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ORIGINAL ARTICLE

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Inhibition of firing rate and changes in the firing pattern of nigral dopamine neurons by γ-hydroxybutyric acid (GHBA) are specifically induced by activation of $GABA_B$ receptors

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Abstract Previous studies have shown that administration of γ-hydroxybutyric acid (GHBA) or the $GABA_B$ receptor agonist baclofen are associated with a decrease in firing rate, a regularisation of firing pattern and a decrease in burst activity of midbrain dopamine (DA) neurons in the substantia nigra (SN).
In the present study we compared the ability of the novel $GABA_B$ receptor antagonist SCH 50911 and the selective antagonist of GHBA binding sites, NCS-382, to antagonise the effects of baclofen or GHBA, respectively, on the neuronal activity of DA neurons in anaesthetised rats. SCH 50911 (75 mg/kg, i.v.) was found to antagonise the decrease in firing rate, the regularisation of firing rhythm and the decrease of burst activity in DA cells, induced by baclofen (1–32 mg/kg, i.v.) or GHBA (12.5–1600 mg/kg, i.v.). NCS-382 (100 mg/kg, i.v.) did not affect the baclofen-induced changes in neuronal activity. Neither was the drug able to influence the GHBA-induced alterations in firing rate or in burst activity, although NCS-382 to some extent antagonised the regularisation of the firing pattern observed following low doses of GHBA (≤100 mg/kg).
The results of the present study give further support for the notion that the GHBA-induced changes in neuronal activity of nigral dopamine neurons are mediated by stimulation of $GABA_B$ receptors.

Key words Substantia nigra · γ-Hydroxybutyrate · Baclofen · SCH 50911 · NCS-382 · Dopamine · Burst firing · $GABA_B$ receptors

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Introduction

γ-Hydroxybutyric acid (GHBA) is an endogenous compound thought to act as a neurotransmitter in mammalian brain (Roth and Giarman 1970; Vayer et al. 1987). GHBA, which is synthesised from GABA (Snead et al. 1989), displays a discrete, uneven distribution with high levels in the substantia nigra (SN; Vayer et al. 1988). Administration of GHBA, or its prodrug γ-butyrolactone (GBL), produces sedation and anaesthesia in humans, although its low potency in this regard has limited its use as an anaesthetic (Snead 1977; Tunnicliff 1992). In animals, the drug has been reported to suppress the spontaneous firing rate of midbrain dopamine (DA) neurons (Roth et al. 1973), to decrease locomotor activity, and to increase brain DA levels, the latter effects being attributed to a reduction in dopamine release (Gessa et al. 1968; Walters and Roth 1972; Stock et al. 1973; Nissbrandt and Engberg 1996). Many effects of GHBA on the central nervous system show a remarkable similarity to the effects produced by the $GABA_B$-receptor agonist baclofen. In this sense, the mode of action by which GHBA produces its central effects remains a matter of controversy. According to previous radioligand binding studies, some actions of GHBA have been suggested to be mediated via activation of highly specific membrane-binding sites without affinity for GABA, GABAergic agonists or GBL (Benavides et al. 1982; Maitre et al. 1983; see Maitre 1997). Furthermore, these binding sites appear to have a different regional, anatomic distribution in rat brain compared to $GABA_B$-binding sites (Snead 1994). On the other hand, many effects produced by GHBA are shown to be attenuated by administration of $GABA_B$-receptor antagonists. Previous studies in our laboratory have shown that systemic administration of GHBA, like baclofen, produces several effects on the neuronal activity of DA neurons in the SN, including a decreased firing rate and a regularisation of the firing rhythm with reduction of burst activity (Engberg and Nissbrandt 1993). These actions of GHBA were attenuated by the specific $GABA_B$-receptor antagonist CGP 35348, indicating a $GABA_B$-receptor-mediated action of GHBA

(Engberg and Nissbrandt 1993). Accordingly, CGP 35348 has been shown to antagonise in vivo the effects of baclofen, GHBA or GBL on striatal DA synthesis/release (Waldmeier 1991; Nissbrandt and Engberg 1996). Moreover, GHBA has been reported to displace [^{3}H]baclofen and the $GABA_B$-receptor antagonist [^{3}H]CGP 27492 from $GABA_B$ sites in cortical and thalamic homogenates (Bernasconi et al. 1992).

In the present study we utilised SCH 50911, a novel selective and potent $GABA_B$-receptor antagonist (Bolser et al. 1995) and NCS-382, a novel compound suggested to act as a specific antagonist of GHBA sites (Maitre et al. 1990), in order to further characterise the receptor involved in the effects of GHBA on the firing of nigrostriatal DA neurons.

Materials and methods

Animals

The experiments were performed on male BKL:SD rats (B&K Universal AB, Sollentuna, Sweden; weighing between 180 and 250 g). The animals were housed in groups of five and kept under constant temperature (25°C), maintained on a 12-h light/dark cycle (lights on at 06.00 h) with ad libitum access to food and water.

Animal surgery

Rats were anaesthetised (chloral hydrate 400 mg/kg, intraperitoneally) and mounted in a stereotaxic frame (David Kopf Instr.,Tujunga, Calif., USA). Additional anaesthesia and drugs were given through a lateral tail vein. Throughout the experiments the body temperature of the animals was maintained at 37°C by means of a heating pad. The skull surface was exposed and a 3-mm burr hole was drilled with its centre located approximately 2 mm anterior to lambda and 2 mm lateral to the midline. The dura was carefully removed and a single-barrel micropipette was lowered about 7 mm into the region of the SN.

Single unit recording

A glass microelectrode with a tip diameter of approximately 1–2 µm (filled with 2 M sodiumacetate saturated with Pontamine Sky Blue) was lowered by means of a hydraulic microdrive (David Kopf Instr., Tujunga, Calif., USA) into the zona compacta-SN. The in vitro impedance of the electrodes was generally 6–9 MΩ, measured in 0.9% saline at 135 Hz. Single unit potentials were passed through a high input-impedance amplifier and filters. Nerve impulses were discriminated from background noise and fed into a computer, and simultaneously displayed on a digital storage oscilloscope, monitored on an audio monitor and on a strip chart recorder (Gould). The position of the electrode was marked at the end of each experiment by iontophoretic ejection of Pontamine Sky Blue. The brains were then removed, buffered in 10% formaldehyde solution and subsequently sliced in 50-µm-thick sections by a microtome and stained with neutral red. Only DA cells within the SN were included in this study and only one neuron per animal was studied.

Data analysis

The temporal distribution of spikes was analysed on-line utilising a Macintosh computer. The software used for the analysis of firing was written in-house using a high level object-oriented programming language called "G" (Lab VIEW, National Instruments, Austin, Tex., USA). The software was designed to sample and analyse the intervals of an arbitrary number of TTL pulses (corresponding to spikes passing through the discriminating filter) using a time resolution of 1 µs. An interspike interval was designated as the time (in ms) elapsed between the rising edge of two sequential TTL pulses. In order to avoid artefacts in the sampling procedure, time intervals below 20 ms were ignored by the spike analyser. The onset of a burst was determined as an interspike time interval shorter than 80 ms and termination of a burst by the next interval longer than 160 ms (Grace and Bunney 1984). Cells were considered to be bursting if at least 1 interspike time interval of 100 recorded spikes was below 80 ms. The intervals were analysed with regard to number of bursts that occurred during each 100-spike sampling period along with a calculation of the percentage of spikes in bursts.

In addition, the software programme sorted the intervals and divided them into 3-ms bins and displayed the results as an interspike time interval histogram (ISH) with regard to the number of intervals corresponding to each bin. The analysis also included calculation of the variation coefficient, which was used as a measure of the regularity of firing (Werner and Mountcastle 1963). Variation coefficient, expressed as the median of at least three consecutive ISHs, was calculated as the ratio between the standard deviation and the mean interval of an ISH. Data collected from the spike analyser obtained less than 1 min after drug administration were not included in the analysis in order to exclude drug wear-on effects.

Chemistry

The preparation of NCS-382 was mainly performed as described previously (Maitre et al. 1990), however, with some modifications given below.

5-Oxo-5,7,8,9-tetrahydrobenzocycloheptene acetic acid

Commercially available1-benzosuberone (12.5 g, 78.1 mmol) was reacted under argon atmosphere with glyoxylic acid as previously described. After the reaction was completed, the mixture was poured into crushed ice. The crystals were filtered off, washed with water and dissolved in diluted NaOH solution (1.5 equivalent NaOH). The aqueous layer was extracted with CH_2Cl_2 and separated. The aqueous solution was poured into diluted HCl (40 ml conc. HCl in 200 g ice-water) under intense stirring. The crystals were filtered off and carefully washed with water and dried at 50°C, yielding 13.3 g (81%) of pure product.

5-Hydroxy-5,7,8,9-tetrahydrobenzocycloheptene acetic acid

To a solution of 5-oxo-5,7,8,9-tetrahydrobenzocycloheptene acetic acid (1 g, 4.6 mmol) inMeOH (30 ml) fresh $NaBH_4$ (1 g, 26.4 mmol) was added. The solution was refluxed for 4 h. Additional $NaBH_4$ (0.5 g, 13.2 mmol) was added and the reaction mixture was refluxed over night. The reaction mixture was poured into H_2O (200 ml) and the pH was adjusted to 1–2 with diluted aqueous HCl and extracted with CH_2Cl_2. The organic layer was washed with water, separated, dried (Na_2CO_3) and filtered, yielding 0.5 g (50%) as crude product. Recrystallisation from trichlorethylene afforded pure compound as crystals. ^{1}H NMR (300 MHz, DMSO) 1.5–1.9 (m, ^{2}H), 2.7–3.1 (m, ^{3}H), 3.4–3.6 (m, ^{1}H), 5.3 (s, ^{1}H), 5.8 (s, br, ^{1}H), 5.9 (s, ^{1}H), 7.1–7.3 (m, ^{3}H), 7.5 (d, ^{1}H). ^{13}C NMR (75.4 MHz, DMSO) 27.83, 29.83, 33.91, 75.69, 114.27, 125.29, 126.37, 126.98, 129.32, 139.79, 141.78, 162.78, 167.85.

The product was converted to the sodium salt (NCS-382) as previously described (Maitre et al. 1990).However, we were unable to use isopropyl alcohol as a tritration agent, since the compound dissolved in this solvent. Instead, diisopropyl ether was used. The compound was finally dried under vacuum at room temperature.

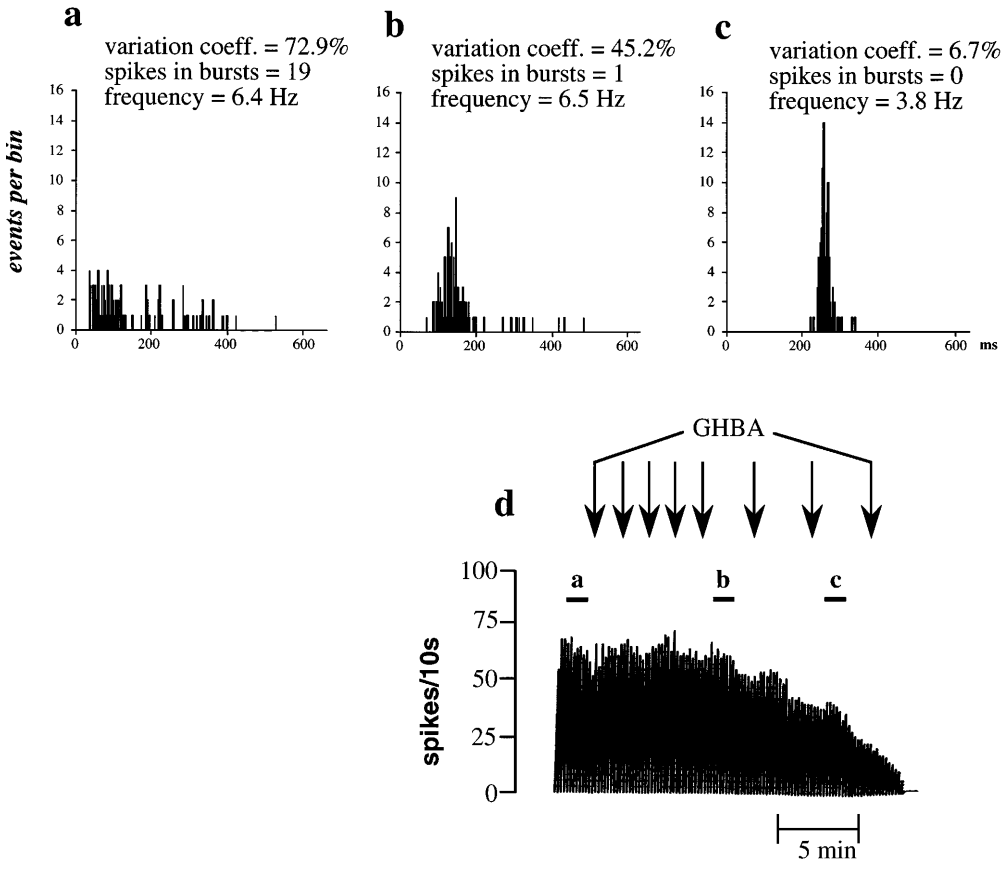


Fig. 1a–d Extracellular recording from a spontaneously bursting dopamine (DA) neuron in the substantia nigra (SN) following intravenous administration of GHBA. **a** Interspike time interval histogram (ISH) before administration of GHBA. **b,c** ISHs after the administration of GHBA. **d** Cumulative rate histogram depicting the action of GHBA (GHBA, 12.5+12.5+25+50+100+200+400+800 mg/kg, at *arrows*) on the firing rate of the same neuron. *Horizontal bars* indicate the time periods where the three ISHs were recorded

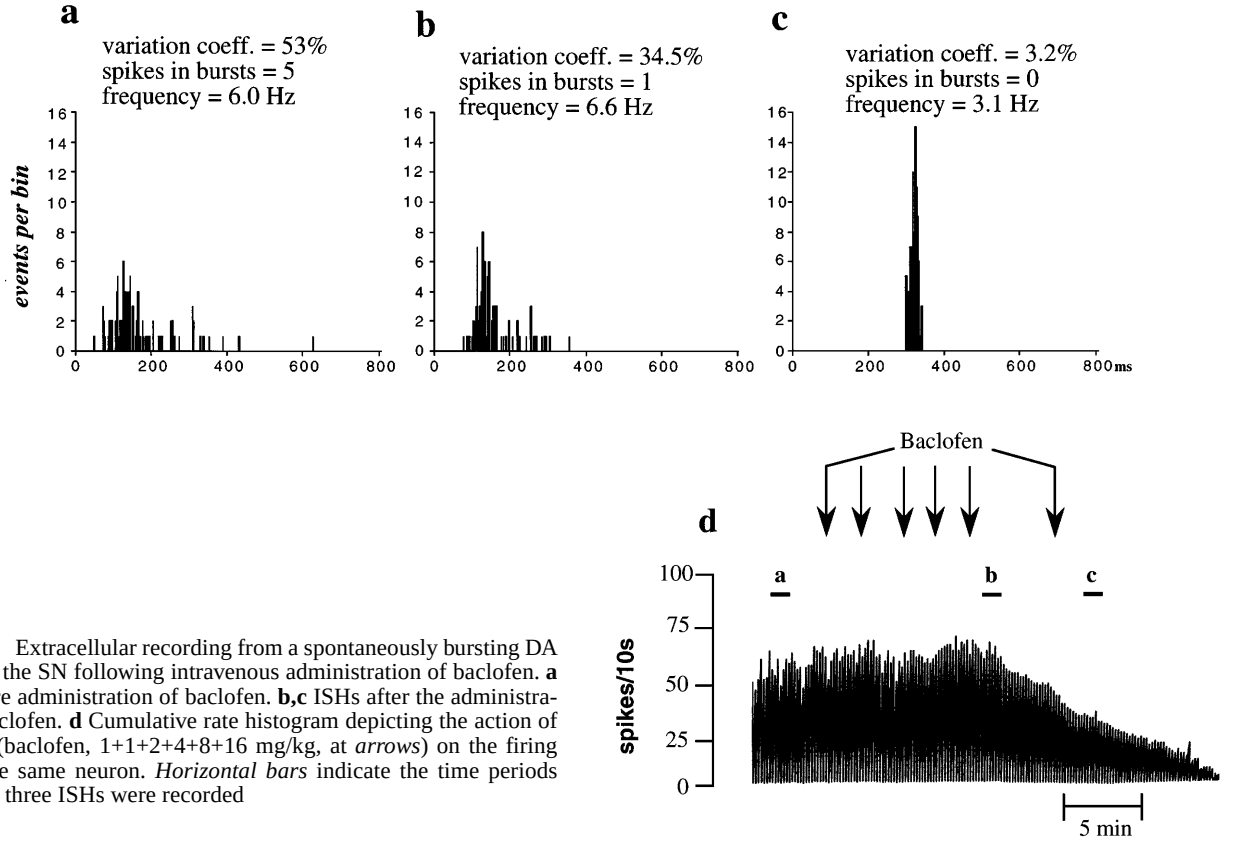


Fig. 2a–d Extracellular recording from a spontaneously bursting DA neuron in the SN following intravenous administration of baclofen. **a** ISH before administration of baclofen. **b,c** ISHs after the administration of baclofen. **d** Cumulative rate histogram depicting the action of baclofen (baclofen, 1+1+2+4+8+16 mg/kg, at *arrows*) on the firing rate of the same neuron. *Horizontal bars* indicate the time periods where the three ISHs were recorded

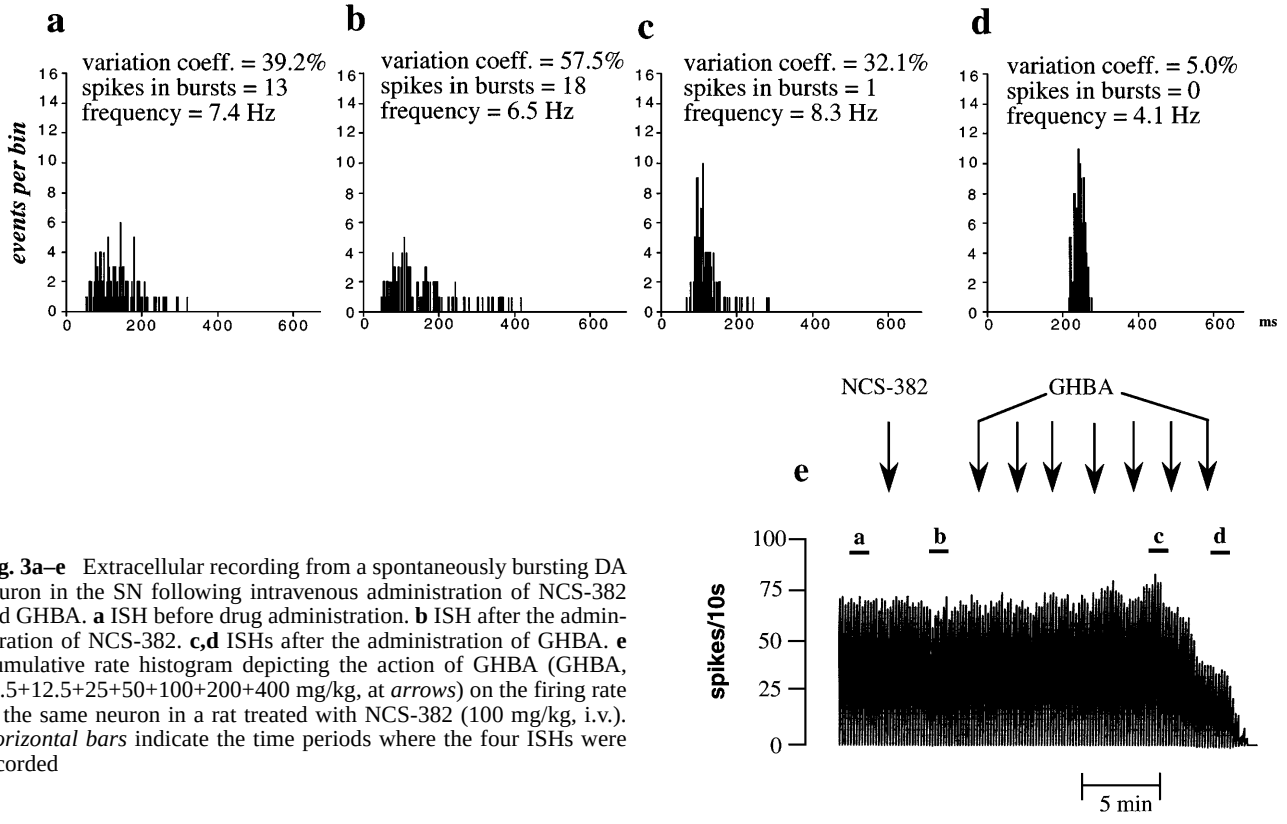


Fig. 3a–e Extracellular recording from a spontaneously bursting DA neuron in the SN following intravenous administration of NCS-382 and GHBA. **a** ISH before drug administration. **b** ISH after the administration of NCS-382. **c,d** ISHs after the administration of GHBA. **e** Cumulative rate histogram depicting the action of GHBA (GHBA, 12.5+12.5+25+50+100+200+400 mg/kg, at *arrows*) on the firing rate of the same neuron in a rat treated with NCS-382 (100 mg/kg, i.v.). *Horizontal bars* indicate the time periods where the four ISHs were recorded

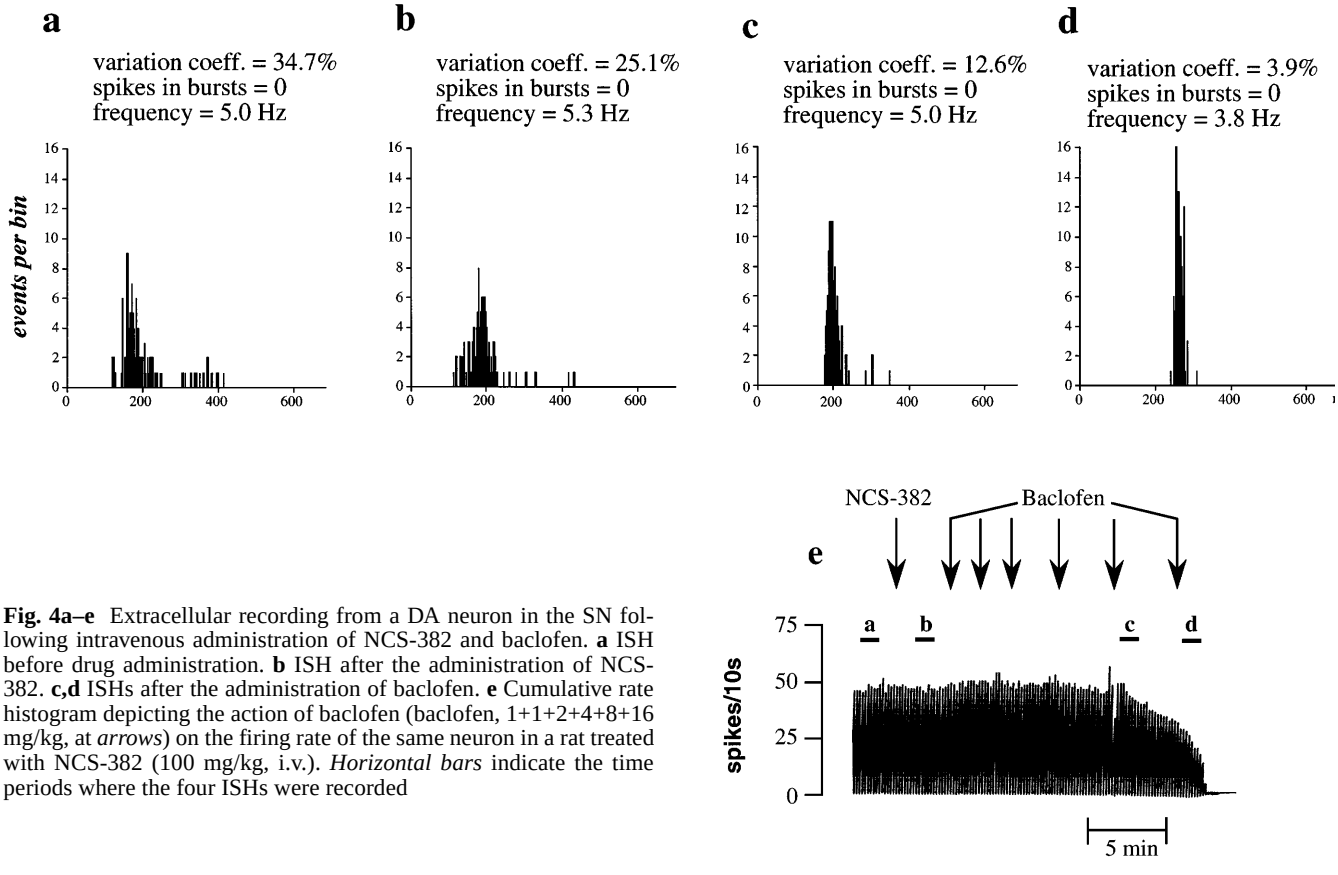
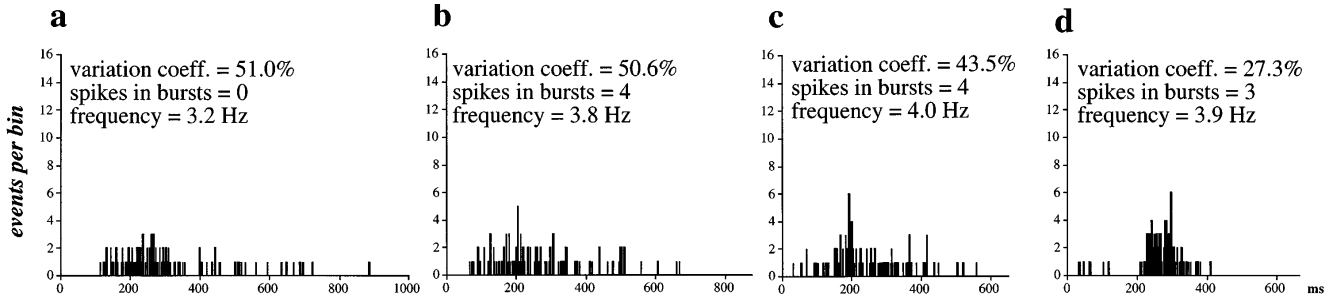


Fig. 4a–e Extracellular recording from a DA neuron in the SN following intravenous administration of NCS-382 and baclofen. **a** ISH before drug administration. **b** ISH after the administration of NCS-382. **c,d** ISHs after the administration of baclofen. **e** Cumulative rate histogram depicting the action of baclofen (baclofen, 1+1+2+4+8+16 mg/kg, at *arrows*) on the firing rate of the same neuron in a rat treated with NCS-382 (100 mg/kg, i.v.). *Horizontal bars* indicate the time periods where the four ISHs were recorded



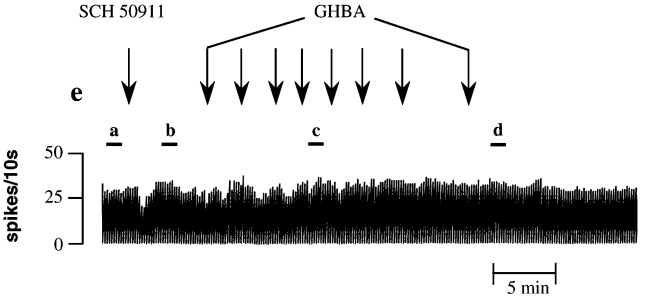
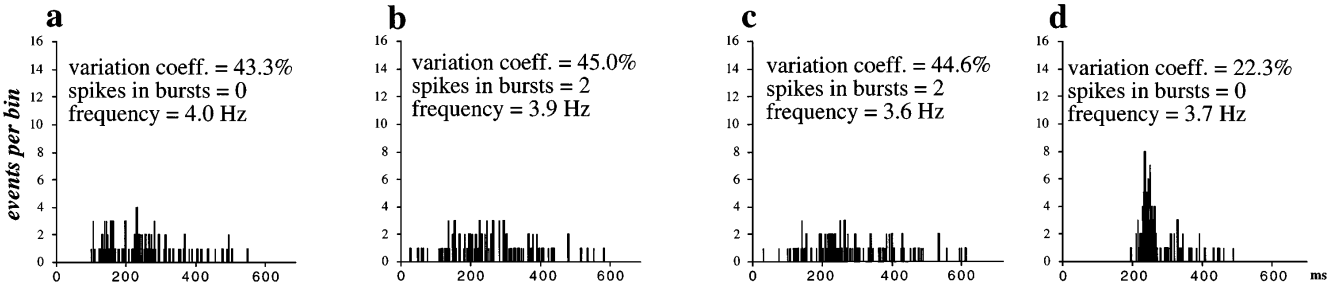


Fig. 5a–e Extracellular recording from a DA neuron in the SN following intravenous administration of SCH 50911 and GHBA. **a** ISH before drug administration. **b** ISH after the administration of SCH 50911. **c,d** ISHs after the administration of GHBA. **e** Cumulative rate histogram depicting the action of GHBA (GHBA, 12.5+12.5+25+50 +100+200+400+800 mg/kg, at *arrows*) on the firing rate of the same neuron in a rat treated with SCH 50911 (75 mg/kg, i.v.). *Horizontal bars* indicate the time periods where the four 15 Hs were recorded



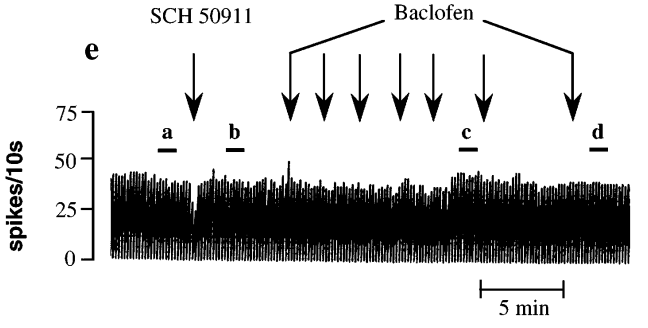


Fig. 6a–e Extracellular recording from a DA neuron in the SN following intravenous administration of SCH 50911 and baclofen. **a** ISH before drug administration. **b** ISH after the administration of SCH 50911. **c,d** ISHs after the administration of baclofen. **e** Cumulative rate histogram depicting the action of baclofen (baclofen, 1+1+2+4+ 8+16+32 mg/kg, at *arrows*) on the firing rate of the same neuron in a rat treated with SCH 50911 (75 mg/kg, i.v.). *Horizontal bars* indicate the time periods where the four ISHs were recorded

Drugs

Chloral hydrate (Merck, Darmstadt, Germany); D-L-baclofen and SCH 50911 (kindly donated by Dr. Kreutner, Schering-Plough, Keningworth, N.J., USA); γ-hydroxybutyric acid (GHBA; Sigma, St. Louis, Mo.,USA).

Results

Intravenous administration of GHBA (12.5–1600 mg/kg, i.v.) or baclofen (1–32 mg/kg, i.v.) induced a dose-dependent decrease in the firing rate of nigral DA neurons (Figs. 1, 2, 7). Furthermore, both drugs dose-dependently induced a regularisation of the firing pattern, expressed as the variation coefficient of an ISH, with a maximal effect (90% decrease with GHBA and 91% decrease with baclofen) obtained at 800 mg/kg or 32 mg/kg, respectively (Figs. 1, 2, 8). In all spontaneously bursting cells tested, both GHBA and baclofen also significantly reduced the percentage of burst firing (Figs. 1, 2, 9a, 10a).

Administration of NCS-382 (100 mg/kg, i.v.), suggested to act as a specific antagonist of GHBA sites, did not affect the regularity of firing or the firing rate of the DA neurons when given alone. The percentage of spikes occurring in

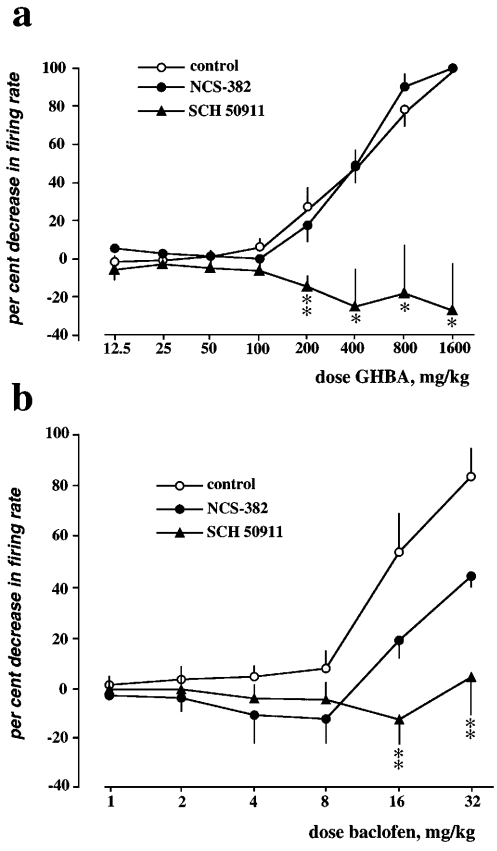


Fig. 7a,b Dose response curves illustrating the action of intravenously administered GHBA (**a**) or baclofen (**b**) on the firing rate of nigral DA neurons in controls, in rats treated with SCH 50911 (75 mg/kg, i.v., 3–8 min before GHBA or baclofen) and in rats treated with NCS-382 (100 mg/kg, i.v., 3–8 min before GHBA or baclofen). Each point represents the mean ± SEM obtained from 6–9 rats. Statistics: $^{*}P<0.05$,$^{**}P<0.01$ vs controls (Mann-Whitney *U*-test)

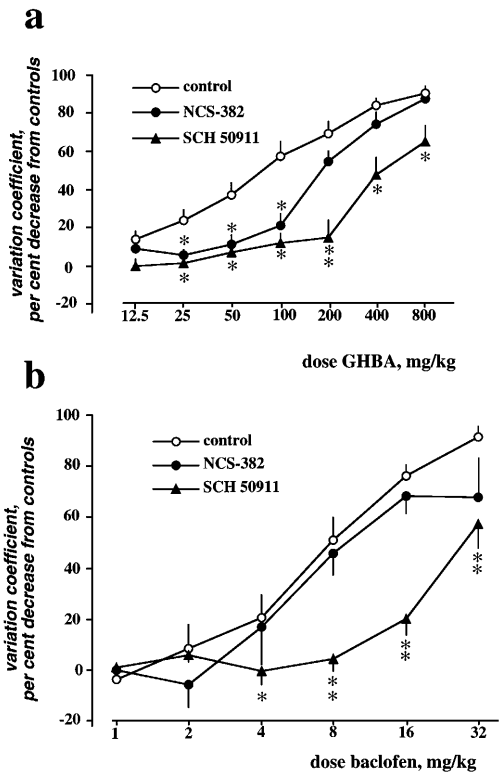


Fig. 8a,b Dose response curves for the change in regularity of firing of nigral DA neurons, induced by GHBA (**a**) or baclofen (**b**), expressed as the variation coefficient, in controls, in rats treated with SCH 50911 (75 mg/kg, i.v., 3–8 min before GHBA or baclofen) and in rats treated with NCS-382 (100 mg/kg, i.v., 3–8 min before GHBA or baclofen). Each point represents the mean ± SEM obtained from 6–9 rats. Statistics: $^{*}P<0.05$,$^{*}P<0.01$ vs controls (Mann-Whitney *U*-test)

bursts was not affected by the drug (data not shown). Furthermore, pretreatment with NCS-382 (100 mg/kg, i.v., 3–8 min) was not found to significantly antagonise the GHBA-induced reduction in firing rate or burst activity, although the drug to some extent antagonised the regularisation of the firing rhythm observed following low doses of GHBA (≤100 mg/kg; Figs. 3, 7a, 8a, 9b). NCS-382 was not able to influence the dose-dependent decrease in variation coefficient, inhibition of firing rate or reduction in burst activity induced by baclofen (Figs. 4, 7b, 8b, 10b).

Pretreatment with the novel, potent, and selective $GABA_B$-receptor antagonist, SCH 50911 (75 mg/kg, i.v., 3–8 min) antagonised all effects, i.e., inhibition of firing rate, regularisation of firing rhythm and burst activity induced by both GHBA and baclofen (Figs. 5–8, 9c, 10c). Systemic administration of SCH 50911 (75 mg/kg, i.v.) alone induced a slight increase in burst activity in spontaneously bursting nigral DA cells, and a vast majority of non-bursting cells were converted to a bursting firing pattern by the drug (Figs. 5, 6).

Discussion

The results of the present study confirm the previous reported action of GHBA to induce an inhibition in firing rate and a regularised firing pattern with a lack of burst activity of nigral DA neurons (Engberg and Nissbrandt 1993; Nissbrandt et al. 1994). In this sense, the actions of GHBA show a remarkable similarity to the effects induced by the specific $GABA_B$-receptor agonist baclofen on these neurons (Engberg et al. 1993). All these electrophysiological effects of baclofen on nigrostriatal DA neurons are suggested to be the consequence of an activation of a somatodendritic $GABA_B$-receptor-mediated input to the SN, which mainly serves to regularise the firing pattern and to prevent burst activity of nigral DA neurons. Functionally, activation of this $GABA_B$-receptor-mediated input decreases DA terminal efflux in the striatum (Nissbrandt et al. 1994). Furthermore, recent studies from our laboratory demonstrate that this input to the SN constitutes the final link in a striatonigral inhibitory control system (Engberg et al. 1997). As discussed below, the results of the present investigation dem-

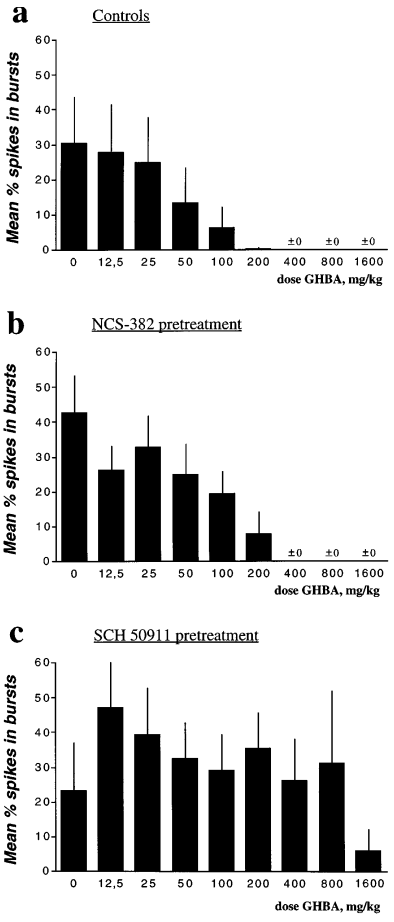


Fig. 9 **a** Effect of GHBA on burst activity of nigral DA neurons in controls. Each *bar* represents the mean ± SEM of 6 spontaneously bursting neurons. **b** Effect of GHBA on burst activity of nigral DA neurons in rats pretreated with NCS-382 (100 mg/kg, i.v., 3–8 min before the first injection of GHBA). Each *bar* represents the mean ± SEM of 9 bursting cells. **c** Effect of GHBA on burst activity of nigral DA neurons in rats pretreated with SCH 50911 (75 mg/kg, i.v., 3–8 min before the first injection of GHBA). Each *bar* represents the mean ± SEM of 6 bursting cells

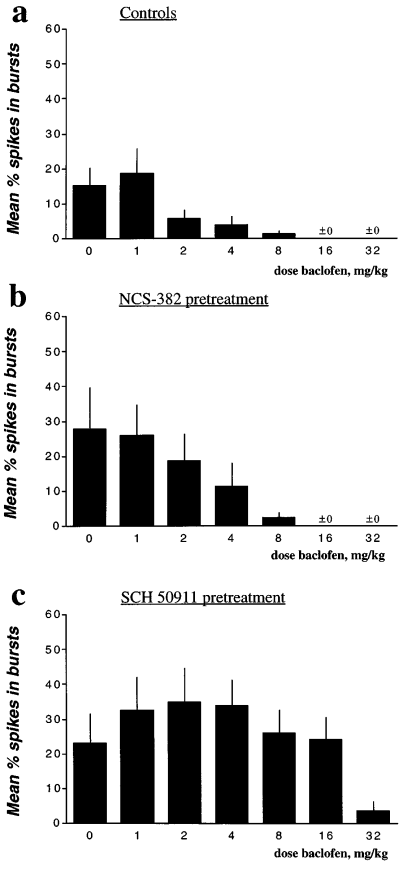


Fig. 10 **a** Effect of baclofen on burst activity of nigral DA neurons in controls. Each *bar* represents the mean ± SEM of 4 spontaneously bursting neurons. **b** Effect of baclofen on burst activity of nigral DA neurons in rats pretreated with NCS-382 (100 mg/kg, i.v., 3–8 min before the first injection of baclofen). Each *bar* represents the mean ± SEM of 7 bursting cells. **c** Effect of baclofen on burst activity of 12 nigral DA neurons in rats pretreated with SCH 50911 (75 mg/kg, i.v., 3–8 min before the first injection of baclofen). Each *bar* represents the mean ± SEM of 5 bursting cells

onstrate that GHBA, like baclofen, produces its actions on nigral DA neurons by affecting the above-described $GABA_B$-receptor-mediated input to the SN.

The major issue of the present study was to evaluate whether the effects of GHBA on the neuronal activity of nigral DA neurons is mediated via $GABA_B$ receptors or via affinity sites for GHBA. With regard to GHBA´s central effects in general, previous studies have formed a picture full of contradictions (cf. Introduction). Based on the present investigation, there are several reasons to believe that GHBA produces its effects on the firing of nigral DA neurons specifically via activation of $GABA_B$ receptors and not via stimulation of GHBA binding sites: (1) the fact that GHBA and baclofen [which is reported devoid of affinity to GHBA binding sites (Snead 1996)] produces almost identical effects on the firing of nigral DA neurons indicates per se that GHBA in this regard displays a similar mode of action as baclofen, i.e. stimulation of $GABA_B$ receptors; (2) in a previous study the effects of baclofen, GHBA and GBL on the firing of nigral DA neurons were antagonised by pretreatment with the specific $GABA_B$-receptor antagonist CGP 35348 (Engberg et al. 1993) which is devoid of affinity for GHBA binding sites (Snead 1996). In addition, in the present study a novel $GABA_B$-receptor antagonist, SCH 50911 (Bolser et al. 1995), was found to antagonise the effects of both baclofen and GHBA. SCH 50911 is reported to act as a potent and highly specific $GABA_B$-antagonist with no affinity for a variety of other receptors including rat $GABA_A$-, histamine H_1-, DA-, D_2-, D_3-, or GHBA receptors (Bolser et al. 1995; Snead 1996). The effectiveness of SCH 50911 in inhibiting the actions of GHBA on the firing of nigral DA neurons principally indicates that GHBA in this regard activates $GABA_B$ receptors. Thirdly, the effects of GHBA and baclofen on nigral DA activity were not reliably antagonised by NCS-382. This drug is reported to selectively block GHBA sites with no antagonistic action at $GABA_B$

receptors (Maitre et al. 1990; Snead 1996) and was in the present study administered (100 mg/kg, i.v.) at a dose twice as high as that required to fully antagonise behavioural effects of GHBA (Colombo et al. 1995). NCS-382 was not able to antagonise the decrease in firing rate as produced by GHBA, nor the GHBA-induced reduction in burst firing. Furthermore, the baclofen-induced alterations in the neuronal activity of nigral DA neurons, i.e. reduction in firing rate, variation coefficient of ISHs and burst firing, was not influenced by pretreatment with NCS-382. Among all electrophysiological parameters measured on nigral DA neurons, the only significant effect observed with NCS-382 was a slight antagonism of the decrease in variation coefficient of ISHs following doses of 100 mg/kg or less of GHBA. This action of NCS-382 is obscure and cannot easily be explained in terms of GHBA- or $GABA_B$-receptor mechanisms.

In all, our results show that GHBA affects DA neuronal activity via activation of $GABA_B$ receptors. A stumbling block with this idea is the lack of reliable binding data showing high affinity of GHBA to $GABA_B$-receptor sites. Rather, some studies show a lack of affinity of GHBA to $GABA_B$-receptor sites (Benavides et al. 1982; Snead and Liu 1984; see Maitre 1997). One may suggest that GHBA could influence the neuronal DA activity indirectly, e.g. by increasing concentrations of GABA, either by a formation of GABA from GHBA or, less likely though, via a release of the transmitter (see Maitre1997). If so, however, then $GABA_A$ receptors, in addition to $GABA_B$ receptors, should be indirectly affected by GHBA. Previous studies have shown that systemic administration of muscimol, a specific $GABA_A$-receptor agonist, is associated with effects on neuronal DA activity opposite to those produced by GHBA (i.e. increased firing rate and burst activity), making this hypothesis less attractive. Furthermore, whereas the actions of GHBA on neuronal DA activity are almost instantaneous in onset, no convincing picture from previous studies has emerged that central endogenous levels of GABA are immediately increased; whereas several studies fail to show increased GABA concentration following GHBA administration (Mitoma and Neubauer 1968; Margolis 1969; Doherty et al. 1975; Möhler et al. 1976; Doherty et al. 1978), others report a slight GABA increase, however, more than 60 min after injection of GHBA (Della Pietra et al. 1966; De Feudis and Collier 1970). In addition, a putative releasing action of GABA by GHBA as induced via GHBA sites is unlikely to occur since such an effect would be blocked by NCS. Consequently, an indirect action of GHBA in affecting neuronal DA activity appears unlikely judging from our results. Rather, a direct action on receptors sensitive to GHBA, i.e. $GABA_B$ receptors, is likely to account for the effects of GHBA in this regard. Such a mode of action of the drug on central mechanisms is also strongly supported by biochemical and behavioural data (Nissbrandt and Engberg 1996), where the $GABA_B$-receptor antagonist CGP 35348 was shown to antagonise the similar action of GHBA and baclofen on striatal DA release and locomotor activity, respectively.

Notably, SCH 50911appeared more potent than CGP 35348 in antagonising the electrophysiological actions of GHBA or baclofen of nigral DA neurons. Thus, pretreatment with SCH 50911 (75 mg/kg, i.v.) almost totally prevented the inhibition of firing rate induced by baclofen. When rats were pretreated with CGP 35348 (200 mg/kg, i.v.), baclofen given in high doses (32 mg/kg, i.v.) still produced about 60% inhibition of firing rate (Engberg et al. 1993).This difference in potency between the two $GABA_B$-receptor antagonists is also in agreement with previous in vivo studies comparing their ability to antagonise inhibition of the antitussive or the respiratory depressant effect of baclofen (Bolser et al. 1995).

Altogether, the results of the present study clearly show that GHBA and the $GABA_B$-receptor agonist baclofen similarly produce their actions on the firing rate and burst activity of nigral DA neurons by specifically inhibiting nigral DA neuronal activity via activation of $GABA_B$ receptors.

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