The Glycine Binding Site of the Synaptic NMDA Receptor in Subpostremal NTS Neurons

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ABSTRACT

The nucleus of the tractus solitarius (NTS) plays an important role in the control of several autonomic reflex functions and has glutamate and GABA as main neurotransmitters. In this work we used patch clamp recordings in transverse slice preparations from rats to investigate whether the glycine binding site of the NMDA receptor is saturated or not in neurons of the subpostremal NTS. Except at hyperpolarized voltages and close to the reversal potential, glycine potentiated the NMDA responses in a concentration-dependent manner. The total charge transferred by glutamatergic currents was enhanced by glycine (500 µM) (from 28 ± 13 pC to 42 ± 18 pC at +50 mV, n = 7, p < 0.05). Glycine increased the conductance of the postsynaptic membrane, without altering its reversal potential, both in the presence (from 2.4 ± 0.06 nS to 3.4 ± 0.09 nS; n = 7) and absence (from 3.1 ± 0.06 nS to 4.4 ± 0.10 nS; n = 8) of Mg^{2+} in the bathing solution. D-serine, in the presence of strychnine, also increased the amplitude of the NMDA component (by 68 ± 19 %, p < 0.05, n = 5). The membrane potential was hyperpolarized (16 ± 6 mV, n = 8) by glycine suggesting the presence of inhibitory glycinergic receptors. Our results indicate that the glycine site of the NMDA receptor in neurons of the subpostremal NTS is not saturated and that glycine may act as a modulator of the NMDA transmission in this nucleus.
INTRODUCTION

The nucleus of the *tractus solitarius* (NTS) is the first synaptic site in the central nervous system (CNS) for a host of peripheral sensory inputs. Several authors have suggested that the NTS is not simply a relay site but can also contribute to the processing and integration of the visceral information through intrinsic neuronal circuitry, and play an important role in the coordination of several autonomic reflex functions (Mifflin and Felder 1990). The subpostremal NTS is defined as the caudal NTS from the level of the obex to the posterior tip of the area postrema (Barraco et al. 1992) and serves as the major NTS region for the integration of cardiopulmonary afferent information (Barraco and El-Ridi 1989; Nelson et al. 1988). In addition, this region is also involved in ingestive reflexes (Altschuler et al. 1989; Spencer and Talman 1986) and in the modulation of the pancreatic insulin release (Dunbar et al. 1992). Although there is a large variety of known neurotransmitters and neuromodulators within the NTS, several studies suggest that glutamate and GABA are the main neurotransmitters (Grabauskas and Bradley 2003; Bonham and Chen 2002; Doyle and Andresen 2001; Fortin and Champagnat 1993; Andresen and Yang 1990). It is accepted that glutamate, released by the first-order visceral afferent fibers, acts on NMDA and non-NMDA ionotropic receptors in second-order neurons within the NTS (Bonham and Chen 2002; Smith et al. 1998, Aylwin et al. 1997; Titz and Keller 1997). Although the synaptic release of glycine in the NTS has not been clearly demonstrated, several lines of evidence suggest the presence of both glycinergic terminals and
receptors in the NTS (Cassel and Talman 2000; Saha et al. 1999; Takahama et al. 1997). Microinjection of glycine into NTS, for example, elicits significant effects in arterial pressure and heart rate (Kubo and Kihara 1987; Talman and Robertson 1989).

The binding of two agonists, glutamate and glycine, is required for activation of the NMDA receptor (Kleckner and Dingledine 1988). While glutamate is the principal neurotransmitter, glycine can play a modulatory function depending on its concentration at the synaptic cleft (for review, see Danysz and Parsons 1998). The modulation of the NMDA response by glycine is therefore unlikely if its concentration is higher than its dissociation constant at the NMDA receptor site (Berger and Isaacson 1999). A number of in vivo and in vitro studies have investigated the glycine binding site in the NMDA receptor and suggest that its saturation depends on the region of the CNS. While some studies have demonstrated that the application of exogenous glycine potentiates the NMDA response (Ahmadi et al. 2003; Martina et al. 2003; Wilcox et al. 1996), others found no modulation (Fletcher et al. 1989; Obrenovitch et al. 1997). Detection of micromolar concentrations of glycine in the extracellular and cerebrospinal fluids (Westergren et al. 1994) suggest that the glycine site in the NMDA receptor may be saturated. However, the local concentration of glycine at glutamatergic synapses is unclear. High-capacity transporters (GLYT1 and GLYT2), can maintain low levels of glycine at the synaptic cleft regulating its concentration in
regions close to the NMDA receptors (Bergeron et al. 1998; Supplisson and Bergman 1997; Zafra et al. 1995).

The aim of this study was to investigate whether exogenous glycine can modulate the NMDA current in subpostremal NTS neurons, thus supporting the hypothesis that glycine concentration in the synaptic cleft is non-saturating.

METHODS

Brain Slices

Transverse brain slices of the medulla oblongata containing the subpostremal NTS were prepared from 30-35 days-old Wistar rats of either sex. The animals were anesthetized with Nembutal (50 mg/Kg, i.p). Following decapitation and craniotomy, the brain and upper cervical spinal cord were removed and submerged in ice-cold (2-3 °C) artificial cerebrospinal fluid (ACSF) pH 7.35-7.4, equilibrated with carbogen (95 % O₂, 5 % CO₂). The ACSF contained (mM): 122 NaCl; 2.5 KCl; 1.0 MgCl₂; 2.0 CaCl₂; 25 NaHCO₃; 1.25 NaH₂PO₄; 25 Glucose and the osmolality was 305-310 mOsm/Kg.H₂O. After the brain stem was dissected, it was glued with cyanoacrylate glue to an L-shaped agar block (4 % agar in ACSF) and two transversal slices (300 µm thick) containing the area postrema were cut using a Vibratome (MA756-Campdem Instruments, England). After cutting, the slices were incubated for 60 minutes at 32 °C in ACSF constantly gassed with carbogen. When currents were measured in the absence of Mg²⁺, the slices were incubated with nominally Mg²⁺-free ACSF for at least 60
minutes before recording. A single slice was transferred to the recording chamber on the stage of an upright microscope (E600 Nikon Inc., Tokyo Japan), held in place with a nylon net mounted on a platinum wire, and continuously superfused with ACSF (or nominally Mg\(^{2+}\) free ACSF depending on the experiment), saturated with carbogen, at a rate of 2-3 ml/min, driven by gravity. All drugs were applied at known concentrations by changing the perfusion line. Experiments were performed at room temperature (23-25 °C). Strychnine was purchased from Sigma Chemical Co (Saint Louis, MO, USA), DL-AP5 (DL-2-Amino-5-phosphonopentanoic acid), DNQX (6,7-Dinitroquinoxaline-2,3 Dione), L-689-560 (trans-2-Carboxy-5,7-dichloro-4-phenylaminocarbonyl amino-1,2,3,4-tetrahydroquinoline) and Bicuculline Methochloride were from TOCRIS Cookson Inc. (Ellisville, MO, USA). All other salts were purchased from Sigma. Efforts were made to minimize the number of animals used and their suffering in accordance with the Guidelines for the Use of Laboratory Animals of the School of Medicine of Ribeirão Preto/USP.

**Electrophysiology**

Patch pipettes were pulled from borosilicate glass tubing (Sutter Instrument Co. Novato, CA, USA) on a P-97 puller (Sutter Instrument Co., Novato, CA, USA) and were fire polished on a microforge (Narishige MF-83, Tokyo, Japan). The internal solutions were (mM): 130 CsF; 10 NaCl; 1 MgCl\(_2\); 3 K-ATP; 10 EGTA; 10 HEPES; pH adjusted to 7.3 with CsOH and osmolality of 295-305 mOsm/Kg.H\(_2\)O for the voltage-clamp experiments or (mM): 115 potassium gluconate; 20 KCl; 2
MgCl₂; 3 K-ATP; 10 EGTA; 10 HEPES; pH 7.3 adjusted with KOH and osmolality 295-305 mOsm/Kg.H₂O for the current-clamp experiments. When filled with the above solutions the pipettes had resistance between 4 and 8 MΩ. Junction potentials were calculated using the Axoscope 1.0 program and the results corrected accordingly. Cells were approached by the ‘blind patch’ method and seal resistances in excess of 2-5 GΩ were obtained prior to entering the whole-cell configuration. Access resistances were corrected between 70-80 %. Recordings were made with an EPC-7 (List Medical - Darmstadt, Germany) patch clamp amplifier. Whole-cell currents and voltages were low-pass filtered at 3 KHz (8 pole Bessel filter - LPF8 - Warner Instruments Corp, Hamden CT, USA) digitized at 10 KHz by a computer driven A/D converter (Digidata 1200B - Axon Instruments – Foster City, USA), and stored on hard disk using the pClamp6 software (Axon Instruments). Data were analyzed off-line using the MiniAnalysis program (Synaptosoft, New Jersey, USA), Clampfit or Axoscope (Axon Instruments). Synaptic responses of the NTS neurons were evoked by electrical stimulation (15 V, 50-100 µs, 0.2-0.5 Hz, stimulus isolation unit-DS2A-Digitimer Ltd., Garden City, England) delivered by a twisted bipolar platinum electrode (100 µm diameter) positioned on the ipsilateral solitary tract (ST). The Glycine concentration-response curve was fitted by the Hill equation:

\[ I = \frac{I_{max} [Glycine]^n}{EC_{50} + [Glycine]^n} \]

where \( I_{max} \) is the maximal response, \( EC_{50} \) is the glycine concentration yielding a current half of the \( I_{max} \) and \( n \) is the Hill coefficient. The
pooled data was expressed as the mean ± SE and statistical significance between values \( (p < 0.05) \) was determined by the Student’s \( t \) test. The F-test was used for comparison of slopes derived from linear fits to the experimental points of the \( I-V \) relationships.

**RESULTS**

Whole cell recordings were made in 68 neurons of the subpostremal NTS located between the solitary tract and the medial line. The ST was stimulated in the presence of 50 µM bicuculline (GABA \(_A\) antagonist) in order to evoke pure glutamatergic excitatory postsynaptic currents (glutamatergic EPSC). Strychnine (5 µM) was also present to block strychnine-sensitive glycine receptors. In some experiments the non-NMDA blocker DNQX (5 µM), was also present in order to evoke pure NMDA EPSCs in the presence \( (n = 7) \) or in the absence \( (n = 8) \) of extracellular \( \text{Mg}^{2+} \). Figure 1A shows the effect of glycine upon the total glutamatergic EPSCs, i.e. NMDA and non-NMDA. At a hyperpolarized voltage (-90 mV), where \( \text{Mg}^{2+} \) blocks the NMDA receptor, glycine had non significant effects on the inward non-NMDA responses, even at the highest concentration used (1000 µM). Conversely, at a holding potential of +50 mV, the NMDA responses were significantly increased by 1000 µM glycine. Application of 10 µM L-689-560 (or 50 µM DL-AP5) blocked the late component of the glutamatergic EPSC, confirming that it is mediated by the NMDA receptor (figure 1A). In order to evaluate the effect of glycine over a wide range of voltages, the current amplitude of the NMDA component was measured 60 ms after the peak of the
glutamatergic EPSC, at which point the non-NMDA currents have already decayed. The $I-V$ plot of figure 1B shows that 500 µM glycine enhanced the amplitudes of the NMDA currents without shifting the reversal potential. Fitting a linear equation to the control and experimental points between -20 and +50 mV (where rectification is negligible) results in significantly different slopes ($2.4 \pm 0.06$ to $3.4 \pm 0.09$ nS, $n = 7$, $p < 0.0001$; F test), indicating that glycine leads to an increase in the maximal conductance of the post synaptic membrane. Figure 1C shows the relationship between the percentage of increase in the amplitude of the current and the different glycine concentrations, for a holding potential of +50 mV. The potentiating effect of glycine was concentration-dependent over the range 10 µM to 1000 µM. The $EC_{50}$ for glycine was 130 µM and $n$ was 0.74, as deduced from a fitting of the Hill equation (see Methods) to the experimental points. Considering the long time course of the NMDA current, we also analyzed the effect of glycine on the transfer of charge. The time integral of the glutamatergic EPSC, taken between the onset of the response and 225 ms later (total charge transferred), was plotted against the holding potentials (-90 mV to +50 mV, in 10 mV steps). Figure 1D shows that 500 µM glycine significantly increased the amount of total charge transferred by the glutamatergic EPSC at -50, -40, -30, +30, +40 and +50 mV. At +50 mV the values are $28 \pm 13$ pC in control and $42 \pm 18$ pC ($n = 7$) in the presence of glycine. Note that glycine does not increase the NMDA current (figure 1B) nor its counterpart of charge transferred (figure 1D) at voltages close to the reversal potential and at
hyperpolarized voltages. In the first case, since the reversal potential is not changed, the points tend to converge to the same value close to 0 mV. What glycine really does is to increase the conductance of the postsynaptic membrane. In the second case, at hyperpolarized voltages, we can assume that glycine cannot overcome the Mg$^{2+}$ blockage or, in a less probable view, that the glycine effect is voltage-dependent. To test this last possibility, and to confirm that glycine is acting on the NMDA component of the glutamatergic EPSC, we analyzed the isolated NMDA current in the absence of extracellular Mg$^{2+}$. The slices were incubated for at least one hour in Mg$^{2+}$-free ACSF before electrophysiological recordings. The NMDA current was evoked in the presence of 5 µM strychnine, 50 µM bicuculline and 5 µM DNQX. The inset in figure 2 shows isolated NMDA EPSC evoked by ST stimulation in the control and in the presence of glycine 500 µM at holding potentials of -90 mV (inward traces) and +50 mV (outward traces). The potentiating effect of glycine is clear. As expected, in the absence of extracellular Mg$^{2+}$, the NMDA I-V relationship (figure 2) becomes linear and the experimental points, measured now at the peak of the current, can be well fitted by a linear function throughout the voltage range from -90 mV to +50 mV. The slopes of the lines (i.e. the conductance of the postsynaptic membrane) in control and in the presence of 500 µM glycine are statistically different (from 3.1 ± 0.06 nS to 4.4 ± 0.10 nS; n = 8; p < 0.0001; F test) leading to the conclusion that glycine is acting on the NMDA receptor to increase the maximal conductance of the postsynaptic membrane. Furthermore,
in the absence of extracellular Mg\(^{2+}\), the effect of glycine is also present at hyperpolarized voltages, showing that it is not voltage-dependent.

The effect of glycine was also observed on spontaneous excitatory postsynaptic currents (sEPSC) recorded in the presence of bicuculline (50 µM) and strychnine (5 µM). In Mg\(^{2+}\)-free ACSF, the glutamatergic sEPSC presented both the NMDA and non-NMDA components (figure 3C). Figures 3A and 3B show a recording at a holding potential of −70 mV, in control and in the presence of 500 µM glycine, respectively. Glycine (500 µM) prolonged the decay time constant of the sEPSC as can be seen from the averaged traces shown in figure 3C. In 6 cells the mean decay time constants, changed from 5.2 ± 0.3 ms in the control to 8.8 ± 1.3 in the presence of 500 µM glycine (p<0.05, n=6). The average peak amplitude of the sEPSCs changed from 25.3 ± 1.6 pA to 30.6 ± 2.16 pA, not significantly different (p>0.05). The increment in the time constants resulted in an increase (59 ± 22 %, n = 6) in the total charge transferred (figure 3D). These effects were reversible and 1 mM Mg\(^{2+}\) abolished the NMDA component of the sEPSC. Addition of 5 µM DNQX blocked the remaining synaptic transmission (not shown). Glycine did not alter the frequency of appearance of the events (1.1 ± 0.2 Hz in control condition and 1.2 ± 0.4 Hz in the presence of glycine, n = 6), suggesting a postsynaptic action.

To check for the specificity of the glycine effect we used D-serine, a glycine-site NMDA receptor agonist, which is ineffective at the strychnine-sensitive glycine
receptor and, contrary to glycine, blocks the NMDA receptors containing NR3A or NR3B subunits (Chatterton et al. 2002). Figure 4A shows that 50 \( \mu \text{M} \) D-serine potentiates the NMDA component (outward current) and did not change the non-NMDA component (inward current) of the glutamatergic EPSC. This effect was reversible. At a holding potential of +50 mV, D-serine significantly enhanced (68 ± 19 %; \( p < 0.05; n = 5 \)) the amplitude of the NMDA currents measured 60 ms after the peak of the response (figure 4B). It also increased the total charge transferred (measured from the onset of the response to 225 ms later) (66 ± 11 %, \( p < 0.05; n = 5 \); figure 4C).

We investigated, at a holding potential of +50 mV, the effect of 500 \( \mu \text{M} \) glycine on the time course of NMDA currents evoked by ST stimulation, in the presence of DNQX (5 \( \mu \text{M} \)), bicuculline (50 \( \mu \text{M} \)) and strychnine (5 \( \mu \text{M} \)). In this condition, the charge transferred, measured from the onset of the response to 570 ms later, was increased by 42% (from 69.2 ± 19 pC to 98.4 ± 24 pC, \( n = 6, p < 0.05 \)). The rise time, measured from the onset of the response to the peak, was not significantly changed and there was a tendency for the decay time constant of the NMDA currents to be slower in the presence of glycine (figures 5A and 5B), although no statistical difference was found.

In current clamp experiments, glycine hyperpolarized the membrane potential by 16 ± 6 mV (\( n = 8 \)). The glycine-induced hyperpolarization was antagonized by 5 \( \mu \text{M} \) strychnine (figure 6).
DISCUSSION

In this work we have shown that the glycine-site of NMDA receptors in subpostremal NTS neurons is not saturated.

Most of our results were obtained by stimulation of the ST and pharmacological isolation of the excitatory postsynaptic currents (EPSC) by adding bicuculline and strychnine to the bath. The antagonist of the glycine-site of the NMDA receptor, L-689-560, blocked the late component of the EPSC, while DNQX abolished the fast component of the response (figures 1A and 5A, respectively). This pharmacological profile, and the time course of the currents, suggests that the EPSCs are mediated by both NMDA and non-NMDA receptors. The $I\text{-}V$ relationship of the late component (60 ms after the peak) shows that the conductance of the postsynaptic membrane is about 40 % larger in the presence of exogenous glycine in both normal or Mg$^{2+}$-free ACSF (figure 2B). The statistically different slopes of the straight lines fitting the experimental points of the $I\text{-}V$ relationships in the absence of extracellular Mg$^{2+}$, in control and in the presence of glycine, and the fact that these lines extend throughout the voltage range from -90 mV to +50 mV, show that the effect of glycine upon the NMDA current is not voltage-dependent. Rather, the absence of a significant effect of glycine at hyperpolarized voltages when the extracellular Mg$^{2+}$ is present is probably due to the fact that glycine can not overcome the Mg$^{2+}$ block.

The experiments with sEPSC (figure 3) reinforce the hypothesis that glycine is acting on the NMDA component of the EPSC and suggest that the NMDA and
non-NMDA receptors are co-localized at the postsynaptic membrane, as also described in the dorsal motor nucleus of the vagus and hypoglossal motoneurons (Travagli et al. 1991; O’Brien et al. 1997).

The potentiation of the NMDA response could have been caused by glycine action (1) on the glycine-site of the NMDA receptor, (2) on the NMDA receptors containing NR3A or NR3B subunits, which are activated by glycine in the absence of glutamate (Chatterton et al. 2002) or (3) presynaptically at the strychnine-sensitive glycine receptors facilitating the release of glutamate (Turecek and Trussell 2001). The fact that also D-serine increased the NMDA current (figure 4) argues against the last two possibilities since D-serine is ineffective at the strychnine-sensitive glycine receptor and, contrary to glycine, blocks the NMDA receptors containing NR3A or NR3B subunits (Chatterton et al. 2002). Moreover, it is important to note that in our experiments the EPSCs were recorded in the presence of strychnine and the non-NMDA component was not altered by glycine. Taken together, these results strongly support the hypothesis that glycine is acting at its site on the NMDA receptor.

Our results also have shown that glycine (500 μM, at +50 mV) does not alter either the rise time or the decay time constant of NMDA currents evoked by ST stimulation (figure 5). This can be taken to suggest that glycine affects the number of channels opening during a synaptic input rather than their kinetics.

The state of saturation of the glycine site appears to depend upon regional differences with respect to NMDA receptor subtype expression, local glycine or
D-serine concentration and the expression of specific types of glycine transporters (Parsons et al. 1998). Some studies have shown immunoreactivity for a high-capacity glycine transporter in NTS (Zafra et al. 1995), which would keep the glycine concentration at the synaptic cleft at low levels, resulting in non-saturation of the glycine-site in NMDA receptors.

Ahmadi et al. (2003) and Berger and Isaacson (1999) suggested that a potential source of glycine for the NMDA receptors is that released synaptically, in a process called spillover. Although terminals from the ST apparently do not release glycine, both glycinergic receptors and terminals have been demonstrated in the NTS (Cassell and Talman 2000; Saha et al. 1999; Takahama et al. 1997). The present results also suggest the presence of strychnine sensitive glycine receptors, since application of glycine to the slice led to a hyperpolarization of the neurons (figure 6). Furthermore, studies by Pickel et al. (1996) suggest that projections from the amygdala to the NTS may be glycinergic, which could be a source of glycine for the NTS neurons.

In summary, we have demonstrated, in vitro, that the glycine-site at NMDA receptors is not saturated in subpostremal NTS neurons, suggesting that glycine can play a role as a modulator in excitatory neurotransmission in this nucleus.
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LEGEND TO FIGURES

FIG. 1. Glycine increases the NMDA component of the glutamatergic postsynaptic currents evoked by ST stimulation. A. Superimposed EPSC at a holding potential of –90 mV (inward currents) and at +50 mV (outward currents) in control condition, in the presence of 1000 µM glycine, after washout and in the presence of L-689-560. B. I-V relationship (n = 7) of the NMDA component of the EPSC, measured 60 ms after the peak, in control condition (filled squares), in the presence of glycine (500 µM; open squares) and L-689-560 (10 µM; filled triangles). The straight lines between -20 and +50 mV are best fits of a linear equation to the experimental points (I=-2.0 + 2.4V, r=0.99. for the control and I=2.6 + 3.2V, r=0.99 in the presence of glycine). The slopes are significantly different (p<0.0001; F test; F=47.9457, DFn=1; DFd=12). C. Glycine concentration-response curve at a holding potential of +50 mV. The numbers in parenthesis indicate the number of cells. D. Relationship between the charge transferred by the EPSC, measured for 225 ms from the onset of the response, and voltage (n = 7) in control and in the presence of 500 µM glycine. * indicates statistically different values (Student’s t-test, p < 0.05).

FIG. 2 – The effect of glycine is voltage independent. The inset shows NMDA EPSCs evoked by solitary tract stimulation in a nominally free-Mg²⁺ ACSF containing 5 µM strychnine, 5 µM DNQX and 50 µM bicuculline at holding potentials of -90 mV (inward currents) and +50 mV (outward currents) in the
absence (traces marked control) and in the presence of 500 μM glycine. The IxV relationships of the EPSCs measured under control condition (filled circles) and in the presence of 500 μM glycine (empty squares) are linear. The straight lines are best fits of a linear equation to the experimental points (I = 14.9 + 3.1V, r=0.99. for the control and I=27.9 + 4.4V, r=0.99 in the presence of glycine). The slopes are significantly different (p<0.0001; F test; F=207.16, DFn=1; DFd=26). Data points are mean ± se of measurements in 8 cells.

FIG. 3. Exogenous application of glycine potentiates the NMDA component of the spontaneous excitatory postsynaptic currents (sEPSC). A. and B. Representative traces in control and in the presence of 500 μM glycine, respectively. sEPSC were recorded with Mg2+-free ACSF at a holding potential of –70 mV. C. Average sEPSC derived from 138, 222, and 48 events in control, in the presence of glycine, and after addition of Mg2+ (1 mM), respectively. The open dots superimposed upon the traces marked control and 500 μM Glycine represent best fits of a monoexponential function to the experimental points. The decay time constants are 5.7 ms for the control and 9.1 ms in the presence of glycine. The average decay time constants measured from 6 cells are: 5.2 ± 0.3 ms and 8.8 ± 1.3 (p<0.05). Average sEPSC shown in C are from the same neuron shown in A and B. D. Charge transferred by the sEPSC at a holding potential of -70 mV, measured from the peak to 150 ms, in control (464 ± 157 fC) and in the presence of 500 μM glycine (703 ± 299 fC; n=6).
FIG. 4. Exogenous application of D-serine increases the NMDA component of the glutamatergic postsynaptic current (EPSC) evoked by ST stimulation. A. EPSCs superimposed at holding potentials of −90 mV (inward currents) and at +50 mV (outward currents) in control condition, in the presence of D-serine and after washout. B. Amplitude of the NMDA component (n = 5) at a holding potential of +50 mV, measured 60 ms after the peak, in control and in the presence of D-serine (50 µM). C. Total charge transferred (n = 5) by the EPSC at a holding potential of +50 mV, measured for 225 ms from the onset of the response, in control and in the presence of D-serine (50 µM). (Students t-Test, * p < 0.05).

FIG. 5. Glycine does not alter the time course of the NMDA currents evoked by stimulation of the solitary tract. A. NMDA currents normalized to unitary amplitude at a holding potential of +50 mV in the control and in the presence of glycine. The broken lines over the decay phase of the currents are monoexponential function fittings with decay time constants of 378 ms and 394 ms for control and glycine conditions, respectively. The currents were recorded in the presence of DNQX (5 µM), bicuculline (50 µM) and strychnine (5 µM). B. Rise time (n = 7) of the NMDA currents at a holding potential of +50 mV, measured from the onset of the response to the peak, in control and glycine (500 µM). C. Decay time constants (τ) of the NMDA currents at a holding potential of +50 mV in control and in the presence of glycine (500 µM). (n=7 cells).
FIG. 6. Strychnine-sensitive glycinergic receptors. Bath application of 500 µM glycine (arrowhead marked G) induced a hyperpolarization of the membrane potential (-15 mV) and cessation of action potential firing. Washout (arrowhead marked W) of the drug reverts to the control situation. Strychnine (arrowhead marked S; 5 µM) slightly increased the firing rate and blocked the effect of glycine (arrowhead marked S+G). This cell had a resting potential around -62 mV and was spontaneously firing action potentials at the beginning of the experiment.
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Figure 2
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