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# Glycine receptor-mediated inhibition of medial prefrontal cortical pyramidal cells

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ABSTRACT

Using whole-cell patch clamp recording on medial prefrontal cortical slices of rats aged 17–33 postnatal days, we demonstrated the glycine-induced strychnine-sensitive outward currents. The amplitude of the peak current increased with the concentrations of glycine with an $EC_{50}$ of 74.7 μM. Application of 1 μM strychnine alone to cells caused a slight inward current without blocking the sIPSCs, indicating that GlyRs in the mPFC are activated by an endogenous ligand that can be released tonically. Glycine reversibly depressed firing rate in cells from both layer 6 and layer 3, with significantly greater inhibition on the former than the latter ($EC_{50}$ 12.9 *vs* 85.6 μM). Glycine hyperpolarized membrane potential in cells of both layer 6 and layer 3 depending on its concentrations, with an $IC_{50}$ of 99.1 and 207.2 μM, respectively. We propose that GlyRs participate in a novel inhibitory mechanism in mPFC, modulating neuronal activity. This finding further supports an important role of GlyR in cortical function and dysfunction.



## 1. Introduction

Emerging information indicates that GlyRs are not restricted to the spinal cord and the brain stem, but are distributed more extensively in the CNS, including many forebrain areas [1–3]. An extensive study of glycine-IR in rats reports that glycine-containing cells are also present in the upper brainstem and the forebrain [4,5]. Functional GlyRs have also been found in other brain areas such as cortex [6,7]. The last few years have seen a surge in interest in the GlyRs in the upper brain areas [8–10]. Consequently, a wealthy of information has recently emerged concerning the properties and the role of the GlyRs located outside of the spinal cord and brain stem [1,7,11].

The medial prefrontal cortex (mPFC) is critically involved in many higher brain functions [12,13], it is an important part of the reward circuit in the rat brain, with strong reciprocal interactions with the ventral tegmental area (VTA) and nucleus accumbens [14–16]. Most receptor researches of mPFC were forced on *N*-methyl-D-aspartate (NMDA) receptor [17–19].

In the present study, we used patch-clamp electrophysiology to provide evidence that both layer 6 and layer 3 mPFC neurons express functional GlyRs. Futhermore, layer 6 cells are more sensitive to glycine inhibition. Our findings indicate that GlyRs are expressed by neurons in mPFC, especially of layer 6 neurons, and their activation provides an important inhibitory function that is required to maintain the normal excitatory balance for proper function of mPFC.

## 2. Materials and methods

### 2.1. Slice preparation

The medial PFC was prepared as described previously by Liu et al. [20]. Rats were anesthetized with ketamine/xylazine and then sacrificed by decapitation. The medial PFC was identified according to the stereotaxic coordinates [21]. Coronal midbrain slices (250 μm thick) were cut using a VF-300 slicer (Precisionary Instruments Inc., Greenville, NC) in ice-cold glycerol-based artificial cerebrospinal fluid (GACSF) containing (in mM): 250 glycerol, 1.6 KCl, 1.2 $NaH_2PO_4$, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 25 $NaHCO_3$, and 11 glucose, and saturated with 95%$O_2$/5%$CO_2$ (carbogen). Two slices per animal were allowed to recover for at least 1 h in a holding chamber at room temperature (22–24 °C) in carbogen-saturated regular artificial cerebrospinal fluid (ACSF), which has the same composition as GACSF, except that glycerol was replaced by 125 mM NaCl.

### 2.2. Electrophysiological recordings

Electrical signals were obtained in whole-cell patch clamp configurations with an Axon 700B amplifier (Molecular Devices Co., Union City, CA, USA), a Digidata 1440A A/D converter (Molecular

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Devices Co.) and pCLAMP 10.2 software (Molecular Devices Co.). Data were filtered at 1 kHz and sampled at 5 kHz. The patch electrodes had a resistance of 4–6 M when filled with the pipette solution containing (in mM): 135 K gluconate, 5 KCl, 2 $MgCl_2$, 10 HEPES, 2 Mg ATP, 0.2 GTP. The pH was adjusted to 7.2 with Tris base. A single slice was transferred to a 0.4 ml recording chamber, where it was held down by a platinum ring. Layers 6 or 3 medial PFC pyramidal neurons were identified under visual guidance using infrared-differential interference contrast (IR–DIC) video microscopy with a 40× water immersion objective. The image was detected with an IR-sensitive CCD camera and displayed on a monitor. Throughout the experiments, the bath was continually perfused with carbogenated ACSF (1.5–2.0 ml/min).

### 2.3. Chemicals and applications

The chemicals, including glycine, strychnine and other standard chemicals were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA).

### 2.4. Data analysis

Average values are expressed as the mean ± SEM, with $n$ equal to the number of cells studied. Statistical significance of results was assessed using Student's $t$-test. Statistical analysis of concentration–response data was performed using the nonlinear curve-fitting program ALLFIT [22], which uses an ANOVA procedure. Values reported for concentration–response analysis are those obtained by fitting the data to the equation:

$$Y = E_{max}/[1 + (EC_{50}/X)^n]$$

where X and Y are concentration and response, respectively, $E_{max}$ is the maximal response, $EC_{50}$ is the concentration yielding 50 percent of maximal effect ($EC_{50}$ for activation, $IC_{50}$ for inhibition), and $n$ is the Hill coefficient.

## 3. Results

### 3.1. Glycine induced strychnine-sensitive outward currents in mPFC neurons

To characterize the role of GlyRs in regulating the function of mPFC neurons, we recorded whole-cell currents from mPFC neurons in slices of rats aged 17– 33 postnatal days. The application of glycine (3–1000 μM) slightly decrease sIPSCs frequency and has no significant effect on the amplitude (data not shown). Under voltage-clamp, bath-applied glycine (3–1000 μM) induced outward currents at a holding potential of 0 mV (Fig. 1A). The amplitude of the peak current increased with the concentrations of glycine. A fit of the dose–response curve to the ALLFIT equation (see method for details) obtained an $EC_{50}$ of 74.7 ± 8.3 μM and Hill coefficient of 1.4 ($n$ = 7; Fig. 1B).

The current-elicited by glycine was sensitive strychnine, a GlyR antagonist. Strychnine (1 μM and 10 μM) attenuated the peak amplitude of glycine-induce current to 32.7% and 2.5%, respectively (control, 147 ± 32 pA; 1 μM strychnine, 48 ± 7 pA, $p < 0.05$; 10 μM strychnine, 4 ± 2 pA, $p < 0.01$; Fig. 1A(b)).

As the Fig. 1C shows, application of 1 μM strychnine alone to cells displaying spontaneous IPSCs caused a slight inward current without blocking the sIPSCs, indicating that GlyRs in the mPFC are activated by an endogenous ligand that can be released tonically.

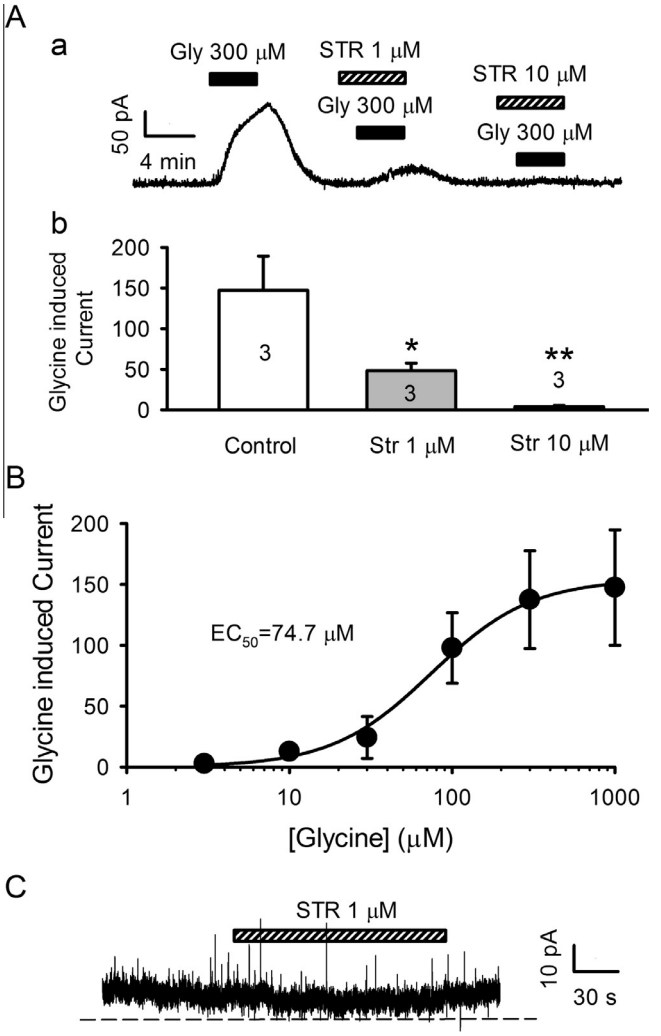


**Fig. 1.** Glycine induced outward currents recorded in mPFC neurons. (A) (a) Records are current induced by 300 μM glycine and its inhibition by 1 μM and 10 μM strychnine, respectively, from a single neuron in slice from a P17 rat. (b) Bar graph showing inhibition by 1 μM and 10 μM strychnine, respectively, of current activated by 300 μM glycine. The percentage inhibition of glycine-activated current by strychnine were significantly different ($^*p < 0.05$ and $^{**}p < 0.01$, respectively for strychnine *versus* prestrychnine). (B) Concentration–response curves of glycine-induced current for the layer 6 mPFC neurons. Each point is the average of 6–11 cells; error bars not visible are smaller than the size of the symbols. The curve shown is the best fit of the data to the equation described in Materials and methods. Fitting the data to this equation yielded the $EC_{50}$ value of 74.7 ± 8.3 μM and the Hill coefficient of 1.4. (C) Application of 1 μM strychnine alone to a neuron in a brain slice of a P21 rat produced a slight inward current without blocking the sIPSCs.

### 3.2. Glycine inhibits more potently the activity of layer 6 neurons than layer 3 ones in the mPFC

The activity of mPFC neurons was recorded from both layer 6 and layer 3 under current-clamp conditions. The average resting potential (RP) of mPFC neurons is – 59.3 ± 1.2 mV (between −50 and −78 mV, $n$ = 37), and only 3 of 82 cells tested had spontaneous firings under these conditions. Therefore, we applied a constant depolarizing current (50–100 pA), which depolarized the membrane potentials by 8–18 mV and induced firings. When firings reached a relatively stable level, glycine was bath-applied for 4 min, and then washout. Glycine reversibly depressed firing rate in cells from both layer 6 and layer 3, but glycine had significantly greater inhibition on the former than the latter. Specifically, 30 μM glycine suppressed the firing rate by 59.3% ($n$ = 6) of layer 6

neurons, which was significantly greater than 29.6% ($n = 5$) of layer 3 neurons (Fig. 2A). The fit to the dose–response curves obtained an $EC_{50}$ of 12.9 ± 1.5 μM and Hill coefficient of 0.6 for layer 6 cells, and an $EC_{50}$ of 85.6 ± 7.9 μM and Hill coefficient of 0.8 for layer 3 cells, respectively ($p < 0.01$, Fig. 2B).

### 3.3. Glycine hyperpolarizing membrane potential in cells of both layer 6 and layer 3

As illustrated in Fig. 3, bath-applied glycine (1000 μM) reduced the firing rate and induced membrane hyperpolarization. On average, 1000 μM glycine reduced the firing rates from 5.2 ± 1.2 to 0 Hz in 100% (7 of 7) of layer 6 neurons and from 4.5 ± 1.1 to 0 Hz in 67% (6 of 9) of layer 3 ones, respectively. In addition, 1000 μM glycine induced membrane hyperpolarization of 9.6 ± 1.3 mV ($n = 5$) of layer 6 cells and of 9.0 ± 1.6 mV ($n = 5$) of layer 3 cells, respectively. Glycine-induced hyperpolarization depended on its concentrations with an $IC_{50}$ of 99.1 ± 5.8 μM and Hill coefficient of 1.1 for layer 6 cells, and $IC_{50}$ of 207.2 ± 11.6 μM and Hill coefficient of 1.1 for layer 3, respectively ($p < 0.05$, Fig. 3B). In 11 of 78 cells tested, low concentration glycine (⩽30 μM) slightly depolarized (less than 1.2 mV) the cells. Interestingly, this depolarization was associated with a slightly reduction in firing rate.

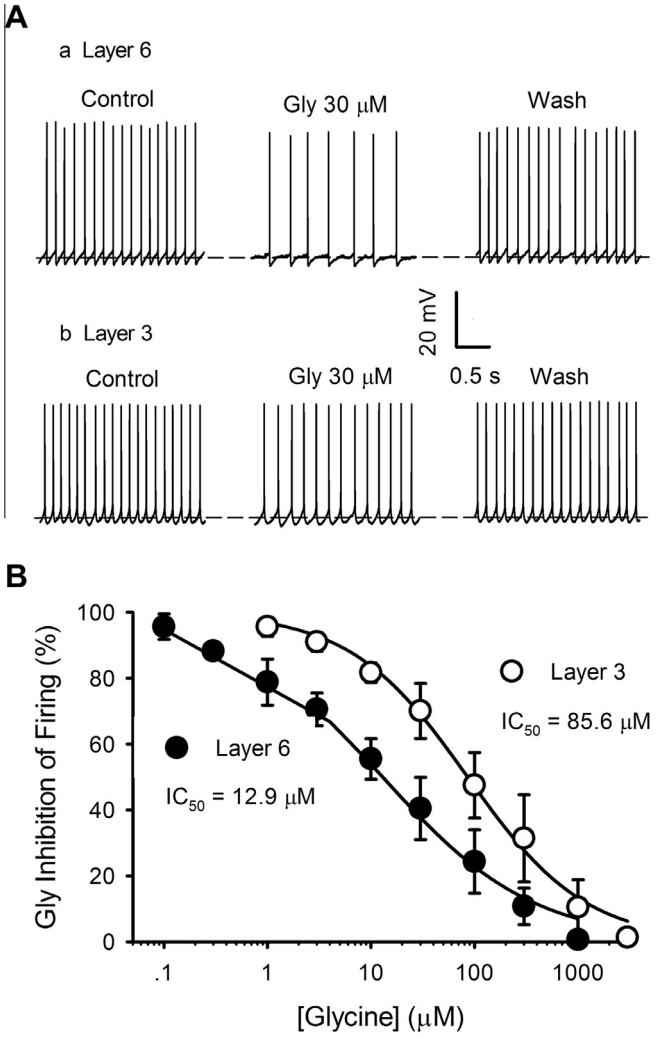


**Fig. 2.** Neurons in layer 6 of mPFC are more sensitive to glycine inhibition compare to layer 3. (A) Ongoing firing, recorded from current-clamped mPFC neurons in slices from a P24 (a) and a P25 (b) rat, respectively, were decreased by 30 μM glycine. (B) Concentration–response curves of glycine inhibition of sAPs for the layer 6 (●) and layer 3 (○) mPFC neurons. Each point is the average of 4–10 cells; error bars not visible are smaller than the size of the symbols. The curve shown is the best fit of the data to the equation described in Materials and methods. Fitting the data to this equation yielded the $IC_{50}$ value and the Hill coefficient of 12.9 ± 1.5 μM and 0.6 for the layer 6, and 85.6 ± 7.9 μM and 0.8 for the layer 3 neurons, respectively.

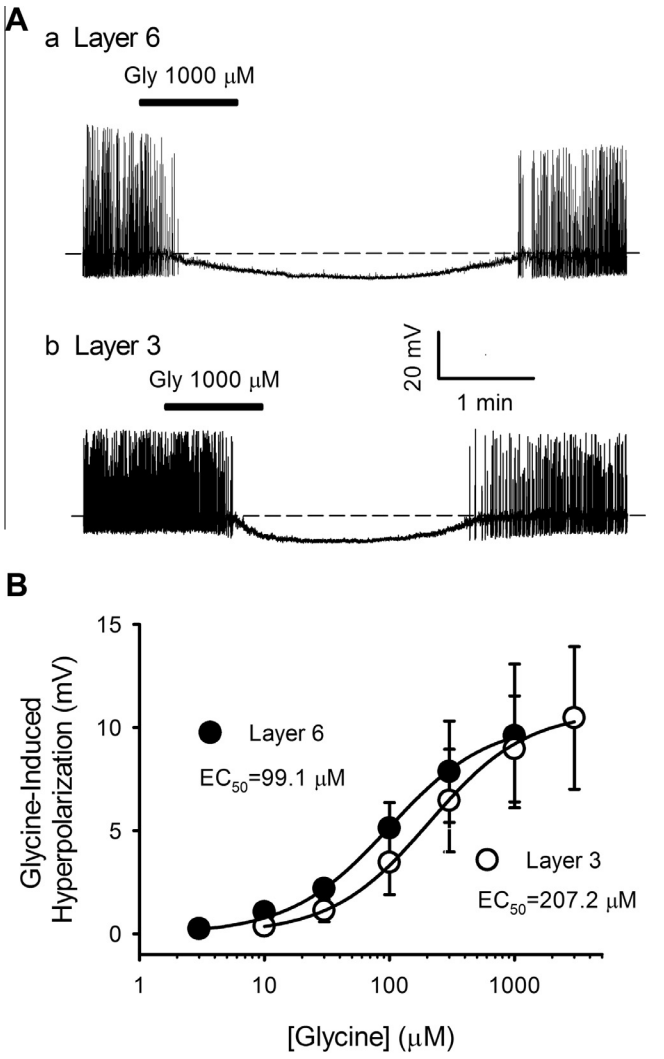


**Fig. 3.** Glycine-induced membrane hyperpolarizatio on mPFC neurons. (A) Glycine stops the firing of action potentials and causes membrane hyperpolarization in both layer 6 and layer 3 cells, recorded from current-clamped mPFC neurons in slices from a P27 (a) and a P30 (b) rat, respectively. (B) Glycine produces concentration-dependent membrane hyperpolarization in layer 6 and layer 3 neurons. Error bars not visible are smaller than the size of the symbols. Fitting the data to this equation yielded the $IC_{50}$ value and the Hill coefficient of 99.1 ± 5.8 μM and 1.1 for the layer 6, and 207.2 ± 11.6 μM and 1.1 for the layer 3 neurons, respectively.

## 4. Discussion

The strychnine-sensitive GlyR is a ligand-gated anionic channel that is primarily involved in fast inhibitory synaptic transmission [8,11]. GlyRs are members of the pentameric Cys-loop receptor superfamily [23,24]. Two different subunits of GlyR have been characterized so far, an α subunit (48 kDa) and a β subunit (58 kDa) [25,26]. GlyRs are formed either from α subunits alone or from both α and β subunits [27]. Synaptically localized heteromeric GlyR consists of three α and two β subunits, which combine to form a pentameric receptor complex [1]. GlyRs in many of the

forebrain areas are not transient and limited to early development but persist through mature developmental stages [1,28]. The current study show that strychnine-sensitive glycine currents recorded from layer 6 and layer 3 mPFC neurons of weanling rats, indicating the expression of glycine-gated chloride channels (GlyRs) in the mPFC. In addition, our data show clearly that glycine, via GlyR activation, depresses activity in the mPFC synaptic network, showing that GlyR-mediated inhibition is an alternative and important mechanism, maybe especially in pathological conditions, such as hyperexcitation, epilepsy, GlyR activation is capable of controlling the activity of excitatory circuits in mPFC.

A major finding in our study is that GlyR activation depresses synaptically generated actional potentials recorded from both layers of mPFC neurons, indicating that these receptors have a general function to limit activity in mPFC circuits. These receptors, especially in layer 6 mPFC neurons, are likely of fundamental and extensive importance in the control of neuronal excitability in this region. In addition, application of 1 μM strychnine alone to cells displaying spontaneous IPSCs caused a slight outward current without blocking the sIPSCs, indicating that GlyRs in the mPFC are activated by an endogenous ligand that can be released tonically. For cortex GlyRs appear to be activated by nonsynaptically released taurine, suggesting an extrasynaptic location of GlyRs, thus, tonically active GlyRs are likely to be extrasynaptic [6,28].

Since our study was performed in acutely prepared slice from P17-P33 rats, our data demonstrate that GlyR expression by mPFC neurons is not transient and limited to early development but persistes through mature developmental stages.

In conclusion, we propose that GlyRs participate in a novel inhibitory mechanism in mPFC, modulating neuronal activity. This finding further supports an important role of GlyR in cortical function and dysfunction.

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## References

[1] J.H. Ye, Regulation of excitation by glycine receptors, Results Probl. Cell Differ. 44 (2008) 123–143.
[2] F. Weltzien, C. Puller, G.A. O'Sullivan, I. Paarmann, H. Betz, Distribution of the glycine receptor beta-subunit in the mouse CNS as revealed by a novel monoclonal antibody, J. Comp. Neurol. 520 (2012) 3962–3981.
[3] S. Jonsson, J. Morud, C. Pickering, L. Adermark, M. Ericson, B. Soderpalm, Changes in glycine receptor subunit expression in forebrain regions of the Wistar rat over development, Brain Res. 1446 (2012) 12–21.
[4] L. Danglot, P. Rostaing, A. Triller, A. Bessis, Morphologically identified glycinergic synapses in the hippocampus, Mol. Cell. Neurosci. 27 (2004) 394–403.
[5] K. Baer, H.J. Waldvogel, R.L. Faull, M.I. Rees, Localization of glycine receptors in the human forebrain, brainstem, and cervical spinal cord: an immunohistochemical review, Front. Mol. Neurosci. 2 (2009) 25.
[6] A.C. Flint, X. Liu, A.R. Kriegstein, Nonsynaptic glycine receptor activation during early neocortical development, Neuron 20 (1998) 43–53.
[7] P.A. Kunz, A.C. Burette, R.J. Weinberg, B.D. Philpot, Glycine receptors support excitatory neurotransmitter release in developing mouse visual cortex, J. Physiol. (2012).
[8] J.W. Lynch, Molecular structure and function of the glycine receptor chloride channel, Physiol. Rev. 84 (2004) 1051–1095.
[9] J.H. Ye, K.A. Sokol, U. Bhavsar, Glycine receptors contribute to hypnosis induced by ethanol, Alcohol Clin. Exp. Res. 33 (2009) 1069–1074.
[10] M. Tanabe, A. Nitta, H. Ono, Neuroprotection via strychnine-sensitive glycine receptors during post-ischemic recovery of excitatory synaptic transmission in the hippocampus, J. Pharmacol. Sci. 113 (2010) 378–386.
[11] P. Legendre, The glycinergic inhibitory synapse, Cell. Mol. Life Sci. 58 (2001) 760–793.
[12] W.H. Alexander, J.W. Brown, Medial prefrontal cortex as an action-outcome predictor, Nat. Neurosci. 14 (2011) 1338–1344.
[13] J. Kim, A.H. Jung, J. Byun, S. Jo, M.W. Jung, Inactivation of medial prefrontal cortex impairs time interval discrimination in rats, Front. Behav. Neurosci. 3 (2009) 38.
[14] R. van Zessen, G. van der Plasse, R.A. Adan, Contribution of the mesolimbic dopamine system in mediating the effects of leptin and ghrelin on feeding, Proc. Nutr. Soc. (2012) 1–11.
[15] S. Ikemoto, Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory, Neurosci. Biobehav. Rev. 35 (2010) 129–150.
[16] T.M. Tzschentke, W.J. Schmidt, Functional relationship among medial prefrontal cortex, nucleus accumbens, and ventral tegmental area in locomotion and reward, Crit. Rev. Neurobiol. 14 (2000) 131–142.
[17] C.C. Huang, K.S. Hsu, The role of NMDA receptors in regulating group II metabotropic glutamate receptor-mediated long-term depression in rat medial prefrontal cortex, Neuropharmacology 54 (2008) 1071–1078.
[18] M. Davis, NMDA receptors and fear extinction: implications for cognitive behavioral therapy, Dialogues Clin. Neurosci. 13 (2011) 463–474.
[19] X. Lopez-Gil, F. Artigas, A. Adell, Unraveling monoamine receptors involved in the action of typical and atypical antipsychotics on glutamatergic and serotonergic transmission in prefrontal cortex, Curr. Pharm. Des. 16 (2010) 502–515.
[20] Y.W. Liu, J. Li, J.H. Ye, Histamine regulates activities of neurons in the ventrolateral preoptic nucleus, J. Physiol. 588 (2010) 4103–4116.
[21] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, 6th ed., Academic Press/Elsevier, Amsterdam, Boston, 2007.
[22] A. DeLean, P.J. Munson, D. Rodbard, Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose–response curves, Am. J. Physiol. 235 (1978) E97–E102.
[23] L.M. McCracken, M.L. McCracken, D.H. Gong, J.R. Trudell, R.A. Harris, Linking of glycine receptor transmembrane segments three and four allows assignment of intrasubunit-facing residues, ACS Chem. Neurosci. 1 (2010) 482.
[24] C. Villmann, J. Oertel, N. Melzer, C.M. Becker, Recessive hyperekplexia mutations of the glycine receptor alpha1 subunit affect cell surface integration and stability, J. Neurochem. 111 (2009) 837–847.
[25] R. Bluem, E. Schmidt, C. Corvey, M. Karas, A. Schlicksupp, J. Kirsch, J. Kuhse, Components of the translational machinery are associated with juvenile glycine receptors and are redistributed to the cytoskeleton upon aging and synaptic activity, J. Biol. Chem. 282 (2007) 37783–37793.
[26] B. Laube, G. Maksay, R. Schemm, H. Betz, Modulation of glycine receptor function: a novel approach for therapeutic intervention at inhibitory synapses?, Trends Pharmacol Sci. 23 (2002) 519–527.
[27] D. Langosch, L. Thomas, H. Betz, Conserved quaternary structure of ligand-gated ion channels: the postsynaptic glycine receptor is a pentamer, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 7394–7398.
[28] S.C. Chattipakorn, L.L. McMahon, Strychnine-sensitive glycine receptors depress hyperexcitability in rat dentate gyrus, J. Neurophysiol. 89 (2003) 1339–1342.