Distinctive glycinergic currents with fast and slow kinetics in thalamus

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Running Head: Synaptic and extrasynaptic glycine receptors in thalamus.

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ABSTRACT

We examined functional properties of inhibitory postsynaptic currents (IPSCs) evoked by medial lemniscal stimulation, spontaneous IPSCs (sIPSCs), and single channel, extrasynaptic currents evoked by glycine receptor agonists or γ-aminobutyric acid (GABA) in rat ventrobasal thalamus. We identified synaptic currents by reversal at $E_{Cl}$ and sensitivity to elimination by strychnine, GABA_A antagonists, or combined application. Glycinergic IPSCs featured short (~12 ms) and long (~80 ms) decay time constants. These fast and slow IPSCs occurred separately with mono-exponential decays, or together with bi-exponential decay kinetics. Glycinergic sIPSCs decayed mono-exponentially with time constants, matching fast and slow IPSCs. These findings were consistent with synaptic responses generated by 2 populations of glycine receptors, localized under different nerve terminals. Glycine, taurine or β-alanine applied to excised membrane patches evoked short- and long-duration current bursts. Extrasynaptic burst durations resembled fast and slow IPSC time constants. The single, intermediate time constant (~22 ms) of GABA_Aergic IPSCs co-transmitted with glycinergic IPSCs, approximated the burst duration of extrasynaptic GABA_A channels. We noted differences between synaptic and extrasynaptic receptors. Endogenously activated glycine and GABA_A receptor channels had higher Cl⁻ permeability than their extrasynaptic counterparts. The β-amino acids activated long-duration bursts at extrasynaptic glycine receptors, consistent with a role in detection of ambient taurine or β-alanine. Heterogenous kinetics and permeabilities implicate molecular and functional diversity in thalamic glycine receptors. Fast, intermediate, and slow inhibitory postsynaptic potential decays mostly due to co-transmission by glycinergic and
GABAergic pathways, allow for discriminative modulation and integration with voltage-dependent currents in ventrobasal neurons.
INTRODUCTION

Since the discovery of glycinergic transmission in the spinal cord, several studies have demonstrated that glycine mediates neurotransmission above the level of the cord (Fatima-Shad and Barry 1998; Chery and De Koninck 1999; Donato and Nistri 2000; Dumoulin et al. 2001). Recently, glycinergic inhibition has become recognized in the ventrobasal thalamus (Ghavanini et al. 2005; cf. Zeilhofer et al. 2005). Medial lemniscal stimulation produced inhibitory postsynaptic potentials (IPSPs) mediated only in part by the prevalent inhibitory transmitter, $\gamma$-aminobutyric acid (GABA; Steriade et al. 1997). A combination of strychnine with a GABA\textsubscript{A} antagonist completely blocked this inhibition (Ghavanini et al. 2005). Unexpectedly, glycinergic or GABAergic IPSPs occurred independently in many neurons, which tended to favor co-transmitting pathways rather than co-release of these amino acids.

Glycine receptors are pentameric complexes containing pore-forming $\alpha$ subunits, with or without accessory $\beta$ subunits (Lynch 2004). Heteromeric ($\alpha/\beta$) receptors localize to the synaptic membrane (cf. Lynch 2004), consistent with the punctate $\alpha$ subunit staining in the thalamus (Ghavanini et al. 2005). In the caudal central nervous system (CNS), glycinergic inhibitory postsynaptic currents (IPSCs) exhibit diverse decay kinetics that correlate to receptor subunit expression. In brainstem and spinal neurons, glycine receptors with $\alpha_1$ or $\alpha_2$ subunits have different kinetic properties (Takahashi et al. 1992, Singer et al. 1998). The $\alpha_1$ subunit predominance in the adult rat bestows fast decay rate for IPSCs. The $\alpha_2$ subunit confers slow IPSC decay, often seen in developing neurons (Takahashi et al. 1992; Ali et al. 2000). Hence, the first objective of the present
study was to examine glycinergic IPSC decay in ventrobasal neurons of the juvenile rat for evidence of kinetic heterogeneity. We also searched for evidence of biphasic decays in spontaneous IPSCs (sIPSCs) sensitive to complete blockade by strychnine with GABA_A antagonists, indicative of co-release from glycinergic and GABAergic pathways (cf. Dumoulin et al. 2001).

Previously, we observed strychnine antagonism of responses to exogenous glycine agonists and diffuse staining for glycine receptor α1 and α2 subunits (Ghavanini et al. 2005). These observations were consistent with extrasynaptic receptor populations. However, functional receptors on extrasynaptic membranes of thalamocortical neurons would require direct demonstration. The second objective of the present studies was to determine if extrasynaptic glycine receptors existed and exhibited the expected kinetic diversity.

For the thalamus as elsewhere, it is not known whether extrasynaptic glycine receptors exhibit differences from synaptic glycine channels. In GABAergic pathways, extrasynaptic GABA_A receptor channels exhibit lower Cl⁻ conductance than synaptic channels (Yeung et al. 2003). Using fluctuation analysis on evoked and spontaneous IPSCs, our third objective was to compare the conductance properties of synaptic and extrasynaptic receptor channels. These studies delineate some unusual facets of glycine receptors and inhibitory transmission in the thalamus.
MATERIALS AND METHODS

Tissue slice preparation

The experimental procedures received approval by Animal Care Committee of University of British Columbia. Sprague-Dawley rats (13-15 day-old) were decapitated while under deep halothane anaesthesia. The brain was rapidly removed and submerged in oxygenated solution at 4 °C containing (in mM): 26 NaHCO$_3$; 1.25 NaH$_2$PO$_4$; 2.5 KCl; 2 MgCl$_2$; 2 CaCl$_2$; 25 dextrose; and 250 sucrose. The solution had an osmolality of 330 mOsmoles. The brains were dissected into two blocks. Using a Vibroslicer (Campden Instruments, London, UK), the block was sectioned into 250-300 µm thick sagittal slices, showing landmarks of the scaphoid nucleus and medial lemniscus (Paxinos and Watson 1986). The slices were incubated for >3 h in artificial cerebrospinal fluid (aCSF) at room temperature (23-25°C), saturated with 95% O$_2$: 5% CO$_2$. The aCSF contained (in mM): 124 NaCl; 26 NaHCO$_3$; 1.25 NaH$_2$PO$_4$; 4 KCl; 2 MgCl$_2$; 2 CaCl$_2$; and 10 dextrose. The aCSF had a pH of 7.3-7.4 and an osmolality of 305 mOsmoles.

IPSC recording

For recording, the slices were placed in a Perspex recording chamber with a ~2 ml volume and were immobilized with a polypropylene mesh. They were perfused with oxygenated aCSF at room temperature, at a rate of 1.5-2 ml/min. Ventrobasal neurons were identified under a differential interference contrast microscope at ×400 (Axioskop 2, Carl Zeiss, Germany). Recording microelectrodes were made using a Narishige puller from thin-wall borosilicate glass tubing (World Precision Instruments, Sarasota, U.S.A.), and filled with a solution containing (in mM): 133 K gluconate; 12 KCl; 4 NaCl; 0.5
CaCl$_2$; 10 EGTA; 3 Mg-ATP; 0.3 Na$_2$-GTP; 2.7 Na$_2$-phosphocreatine, and 10 N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid] (HEPES). The pH was adjusted to 7.3-7.4. The calculated Nernst potentials for this normal patch solution were -53 mV for Cl$^-$ ($E_{Cl}$) and -84 mV for K$^+$ ($E_K$). Electrode resistances ranged between 4-5 MΩ.

Whole-cell recording of IPSCs was performed using amplifiers (Axoclamp 2A; Axon Instruments, Foster City, U.S.A. and List EPC-7; HEKA, Lambrecht, Germany) in the current- and voltage-clamp modes. The neurons were voltage-clamped at $V_h = -80$ mV to minimize contributions of infrequently occurring GABA$_B$ currents. As previously (Ghavanini et al. 2005), ionotropic glutamatergic currents were blocked with kynurenate (1 mM). In 14 neurons, Cs$^+$ (145 mM) and QX-314 (3 mM) were applied intracellularly to suppress K$^+$ and Na$^+$ currents, and Ni$^{2+}$ (500 µM) was applied extracellularly to block T-type Ca$^{2+}$ currents. In such experiments, the [Cl$^-$] was adjusted such that $E_{Cl}$ was zero millivolts. Signals, filtered at 3 kHz and digitized at 10 kHz with a 16-bit data acquisition system, were analyzed using pClamp software (Axon Instruments).

IPSCs were evoked by stimulation at <0.5 Hz with a bipolar electrode placed in the medial lemniscus outside the thalamus, at 1-2 mm from the ventrobasal nuclei. The resistance of the stimulating electrode was 5.5 MΩ. We activated glycine receptors by electrical stimulation of the medial lemniscus, evoking IPSCs during ionotrophic glutamate receptor blockade. The stimulus intensity was adjusted to evoke stable amplitude responses, without failures. The stimulus parameters were a rheobasic current of $3.9 \pm 0.9 \mu$A and a chronaxie of $245 \pm 37 \mu$s (n = 21).
Spontaneous IPSCs were recorded during intracellular application of Cs\(^+\) and QX-314 in neurons that were voltage clamped at -60 mV. Single sIPSCs were visually selected for averaging and creation of search templates. We used the sliding-template procedure of pClamp software, setting the template match stringency to a medium level. Given the observed variability of sIPSC time courses, multiple template searches were required for precise detection of all sIPSCs. The events were monitored visually during the entire procedure, for rejection of sIPSPs with more than a single peak and noise.

We used pClamp, Prism GraphPad, and CorelDraw software for analysis. Exponential functions were fitted to the decay phase of single sIPSCs, and evoked IPSCs averaged from 5-10 individual currents. The double exponential function was the sum of two terms, \(A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)\) where \(A_1\) and \(A_2\) were the amplitudes with time constants \(\tau_1\) and \(\tau_2\), respectively.

Non-stationary noise analysis

We subjected the IPSCs to non-stationary fluctuation analysis to reveal the properties of synaptic channels activated by endogenous transmitters (Traynelis et al. 1993; De Koninck and Mody 1994). We confirmed the stability of quantal release in the IPSCs before subjecting them to non-stationary noise analysis. The amplitude of evoked IPSCs did not significantly change with the stimulus number, which implied no change in quantal release at stimulation frequencies below 0.5 Hz. We grouped 3 successive responses and calculated the coefficients of variation for each triplet. The coefficients of
variation did not change with triplet number, confirming stable quantal release (cf. Scheuss and Neher 2001).

We averaged 10 successive IPSCs, after aligning their peaks in time. Starting at the IPSC peak, the decays were binned at 1.5 ms. $I_{\text{mean}(t)}$, or the average current of each bin (t) was calculated from the relationship, $I_{\text{mean}(t)} = \frac{\sum I_{(t)j}}{n}$, where $I_{(t)j}$ was the current amplitude for trial j for bin t, and n was the number of trials.

The variance ($\sigma^2$) of each bin was calculated from the difference between the scaled average evoked or spontaneous IPSCs, and the individual currents (cf. Traynelis et al. 1993). Using a least squares algorithm, the resulting plot was fitted with a quadratic function, $\sigma^2(t) = i_{\text{Cl}} - I_{\text{mean}(t)} - \frac{I^2_{\text{mean}(t)}}{N} + \sigma_{\text{th}}^2(t)$ where $\sigma_{\text{th}}^2(t)$ denoted residual noise and $i_{\text{Cl}}$ was the elementary current through the agonist-gated channel. The parameter N (total number of channels at the synaptic site) was not considered further since scaling the average IPSC to individual IPSC increases the accuracy of $i_{\text{Cl}}$ but decreases accuracy of N (Traynelis et al. 1993). We also obtained $i_{\text{Cl}}$ as the slope of the initial part of the variance-to-mean current relationship, fitted by linear regression. The results of the two estimates were in good agreement.

The channel Cl$^-$ permeability ($P_{\text{Cl}}$) was calculated from the Goldman-Hodgkin-Katz (GHK) constant-field relationship, $P_{\text{Cl}} = i_{\text{Cl}}(RT/VF^2) \cdot \{ (1 - e^{VF/RT})/([Cl^-]_i - [Cl^-]_o \cdot e^{VF/RT}) \}$, where R, T, and F had their usual meanings, V was membrane potential, and
[Cl\textsubscript{i}] and [Cl\textsubscript{o}] were the intracellular and extracellular Cl\textsuperscript{-} concentrations, respectively. We applied a similar procedure to calculate the P\textsubscript{Cl} from single channel currents.

\textit{Dissociated neuron preparation}

For single channel recording, acutely dissociated neurons were prepared from horizontal slices containing the ventrobasal complex. The slices were initially incubated at room temperature for 10 min in oxygenated, Ca\textsuperscript{2+}-free media composed of (in mM): 120 NaCl; 5 KCl; 1 MgCl\textsubscript{2}; 5 d-glucose; 20 1,4 piperazine-bis-(2-ethanesulfonic acid) (PIPES); ethylene-glycol-bis(2-aminoethylether)-N, N, N', N'-tetraacetic acid (EGTA), and 2 mg/ml bovine serum albumin (BSA) at pH = 7.3. The tissue was then stirred at 32\textdegree C for 45 minutes in a solution of composition (in mM): 120 NaCl; 5 KCl; 1 MgCl\textsubscript{2}; 1 CaCl\textsubscript{2}; 20 PIPES; 2 mg/ml BSA; and, 14 units/ml papain at pH = 7.0. The tissue was rinsed and left for 15 min at room temperature. The cells were mechanically dispersed in 2 ml of Ca\textsuperscript{2+}- and BSA-free PIPES solution and plated on uncoated 35 mm tissue culture dishes. The cells remained in PIPES buffered solution at room temperature until needed for recording.

\textit{Single channel recording and data analysis}

We recorded single channel currents at room temperature (Kim et al. 2004). Dispersed ventrobasal neurons were bathed in a saline containing (in mM): 4 KCl; 135 NaCl; 10 CaCl\textsubscript{2}; 1 MgCl\textsubscript{2}; 10 HEPES; and, 5 d-glucose. The pH was 7.3. Patch pipettes (10-15 M\textOmega) contained a solution (pH 7.3) composed of (in mM): 135 CsCl; 1 MgCl\textsubscript{2};
0.267 CaCl₂; 10 HEPES; 3 EGTA; and 5 D-glucose. $E_{Cl}$ was zero millivolts in these recordings.

Outside-out membrane patches were voltage-clamped with a List EPC-7 amplifier at a holding potential, $V_h = -60$ mV. Amino acids were applied by exchange and perfusion in bath. Responses to agonists reached a steady state within 30 s of switching from control to agonist solutions. The duration of each application was ~5 min. The currents were filtered at DC to 1 kHz, digitized (8 kHz) and analyzed off-line with commercial software (Instrutech Corp., New York, U.S.A.). Single channel openings were detected as transients exceeding 50% of the difference between the averaged baseline and open channel currents, disregarding events briefer than 180 µs. Channel open probability, $P_o$, was calculated as $P_o = (T_1 + 2.T_2 + \ldots N.T_N)/T_{tot}.N$ where $N$ was the number of channels in the patch, $T_{tot}$ the total duration of the record, and $T_1 + 2.T_2 + \ldots N.T_N$ were the times when at least 1,2…$N$ channels were open.

Distributions of open channel times were fitted by a triexponential function. Closed time distributions were fitted by 4 exponential terms. Exponential fitting was performed using Simplex maximization of likelihood. We defined groups of openings as bursts, provided that the openings were separated by gaps shorter than $t_c$, a specified critical time. We calculated $t_c$ by solving $1 - \exp(-t_c/\tau_{c3}) = \exp(-t_c/\tau_{c2})$. Here, $\tau_{c2}$ and $\tau_{c3}$ were the time constants of the second and third fastest components in closed time distributions (Colquhoun and Sakmann 1985; Twyman and Macdonald 1991), as appropriate for a DC to 1 kHz recording bandwidth. Using Simplex methods, we fitted 1
or 2 Gaussian terms to the amplitude distributions of single channel currents. Mean channel conductance was calculated as the weighted sum of the Gaussian fit components.

**Chemicals and drugs**

All chemicals, including glycine, β-alanine, taurine, strychnine, GABA, bicuculline methiodide, gabazine, kynurenate, and QX-314 were purchased from Sigma (Sigma Chemical Co., St. Louis, U.S.A.). Drugs were applied by perfusion of slices, or to the external face of outside-out membrane patches.

**Statistics**

Using bootstrap methods, we estimated the 95% confidence interval for parabolic fits to variance-to-mean current relationships. Data are expressed as mean ± S.E.M. and \( n \) denotes number of neurons or patches. The Kolmogorov-Smirnov test was used to assess goodness-of-fit to normal and Gaussian distributions. For normally distributed data, we used ANOVA for multiple comparisons, and a Tukey post-hoc test for comparing group pairs. Student’s \( t \)-test was used for comparing two groups. For non-normally distributed data, Wilcoxon and Kruskal-Wallis tests were used for comparing two groups, or for multiple comparisons, respectively. Significance was defined as \( P < 0.05 \).
RESULTS

Characteristics of IPSCs

We studied IPSCs evoked by medial lemniscal stimulation in 21 neurons, recorded with the normal solution in the patch electrode. Complete blockade of IPSCs in 14 neurons required co-application of strychnine (1 µM) with a GABA_A antagonist, either bicuculline (25 µM) or gabazine (10 µM; Fig. 1A). The IPSCs in 7 remaining neurons showed exclusive sensitivity to either strychnine or GABA_A antagonist. Strychnine eliminated the IPSCs in 3 neurons, unaffected by prior bicuculline application. Bicuculline or gabazine eliminated IPSCs in 4 neurons, unaffected by strychnine. We refer to currents requiring both strychnine and GABA_A antagonists for elimination, as “mixed IPSCs”.

We first compared the rise times of glycinergic and GABA_Aergic components in the mixed IPSCs. The mean rise time for the glycinergic currents isolated by application of a GABA_A antagonist in neurons held at V_h = -80 mV was 2.6 ± 0.5 ms (n = 7). This value did not differ from the mean rise time (3.0 ± 0.5 ms, n = 7) for GABA_Aergic currents isolated by strychnine application (t-test, P > 0.05). Figure 1A shows the glycinergic and GABA_Aergic currents of 2 mixed IPSCs, obtained by subtraction of the currents during glycine- and GABA_A-receptor antagonism from the control. The peak amplitudes of glycinergic and GABA_Aergic currents often differed between neurons (cf. Fig. 1A). The average peak amplitudes were not different between the two groups at 6 tested holding potentials (ANOVA, P < 0.05; cf. Fig. 1B). The resolved glycinergic and
GABA<sub>A</sub>ergic currents had reversal potentials at $E_{\text{Cl}}$ (Fig. 1B), implicating Cl$^-$ dependence with little or no contribution from other ion species (cf. Bormann et al. 1987).

**Decay kinetics of IPSCs**

Glycinergic currents often displayed more complex decay kinetics than the GABA<sub>A</sub>ergic currents which all decayed monoexponentially with a normal distribution of constants (Fig. 2A,C). Eleven out of 17 glycinergic IPSCs exhibited a monoexponential decay (cf. Fig. 2A). The decay time constants for these IPSCs were not well-fitted by a normal distribution ($P < 0.05$, Kolmogorov-Smirnov test), and likely represented 2 populations. One population had a short decay time constant, $\tau_{\text{str(short)}} = 10 \pm 1.4$ ms ($n = 8$), whereas the other had a long decay time constant, $\tau_{\text{str(long)}} = 70 \pm 4.0$ ms in 3 neurons (ANOVA, $P < 0.01$). These values remained stable over a period of 1.5 h, indicating stationarity of decay kinetics.

Four glycinergic IPSCs decayed with a biexponential time course (cf. Fig. 2B and Ghavanini et al. 2005). Their biexponential time course remained stable over a period of 1.5 h, indicating stationarity of decay kinetics. The 2 remaining IPSCs had discernible fast and slow components, but additionally exhibited long tails. We did not further study these IPSCs because of uncertainties in their exponential fits. The decay time constants ($\tau_1$ and $\tau_2$) for biexponential IPSCs had means of $13 \pm 2.1$ ms and $93 \pm 10$ ms, respectively ($n = 4$). These values do not differ from $\tau_{\text{str(short)}}$ and $\tau_{\text{str(long)}}$, obtained from monoexponential fits ($t$-test, $P > 0.05$). On pooling the data obtained from mono- and biexponential fits, $\tau_{\text{str(short)}}$ was $12 \pm 1.1$ ms ($n = 12$) and $\tau_{\text{str(long)}}$ was $80 \pm 6.8$ ms ($n = 7$),

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as shown in the frequency histogram of Figure 2C. These time constants differed from each other (ANOVA, \( P < 0.01 \)), as well as from \( \tau_{\text{GABA}} = 22 \pm 1.5 \text{ ms} \) (\( n = 18 \); Fig. 2C).

**Decay kinetics of spontaneous IPSCs**

We studied spontaneous IPSCs in 7 neurons, recorded with Cs\(^+\) and QX-314 in the patch electrode. We observed spontaneous inward currents of small amplitude in all neurons. These events occurred at an average frequency of \( 3.8 \pm 0.9 \text{ Hz} \) (cf. Fig. 3A). These events reversed at a \( V_h \) near \( E_{\text{Cl}} \). There was no correlation between the amplitude, rise time, and decay time constant of the events (\( R^2 < 0.01 \)). Hence, the events likely represented genuine sIPSCs rather than random noise.

Strychnine application (1 \( \mu \text{M} \)) decreased the frequency of sIPSCs to \( 0.8 \pm 0.3 \text{ Hz} \) in all neurons (\( P < 0.05 \), paired \( t \)-test). Application of strychnine or gabazine occasionally produced outward changes in the holding current. The average change was \( 20 \pm 15 \text{ pA} \) for strychnine (\( n = 4 \)) and \( 52 \pm 54 \text{ pA} \) for gabazine (\( n = 4 \)). The means did not significantly differ from zero (\( P > 0.05 \), Student’s \( t \)-test). Co-application of strychnine and gabazine (10 \( \mu \text{M} \)) abolished the sIPSCs (Fig. 3A). The average amplitude of the sIPSCs decreased from \(-33 \pm 0.5 \text{ pA} \) to \(-26 \pm 1.0 \text{ pA} \) following the application of strychnine (\( P < 0.05 \), paired \( t \)-test). The average rise time of the sIPSCs was \( 0.9 \pm 0.02 \text{ ms} \) before, and \( 1.1 \pm 0.05 \text{ ms} \) after strychnine. This rise time was significantly shorter than the average for synaptically evoked IPSCs (\( P < 0.05 \), ANOVA). The longer rise times of evoked responses were possibly due to temporal dispersion among developing
pathways. In contrast to medial lemniscal IPSCs, therefore, the sIPSCs were not likely multi-quantal events.

After we aligned the peaks and scaled the sIPSCs to the same amplitude, 3 distinct time courses were evident in all neurons (cf. Fig. 3C). Fast sIPSCs completely decayed within 100 ms, whereas intermediate sIPSCs required 100 to 200 ms and slow sIPSCs required 500 to 1000 ms for complete decay. The majority of sIPSCs had a decay phase that was well fitted with a single exponential function (Fig. 3C). A bi-exponential function was required for an appropriate fit in <6% of sIPSCs. The mean amplitude of bi-exponential sIPSCs (-40 ± 1.9 pA) was slightly higher than the mean for mono-exponential IPSCs (-32 ± 0.5 pA) IPSCs (P <0.05, unpaired t-test). We observed a higher percentage of bi-exponential decays in IPSCs evoked by medial lemniscal stimulation (~24%) of neurons displaying mixed IPSCs, than in sIPSCs (<6%). The reasons for the higher percentage are unclear. One possibility is that electrical stimulation co-activated different nerve fibers, producing more biphasic responses.

Figure 3C illustrates the frequency distribution of decay time constants for the sIPSCs. Before strychnine application, the distribution was well described with sum of 3 Gaussian functions (P < 0.05, Kolmogorov-Smirnov test). This fit implied 3 populations of sIPSCs. Strychnine abolished the fastest and the slowest sIPSCs with average time constants of 11 ± 0.1, 22 ± 0.1 and 74 ± 2.4 ms, but not the GABAergic sIPSCs with an average time constant of 22 ± 0.2 ms (Fig. 3B). Hence, glycinergic sIPSCs had distinct fast and slow decay kinetics. The decay time constants of glycinergic and GABAergic
sIPSCs matched the respective time constants of evoked IPSCs (Fig. 3D, \( P > 0.05 \), ANOVA). Hence, the 3 decay time constants of sIPSCs likely represent genuine findings and decay kinetics of synaptic receptors.

**Kinetic properties of extrasynaptic receptors**

Application of glycine, taurine, and β-alanine induced inward, single channel currents in membrane patches (Fig. 4A). Prior to agonist application, the outside-out patches infrequently displayed spontaneous currents at -60 mV. Glycine (20 µM) activated currents in 11 out of 27 patches. In separate experiments, taurine (20 µM) activated currents in 12 out of 28 patches, and β-alanine (20 µM) activated currents in 8 out of 30 patches. The means of \( P_o \) for activations by glycine (0.029 ± 0.018, \( n = 11 \)), taurine (0.029 ± 0.031, \( n = 10 \)), and β-alanine (0.042 ± 0.013, \( n = 7 \)) were not different (\( P > 0.05 \), ANOVA). \( P_o \) had a tendency to decline during agonist application and hence, we did not test more than one agonist on individual patches. When strychnine (1 µM) was co-applied with glycine, taurine, or β-alanine, single channel currents occurred very infrequently (overall \( P_o <0.001 \)). An observed reversal potential near \( E_{Cl} \) and sensitivity to strychnine implicated glycine receptors in the agonist-evoked currents.

To compare the burst durations of agonist-induced currents, we calculated the critical time, \( \tau_c \), from channel closed time distributions. The closed time distributions were well described by the sum of 4 exponentials, as exemplified for glycine in Figure 4B. The calculated values of \( \tau_c \) did not differ between channels that displayed short- or long-duration bursts (\( P > 0.05 \), ANOVA). The mean \( \tau_c \) for glycine (7.3 ± 0.8 ms, \( n = \))
11), taurine (8.3 ± 0.8 ms, n = 10), and β-alanine (8.9 ± 0.9 ms, n = 7) did not differ (P > 0.05, ANOVA).

The currents activated by the β-amino acids displayed either short or long-duration bursts (Fig. 4A). Glycine activated short-duration bursts in 10 out of 11 patches (Fig. 4A), and long-duration bursts only in 1 patch. Taurine-activated currents were characterized by short-duration bursts of openings in 6 out of 10 patches, and long-duration bursts in the remaining 4 patches. β-alanine activated short-duration bursts in 4 out of 7 patches, and long-duration bursts in the remaining 3 patches.

As exemplified by taurine (Fig. 4C), burst-duration distributions were well described by the sum of 3 exponentials. The mean burst duration for glycine-activated channels was 19 ± 4 ms, whereas the sole long-duration burst averaged 87 ms. The taurine-activated short-duration bursts had a mean of 26 ± 4 ms, whereas long-duration bursts averaged 88 ± 8 ms. The β-alanine activated short-duration bursts had a mean of 21 ± 4 ms, whereas long-duration bursts averaged 137 ± 5 ms. The average lifetimes of short-duration bursts did not depend on the nature of the agonist (cf. Fig. 4D). The average lifetimes of long-duration bursts activated by taurine or β-alanine did not differ (P > 0.05, ANOVA). For the β-amino acids, short and long bursts differed significantly from each other in duration, and likely represented 2 populations (cf. Fig. 4D, P < 0.05, ANOVA).
**Conductance of synaptic and extrasynaptic receptors**

To estimate the Cl⁻ permeability of synaptic receptor channels for comparison with the extrasynaptic channels, the first step was to determine the elementary current, $i_{Cl}$, during synaptic activation. The variance-to-mean current relationships for both short- and long-duration glycinergic IPSCs were well-described by a quadratic function (Fig. 5A). From these fits, the mean $i_{Cl}$ for short-duration IPSCs (-0.6 ± 0.2 pA; n = 12) did not differ from the mean $i_{Cl}$ for long-duration IPSCs (-0.8 ± 0.2 pA, n = 8; ANOVA, $P > 0.05$). These means were not significantly different from the mean $i_{Cl}$ for GABAₐergic IPSCs (-0.6 ± 0.1 pA, n = 18; $P > 0.05$, ANOVA).

Estimates of $i_{Cl}$ from multi-quantal IPSCs can undergo distortion from fluctuations in transmitter release on a trial-to-trial basis, as well as from other factors (cf. Diamond and Jahr 1995). We sought to overcome this limitation by calculating $i_{Cl}$ from sIPSCs with $E_{Cl} = 0$ mV, which have a predominantly mono-quantal nature (cf. Fig. 5A). For short-duration sIPSCs, we found a mean $i_{Cl} = -3.5 ± 0.6$ pA (n = 6). For long-duration sIPSCs, the mean $i_{Cl}$ was -2.8 ± 0.8 pA (n = 6). These values did not differ from each other or from the value of -2.5 ± 0.5 pA (n = 7) obtained for GABAₐergic sIPSCs ($P > 0.05$, ANOVA). I-V relationships for the $i_{Cl}$s calculated from glycinergic and GABAₐergic sIPSCs were linear and showed apparent reversal potentials near $E_{Cl}$ (Fig. 5B). The single channel conductances for fast (58 ± 10 pS, n = 6), and slow (46 ± 14 pS, n = 6) glycinergic sIPSCs did not differ from each other, or from the conductance (41 ± 9 pS, n = 7) from GABAₐergic sIPSCs ($P > 0.05$, ANOVA).
For comparison of $i_{\text{Cl}}$s obtained from IPSCs and sIPSCs under differing holding potentials and $E_{\text{Cl}}$s, we used the GHK equation to convert the values to chloride permeability, $P_{\text{Cl}}$. Mean $P_{\text{Cl}}$ for short-duration glycinergic IPSCs was $1.6 \pm 0.5 \times 10^{-13}$ cm$^3$/s for evoked ($n = 12$), and $1.1 \pm 0.2 \times 10^{-13}$ cm$^3$/s for spontaneous ($n = 6$) responses. Mean $P_{\text{Cl}}$ for long-duration glycinergic IPSCs was $1.7 \pm 0.4 \times 10^{-13}$ cm$^3$/s for evoked ($n = 8$), and $0.9 \pm 0.3 \times 10^{-13}$ cm$^3$/s for spontaneous ($n = 6$) responses. As shown in Fig. 5C, $P_{\text{Cl}}$s from evoked and spontaneous IPSCs were not different ($P > 0.05$, unpaired $t$-test). The glycinergic $P_{\text{Cl}}$s did not differ from evoked ($1.5 \pm 0.3 \times 10^{-13}$ cm$^3$/s, $n = 18$) and spontaneous ($0.8 \pm 0.2 \times 10^{-13}$ cm$^3$/s, $n = 7$) GABA$\text{A}$ergic $P_{\text{Cl}}$s ($P > 0.05$, ANOVA).

All 3 agonists evoked extrasynaptic currents of small and large amplitude (arrowheads, Figs. 4A and 6A,B). Smaller currents were seen only in the presence of larger amplitude currents. Hence, the small currents likely reflected openings to a substate conductance, appropriately 70% of the full conductance. Amplitude distributions for the currents were well described by the sum of 2 Gaussian terms (Fig. 6A). The current-voltage (I-V) relationships were linear over the range of 0 to -60 mV (Fig. 6B). From these relationships, the mean conductances from short-duration bursts were $15 \pm 1$ pS ($n = 10$), $21 \pm 2$ pS ($n = 6$) and $22 \pm 3$ pS ($n = 4$) for glycine, taurine and $\beta$-alanine, respectively. The mean conductances from long-duration bursts were $19$ pS ($n = 1$), $33 \pm 1$ pS ($n = 4$) and $30 \pm 4$ pS ($n = 3$) for glycine, taurine and $\beta$-alanine, respectively. These conductances were not different ($P > 0.05$, ANOVA).

$P_{\text{Cl}}$ of synaptic and extrasynaptic receptors

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For comparison, we converted extrasynaptic single channel currents to P_{Cl}s. In view of the similar P_{Cl}s from evoked and spontaneous IPSCs, as well as the similar extrasynaptic P_{Cl}s for the agonists, we pooled the data into synaptic and extrasynaptic categories. Table 1 shows the distinct nature of P_{Cl}s of synaptic and extrasynaptic channels. The short- and long-duration, glycinergic synaptic channels yielded P_{Cl}s that were higher than the estimates from short- and long-duration bursts activated by the agonists (P < 0.05, unpaired t-test). The GABAergic synaptic channels yielded P_{Cl}s that were higher than the estimates from extrasynaptic channels (P < 0.05, unpaired t-test) obtained from Kim et al. (2004).

We tested the possibility that voltage-dependent currents contributed to a higher estimate of P_{Cl}, by blocking voltage-dependent currents with Ni^{2+}, QX-314, and Cs^+ in 7 neurons. sIPSCs were observed only infrequently during extracellular Ni^{2+} application in all neurons. No glycinergic IPSCs were observed in these neurons, possibly due to pre- and postsynaptic blocking actions of Ni^{2+} (e.g., postsynaptic, Doi et al. 1999). The average P_{Cl} of GABAergic IPSCs was 1.7 ± 0.8 x 10^{-13} cm^3/s and did not differ from P_{Cl} in the absence of Ni^{2+}, QX-314 and Cs^+ (t-test, P > 0.05).
DISCUSSION

These studies have revealed some unusual facets of inhibitory transmission in thalamus. Spontaneous IPSCs mediated by glycine- and GABA_A-receptors were monophasic, showing fast, intermediate, and slow decays. IPSCs evoked by medial lemniscal stimulation also showed fast, intermediate, or slow decays, alone or in combination. Currents with intermediate decays were due to co-transmission by a GABA_A-receptor mediated pathway. Strychnine antagonized the fast and slow synaptic currents, mediated by glycine-like amino acids. Extrasynaptic receptors, activated by glycine agonists in membrane patches, displayed the predicted short- or long-duration burst openings (cf. Introduction). The dual kinetics of synaptic and single channel currents implicate functional diversity in glycine receptors at a juvenile stage of rat development.

The chief finding of these studies was glycinergic sIPSCs and components of mixed IPSCs, decaying with fast or slow kinetics. Most sIPSCs (>94%) exhibited mono-exponential decays with fast (11 ms) or slow (74 ms) time constants. The fast and slow time constants of sIPSCs, which largely represented mono-quantal packets of transmitter, matched the fast (12 ms) and slow (80 ms) time constants of evoked IPSCs. This finding provided assurance that spontaneous and evoked IPSCs were attributable to the same glycine-receptor populations. The observations were consistent with the activation of 2 kinetically distinct populations of glycine receptors. Another important finding was that fast or slow synaptic currents occurred separately in different neurons. These IPSCs also decayed in a mono-exponential manner, suggesting that the slow IPSCs were not likely
due to a spillover of transmitter to peri-synaptic receptors (cf. Chery and De Koninck 1999). Based on the observations on spontaneous and evoked IPSCs, we suggest that the receptor populations are predominantly localized under separate nerve terminals.

Our observations of fast and slow mono-quantal sIPSCs contrast with the literature. In embryonic zebrafish (Ali et al. 2000), sIPSCs have bi-exponential decay due to co-localization of receptors with fast and slow kinetics at the same synaptic sites. Spontaneous IPSCs decay mono-exponentially with a fast (4-8 ms) time constant in rat spinal neurons (Chery and De Koninck, 1999; Gonzáles-Forero and Alvarez, 2005) and with a slow (~63 ms) time constant in mouse retinal ganglion cells (Tian et al. 1998). Apparently, thalamic neurons in juvenile rats have a predominant ability to segregate 2 populations of glycine receptors with fast and slow kinetics.

The fast and slow kinetics of the synaptic currents were likely due to structurally distinct receptor populations. The α₁ and α₂ receptor subunits (Ghavanini et al. 2005) determine synaptic decays of fast and slow IPSCs (Takahashi et al. 1992; Singer and Berger 1999). Given their very long burst duration, receptors containing α₂, but not α₁ subunits (Mangin et al. 2003), may account for the long decay tails of 2 atypical IPSCs in this study. Co-assembly of α₁ and α₂ subunits likely occurs in developing neurons, where slow IPSCs are common (Takahashi et al. 1992; Ali et al. 2000). Hence, persistence of α₁/α₂ receptors in thalamic neurons may have resulted in the slow kinetics. Slow glycinergic inhibition contrasts with metabotropic GABAergic inhibition (Browne et al. 2001), mostly suppressed in our recordings. Another possibility is that post-translational...
phosphorylation of glycine receptor channels (Agopyan et al. 1993), produced diverse kinetics.

The kinetics of extrasynaptic glycine receptor channels resembled the decays of glycinergic currents. The average lifetimes of short- and long-duration bursts activated by glycine, taurine, and β-alanine were close to decay time constants for fast and slow IPSCs. The multiple congruencies in kinetics seem unlikely to have occurred by chance although burst duration may depend on high agonist concentrations (cf. Beato et al. 2002) at glycinergic synapses.

There are reasons for postulating differences between synaptic and extrasynaptic glycine receptors. We observed that synaptic channels had higher $P_{\text{Cl}}$s than extrasynaptic channels. The $P_{\text{Cl}}$ estimates were similar when measured with an optimized space-clamp and were not likely due to vagaries in fluctuation analysis (cf. Benke et al. 2001). The unitary conductance obtained from sIPSCs was in the same range as in other preparations under similar conditions (cf. Poncer et al. 1996; Singer and Berger 1999). The synaptic GABA$_A$ergic channels had a higher conductance compared to extrasynaptic channels, as found elsewhere (Yeung et al. 2003). The low conductances of extrasynaptic glycine receptors were compatible with embryonic receptor channels (Rajendra et al. 1997) and extrasynaptic receptors on hippocampal neurons (Fatima-Shad and Barry 1995). Given these considerations, we suggest that the conductance differences were genuine. Extrasynaptic receptors, usually thought as high conductance homomers (Lynch 2004), in
this case may have reduced conductance, reflecting post-translational modification (cf. Caraiscos et al. 2002).

The present results are compatible with co-transmission by glycinergic and GABAergic pathways, rather than co-release of glycine-like amino acids and GABA. An appreciable number of neurons showed exclusively glycinergic or GABAergic responses to medial lemniscal stimulation, consistent with co-transmission by independent pathways. If co-release of glycine-like amino acids and GABA were to occur (Jonas et al. 1998), we would expect a prevalence of multiphasic sIPSCs in each neuron, converting on strychnine application to monophasic GABAergic currents (cf. Dumoulin et al. 2001). In contrast, the majority of sIPSCs in 7 tested neurons showed a monophasic decay, with or without strychnine application. We conclude that if present in thalamic inhibition, co-release was a less common occurrence than co-transmission.

**Physiological implications**

Fast synaptic kinetics allow rapid phasic transfer of information for somatotopic representations of rapidly adapting receptors (cf. Tsumoto and Nakamura 1974). Slow IPSP decays affect hyperpolarization-activated currents, remove Ca\(^{2+}\) channel inactivation, and promote low threshold Ca\(^{2+}\) bursting (cf. Steriade et al. 1997). The glycinergic IPSC components were kinetically distinct from GABAergic IPSCs (~22 ms; cf. Dumoulin et al. 2001). The co-occurrence of fast and slow glycinergic IPSPs with intermediate GABAergic IPSPs would confer fine tuning of inhibitory transmission
by modulation of voltage-dependent currents in somatosensory thalamus. The higher $P_{Cl}$ of synaptic receptors ensures high transmission efficacy.

Despite the differences in $P_{Cl}$, the striking similarities between IPSC decay and extrasynaptic channel burst duration imply that glycine, taurine, and $\beta$-alanine each could mediate inhibition. When applied at the same concentration, glycine, taurine and $\beta$-alanine activated channels with comparable open probabilities. The abilities of $\beta$-amino acids, relative to glycine, to activate long-duration bursts was greater at extrasynaptic receptors than most receptor variants (cf. Flint et al. 1998; Martin & Siggins, 2002). The lower Cl$^-$ permeability may suit extrasynaptic receptors for the detection of ambient $\beta$-amino acids, tonic inhibition, and receptor modulation (cf. Berger et al. 1998; Flint et al. 1998; Mori et al. 2002).

Thalamocortical neurons segregate ionotropic glycine receptors showing fast and slow decay kinetics. Co-transmission with $\text{GABA}_A$ receptors showing intermediate kinetics, and known metabotropic $\text{GABA}_B$ receptors, facilitate postsynaptic discrimination of inputs in neurons of somatosensory nuclei.
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Figure Legends

Figure 1. Medial lemniscal mixed IPSCs resolved into glycinergic and GABA<sub>A</sub>ergic currents reversed at \( E_{Cl} \). A (upper panel): 10 µM gabazine slightly reduced control IPSC, yielding a large glycinergic current which was eliminated by co-applied gabazine and 1 µM strychnine (GbZ and Str). Subtraction of current during gabazine from control IPSC yielded a small GABA<sub>A</sub>ergic current. A (lower panel): 10 µM gabazine greatly suppressed control IPSC in a second neuron, yielding a small glycinergic current eliminated by co-applied gabazine and 1 µM strychnine. Subtraction of current during gabazine from control IPSC yielded a large GABA<sub>A</sub>ergic current. Currents were averaged from 10 IPSCs. B: reversal potentials for glycinergic (11 neurons) and GABA<sub>A</sub>ergic (14 neurons) components of mixed IPSCs were identical to \( E_{Cl} \) (arrows). Mean peak values of glycinergic and GABA<sub>A</sub>ergic components did not differ at \( V_h = -80 \) mV (ANOVA, \( P > 0.05 \)).
Figure 2. Resolved currents had different decays. A: glycinergic and GABAergic currents were peak aligned and scaled to the same amplitude for comparison of time courses in different neurons. The glycinergic current decayed faster in the neuron of Aa, and slower in the neuron of Ab, when compared to the GABAergic currents. B: the decay phase of the glycinergic current in another neuron was well-fitted by the sum of two exponential terms (upper trace, smooth curve). The lower trace shows these terms and their time constants. C: frequency distribution histograms of decay time constants for glycinergic and GABAergic currents. The arrowheads indicate mean values. $V_h = -80 \text{ mV}, E_{Cl} = -53 \text{ mV}$. A and B show averages of 10 IPSCs.
Figure 3. sIPSCs recorded with Cs\(^+\) and QX-314 in patch electrode. A: Sample records of ~1 min duration show currents from neuron in control, strychnine (Str, 1 \(\mu\)M), as well as strychnine and gabazine (Gbz, 10 \(\mu\)M) conditions. Strychnine decreased frequency of sIPSCs. Co-application of Str and Gbz abolished the sIPSCs. B: Frequency distribution of decay time constants from 7 neurons. Before strychnine application, the distribution was well-described with sum of 3 Gaussian functions with mean time constants (arrowheads) of 11 ± 0.1, 22 ± 0.1, and 74 ± 2.4 ms. Strychnine abolished fast and slow sIPSCs, but not the sIPSCs, fitted with a single intermediate mean time constant (arrowhead) of 22 ± 0.2 ms. C: Examples of unaveraged sIPSCs, scaled to the same amplitude, show fast, intermediate, and slow decay time courses. The smooth curves represent single exponential fits to the peak-aligned sIPSCs (indicated decay time constant, \(\tau\)). The slow sIPSC that had a longer decay time constant than the average time constant of the slow sIPSC (cf. panel D) was selected for illustrative clarity. D: Mean decay time constants for glycinergic sIPSCs did not differ from evoked IPSCs \((P > 0.05, \text{ANOVA})\). Numbers of neurons are indicated in parentheses. For sIPSCs, \(V_h = -60\) mV and \(E_{Cl} = 0\) mV.
**Figure 4.** Glycine receptor agonists activate short- or long-duration bursts of channel openings. A: representative currents obtained from separate membrane patches. Channel openings appear as downward deflections from baseline (inward currents). Arrows indicate currents associated with a substate conductance. The insert under the long-duration bursts activated by β-alanine shows a segment of the main trace with higher amplification and time-base. Current levels indicative of closed channel (c), substate conductance (s, arrow) and full open conductance (o) states are indicated by dashed lines. B: distribution of closed times (n = 2,742 events) for glycine-activated currents from an additional patch showing fit by the sum of four exponential terms, with areas and time constants (arrowheads): A$_1$ = 0.45, $\tau_1$ = 0.2 ms, A$_2$ = 0.28, $\tau_2$ = 8.1 ms, A$_3$ = 0.19, $\tau_3$ = 19.8 ms, A$_4$ = 0.08, $\tau_4$ = 173 ms. C: distributions of burst durations for taurine-activated currents in two additional patches showing short- and long-duration bursts. Each distribution was well-fitted by the sum of 3 exponentials with A$_1$ = 0.03, $\tau_1$ = 3.0 ms, A$_2$ = 0.88, $\tau_2$ = 20.1 ms, A$_3$ = 0.09, $\tau_3$ = 103 ms, $\tau_c$ = 9.0 ms, and mean duration = 26.7 ms for short-duration bursts (n = 552 bursts), and A$_1$ = 0.42, $\tau_1$ = 15.7 ms, A$_2$ = 0.40, $\tau_2$ = 84.7 ms, A$_3$ = 0.18, $\tau_3$ = 730 ms, $\tau_c$ = 5.7 ms, and mean duration = 172 ms for long-duration bursts (n = 506 bursts). Arrowheads indicate time constants. D: mean durations of short- and long-duration bursts activated by glycine receptor agonists. Numbers in parentheses indicate patches. Mean durations of short-duration bursts activated by taurine and β-alanine were significantly different from long-duration bursts activated by the same agonist (*†$P < 0.05$, ANOVA). Agonists were applied at 20 µM to outside-out patches. $V_h$ = -60 mV, $E_{Cl}$ = 0 mV.
Figure 5. Estimation of elementary channel currents from non-stationary noise analysis of glycinergic currents. A: 10 successive, short- and long-duration glycinergic IPSCs in 2 neurons, and 37 short-duration sIPSCs in 1 neuron. Current variance was plotted as a function of mean current for the neurons. Variance-mean relationships were fitted with

\[ \sigma^2(t) = i_{Cl} I_{\text{mean}(t)} - I_{\text{mean}(t)}^2/N + \sigma_{\text{th}}^2(t) \] (dark curves). \( i_{Cl} \) was obtained from the slopes of the initial part in the variance-average current relationships, fitted by linear regression. The two methods yielded similar values of \( i_{Cl} \). The estimates were \( i_{Cl} = -0.9 \) pA for a short-duration evoked IPSC (left), \( i_{Cl} = -0.5 \) pA for a long-duration evoked IPSC (middle), \( i_{Cl} = -3.3 \) pA for a short-duration sIPSC (right). When averaged, \( i_{Cl} \) did not differ for short and long IPSCs (\( n = 20; P > 0.05, \text{ANOVA} \)). The gray lines show 95% confidence intervals. For evoked IPSCs, \( V_h = -80 \) mV, \( E_{Cl} = -53 \) mV. For sIPSCs \( V_h = -60 \) mV, \( E_{Cl} = 0 \) mV.

B: \( I-V \) relationship for glycinergic and GABA\( \alpha \)ergic sIPSCs (3 neurons). \( E_{Cl} = 0 \) mV.

C: Comparison of \( P_{Cl} \) values estimated for short and long duration sIPSCs and evoked IPSCs. Numbers in parentheses indicate numbers of neurons. There were no differences between fast and slow responses, or between spontaneous and evoked responses (\( P > 0.05, \text{ANOVA} \)).
Figure 6. Conductance of single channels activated by glycine receptor agonists. A: amplitude distributions for agonist-activated currents (V_h = -60 mV) were fitted by the sum of 2 Gaussian terms (smooth curves). Means (arrowheads) were -1.2 pA and -0.96 pA for glycine (n = 1473 transitions), -1.6 pA and -1.2 pA for taurine (n = 2465 transitions), and -1.7 pA and -1.3 pA for β-alanine (n = 1144 transitions). B: single channel I-V relationships for the full and substate conductances of glycine-activated receptor channels. Data were obtained from 12 patches. Linear regression fits indicated mean values of 13 pS and 17 pS for the substate and full conductances, respectively. In all panels, agonists were applied at 20 µM to outside-out patches with E_{Cl} = 0 mV. C: comparison of P_{Cl} values for short and long duration channel bursts, activated by glycine receptor agonists. Numbers in parentheses indicate number of patches. There were no significant differences in values obtained (P >0.05, ANOVA).
TABLE 1. *Chloride permeability of synaptic and extrasynaptic glycine and GABA<sub>A</sub> receptors*

<table>
<thead>
<tr>
<th></th>
<th>P&lt;sub&gt;Cl&lt;/sub&gt; ((×10^{-13} \text{ cm}^3/\text{s}))</th>
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<td><strong>Fast glycinergic</strong></td>
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<tr>
<td>Synaptic (18)</td>
<td>1.4 ± 0.3*</td>
</tr>
<tr>
<td>Extrasynaptic (20)</td>
<td>0.4 ± 0.02</td>
</tr>
<tr>
<td><strong>Slow glycinergic</strong></td>
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</tr>
<tr>
<td>Synaptic (13)</td>
<td>1.4 ± 0.3*</td>
</tr>
<tr>
<td>Extrasynaptic (8)</td>
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<tr>
<td><strong>GABA&lt;sub&gt;A&lt;/sub&gt;ergic</strong></td>
<td></td>
</tr>
<tr>
<td>Synaptic (25)</td>
<td>1.3 ± 0.2*</td>
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<tr>
<td>Extrasynaptic (8)</td>
<td>0.3 ± 0.01†</td>
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Data are Means ± SEM and number of observations in parentheses. See text for explanation.

*Significant \((P < 0.05, \text{ unpaired } t\)-test).† Data from Kim et al. (2004).
Figure 1
Figure 2
Figure 3
Figure 4

A

Short-Duration Bursts

Glycine

Taurine

L-Alanine

Long-Duration Bursts

Taurine

β-Alanine

B

Closed

Number of events

Log closed time (s)

C

Short-duration

Log burst duration (s)

D

Long-duration

Log burst duration (s)

Burst duration (ms)

Gly Taur β-Ala Short Taur β-Ala Long

(1) (6) (4)
Figure 5
Figure 6