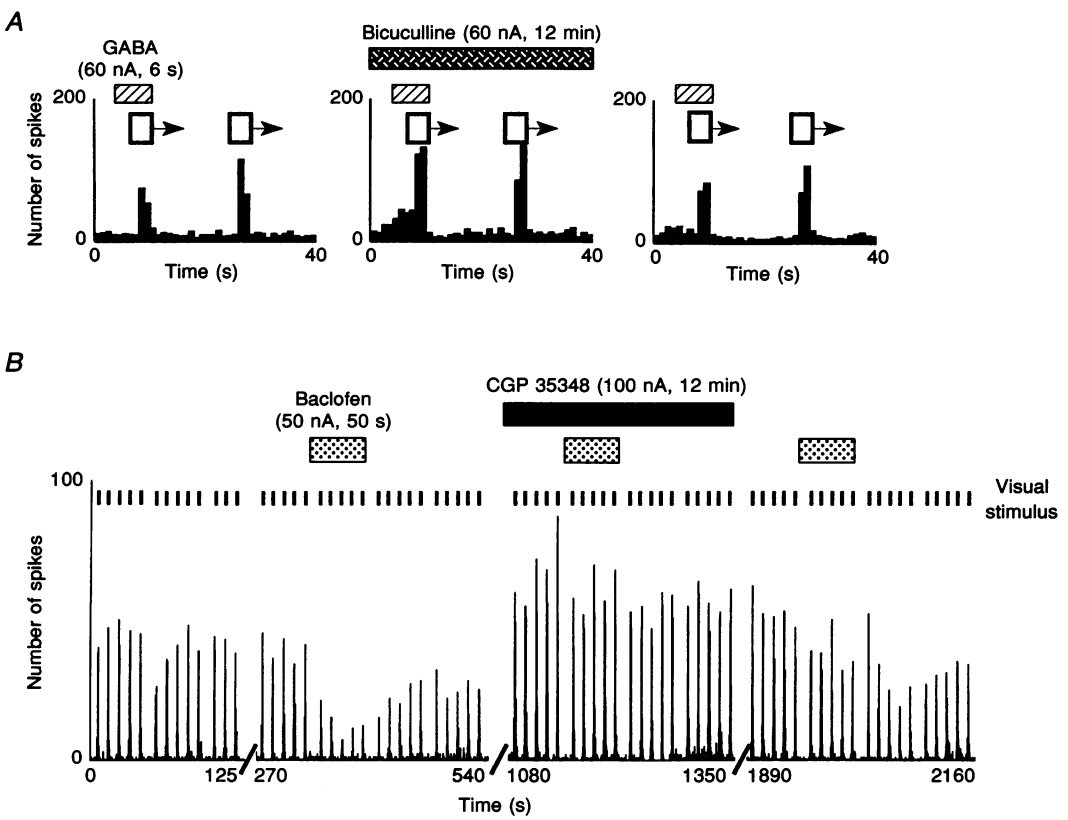

Figure 1. Data from individual neurones illustrating the typical effects of GABA agonists and antagonists

A, PSTHs showing action potential spikes evoked by pairs of visual stimuli (a 10 × 20 deg moving bar making a 40 deg excursion, duration of stimulus presentation = 2 s, interstimulus interval = 16 s; times as shown by the open bar). In each case GABA (60 nA, 6 s) was ejected prior to and during the first presentation (hatched bar above). The graphs show cumulative data from 4 consecutive pairs of stimulus presentations. Action potentials were collected in 1 s bins. The plots show GABA-reduced response and control visual response (left), equivalent data collected during bicuculline ejection (60 nA, 12 min; dark-weave bar) (centre), and recovery data collected 12 min after cessation of bicuculline ejection (right). *B*, PSTH showing excerpts of the response evoked by continuous presentation of a visual stimulus (10 × 20 deg moving bar, out 2 s, still 8 s, 40 deg excursion). Action potential spikes were collected in 1 s bins. Ejections of baclofen (50 nA, 50 s; dotted bar) reduced the response to visual stimulation, but in the presence of CGP 35348 (100 nA, 12 min; filled bar) baclofen no longer caused any reduction. Nine minutes after cessation of CGP 35348 ejection baclofen inhibits the visual responses again.

statistics (Mann–Whitney U tests) were used to test for significant differences between control data and antagonist treated groups.

RESULTS

Effects of $GABA_A$ and $GABA_B$ agonists and antagonists on SGS visual responses

Both GABA and the $GABA_B$ agonist baclofen (Seabrook, Howson & Lacey, 1990) reduced the visual responses of SGS neurones. GABA was most effective when ejected for short (4–6 s) periods just prior to and during stimulus presentation, and reduced the visual response to 44 ± 5% ($n = 14$) of the control within the same cycle. The effects of GABA could normally be blocked by ejection of the $GABA_A$ antagonist bicuculline (Curtis, Duggan, Felix & Johnston, 1970), such that the response in the presence of GABA was 73 ± 5% of the control. Bicuculline often also increased the level of background activity severalfold (363 ± 96%) and caused a substantial increase in the response to visual stimulation in the absence of GABA (to 251 ± 50% of the control in preceding cycle where bicuculline was not ejected). An example of the effects of GABA and bicuculline is shown in Fig. 1*A*. These data are similar to those obtained by Sillito & Kemp (1983) in cat lateral geniculate nucleus (LGN) using similar methods.

Much longer durations (50–60 s) of baclofen ejection were necessary to reduce the response to visual stimulation. Baclofen ejection reduced the responses to visual stimulation to a mean of 49 ± 5% ($n = 7$) of control responses. The effects of baclofen were blocked when the $GABA_B$ selective antagonist CGP 35348 was continuously ejected, such that baclofen in the presence of CGP 35348 only reduced the visual response to 90 ± 9% of control levels (Fig. 1*B*).

Electrophysiological (Dutar & Nicoll, 1988; Calabresi, Mercuri, DeMurtas & Beradi, 1990) and anatomical (Price, Kelly & Bowery, 1987) evidence indicates that $GABA_B$ receptors can be located pre- and/or postsynaptically. Thus we attempted to determine whether pre- and/or post-synaptic mechanisms were involved in the baclofen-induced reduction of the visual response in the SC. In four cells, iontophoretic applications of NMDA were used to directly excite the postsynaptic membrane into producing action potential firing and this could be inhibited by baclofen (response reduced to 47 ± 5% of control levels). In each case the same current and duration of baclofen had previously been shown to reduce the visual response of the same neurone. An example is shown in Fig. 2.

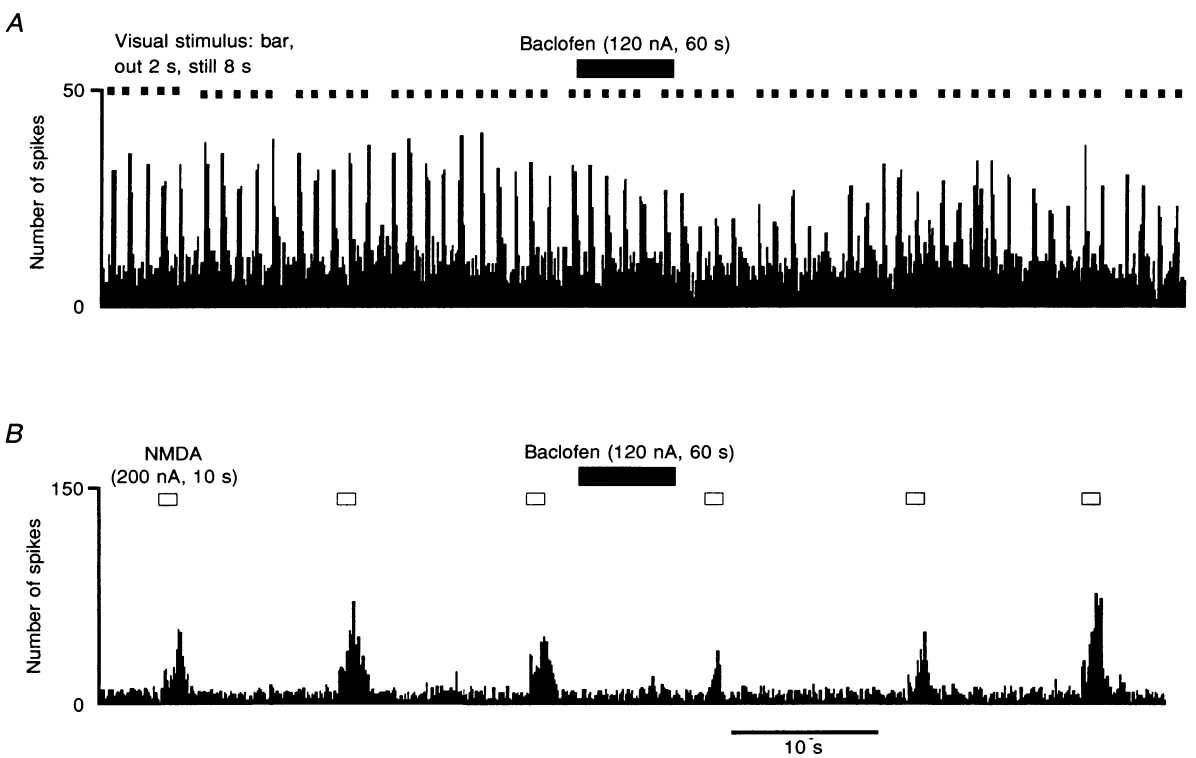

Figure 2. Effects of baclofen on visual and NMDA-evoked activity: data from an individual SGS neurone

A, PSTH of the response to repeated (8 s interstimulus intervals, stimulation and graph construction as Fig. 1*B*) visual stimulation showing the effects of baclofen (120 nA, 60 s). *B*, PSTHs of the response of the same neurone to brief ejections of NMDA (200 nA, 10 s). Baclofen (same ejection current and duration) reduced the response to NMDA. Action potential spikes were collected in 1 s bins.

Centre–surround antagonism in the SC: the role of $GABA_A$ receptors

Spot-diameter tuning curves were plotted for the visual responses of fourteen neurones. Each exhibited the characteristic excitatory response to stimulation of the receptive field centre and the spot diameter evoking the best response was within the range 5–21 deg (mean ± S.E.M. = 9·6 ± 1·2 deg). Neurones responded less well to stimuli of larger diameter. The spot diameters that evoked responses 25 and 50% less than the maximum were measured from the tuning curves (25% less = 14·8 ± 1·8, 50% less = 22·8 ± 3·3). Figure 3*A* shows PSTHs of the response of a typical neurone to spots of increasing diameter and the spot-diameter tuning curve for the same neurone is shown in Fig. 3*B*. The effects of bicuculline on surround antagonism were tested in ten neurones including that shown in Fig. 3. This antagonist did not change the spot diameter that evoked the best response (for details see Fig. 5), but surround inhibition was reduced such that the spot diameters which produced a 25 and 50% reduction of response were significantly greater. In contrast CGP 35348 ($n = 7$) had no effect on surround inhibition and the spot diameters that caused a 25 and 50% reduction of the response remained unchanged from control levels.

Habituation in the SC: the role of $GABA_B$ receptors

During repeated stimulus presentations the responses of SGS neurones habituate. In other species response habituation has been shown to be dependent on stimulus frequency (rabbit – Oyster & Takahashi, 1975; cat – Binns & Salt, 1995). In the rat, SGS neurones responded vigorously when

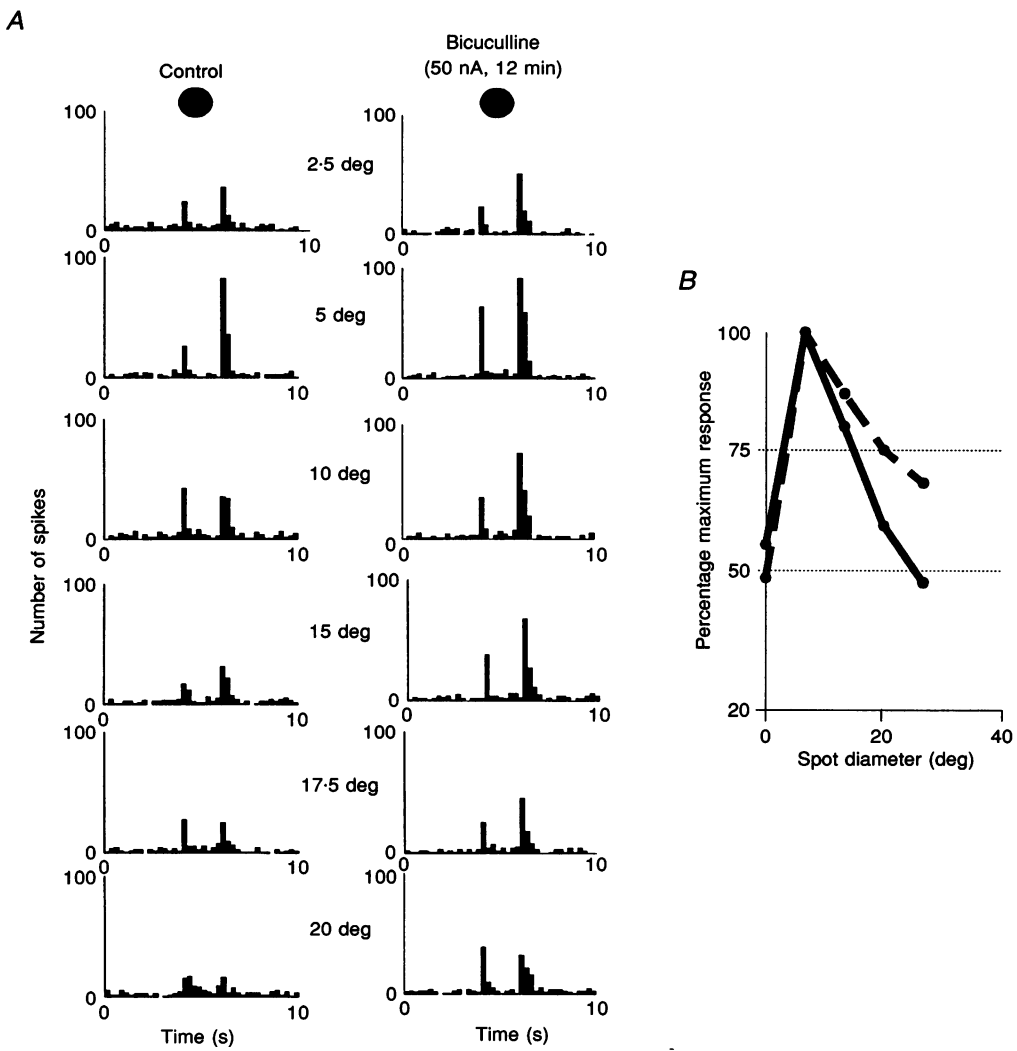

Figure 3. Spot-diameter tuning curves and the effect of bicuculline

A, data from a typical neurone. Each PSTH shows spikes evoked (cumulative response to 3 presentations) by the presentation of a spot (off 4 s, on 2 s, off 4 s) collected in 640 ms bins. The response to spots of increasing diameter (2·5–20 deg) are shown during the control period (left) and during a 12 min ejection of bicuculline (50 nA, a current and duration which for the same neurone blocked a GABA-induced reduction of the visual response as in Fig. 1*A*). *B*, spot-diameter tuning curves in the absence (continuous line) and presence (dashed line) of bicuculline plotted using data (summed on + off response) for the same neurone.

a stimulus was first presented, but when the interstimulus interval was short the response was reduced by about half within five repeats (% mean ± S.E.M., % fifth/first: 44 ± 3% at 0·5 s intervals and 59 ± 5% at 1 s intervals; $n = 22$). Response habituation was less severe when 2 s intervals were used (63 ± 6%) and did not occur with longer interstimulus intervals (4 s intervals, 93 ± 4%; 8 s intervals, 100 ± 3%). Figure 4*A* shows the effect of increasing the interstimulus interval on the habituation of a typical neurone. To test the effects of GABA receptor antagonists on response habituation a repetitive stimulus (five repeats, interval 0·5 s) was used to induce habituation in control cycles. In controls this stimulus reduced the response evoked by the fifth stimulus presentation to a mean of 43 ± 3% of the response evoked by the first, but during CGP 35348 ejection this value was increased to 74 ± 7%, ($n = 10$). Hence CGP 35348 reduced response habituation (for typical example see example in Fig. 4*B*). Bicuculline had no such effect, and during its ejection visual responses continued to habituate (% fifth/first: 44 ± 6%, control and 49 ± 17% during bicuculline ejection; $n = 9$).

Summary of data

The histograms in Fig. 5 summarize the effects of bicuculline and CGP 35348 on surround inhibition and response habituation. Overall, CGP 35348 significantly reduced response habituation but had no effect on either the spot diameter that evoked the maximum response or the spot diameters that caused 25 and 50% reductions of the maximum response to a flashed spot. In contrast, bicuculline failed to reduce habituation, but significantly increased the diameter of spots that caused 25 and 50% reductions of the response. The experimental protocol was time consuming and it was rarely possible to obtain a complete set of data from an individual neurone. On those occasions where the same current and duration of bicuculline were tested against both ($n = 7$) surround inhibition and habituation only surround inhibition was reduced. Conversely, when CGP 35348 was tested against both ($n = 4$) physiological protocols only habituation was effected. A complete set of data was obtained from three neurones and in each case the effects of bicuculline and CGP 35348 were mutually exclusive.

DISCUSSION

The superficial grey layer of the superior colliculus contains the highest levels of GABAergic neurones (Okada, 1974) found in the CNS and a correspondingly high density of

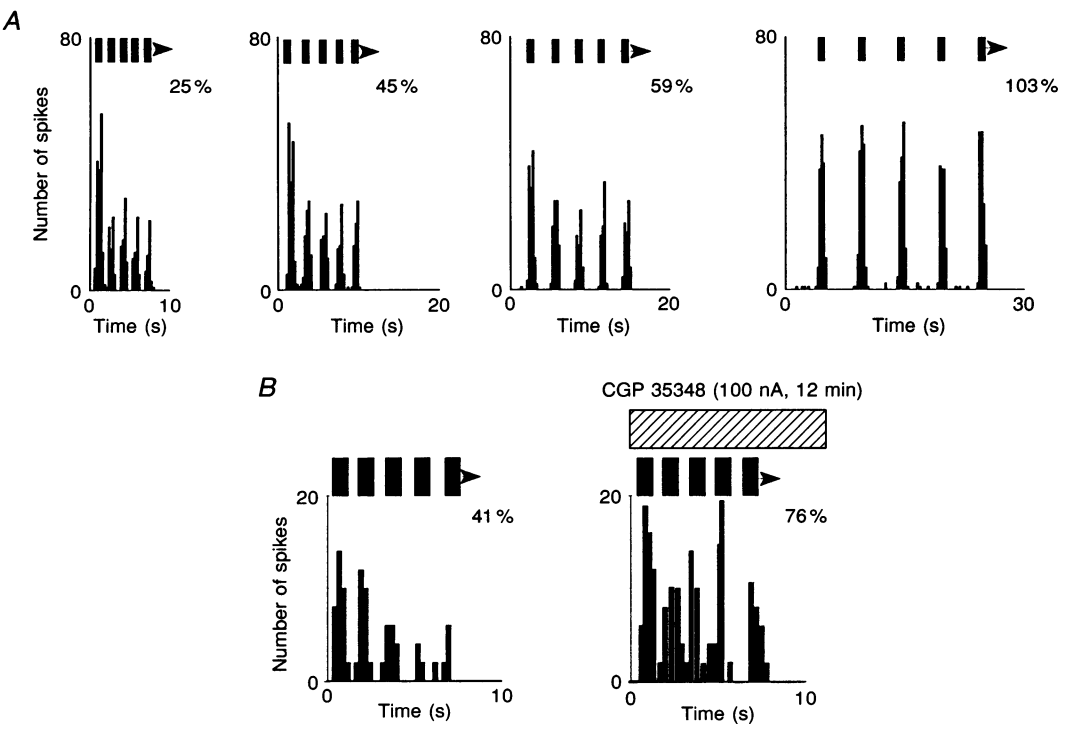

Figure 4. Habituation in SGS and the effect of CGP 35348

A, PSTHs showing spikes evoked by 5 identical stimulus (10 × 20 deg bar, out 1, 40 deg excursion) presentations. Spikes were collected in 0·25 s bins and cumulative data from 5 repeat trials are shown. The effects of increasing the interstimulus interval from 0·5 s (left) to 4 s (right) are shown. *B*, PSTHs showing spikes evoked by 5 identical stimulus (10 × 20 deg bar, out 1, 40 deg excursion) presentations with interstimulus intervals of 0·5 s. Spikes were collected in 0·25 s bins and cumulative data from 2 repeat trials are shown. Left, control response; right, response during ejection of CGP 35348 (100 nA, 12 min). The percentages in each PSTH indicate the fifth response compared with the first response.

GABA receptors (Bowery *et al.* 1987). Thus it is reasonable to question the functional significance of this inhibitory transmitter for visual processing in the SGS.

In other visual nuclei GABA has a key role in the shaping of receptive field properties. For example, in the LGN iontophoretic injections of bicuculline reduce surround inhibition (Sillito & Kemp, 1983), and in the visual cortex it is reported to cause the loss of directional specificity, orientation bias and length tuning (Sillito, 1992). All of these effects are attributable to blocking $GABA_A$ receptors. SGS neurones exhibit many of the same receptive field properties; however, not all these aspects of the visual response are generated locally, rather they are assumed to be conferred on SGS neurones by their convergent retino-collicular and cortico-collicular inputs. Response properties like direction and orientation tuning are probably reliant on extra-collicular mechanisms but there is some debate as to whether these properties are exported to the SC from the cortex (Wicklegren & Sterling, 1969; Berman & Cynader, 1975; Ogasawara, McHaffie & Stein, 1984) or retina (Weyand, Maleli, Lee & Schwartz, 1986; Mendola & Payne, 1993; Binns & Salt, 1996). The retina (Hammond, 1973) certainly contributes to the mechanisms that produce surround inhibition but the effect is probably enhanced at the local level in the SGS as in the LGN. Response habituation (Oyster & Takahashi, 1975) in the SGS is defined as a decrement in the response of single neurones resulting from repeated stimulus presentation, which is dependent on the frequency of stimulus presentation and which recovers if stimulus presentation is withheld for some time. Response habituation is likely to be intrinsic to the SC, since it can be modified by the local iontophoretic ejection of glutamate receptor antagonists (Binns & Salt, 1995). Given the likelihood that response habituation and surround inhibition are largely reliant on intrinsic inhibitory mechanisms we decided to focus our study of GABAergic activity in the SGS on these two aspects of the visual response.

Intrinsic and extrinsic sources of GABA in the SGS

Before going on to discuss the results of this study it is necessary to give a brief description of the sources of GABA that may contribute to visual processing in the SC. External sources of GABA arise from several areas including the zona incerta, substantia nigra, pretectum and contralateral SC (Araki, McGeer & McGeer, 1984; Ficalora & Mize, 1989; Appell & Behan, 1990). Of these, the projections from the substantia nigra and zona incerta are non-visual and terminate in the deeper layers of the SC and thus are

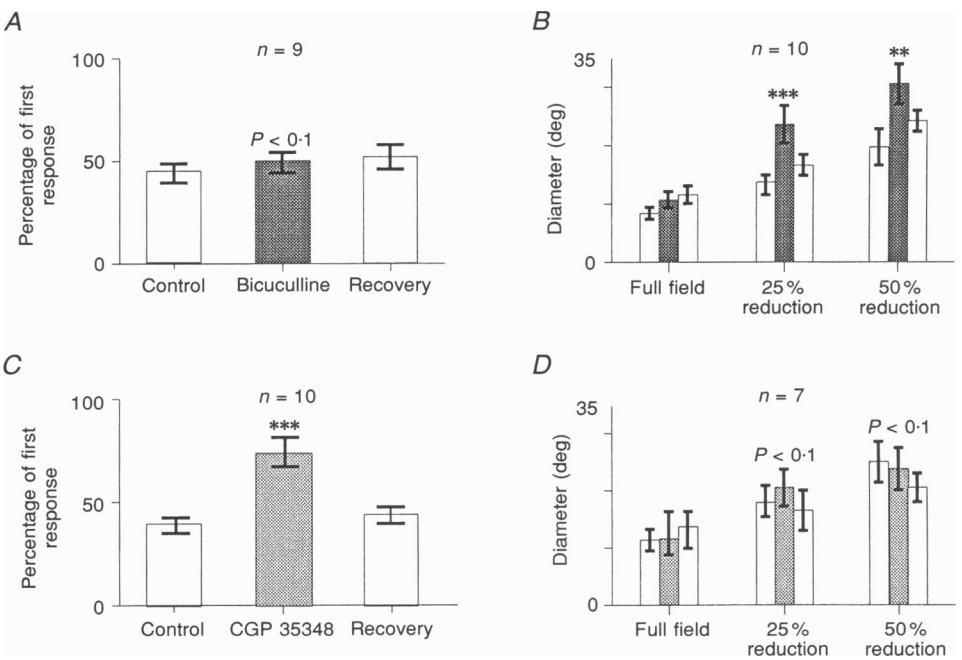

Figure 5. Summary of data

A and *C*, histogram showing the effects of bicuculline (*A*) and CGP 35348 (*C*) on response habituation. The bars show the means ± S.E.M. of the fifth response to 5 repeated stimuli expressed as a percentage of the first response during the control period, antagonist ejection and recovery. Bicuculline had no effect on habituation but CGP 35348 reduced habituation. *B* and *D*, histograms showing the effect of bicuculline (*B*) and CGP 35348 (*D*) on surround inhibition. Mean ± S.E.M. of the spot diameter evoking the maximum (full field) response and 25 and 50% reductions of the maximum response are shown. Each group of bars shows spot diameters during the control period, antagonist ejection and recovery (left to right). Neither antagonist changed the diameter of the full field. Bicuculline increased the spot diameters producing a 25 and 50% reduction of the response. CGP 35348 had no effect. Significantly different from control: ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

unlikely to influence visual processing in the SGS. Afferents from the pretectum and contralateral colliculus terminate in the SGS, carry visual information and could influence the response to visual stimulation.

As surround inhibition and response habituation are generated within the SC the probable sources of GABA for these processes are its intrinsic inhibitory neurones. Approximately 50% of the total cell population of the superficial SC are GABAergic and of the three morphologically different types described (Mize, 1992; horizontal, stellate and pyriform), horizontal neurones are best placed to mediate surround inhibition. These cells have fusiform cell bodies and large calibre dendrites, which extend some distance in the horizontal dimension, and they receive a substantial part of their innervation from the retina and visual cortex. Given the topographic precision of the retino-collicular and cortico-collicular projections, the activation of horizontal neurones could allow an inhibitory interaction between regions of the SC that have different visual receptive fields. Physiological evidence of such an interaction has been demonstrated (Rizzolatti *et al.* 1974).

Response habituation might be mediated by either stellate or pyriform cells. These receive little or no cortical input and their dendrites are closely associated with retinal terminals.

The effects of GABAergic agonists

When GABA is briefly ejected onto visually stimulated SGS neurones the response to an infrequent moving bar is much smaller than that observed in the absence of GABA. Interestingly, GABA ejection (regardless of the current and/or duration used) did not normally abolish the neurones response to visual stimulation in SGS as occurs using similar ejection currents of GABA in the LGN or cortex (Sillito & Kemp, 1983; Sillito, 1992). This difference is unlikely to be due to the use of an inadequate GABA ejection current, rather these data imply that the GABAergic inhibitory circuitry of the SGS differs from that in the LGN and cortex.

It is now clear that there are many subtypes of GABA receptor, which are broadly divided into three groups ($GABA_A$, $GABA_B$ and $GABA_C$), and which differ in their distribution and association with ion channels or second messenger systems. $GABA_A$ receptors form a complex containing binding sites for barbiturates, benzodiazepine and other modulators (Sieghart, 1992), which gate a chloride channel. The activation of $GABA_A$ receptors results in a fast IPSP which reaches peak conductance at less than 30 ms. $GABA_A$ receptors have a much higher affinity for GABA than $GABA_B$ receptors and are specifically antagonized by bicuculline (Curtis *et al.* 1970). The lower affinity $GABA_B$ receptors preferentially bind the agonist baclofen (Bowery, 1993). The binding of either GABA or baclofen to these receptors activates second messenger systems and results in much longer (250–1000 ms) IPSPs (Connors, Gutnick & Prince, 1982). $GABA_B$ receptor-selective antagonists include phaclofen, saclofen and CGP 35348 (Olpe *et al.* 1990).

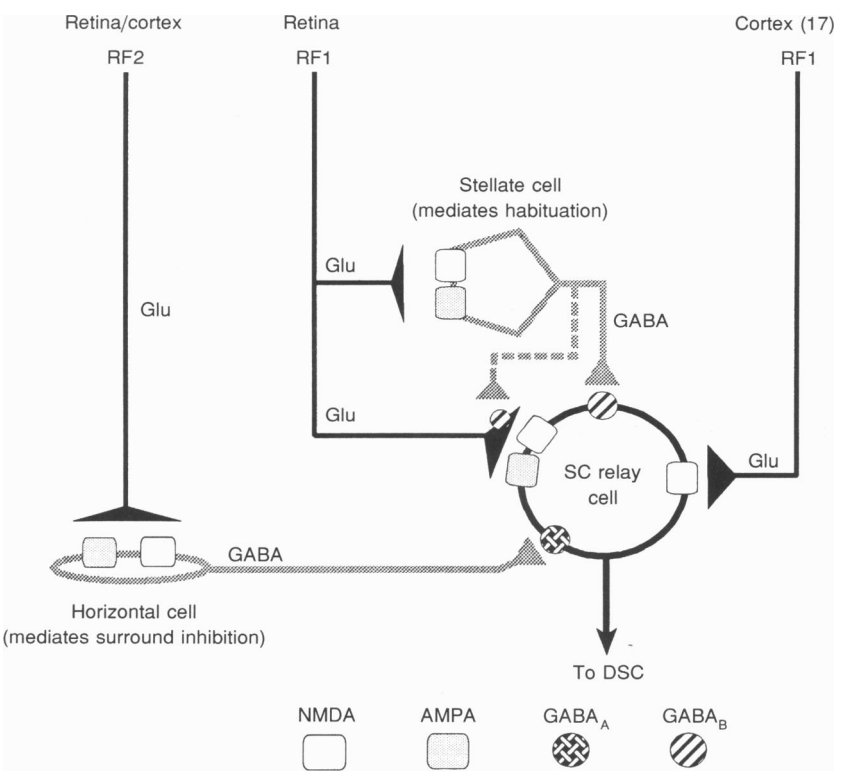

Figure 6. Possible GABAergic circuits for response habituation and surround inhibition in the SGS

See text for details. Glu, glutamate; DSC, deep superior colliculus; RF, receptive field.

We can assume that the effects of $GABA_A$ activation in the SGS are the result of postsynaptic inhibition since these receptors are not found on presynaptic membranes. $GABA_B$ receptors are located either pre- and/or postsynaptically in several brain areas (Dutar & Nicoll, 1988; Calabresi *et al.* 1990). Glutamate is the putative excitatory transmitter in the visual pathway and in many visual areas including the SGS (Binns & Salt 1994, 1996) iontophoretically applied NMDA and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor antagonists such as D-aminophonovalerate (AP5) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) reduce the response to visual stimulation. Presumably, AP5 and CNQX reduce visual activity as a consequence of blocking receptors on the postsynaptic surface and thus the effects of iontophoretically applied NMDA and AMPA are mediated by these receptors. Given that baclofen reduced the response evoked by NMDA ejection it is probable that at least some of its effects are mediated by postsynaptic events, but this does not exclude the possibility that it may also have presynaptic actions.

The effects of GABAergic antagonists

Bicuculline, $GABA_A$ receptors and surround inhibition. Visual neurones in the SGS exhibit surround inhibition (Wicklegren & Sterling, 1969; Berman & Cynader, 1975). Using ejection currents and durations of bicuculline, which block GABA-induced reductions of the visual response, we were able to reduce the surround inhibition. These data confirm the results of earlier studies (Ma, Li, Sun & Diao, 1991), are consistent with studies of the effects of bicuculline on surround inhibition in the LGN (Sillito & Kemp, 1983) and visual cortex (Sillito, 1992) and imply that similar $GABA_A$-mediated mechanisms produce this effect in all three areas.

CGP 35348, $GABA_B$ receptors and response habituation. $GABA_B$ receptors are present throughout the visual pathway (Bowery *et al.* 1987). There is ample evidence that $GABA_B$ receptors mediate slow IPSPs following the electrical stimulation of afferents to LGN X and Y cells *in vitro* (Soltesz, Lightowler, Leresche & Crunelli, 1989) but there have been few attempts to ascribe a function in visual processing to $GABA_B$ receptors. One recent report (Allison, Kabara, Snider, Casagrande & Bonds, 1996) suggests that $GABA_B$ receptors participate in the generation of orientation tuning in conjunction with $GABA_A$ receptors in the visual cortex.

The finding that $GABA_B$ receptors are involved in response habituation is quite novel. Response habituation is a commonly described property of SGS neurones (Harutinian-Kozak, Dec & Dreher, 1971; Oyster & Takahashi, 1975) and is suggested to underlie their novelty detection function. Ejection currents (and durations) of CGP 35348, which reverse the effects of baclofen on the response to infrequent stimulus presentation, also greatly reduce response habituation when stimuli are presented with small interstimulus intervals (0·5 s). As bicuculline did not reduce response habituation it appears that $GABA_A$ receptors do not contribute to this mechanism.

These data add significantly to our understanding of the mechanisms which generate response habituation in the SGS. It is already known that response habituation is dependent on the frequency of stimulation (Oyster & Takahashi, 1975) and can be modulated by local iontophoretic ejection of either AP5 or CNQX, which block glutamate receptor-mediated activity (Binns & Salt, 1995). It has been proposed that response habituation in the SGS arises as a result of coactivation of excitatory and inhibitory neurones by the same retinal afferents (Oyster & Takahashi, 1975). These latest data imply that the inhibitory neurones are GABAergic and that their activation results in the release of GABA, which blocks the excitatory response to subsequent presentations of the stimulus via $GABA_B$ receptors. The inclusion of $GABA_B$ receptors in this mechanism provides for the initial maximal response, which is mediated by ionotropic glutamate receptors and the progressive reduction of the response to subsequent stimulus presentations as slow IPSPs counteract the excitation. The long duration of the IPSP (250–1000 ms) may determine the length of the inter-stimulus interval required to avoid response habituation, which in the case of neurones in the rat SGS is 1–2 s. In addition, we were able to demonstrate that iontophoretically applied baclofen inhibits both visual responses and the response of SGS neurones to iontophoretically applied NMDA. While these data imply that postsynaptic $GABA_B$ receptors are capable of reducing the response, they do not negate a possible involvement of presynaptic $GABA_B$ receptors, and in this respect it is interesting to note that many presynaptic dendrites are GABAergic and are closely associated with the retinal axons (Behan, 1981; Mize & Norton, 1985).

GABA circuitry in the SGS

The results of the present study on the role of GABA, together with data from our previous work on the role of glutamate in the SGS (Binns & Salt, 1994, 1995, 1996) and the relevant anatomical information (Mize, 1992), allows the proposal of neuronal circuits that might produce surround inhibition and response habituation (see Fig. 6).

The projections from the retina and visual cortex are glutamatergic and are strictly retinotopic. Their activation via a stimulus in the centre of the receptive field directly activates the relay cells on which they converge. Input from the retina is probably mediated by both AMPA and NMDA receptors, but cortical activation uses NMDA receptors (Binns & Salt, 1996). The same retino-collicular afferents activate inhibitory stellate cells, which release GABA onto postsynaptic (and possibly presynaptic) $GABA_B$ receptors: the subsequent slow IPSP limits the response to later stimuli presented in the same spatial location, and in so doing produces habituation. Stimuli remote to the receptive field activate retinal and cortical afferents, which terminate some distance from the relay cell on horizontal cells. GABA

released from horizontal cells generates surround inhibition by the activation of $GABA_A$ receptors. Together these GABAergic inhibitory circuits make a significant contribution to two of the most characteristic features of SGS visual responses and promote accurate orientation responses towards novel visual targets.

ALLISON, J. D., KABARA, J. F., SNIDER, R. K., CASAGRANDE, V. A. & BONDS, A. B. (1996). $GABA_B$ receptor mediated inhibition the orientation selectivity of the sustained response of striate cortical neurons in cats. *Visual Neuroscience* **13**, 559–566.

APPELL, P. P. & BEHAN, M. (1990). Sources of sub-cortical projections to the superior colliculus in the cat. *Journal of Comparative Neurology* **302**, 143–158.

ARAKI, M., MCGEER, P. L. & MCGEER, E. G. (1984), Presumptive gamma-aminobutyric acid pathways from the mid brain to the superior colliculus studied by a combined horse raddish peroxidase-gamma-aminobutyric acid transaminase pharmaco-histochemical method. *Neuroscience* **13**, 433–439.

BEHAN, M. (1981). Identification and distribution of retinocollicular terminals in the cat: An electron-microscopic autoradiographic analysis. *Journal of Comparative Neurology* **199**, 1–16.

BERMAN, N. & CYNADER, M. (1975). Receptive fields in cat superior colliculus after visual cortex lesions. *Journal of Physiology* **245**, 261–270.

BINNS, K. E. & SALT, T. E. (1994). Excitatory amino acid receptors participate in synaptic transmission of visual responses in the superficial layers of the cat superior colliculus. *European Journal of Neuroscience* **6**, 161–169.

BINNS, K. E. & SALT, T. E. (1995). Excitatory amino acid receptors modulate habituation of the response to visual stimulation in the cat superior colliculus. *Visual Neuroscience* **12**, 563–571.

BINNS, K. E. & SALT, T. E. (1996). Cortico-fugal influences on visual responses in cat superior colliculus: The role of NMDA receptors. *Visual Neuroscience* **13**, 683–694.

BOWERY, N. G. (1993). $GABA_B$ receptor pharmacology. *Annual Review of Pharmacology and Toxicology* **33**, 109–147.

BOWERY, N. G., HUDSON, A. L. & PRICE, G. W. (1987). $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system. *Neuroscience* **20**, 365–383.

CALABRESI, P., MERCURI, H., DEMURTAS, M. & BERADI, N. (1990). Endogenous GABA mediates presynaptic inhibition of spontaneous and evoked excitatory synaptic potentials in the rat neostriatum. *Neuroscience Letters* **118**, 99–102.

CHALUPA, L. M. & RHOADES, R. W. (1977). Responses of visual, somatosensory and auditory neurones in the golden hamster superior colliculus. *Journal of Physiology* **270**, 595–625.

CONNORS, B. W., GUTNICK, M. J. & PRINCE, D. A. (1982), Electrophysiological properties of neocortical neurones *in vitro*. *Journal of Neurophysiology* **48**, 1302–1320.

CURTIS, D. R., DUGGAN, A. W., FELIX, D. & JOHNSTON, G. A. R. (1970). GABA, bicuculline and central inhibition. *Nature* **226**, 1222–1224.

DRAGER, U. C. & HUBEL, D. H. (1975). Responses to visual stimulation and relationship between visual, auditory and somatosensory inputs in mouse superior colliculus. *Journal of Neurophysiology* **38**, 690–713.

DUTAR, P. & NICOLL, R. A. (1988). Pre and post-synaptic $GABA_B$ receptors in the hippocampus have different pharmacological properties. *Neuron* **1**, 585–598.

FICALORA, A. S. & MIZE, R. R. (1989). The neurons of the substantia nigra and zona incertia which project to the cat superior colliculus are GABA immuno reactive: a double label study using GABA immunocytochemistry and lectin retrograde transport. *Neuroscience* **29**, 567–581.

HAMMOND, P. (1973). Contrast in spatial organization of receptive fields at the geniculate and retinal levels: centre, surround and outer surround. *Journal of Physiology* **228**, 115–137.

HARUTINIAN-KOZAK, B., DEC, K. & DREHER, B. (1971). Habituation of unitary responses in the superior colliculus of the cat. *Acta Neurobioliae Experimentalis* **31**, 213–217.

KANEKO, T. & HICKS, T. P. (1990). $GABA_B$-related activity involved in synaptic processing of somatosensory information in S1 cortex of the anaesthetized cat. *British Journal of Pharmacology* **100**, 689–698.

MA, M. H., LI, B., SUN, J. S. & DIAO, Y. C. (1991). Effects of GABA and bicuculline on the properties of visual superior colliculus neurons in golden hamsters. *Acta Physiologica Sinica* **43**, 573–579.

MENDOLA, J. D. & PAYNE, B. R. (1993). Directional selectivity and physiological compensation in the superior colliculus following removal of areas 17 and 18. *Visual Neuroscience* **10**, 1019–1026.

MIZE, R. R. (1992). The organization of GABAergic neurones in the mammalian superior colliculus. In *GABA in the Retina and Central Visual System*, ed. MIZE, R. R, MARC, R. E. & SILLITO, A. M., *Progress in Brain Research*, vol. 90, pp. 219–248. Elsevier, Amsterdam.

MIZE, R. R. & NORTON, T. T. (1985). GABA antiserum reactivity in the superior colliculus of the tree shrew (*Tupaia Belangeri*). *Investigative Opthalmology and Visual Science* (Suppl. 26) 163.

OGASAWARA, K., MCHAFFIE, J. G. & STEIN, B. E. (1984). Two visual corticotectal systems in the cat. *Journal of Neurophysiology* **52**, 1226–1245.

OKADA, Y. (1974). Distribution of γ-aminobutyric acid (GABA) in the layers of the superior colliculus of the rabbit. *Brain Research* **75**, 362–365.

OLPE, H.-R., KARLSSON, G., POZZA, M. F., BRUGGER, F., STEINMANN, M., RIEZEN H.-V., FAGG, G., HALL, R. G., FROESTL, W. & BIFFIGER, H. (1990). CGP 35348: a centrally active blocker of $GABA_B$ receptors. *European Journal of Pharmacology* **187**, 27–38.

OYSTER, C. W. & TAKAHASHI, E. S. (1975). Responses of rabbit superior colliculus to repeated visual stimuli. *Journal of Neurophysiology* **38**, 301–312.

PRICE, G. W., KELLY, J. S. & BOWERY, N. G. (1987). The location of $GABA_B$ receptor binding sites in mammalian spinal cord. *Synapse* **1**, 530–538.

RIZZOLATTI, G., CAMARDA, R., GRUPP, L. A. & PISA, M. (1974). Inhibitory effect of remote visual stimuli on visual responses of cat superior colliculus: Spatial and temporal factors. *Journal of Neurophysiology* **37**, 1262–1275.

SEABROOK, G. R., HOWSON, W. & LACEY, M. G. (1990). Electrophysiological characterization of potent agonists and antagonists at pre and postsynaptic $GABA_B$ receptors on neurones in rat brain slices. *British Journal of Pharmacology* **101**, 949–957.

SIEGHART, W. (1992). $GABA_A$ receptor ligand-gated Cl^- ion channels modulated by multiple drug binding sites. *Trends in Pharmacological Sciences* **13**, 446–450.

SILLITO, A. M. (1992). GABA mediated inhibitory processes in the function of the geniculo-striate system. In *GABA in the Retina and Central Visual System*, ed. MIZE, R. R., MARC, R. E. & SILLITO, A. M., *Progress in Brain Research*, vol. 90, pp. 219–248. Elsevier, Amsterdam.

SILLITO, A. M. & KEMP, J. A. (1983). The influence of GABA-ergic inhibitory processes on receptive field structure of X and Y cells in the cat dorsal lateral geniculate nucleus (dLGN). *Brain Research* **277**, 63–77.

SIMINOFF, R., SCHWASSMANN, H. O. & KRUGER, L. (1966). An electrophysiological study of the superior colliculus of the rat. *Journal of Comparative Neurology* **127**, 435–444.

SOLTESZ, I., LIGHTOWLER, S., LERESCHE, N. & CRUNELLI, V. (1989). On the properties and origin of the $GABA_B$ inhibitory postsynaptic potential recorded in morphologically identified projection cells of the cat dorsal lateral geniculate nucleus. *Neuroscience* **33**, 23–33.

SPARKS, D. L. & NELSON, J. S. (1987). Sensory and motor maps in the mammalian superior colliculus. *Trends in Neurosciences* **10**, 312–317.

WEYAND, T. G., MALPELI, J. G., LEE, C. & SCHWARK, H. D. (1986). Cat area 17. III. Response properties and orientation anisotropies of corticotectal cells. *Journal of Neurophysiology* **56**, 1088–1101.

WICKELGREN, B. G. & STERLING, P. (1969). Influence of visual cortex on receptive fields in the superior colliculus of the cat. *Journal of Neurophysiology* **32**, 16–23.

Acknowledgements

K. E. Binns is supported by The Wellcome Trust. CGP 35348 was a gift from Ciba Geigy.

Author's email address

K. E. Binns: K.Binns@ucl.ac.uk

Received 11 March 1997; accepted 15 July 1997.