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# Glycine induces a novel form of long-term potentiation in the superficial layers of the superior colliculus

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**1** The mammalian superior colliculus (SC) is a midbrain nucleus containing space maps of different sensory modalities which show various forms of age- and activity-dependent plasticity *in vivo* and *in vitro*. In the present study, we aimed to characterize the role of glycine (Gly) receptors in the SC, and we observed that application of glycine (Gly; 500 $\mu$M and 3 mM) for 7 min to SC slices of adult guinea-pigs caused a novel form of long-term potentiation (termed $LTP_{gly}$) of evoked excitatory postsynaptic potentials recorded in the superficial layers.

**2** The strength of potentiation was found to be concentration-dependent and partially independent from synaptic stimulation.

**3** $LTP_{gly}$ did not involve NMDA receptor activation as proven by the lack of inhibition by 100 $\mu$M D,L-2-amino-5-phosphonovaleric acid (APV) and 10 $\mu$M MK-801.

**4** $LTP_{gly}$ could only be masked but not prevented by strychnine (100 $\mu$M) and remained undisturbed in the presence of picrotoxin (100 $\mu$M).

**5** Inhibition of carbonic anhydrase by acetazolamide (20 $\mu$M) had no effect on $LTP_{gly}$ suggesting that the excitatory action of Gly is not due to a differential breakdown of the $Cl^-/HCO_3^-$ gradients.

**6** As indicated by the inhibition of $LTP_{gly}$ of the fEPSP slope by the L-type calcium channel blocker nifedipine (20 $\mu$M), voltage-dependent calcium channels are the source for $Ca^{2+}$ elevation as the intracellular trigger.

**7** Our data provide the first evidence for a role of Gly in SC synaptic transmission. They illustrate a so far unknown action of Gly which can lead to long-lasting changes of synaptic efficacy and which is not mediated *via* NMDA-related or strychnine-sensitive binding sites.

**Keywords:** Glycine; superior colliculus; long-term potentiation; plasticity; slice; NMDA; strychnine; voltage-dependent calcium channels

## Introduction

Glycine (Gly) and $\gamma$-aminobutyric acid (GABA)-A receptors are coupled to a chloride ($Cl^-$) channel and provide the major source of inhibition in the central nervous system (CNS) (Betz, 1991; Bormann & Feigenspann, 1995). As reported by Eccles *et al.* (1954), glycine-mediated inhibitory postsynaptic potentials (IPSPs) in the spinal cord can be abolished by strychnine, whereas GABA-A mediated inhibition can be specifically antagonized by bicuculline. The distribution pattern of GABA and Gly receptors indicates that GABA is predominant in rostral areas of the brain whilst inhibitory Gly receptors are more prevalent in the hindbrain. Nevertheless, although earlier reports indicated that Gly receptors are absent in the cortex of adult rats (Araki *et al.*, 1988, Zabrin *et al.*, 1981), distinct GABA- and Gly-mediated $Cl^-$ responses from various parts of the adult rat brain including cortex, hippocampus and medulla have been reported (Engblom *et al.*, 1996). Moreover, strychnine binding in some parts of the brain is nearly absent, but antibody staining provided evidence for expression of the $\beta$ subunit of Gly receptors (Betz, 1991).

Although mainly inhibitory in nature, GABA and Gly have also been reported to result in depolarizing actions in central and peripheral neurones of embryonic and neonatal rats (e.g. Cherubini *et al.*, 1991). This depolarization may play a trophic role (LoTurco *et al.*, 1995), for instance by providing the excitatory drive required for growth and differentiation (Wang *et al.*, 1994). Accordingly, glycinergic transmission has been demonstrated to regulate dendritic growth in the lateral superior olive (Sanes & Hafidi, 1996) and a recent report suggests the involvement of nonsynaptic glycine receptors in neocortical development (Flint *et al.*, 1998).

Beside the strychnine-sensitive Gly receptor, a strychnine-insensitive, co-agonistic binding site exists on the N-methyl-D-aspartate (NMDA) receptor, which is known to be crucial for certain forms of neuronal plasticity such as long-term potentiation (LTP; Bliss & Collingridge, 1993). Although glycine concentrations present in the extracellular space appear to be sufficient to saturate this binding site (Johnson & Ascher, 1987, but see Wilcox *et al.*, 1996), application of Gly causes LTP via an action on NMDA receptors (Shahi *et al.*, 1993).

The mammalian superior colliculus (SC) is a multilayered midbrain nucleus which can be functionally divided into superficial (visual) layers and the deep (multimodal) layers. It contains topographic maps of sensory space with a distinct age- and experience-dependent maturation which remain partially plastic in the adult (e.g. Withington *et al.*, 1994). Both short- and long-term plasticity have been described *in vitro* in slices of adult guinea-pigs (e.g. Platt & Withington, 1997a,b; Miyamoto *et al.*, 1990). The function of Gly receptors has not been studied in detail in the SC, although Gly and strychnine labelling has been reported throughout all SC layers (Pourcho *et al.*, 1992; Bristow *et al.*, 1986, Zabrin *et al.*, 1981). Some evidence for a physiological role of Gly arises from studies in the optic tectum (the structural homologue of the SC) of pigeons (Barth & Felix, 1974) and frogs (Sivilotti & Nistri, 1986). The latter report showed that Gly causes a

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biphasic, i.e. enhancing and depressing, action on synaptic transmission in a strychnine-sensitive manner.

In the present study, we aimed to investigate the role of Gly receptors in the SC. We demonstrate that Gly causes a novel form of LTP (termed $LTP_{gly}$) which is neither mediated nor dependent on NMDA receptor activation. We provide evidence for an excitatory action of glycine in the SC which is strychnine-insensitive and causes a potentiation of evoked potentials via the activation of voltage-dependent calcium channels (VDCCs).

## Methods

### *Tissue preparation and recording procedure*

Adult guinea-pigs (>3 months) were deeply anaesthetized (2 ml $kg^{-1}$ Hypnorm, i.m.; 4 ml $kg^{-1}$ Hypnovel, i.p.) and decapitated. The brain was removed quickly and transferred into ice-cold Ringer solution (composition (in mM): NaCl, 125; KCl, 5; $MgCl_2$ 1.3; $CaCl_2$, 2.5; $KH_2PO_4$, 1.2; Glucose, 10; $NaHCO_3$ 25; saturated with 95% $O_2$/ 5% $CO_2$). Sagittal SC slices (400 $\mu$M) were prepared using a vibratome and equilibrated at 32°C for at least 30 min after the preparation. For recordings, slices were placed on a Nylon net in a temperature-controlled (32°C) submersion chamber and superfused continuously with pre-warmed Ringer solution. A stainless steel stimulation electrode was positioned in the optic layer, a glass electrode (~2 MΩ) filled with Ringer solution was used for extracellular recordings in the superficial grey layer.

Responses were elicited every 60 s at ~50% of maximum of the fEPSP slope by test pulses of 50 $\mu$ s duration. After a stable baseline was recorded, standard Ringer containing glycine (500 $\mu$M and 3 mM; taken from a 1 M stock solution) was superfused for 7 min and washed out subsequently.

### *Pharmacological agents*

All drug-containing solutions were prepared briefly before the experiment started and bath-applied *via* superfusion. Glycine, D,L-2-amino-5-phosphonovaleric acid (APV) and MK-801 (as (+)-MK-801 maleate) were purchased from Tocris Cookson (Bristol, U.K.) Nifedipine, picrotoxin, strychnine and acetazolamide were obtained from Sigma (Dorset, U.K.).

### *Data analysis*

In all experiments, the early amplitude and slope function of the fEPSP were analysed and given as means ± s.e.mean in % relative to baseline. Additionally, the late amplitude of the fEPSP was determined 6 ms after the stimulus artefact for control experiments and those on the action of NMDA receptors antagonists since this part of the fEPSP was found to be affected the most by those antagonists and showed the strongest enhancement during Gly application. The Gly antagonist strychnine caused the strongest action on a later part of the fEPSP (~15 ms after the stimulus artefact) which was analysed for this subset of data.

Statistical significance was assessed by unpaired $t$-test for between group comparison during Gly application and 63 min after Gly application (i.e. $t=80$ min in control experiments). Paired $t$-test was performed for within group comparison between $t=10$ min (baseline) and $t=80$ min. $P$ values $\leqslant 0.05$ and 0.01 are defined as significant and highly significant, respectively.

## Results

### *Glycine causes LTP in a concentration- and partially use-dependent manner*

In control experiments, the field excitatory postsynaptic potentials (fEPSPs) extracellularly recorded in the superficial grey layer (SGL) of the SC and elicited by stimulation of the optic layer (OL) responded with an enhancement of the evoked response when 500 $\mu$M Gly was bath-applied for 7 min (circles, $n=8$), corresponding data are shown for the fEPSP slope (Figure 1A), early amplitude (Figure 1B) and late amplitude

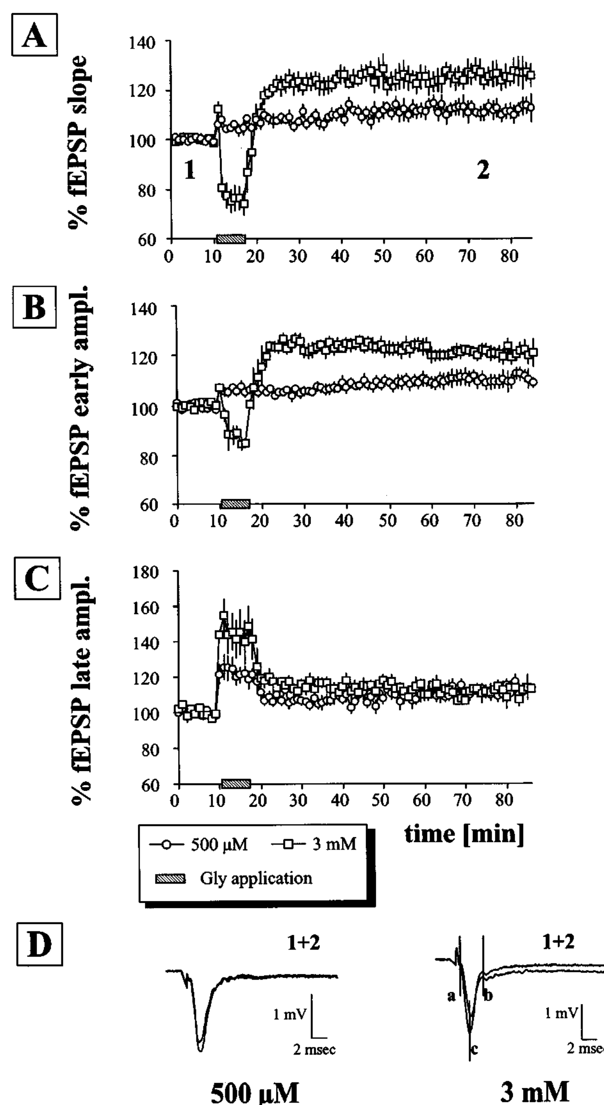


**Figure 1** Long-lasting effects of Gly application on synaptic transmission in the superficial SC. Data are shown as means ± s.e.mean of the fEPSP slope, early and late amplitude in % relative to baseline. Bath application of 500 $\mu$M Gly for 7 min ($n=8$) induced a weak but significant potentiation of the fEPSP slope (A) and early amplitude (B). When 3 mM Gly ($n=8$) was applied a transient enhancement followed by a depression of the early fEPSP occurred. During wash, a stable potentiation of slope and early amplitude developed, termed $LTP_{gly}$. (C) illustrates the time course of the late amplitude which showed a strong increase during Gly application but declined subsequently to values not different from baseline. (D): Representative sample traces for time points 1 and 2 indicated in part A. The right hand traces also illustrate the marker positions for analysis of early slope and amplitude (between markers a and b), and measurement of the late amplitude (between baseline and marker c).

(6 ms after stimulus artefact, Figure 1C). Slope and early amplitude increased slightly during wash reaching a weak but stable potentiation (slope: $111 \pm 3\%$; amplitude: $110 \pm 3\%$ at $t = 80$ min) which was found to be significantly different from baseline for both parameters ($P < 0.05$). The late amplitude showed a strong enhancement during Gly application ($+25\% \pm 10\%$) but declined during wash and reached a value of $110 \pm 4\%$ at $t = 80$ min which was found not to be significantly different from baseline ($P > 0.05$). In general, values of the late fEPSP proved to be more variable than the early rising phase due to more pronounced differences in the waveform after the initial peak of the evoked response. Thus, the higher variability of the data may account for the lack of significance of potentiation obtained for this parameter.

Also illustrated in Figure 1 is the action of 3 mM Gly on evoked fEPSPs in the SGL (squares, $n = 8$). Since 3 mM Gly was also used for subsequent experiments, this group will be referred to as the 'Gly-control group'. During superfusion of this concentration the slope function (Figure 1A) increased initially ($111 \pm 4\%$) and declined thereafter below baseline ($-26\%$). With the onset of the wash the slope function of the fEPSP recovered and rose up to 125%. During washout the enhancement remained stable, with a slope of $125 \pm 3\%$ at $t = 80$ min which was highly significantly different from baseline ($P < 0.01$). The amplitude of the early fEPSP showed a similar time course (Figure 1B), only the depression during Gly application was observed to be slightly but not significantly weaker and a potentiation of $120 \pm 3\%$ was reached at $t = 80$ min. The enhancement of fEPSP slope and early amplitude was found to be concentration-dependent since the potentiation was significantly stronger when induced by 3 mM compared to 500 $\mu$M at $t = 80$ min ($P < 0.05$). For the late amplitude (Figure 1C), a strong increase was observed during superfusion of 3 mM Gly ($+45\%$) but values declined during wash and were not found to be significantly enhanced at $t = 80$ min ($112 \pm 7\%$, $P > 0.05$).

Since our data show that Gly can induced a long-lasting potentiation of synaptic transmission we named this phenomenon glycine-induced LTP, or $LTP_{gly}$. Although the concentrations used in this study are relatively high, they are in agreement with reports of others concerning the optimal range of Gly receptor activation (e.g. Shirasaki *et al*., 1991; Ito & Cherubini, 1991). Moreover, it has been suggested that transmitter concentrations may reach 5 mM during normal synaptic transmission (Clements, 1996). In the hippocampal slice preparation, perfusion of 10 mM glycine for 10 min was used to induce a form of long-lasting potentiation of transmission (Shahi *et al*., 1993).

In Figure 2, another set of control experiments is illustrated showing the partial use-dependence of $LTP_{gly}$: in this case, 3 mM Gly was applied while stimulation of the OL was discontinued (Gly-ud group; $n = 7$). The application of Gly was followed by a 20 min period of wash, before stimulation was resumed. Interestingly, the fEPSP slope was found to be significantly enhanced compared to baseline ($t = 10$ min *vs* $t = 80$ min: $P < 0.05$) and compared to control experiments (at $t = 80$ min: $P < 0.05$), during which stimulation was stopped for the same period of time without application of Gly ($n = 6$). In the latter experiments, data were not found to be significantly different compared to baseline ($P > 0.05$). The comparison between the Gly-control group and the Gly-ud group revealed a significant difference concerning the strength of $LTP_{gly}$ (at $t = 80$ min: $P < 0.05$).

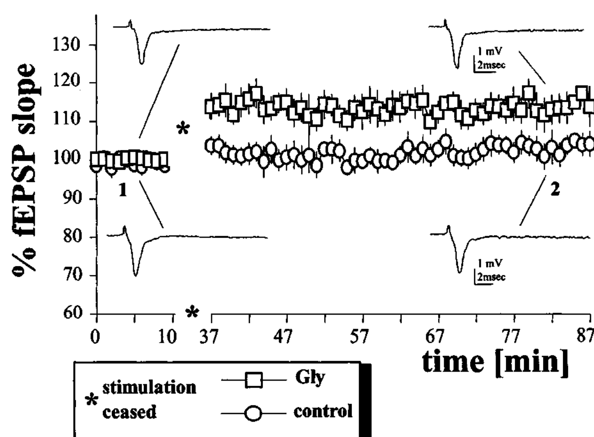


**Figure 2** The action of 3 mM Gly is partially use-dependent: During the time period indicated by the asterisks Gly was applied for 7 min followed by a 20 min period of wash without stimulation of the optic layer ($n = 7$). When stimulation was resumed, the evoked fEPSP was found to be significantly enhanced compared to baseline and control experiments, under which stimulation was discontinued for 27 min without application of Gly ($n = 6$). Under these conditions, $LTP_{gly}$ was nevertheless weaker compared to data shown in Figure 1 where continuous stimulation was performed during application of Gly.

### *$LTP_{gly}$ is strychnine- and picrotoxin-insensitive*

Since it has been reported that Gly can depolarise neurones in a strychnine-sensitive manner for instance in embryonic spinal cord preparations (e.g. Reichling *et al*., 1994) we then aimed to determined the action of the specific Gly antagonist strychnine (str) on both synaptic transmission (Figure 3) and on $LTP_{gly}$ (Figure 4). In a first attempt, 10 $\mu$M strychnine was tested, which enhanced the late part of the fEPSP without affecting the early component, and was found to be ineffective in blocking $LTP_{gly}$ ($n = 2$; data not shown). Although most Gly receptors are blocked by low micromolar or even nanomolar concentrations of strychnine, some receptors show a much lower ($\sim$ 500-fold) affinity for strychnine (Betz, 1991). Thus, to ensure the block of low-affinity Gly receptors, 100 $\mu$M strychnine was used ($n = 9$, Figure 3). When 100 $\mu$M strychnine was bath-applied, the early part (changes of the fEPSP slope and amplitude) and late part (illustrated by measurements of the fEPSP amplitude 15 ms after stimulus artefact, see Figure 3C) of the fEPSP were affected differentially: The late part was briefly depressed ($t = 11$ min: 70%) followed by a dramatic increase up to $350 \pm 32\%$ at $t = 20$ min (Figure 3B), whereas the fEPSP slope (Figure 3A) showed a brief enhancement ($107 \pm 2\%$) and a subsequent depression (at $t = 30$ min: $60 \pm 4\%$). While the fEPSP slope recovered back to baseline at $t = 55$ min, the late amplitude remained enhanced up to the end of the recording ($t = 80$ min: $186 \pm 6\%$). Thus, it appears that the block of strychnine-sensitive IPSPs caused a pronounced disinhibition, which may have reduced the driving force for excitatory synaptic currents hence depressing the early part of the fEPSP.

Although these data prove both the existence and the importance of strychnine-sensitive inhibitory Gly receptors in the SC, we found that $LTP_{gly}$ was not antagonised by strychnine (Figure 4, $n = 13$). The application of Gly in the presence of strychnine did not cause any obvious changes of the fEPSP slope and the slope remained depressed while strychnine was present after the Gly superfusion. After washout of strychnine, a stable potentiation developed (at $t = 90$ min: $135 \pm 6\%$) which was not significantly different from the Gly-control group (Gly-control *vs* Gly-str group 63 min after Gly application: $P > 0.05$). It therefore appears

that high concentrations of strychnine can mask but not prevent the induction of $LTP_{gly}$.

Evidence from molecular studies and electrophysiological investigations in other brain areas have indicated that there is a close relationship between GABA-A and Gly receptors. It appears that Gly may be able to activate GABA-A receptors, and GABA and Gly receptors are sometimes co-localized and a partial occlusion of GABA and Gly responses has also been described (e.g. Lewis & Faber, 1993). In order to clarify whether $LTP_{gly}$ is due to an unspecific action or indirect modulation of Gly on GABA-A receptors, we investigated the action of the GABA-A antagonist picrotoxin (PTX, 100 $\mu$M) on $LTP_{gly}$ (Figure 5). As can be seen from the illustrated sample traces, PTX also enhanced the late part of the fEPSP. Since it did not affect significantly the early part of the fEPSP (data not shown), it was present throughout the experiment. When 3 mM Gly was applied under these conditions ($n=9$), a brief enhancement, similar to the one in the Gly-control group, was obtained ($108 \pm 4\%$). The following depression, however, did not occur indicating that the respective $Cl^-$ conductance was blocked by PTX. During washout, the fEPSP slope increased reaching $130 \pm 5\%$ at $t=80$ min. This potentiation was not significantly different from Gly-control data ($P > 0.05$).

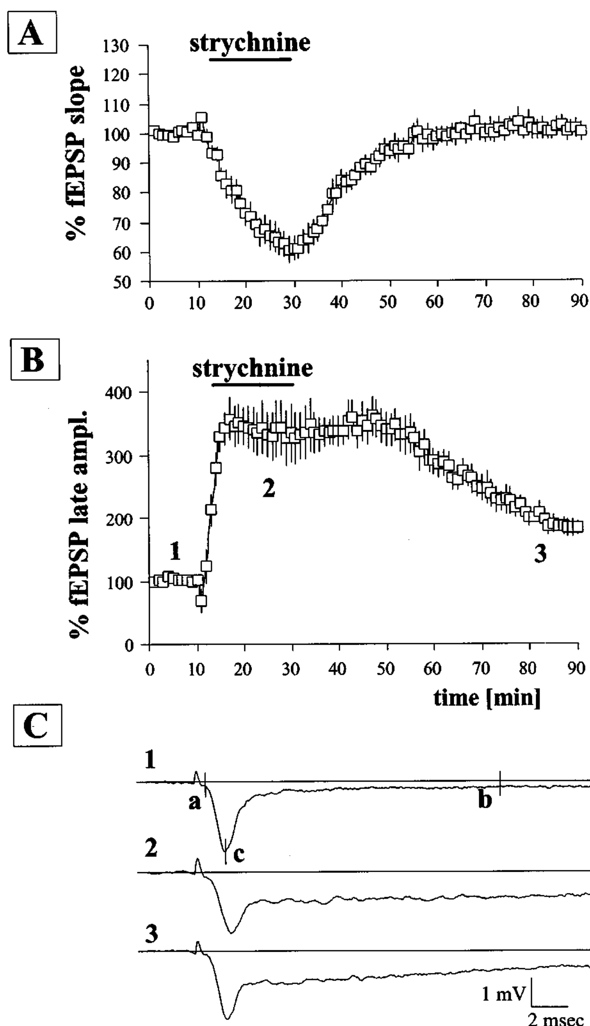


**Figure 3** The action of 100 $\mu$M strychnine on synaptic transmission in SC slices ($n=9$). (A) shows the depression of the fEPSP slope which was found to be fully reversible after wash. In (B) the dramatic enhancement of the amplitude of the late part of the fEPSP measured 15 ms after stimulus artefact is illustrated. The amplitude declined slowly during wash but did not recover back to baseline. Representative sample traces are given in part (C), the marker positions for the analysis of the fEPSP slope (between a and b) and late amplitude (baseline to marker c) are indicated.

### *$LTP_{gly}$ is not due to a differential breakdown of $HCO_3^-$ and $Cl^-$ gradients*

Recently, Staley *et al.* (1995) proposed a model for the excitatory action of GABA in the distal dendrites of the hippocampal CA1 region. The authors show that GABA-A mediated excitation is due to a shift of the reversal potential for GABA caused by a differential breakdown of the $Cl^-$ influx and $HCO_3^-$ efflux. While the $Cl^-$ gradient collapses due to intracellular accumulation and extracellular depletion of $Cl^-$, the $HCO_3^-$ efflux is maintained by the activity of carbonic anhydrase (see Figure 6A). Based on this model, we aimed to investigate whether $LTP_{gly}$ is prevented by acetazolamide (AAA), an inhibitor of carbonic anhydrase which has been reported to abolish GABA-mediated depolarization (Staley *et al.*, 1995). In accordance with this study, we found that AAA (20 $\mu$M) did not cause any baseline effect (tested for 30 min) and was therefore present throughout the experiment. When Gly was applied (Figure 6B, $n=7$) there was again a brief enhancement of the fEPSP slope ($113 \pm 5\%$) followed by a depression ($58 \pm 4\%$). This depression was found to be

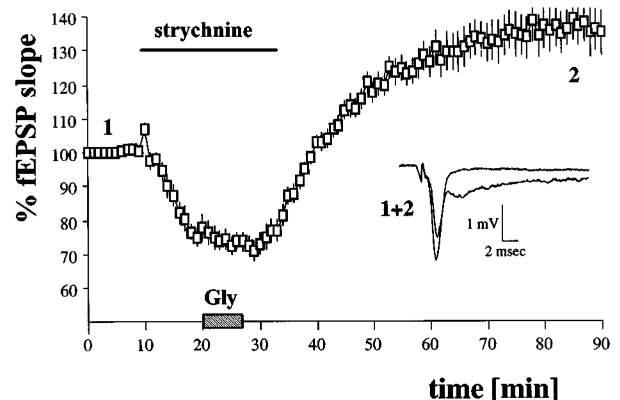


**Figure 4** Strychnine does not block $LTP_{gly}$: 100 $\mu$M Strychnine prevented the depression of the fEPSP slope during application of Gly (3 mM) and masked but did not block the emergence of $LTP_{gly}$ ($n=13$). Sample traces for time points 1 and 2 are illustrated.

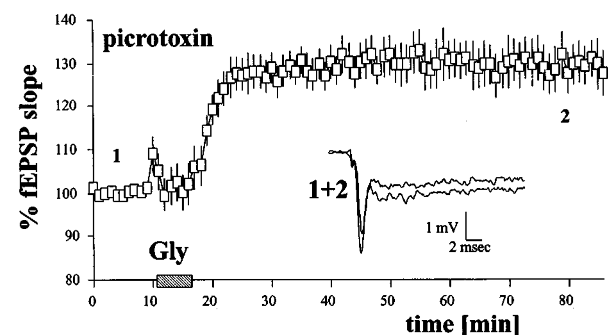


**Figure 5** Picrotoxin has no effect on $LTP_{gly}$: In the presence of picrotoxin (100 $\mu$M), only a brief enhancement of the fEPSP slope occurred during application of 3 mM Gly. A stable potentiation not significantly different from data shown in Figure 1A emerged ($n=9$). Representative raw traces of an fEPSP at time point 1 (baseline) and at $t=80$ min (time point 2) are shown.

significantly stronger compared to the reduction observed in the Gly-control group ($P<0.01$) which provides evidence for a contribution of $HCO_3^-$ efflux during Gly application which partially limits the hyperpolarizing action of Gly under control conditions. Nevertheless, $LTP_{gly}$ emerged undisturbed compared to the Gly-control group with $127\pm4\%$ at $t=$ 80 min ($P=1$).

### *$LTP_{gly}$ is not mediated* via *NMDA receptors*

In order to investigate whether $LTP_{gly}$ is due to an action on the Gly binding site of the NMDA receptor further experiments were performed with Gly being applied in the presence of the NMDA antagonists APV (100 $\mu$M, $n=6$) and MK-801 (10 $\mu$M, $n=7$). The corresponding graphs of both early slope and late amplitude (6 ms after stimulus artefact) are shown in Figure 7. With the onset of APV application a slight drop in the slope ($5\pm3\%$) and a stronger inhibition of the late amplitude ($15\pm5\%$) was obtained (Figure 7A and Figure 7B, respectively). Concomitant application of Gly resulted in a brief enhancement of the fEPSP slope followed by a depression (27%). After Gly application a rapid rise in the slope occurred whilst APV was still present. This was followed by the development of a stable potentiation during wash (at $t=80$ min: $130\pm5\%$). The late amplitude reached values of $153\pm9\%$ during Gly superfusion and declined to $112\pm6\%$ at $t=80$ min. In contrast to APV, MK-801 did not produce any baseline effect on the slope function (Figure 7D) but inhibited the late amplitude to a similar extent (Figure 7E; $14\pm4\%$). With respect to the time course of the fEPSP slope during and after application of Gly, there was no difference compared to the APV group, the slope was potentiated by $22\pm4\%$ 1 h after Gly application. In the presence of MK-801, the late amplitude appeared to be further increased during Gly application ($75\pm8\%$), but this was not found to be significantly different from both the enhancement obtained for the Gly-control and the APV group ($P>0.05$).

As for the Gly-control group, the fEPSP slope was highly significantly potentiated ($P<0.01$) while the late amplitude was not found to be significantly enhanced compared to baseline ($P>0.05$) both for the APV group and the MK-801 group. Moreover, no significant differences between the $LTP_{gly}$ in the absence or presence of either NMDA antagonists were found for both early slope and late amplitude of the fEPSP ($P>0.05$).

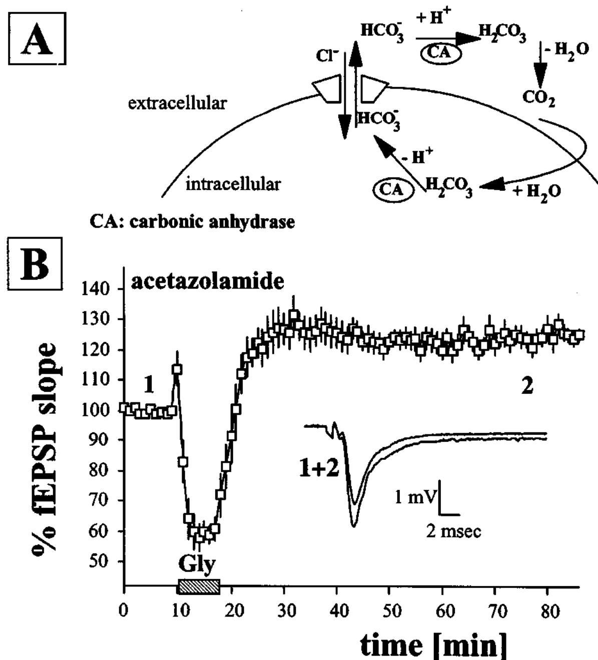


**Figure 6** Effect of the carbonic anhydrase inhibitor acetazolamide on $LTP_{gly}$. (A): A model suggested for GABA-A receptor mediated excitation: While the $Cl^-$ gradient collapses due to intracellular accumulation and extracellular depletion of $Cl^-$, the $HCO_3^-$ efflux is maintained by intra- and extracellular activity of carbonic anhydrase, causing a depolarization of the neurones. (B) When the carbonic anhydrase inhibitor acetazolamide (20 $\mu$M) was present, the depression of the fEPSP slope during Gly application (3 mM) was found to be stronger compared to Gly application in the absence of acetazolamide. $LTP_{gly}$, however, was not affected ($n=7$). Sample traces for time points 1 and 2 are included.

### *$LTP_{gly}$ is caused by activation of voltage-dependent calcium channels*

The next major issue to be addressed was the route by which Gly can cause LTP, since $LTP_{gly}$ did not require the activation of NMDA receptors. While $Ca^{2+}$ appears to be the major trigger for various forms of LTP, we investigated the possibility that VDCCs may be activated by a Gly-induced depolarization thus giving rise to an elevation of intracellular $Ca^{2+}$. We therefore tested the action of the L-type VDCC blocker nifedipine (20 $\mu$M) as illustrated in Figure 8. Control experiments ($n=7$) revealed that nifedipine applied on its own for 22 min (same total time as in experiments where Gly was applied, see below) did not cause any immediate or delayed effect on the fEPSP slope (Figure 8A) or late amplitude (Figure 8B). Throughout the time course illustrated values varied between $95\pm4\%$ and $106\pm3\%$ (slope) and $77\pm8\%$ and $115\pm7\%$ (late amplitude). These control experiments again illustrate the higher variability of late amplitude measurements.

When Gly was applied in conjunction with nifedipine (Figure 8, squares, $n=7$) no initial enhancement of the fEPSP slope was obtained, only a depression of the fEPSP occurred (at $t=19$ min: $77\pm6\%$) which was in the same range as in the Gly-control group ($P>0.05$). While nifedipine was still present after application of Gly, the fEPSP slope recovered back to baseline but no potentiation emerged even after removal of nifedipine from the bath solution. At $t=86$ min (which corresponds to $t=80$ min in Gly-control experiments relative to Gly application) the slope was highly significantly different compared to Gly-control ($P<0.01$) and not significantly different from baseline data ($100\pm5\%$; $P>0.05$).

The late amplitude however showed a time course not significantly different from the Gly-control group (see Figure 1C), i.e. during Gly application an enhancement up to $157\pm5\%$ was observed, and values declined to $100\pm4\%$ at $t=86$ min ($P>0.05$).

## Discussion

While both strychnine-sensitive glycine receptors and strychnine-insensitive Gly binding sites on NMDA receptors have been studied in hindbrain and forebrain, respectively, not much is known about the role of these receptors in the midbrain and in the SC in particular. Whole brain [$^3$H] strychnine autoradiography as well as immunocytochemistry using Gly receptor antibodies have proven their presence in the SC (e.g. Pourcho *et al.*, 1992; Betz, 1991), but their function has not been investigated as yet. In the present study, we provide evidence for inhibitory Gly receptors in the superficial

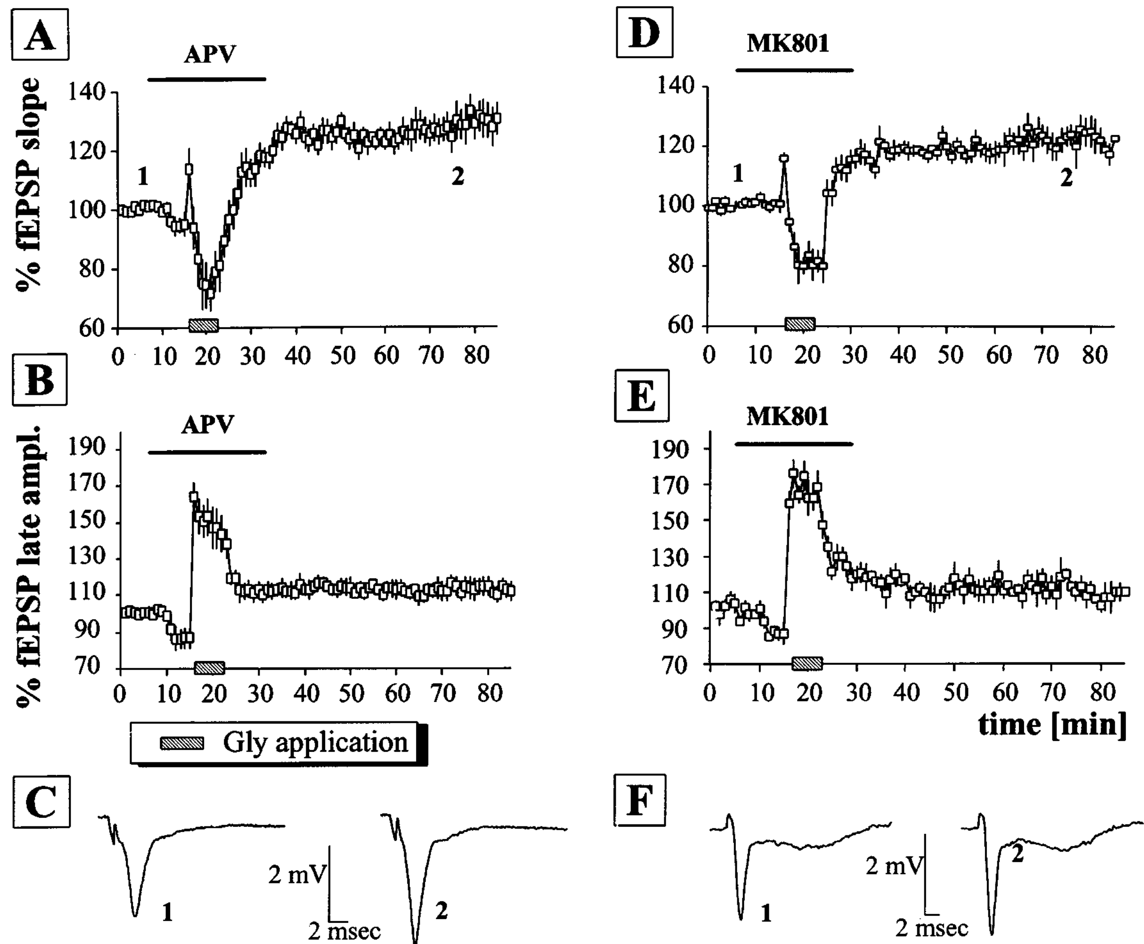


**Figure 7** The NMDA antagonists APV (left column, 100 μM, $n=6$) and MK-801 (right column, 10 μM, $n=7$) had no significant effect on the action of Gly during its application (3 mM), and Gly-induced potentiation of the early fEPSP slope (shown in A and D) was not different compared to the 3 mM Gly group shown in Figure 1. The enhancement of the late amplitude (B and E) during Gly application was also not inhibited by these antagonists. Sample traces of time points 1 and 2 are illustrated in part (C) for the APV group and in part (F) for the MK-801 group, respectively.

SC as indicated by the action of strychnine and the depression of the fEPSP during application of Gly which was abolished by strychnine and PTX. The action of AAA during Gly application suggests that these Gly receptors mediate a (smaller) depolarizing $HCO_3^-$ efflux in addition to a hyperpolarizing $Cl^-$ influx, in agreement with inhibitory Gly receptors described in other brain areas.

Besides this 'classic' action of Gly, we show that Gly causes a long-lasting enhancement of synaptic transmission in the superficial SC. $LTP_{gly}$ represents a novel form of synaptic plasticity which does not require the activation of NMDA receptors. This was emphasized by the lack of inhibition of $LTP_{gly}$ observed with APV and MK-801, since 10 μM MK-801 was found to inhibit NMDA-mediated excitotoxicity induced by 10 mM Gly in the hippocampus (Newell *et al.*, 1997). In addition, Gly-induced LTP observed in hippocampal slices was also found to be mediated via NMDA receptors (Shahi *et al.*, 1993). Therefore, $LTP_{gly}$ in the superficial SC is based on different mechanisms and it is (a) not caused by an action on the Gly binding site of the NMDA receptor and (b) does not depend on the intracellular cascades downstream from NMDA receptor activation. It appears that $LTP_{gly}$ in the SC may be caused by a direct or indirect excitatory action of Gly strongly reflected in the late part of the fEPSP. Excitatory Gly receptor may therefore be similar to excitatory GABA-A and Gly receptors described for instance in the frog optic tectum (Sivilotti & Nistri, 1986) and in the hippocampus of new born rats (Cherubini *et al.*, 1991). The latter study demonstrated that the action of Gly leads to the activation of VDCCs (e.g. Ito & Cherubini, 1991) which is in agreement with our observations. An excitatory, relatively strychnine-insensitive action of Gly has also been observed in a subset of sympathetic preganglionic neurones, co-existing with inhibitory Gly receptors (Spanswick *et al.*, 1994). Moreover, since $\beta$ subunit mRNA has been found in brain areas where no strychnine or $\alpha$ subunit labelling was seen, the presence of strychnine-resistant Gly receptor subunits has been suggested by others (Betz, 1991).

In contrast to our observations, Gly-induced depolarization in the hippocampus was strychnine-sensitive and no long-lasting changes of neuronal excitability by Gly have been reported (Ito & Cherubini, 1991). Our data, on the other hand, suggest that $LTP_{gly}$ is not only strychnine-insensitive but also PTX-insensitive indicating the lack of an involvement of any known ligand-gated $Cl^-$ channel. Since the concentration of both strychnine and PTX were relatively high, it cannot be excluded that these drugs themselves exert additional unspecific effects. Nevertheless, since both antagonists at relatively high concentrations were found to be ineffective in blocking $LTP_{gly}$, this emphasizes even more that none of these

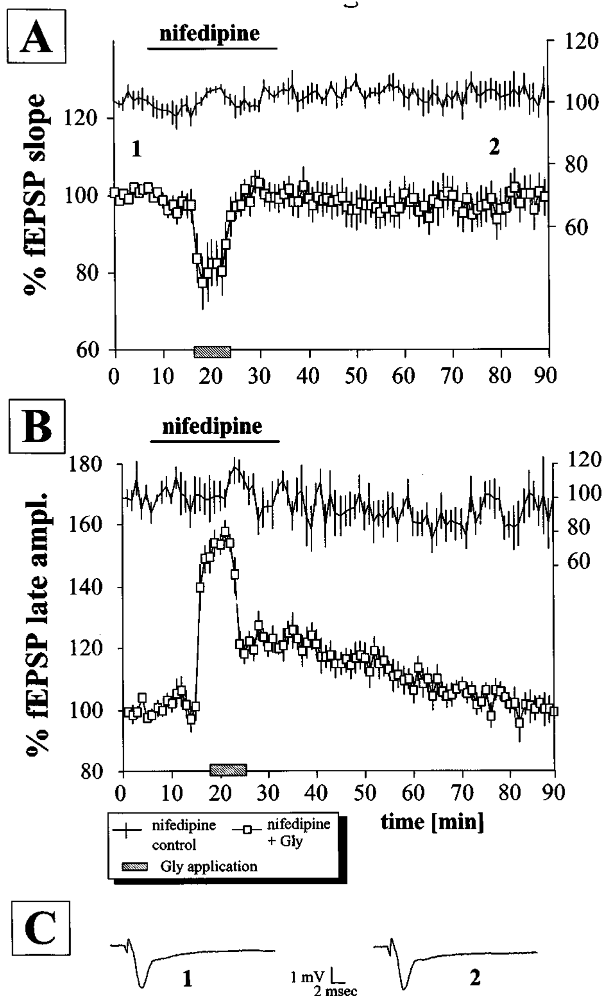


**Figure 8** Nifedipine, an inhibitor of L-type voltage-dependent calcium channels, prevents $LTP_{gly}$ of the fEPSP slope. When nifedipine was applied alone (20 $\mu$M, $n=7$) no baseline effect was observed (right y-axis) for both the fEPSP slope (A) and late amplitude (6 ms after stimulus artefact, B). When Gly (3 mM, $n=7$) was applied in conjunction with nifedipine a depression of the fEPSP slope occurred which recovered during wash while nifedipine was still present (left y-axis). No significant increase above baseline was found during wash. The late fEPSP amplitude showed no differences compared to Gly-control data (Figure 1C). (C) Raw traces of fEPSPs are illustrated for time points 1 and 2.

receptors are required for the expression of $LTP_{gly}$. Furthermore, it can also be excluded that $LTP_{gly}$ is caused by an unspecific or indirect action on GABA-A receptors. Thus, we hypothesize that Gly causes a so far unknown excitation in the superficial SC which is linked to VDCC activation. It has to be considered, however, that the action of Gly may be caused by some unspecific action which may lead to the activation of second-messenger cascades. Although this notion requires clarification in future studies no such unspecific action was reported in other studies using 3-10 mM Gly (e.g. Newell *et al.*, 1997, Shahi *et al.*, 1993) and this concentration was reported to be required for maximum Gly-mediated responses (e.g. Ito & Cherubini, 1991).

Some support for a possible postsynaptic location of the Gly binding site responsible for $LTP_{gly}$ arises from the data obtained with nifedipine. Since nifedipine had no effect on synaptic transmission but inhibited $LTP_{gly}$ it can be concluded that other subtypes of VDCCs are involved in transmitter release, and that $LTP_{gly}$ is not due to a modulation of presynaptic L-type VDCCs. Interestingly, we found that Gly mediated excitation reflected in the late amplitude is not blocked by nifedipine. Thus, this excitation does obviously not involve L-type VDCCs and is not caused by Gly acting on VDCCs, but VDCC activation may be a consequence thereof under control conditions since this appears to be required for $LTP_{gly}$ of the early fEPSP slope. The observation that $LTP_{gly}$ is only partially use-dependent from afferent stimulation shows that the action of Gly is strong enough in itself to cause a partial activation of VDCCs and a (smaller) rise in intracellular $Ca^{2+}$, as a trigger for $LTP_{gly}$. However, the presence of glutamatergic transmission providing additional depolarization is obviously aiding the full induction process of $LTP_{gly}$.

Although evidence for an $HCO_3^-$ efflux was found during Gly application such a conductance did not appear to be involved in $LTP_{gly}$ as illustrated by the lack of action of AAA, an inhibitor of carbonic anhydrase. This enzyme causes the maintenance of the $HCO_3^-$ efflux while a use-dependent breakdown of the $Cl^-$ gradient occurs in parallel, which was found to be the crucial component for GABA-mediated depolarization in the distal dendrites of the hippocampus (Staley *et al.*, 1995). Although AAA enhanced the depression of the fEPSP during Gly application, no change in the expression of $LTP_{gly}$ was obtained. Thus, it can be deduced that this model does not hold true for excitatory actions caused by Gly in the superficial SC. Further investigations on the nature of the Gly binding site and on a putatively excitatory Gly receptor in the SC are therefore required to identify the source and underlying mechanisms of this unusual excitation.

In conclusion, our data provide evidence for the presence of inhibitory Gly receptors in the superficial SC similar to the report of others, based on the data obtained with strychnine, PTX and AAA *during* Gly application. Nevertheless, the action which causes a long-lasting enhancement of synaptic transmission appears to be different in nature since $LTP_{gly}$ is neither strychnine nor PTX sensitive and not influenced in the expected way by carbonic anhydrase inhibition. Thus, $LTP_{gly}$ provides evidence for the presence of an unusual Gly binding site in the SC linked to VDCC receptor activation. Although a broad and long-lasting Gly release is not likely to happen *in vivo*, we hypothesize that $LTP_{gly}$ may occur at distinct synapses causing a long-lasting enhancement of synaptic efficacy. Thus, this novel form of LTP may contribute to plastic events concerning visual processing in the SC and it indicates a possible involvement of excitatory Gly receptors in plasticity which exceeds early phases of synaptogenesis and development.

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