Biphasic modulation of GABA release from stellate cells by glutamatergic receptor sub-types

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ABSTRACT

The release of inhibitory transmitters from CNS neurons can be modulated by ionotropic glutamate receptors that are present in the presynaptic terminals. In the cerebellum, glutamate released from climbing fibres (but not from parallel fibres) activates presynaptic AMPA receptors and suppresses the release of the inhibitory transmitter, GABA, from basket cells onto post-synaptic Purkinje cells. This input specific modulation has been attributed to the close proximity of the climbing fibres to the axons of the basket cells. Our recent work indicates that glutamate released from parallel fibres can “spillover” and reach the axons of stellate cells. Here I test the possibility that this spillover glutamate can activate presynaptic AMPA receptors in stellate cells and in this way modulate their release of GABA. I find that stimulation of parallel fibres activates AMPA receptors and transiently suppresses autoreceptor and autaptic GABAergic currents in stellate cells. Activation of AMPA receptors reduces the release of GABA and the suppression occurs more frequently in immature cells that have a high release probability. By contrast the release of GABA from mature stellate cells that have a low release probability is potentiated by the activation of NMDA-type glutamate receptors on presynaptic terminals. Thus during development, the glutamatergic modulation of GABA release switches from an AMPA receptor-mediated transient suppression to a NMDA receptor-induced lasting potentiation.
INTRODUCTION

Inhibitory synapses control the timing and firing patterns of neurons by limiting the level of synaptic depolarization and by shunting excitatory currents. Excitatory transmitters such as glutamate can directly regulate inhibitory transmission by presynaptically changing the release of inhibitory transmitters (Belan and Kostyuk, 2002; Engelman and MacDermott, 2004). This modulation can change the balance between excitatory and inhibitory inputs and alter the output of a neuronal network.

One example is the GABAergic stellate and basket cells located in the molecular layer of the cerebellum. These interneurons form inhibitory synapses onto Purkinje cells and other stellate cells and suppress their activity (Hausser and Clark, 1997). Glutamate can be released from one of two inputs. The release from climbing fibres activates presynaptic AMPA receptors (AMPARs) on basket cells and transiently reduces the release of GABA onto Purkinje cells (Satake et al., 2000). By contrast stimulation of the second glutamatergic input, the axons of granule cells (parallel fibres), does not decrease GABA release from the basket cells (Rusakov et al., 2005). This input-specific modulation of GABA release from basket cells has been attributed to the close proximity of the climbing fibres to the axons of basket cells. However unlike basket cells that are located near the soma of Purkinje cells, stellate cells are distributed in the upper two thirds of the molecular layer, where the parallel fibres are abundant. Sensory stimulation evokes a burst of action potentials in cerebellar granule cells (Chadderton et al., 2004) and direct stimulation of these neurons evokes glutamate release which activates extrasynaptic AMPARs on stellate cells (Carter and Regehr, 2000). In a recent study we found that stimulation of parallel fibres could activate NMDA-type glutamate receptors on the presynaptic terminals of stellate cells (Liu and Lachamp, 2006). Thus the glutamate that spills over from the parallel fibres can reach the axons of stellate cells, and may therefore also activate AMPARs and suppress their release of GABA.

The mechanism underlying the suppression of GABA release from basket cells involves the AMPA-induced reduction in Ca$^{2+}$-entry through voltage-gated channels at the presynaptic terminal (Rusakov et al., 2005; Satake et al., 2006). Interestingly AMPA reduces the action potential-evoked Ca increase only in ~50% of basket cell terminals (Rusakov et al., 2005). What determines the sensitivity of an axon terminal to AMPAR-mediated modulation is not known.

Stellate cells not only innervate Purkinje cells but also innervate themselves by GABAergic autaptic connections. Additionally GABA released from stellate cells can activate axonal autoreceptors (Pouzat and Marty, 1998; 1999). This autaptic synapse and autoreceptor current has proven to be very useful experimentally since it can be used to detect the evoked secretion of GABA from a single axon. Using this preparation we addressed two questions. First, could physiological-like stimulation of granule cells activate AMPARs and reduce the inhibitory autaptic/autoreceptor current in stellate cells? Second, if modulation was indeed present, did the AMPA-induced suppression of IPSCs occur at all axonal terminals or was it limited to a distinctive subpopulation?
METHODS

Slice preparation: Saggital or horizontal cerebellar slices (250µm) were obtained from P13-P20 C57BL/6 mice with a Leica VT1000S vibrating microslicer in an ice-cold slicing solution as previously described (Liu and Cull-Candy, 2005). The slicing solution contained (in mM) 125 NaCl, 2.5 KCl, 1 CaCl2, 7 MgCl2, 1.25 NaH2PO4, 26 NaHCO3, and 25 glucose, (pH 7.4) and was continuously bubbled with 95% O2 and 5% CO2. Slices were maintained at room temperature for at least 1 hour before recording.

Electrophysiological recordings:

Voltage-clamp recordings were made using an Axopatch 700A amplifier (Axon Instruments, Foster City, CA) in an extracellular solution (in mM: 125 NaCl, 2.5 KCl, 2 CaCl2, 1 MgCl2, 1.25 NaH2PO4, 26 NaHCO3, and 25 glucose, pH 7.4) saturated with 95% O2 and 5% CO2. Recordings were made from visually identified neurons located in the outer two-thirds of the molecular layer. Stellate cells were identified by their ability to fire spontaneous action potentials in the cell-attached configuration and by the presence of spontaneous excitatory and inhibitory synaptic currents in the perforated patch configuration.

Autaptic and autoreceptor currents (aIPSCs) were evoked by a 1 ms depolarization to 0 mV from a holding potential of -70 mV in a voltage-clamp configuration at 0.3 Hz. aIPSCs were filtered at 2 kHz and digitized at 10 kHz. Electrodes with a resistance of 4-8MΩ were filled with a pipette solution (in mM: 150 KCl, 4.6 MgCl2, 0.1 CaCl2, 10 HEPES, 1 EGTA, 4 Na-ATP, and 0.4 Na-GTP, pH 7.4) that included amphotericin B (300µg/ml). Series resistance was monitored throughout the experiment. If this changed by more than 20%, the experiment was terminated.

Stimulation of parallel fiber inputs: parallel fibres in horizontal slices were stimulated by a train of 4 depolarizations at 100Hz using a bipolar electrode (stimulation strength: 6-17 V; stimulation duration 20-140 µs) placed across the molecular layer about 200µm from the recording electrode. An aIPSC was then evoked 100 ms after each burst. Since presynaptic AMPA receptors in stellate cells are activated by “spillover” glutamate released from PFs, activation of these AMPARs might depend strongly on temperature. Thus recordings were made at 36ºC. Recordings at 36ºC were stable for 10-15 minutes and therefore were not ideal for longer-term recordings.

Application of AMPA: aIPSCs were recorded at room temperature prior to and during the application of AMPA (0.3µM AMPA was applied for 10 minutes). At room temperature perforated patch recordings lasted for 20 – 60 minutes (series resistance was found to remain stable for 1 hour in ~20% of the recorded cells). This was sufficient time to monitor the long-term change in aIPSC amplitude.

Recordings of miniature spontaneous IPSCs (mIPSCs) were made in the presence of 0.4 µM TTX (tetrodotoxin), 10 µM CPP, 1 µM AM-251 and 10 µM SCH50911 in the whole
cell configuration. mIPSCs were recorded for 4 minutes before, during and after AMPA application.

Data analysis: average aIPSCs were obtained from 10 consecutive sweeps using Clampfit (version 9.0, Axon Instruments). Each mean paired-pulse response was constructed from 50–70 events. The paired pulse ratio was calculated as mean aIPSC$_2$ amplitude/mean aIPSC$_1$ amplitude. The coefficient of variation of synaptic transmission was calculated from the peak amplitude of at least 70 events. Data are expressed as mean±SEM. A two-tailed Student’s t test was used to assess statistical significance.
RESULTS

The release of GABA from single axons of cerebellar stellate cells was examined by measuring the autaptic/autoreceptor currents (aIPSCs) that were evoked by a 1 ms depolarization. This current was inhibited by SR95531 and therefore is mediated by the activation of GABA_A receptors (Supplemental Figure 1).

**Burst stimulation of parallel fibre inputs transiently suppressed aIPSCs.**

Autoreceptor currents were recorded at 36°C in a perforated patch configuration from P12-13 stellate cells. To determine the effects of glutamate released from parallel fibres (PFs), an aIPSC was evoked 100 ms following a train of PF stimulation. The ACSF contained 10 μM CPP, 1 μM AM-251 and 10 μM SCH50911 to block NMDA, CB1 and GABA_B receptors, respectively (Figure 1A and 1B). The aIPSC was compared with the control aIPSC without PF stimulation.

As shown in Figure 1, PF stimulation reduced the amplitude of aIPSCs by 14.1±2.9% (n=5, p<0.03, paired t-test). The suppression was transient as the aIPSC amplitude recovered within one minute. This activity-dependent suppression was prevented by NBQX, a non-NMDAR blocker (aIPSC_control: 36.2±8.0 pA; aIPSC_Stimulation: 35.8±7.9 pA, n=5, p=0.39). No difference in the aIPSC amplitude and in the decay time constant was observed between controls with and without NBQX. Thus the PF stimulation-induced transient depression required the activation of non-NMDA receptors.

**Exogenous AMPA application suppressed aIPSCs in immature stellate cells via a presynaptic mechanism.**

A recent study showed that activation of AMPARs suppressed Ca^{2+} entry in 50-60% of basket cells in the third postnatal week (Rusakov et al, 2006). This suggests that only a subset of presynaptic terminals are regulated by AMPARs. The probability of GABA release from basket/stellate cells is known to decrease during development (Pouzat and Hestrin, 1997; Liu and Lachamp, 2006). Thus one possibility is that the AMPAR-mediated inhibition of GABA release from stellate cells occurs preferentially in presynaptic terminals that have a high release probability. I therefore examined the effect of activating AMPARs on the aIPSCs recorded in P12-20 stellate cells. To determine whether the extent of suppression was developmentally regulated and correlated with the release probability, I exogenously applied AMPA to obtain a maximal AMPAR-induced suppression of aIPSCs. The amplitude of aIPSCs recorded in the perforated patch configuration was stable for at least 50 minutes (see Supplemental Figure 2).

The aIPSC amplitude was determined in stellate cells prior to and during the application of a low concentration of AMPA. The amplitude of aIPSCs in P12-15 cells decreased by 25.1±4.0% during AMPA application (from -46.5±8.2 to -33.6±5.1 pA, n = 16; p<0.005; paired t-test; Fig 2A and 2B) and returned to 43.2 ± 9.7 pA following the removal of AMPA (5 min washout). Of the 16 P12-15 cells recorded, 14 cells showed a greater than 10% depression (Supplemental Figure 3). AMPA application in the presence
of CB1 and GABAB receptor blockers also suppressed the aIPSC amplitude by 26.5 ± 4.3% (P12-13, n = 9; ranging from 11% to 49%). Thus AMPA application indeed produced a greater reduction in aIPSC amplitude than did PF stimulation (Fig 1). The activation of AMPARs appeared to cause this inhibition of aIPSCs because NMDAR blockers did not prevent the AMPA-induced suppression of aIPSCs (Fig 2A). By contrast, the average current amplitude in P17-19 cells (n=6) did not change during AMPA application. Thus exogenous AMPA application suppressed aIPSCs in immature but not mature stellate cells.

I next determined whether the AMPA-induced suppression in aIPSC amplitude was due to a decrease in the release of GABA. If this were the case one would expect that the suppression would be associated with an increase in the paired pulse ratio (PPR) and a decrease in 1/CV². The PPR was determined prior to and during the application of AMPA. In P12-15 cells there was an increase in the PPR during the application of AMPA (from 0.30±0.08 to 0.41±0.10; n=13, p<0.04; by paired t-test; Fig 2C), indicating a reduction in GABA release. However no significant change in PPR was found in P17-19 cells. Thus an increase in PPR (i.e. a decrease in release probability) occurred predominantly in immature cells that displayed the depression. The 1/CV² of aIPSCs was also found to decrease during the AMPA-induced suppression of aIPSC amplitude. This was illustrated by the correlation between 1/CV² and the change in the current amplitude (Fig 2D; R² = 0.43, n = 26, slope = 1.04 ± 0.24; p<0.0004). Together these results suggest that AMPA application transiently reduced the secretion of GABA from stellate cells.

In further support of a presynaptic mechanism, mIPSC frequency in P12-13 stellate cells increased during AMPA application by ~11 fold (from 0.4 ± 0.2 to 4.3 ± 1.5 Hz; n=6; p<0.05) and returned to the control level following the removal of AMPA (Fig 2E). The amplitude of mIPSCs did not change. This result is consistent with a previous report by Bureau and Mulle (1998) and supports the idea that the expression site of this plasticity is presynaptic.

Modulation of GABA release by glutamate depends on developmental age and the initial synaptic release probability.

To confirm that the probability of GABA release from basket/stellate cells decreased during development, I examined the aIPSCs in response to two depolarizations separated by 20 ms in P12-20 stellate cells. Consistent with previous observations (Pouzat and Hestrin, 1997; Liu and Lachamp, 2006), the paired pulse ratio increased with age (R²=0.53; n = 37; slope = 0.12 ± 0.02; p < 0.0001), indicating that the GABA release probability decreased during development (Fig 3A).

To address the question of why AMPA application suppressed aIPSCs mainly in immature neurons, I tested the hypothesis that the depression of GABA release occurred preferentially at those synapses that had a high release probability (i.e. a low PPR). As shown in Fig 3B, depression was more frequently observed in cells with a low PPR than in those with a higher PPR (low release probability). Thus the AMPA-induced change in the aIPSC amplitude correlated with the initial PPR (R² = 0.34, n=27, slope = 33.5 ± 9.5, p<0.002; Fig 3B). I then determined whether reducing the release probability in immature P12-13 stellate cells by lowering the extracellular Ca concentration from 2 mM to 1 mM prevented the AMPA-induced
suppression of aIPSCs. In low Ca the PPR increased from 0.06 ± 0.04 to 0.47 ± 0.15 (n = 6; p < 0.05) and AMPA application no longer suppressed aIPSC amplitude (Fig 3C). These results are consistent with the idea that activation of AMPA receptors is more likely to induce a transient depression of GABA release at synapses that have a high release probability.

Our recent work showed that burst stimulation of PFs also activates presynaptic NMDA receptors and induces a lasting increase in GABA release from stellate cells (Liu and Lachamp, 2006). Thus glutamate can exert two opposing effects on GABA release. First, a transient suppression of GABA release due to the activation of AMPARs. Second, a lasting enhancement of GABA release that is induced by NMDAR activation. Can a single stellate cell show both responses? We have previously shown that the application of AMPA can induce a lasting increase in GABA release from mature stellate cells and that paradoxically this potentiation can be blocked by NMDAR blockers (Liu and Lachamp, 2006). Thus AMPA application presumably increases the release of glutamate and glycine from other cerebellar cells by activating AMPARs on these cells. While glutamate could be released from cerebellar granule cells, glycine could originate from Bergmann glial cells, Golgi or Lugaro cells (Huang et al., 2004; Zeilhofer et al., 2005). Endogenously released glutamate and glycine could then activate NMDARs on stellate cells. Since AMPA application can produce both a transient suppression and a lasting enhancement of GABA release, we measured the AMPAR-mediated suppression (during AMPA application) and NMDAR-induced lasting potentiation (15-30 minutes after AMPA application) in the same stellate cells (supplemental Fig 4).

As shown in Fig 3D, cells that exhibited an AMPAR-mediated depression did not show long-term potentiation. On the contrary a lasting potentiation occurred only in cells that did not display a transient suppression of GABA release. This is illustrated by the correlation between the change in aIPSC amplitude during AMPA application and the lasting change in aIPSCs observed 15-30 min after AMPA application (R²=0.66, n = 11, slope = 1.55±0.38, n = 11, p<0.003). These results indicate that GABA release from a stellate cell can be either transiently depressed or persistently enhanced by the activation of glutamate receptors.

I then tested the idea that whether a stellate cell undergoes a transient depression or an enduring potentiation is correlated with the initial release probability. Cells were divided into two groups based on their initial PPR (PPR: 0-0.5 and 0.6-1.6). As predicted, the long-term potentiation (but not the transient suppression) was found only in cells with a low release probability (i.e. that exhibited a high PPR, Fig 3E). In contrast, a transient depression (but not a lasting potentiation) was observed in cells that had a high release probability (i.e. a low PPR). Thus the glutamatergic modulation of GABA secretion from stellate cells depends on the initial release probability.

The observation that the probability of GABA release decreased during synaptic maturation raises the possibility that the AMPAR-mediated transient suppression is more likely to occur in immature stellate cells while the NMDAR-induced lasting potentiation of GABA release will occur preferentially in mature neurons. During AMPA application aIPSCs in cells from P13-16 mice displayed depression whereas P17-19 cells showed no change (P13-16: -15±5%, n = 6 vs P17-19: 7±8%, n = 5; p < 0.05; Fig 3F). By contrast, a
lasting increase in aIPSCs was observed only in P17-19 cells, but not in cells from P13-16 mice (P17-19: 59±10% vs P13-16: 13±13%, p < 0.03). Thus during synaptic maturation the glutamatergic modulation of GABA release switches from an AMPA receptor-mediated transient suppression to a NMDA receptor-induced lasting potentiation.
DISCUSSION

In the cerebellar cortex, stellate cells are present in the upper two thirds of the molecular layer whereas basket cells are found close to the somata of Purkinje cells. Here I show that stimulation of the glutamatergic parallel fibres in a manner that mimics physiological activity can transiently reduce the amplitude of autoreceptor/autaptic currents in cerebellar stellate cells by activating AMPA receptors. Thus the glutamate that is released from PFs can activate AMPARs on stellate cells and reduce GABA release. By contrast glutamate released from climbing fibres (but not from PFs) activates AMPARs on the presynaptic terminals of basket cells and suppresses the release of GABA from cerebellar basket cell onto Purkinje cells (Satake et al, 2000; Rusakov et al, 2005). This raises the possibility that two glutamatergic inputs, parallel fibres and climbing fibres, can preferentially modulate GABA release from stellate and basket cells, respectively.

Activation of presynaptic kainate receptors suppresses the PF to stellate cell synapse, but enhances the PF to Purkinje cell synapse, producing a target dependent effect (Delaney and Jahr, 2002). Does the AMPAR-mediated suppression of GABA release from cerebellar interneurons also depend on the target cell? Previous work has shown that GABA release is reduced at the basket to Purkinje cell synapse by activation of AMPARs, but not kainite receptors (Satake et al, 2000). Our results indicate that this also occurs when the postsynaptic cells are stellate cells. Regardless of the type of postsynaptic target cell, activation of AMPARs consistently gives rise to a transient suppression of GABA release from cerebellar interneurons. Therefore AMPA-induced presynaptic short-term plasticity occurs at all synapses innervated by the axons of stellate/basket cells.

What is the physiological relevance of the PF-induced suppression of GABA release from stellate cells? Sensory stimulation evokes a burst of action potentials in cerebellar granule cells (Chadderton et al, 2004). PF activation increases the release of glutamate and glutamate spillover can activate extrasynaptic AMPARs in stellate cells (Carter and Regehr, 2000). Our results suggest that burst stimulation of PFs also induces an AMPAR-dependent suppression of GABA release. These results imply that burst PF stimulation not only activates extra-synaptic AMPARs, but also presynaptic AMPARs on stellate cells. It is known that glutamate spillover is controlled by the activity of glutamate transporters whose transport rate is strongly affected by temperature (Asztely et al., 1997). Thus our PF stimulation experiments that were designed to mimic the high frequency burst of action potentials that are evoked by sensory stimulation were conducted at a near physiological temperature. Hence the burst of PF activity that triggered a suppression of GABA release is likely to occur under physiological conditions. In a variety of systems, inhibitory autaptic and autoreceptor currents are thought to be involved in the regulation of neuronal activity. In neocortical inhibitory interneurons, autaptic currents enhance the precision of spike-timing (Bacci and Huguenard, 2006). The autoreceptor currents in stellate cells generate a depolarization and increase the firing probability, producing burst firing (Mejia-Gervacio and Marty,
Thus the AMPAR-mediated suppression of the autoreceptor current may alter the firing pattern of stellate cells.

Glutamate/AMPA receptors modulate the release of many neurotransmitters by depolarizing the presynaptic membrane thus altering the excitability of the presynaptic terminals (Rusakov 2005; Satake et al, 2004, 2006; Engelman et al, 2006; 2004; Lee et al., 2002). One unusual feature of the AMPAR-mediated suppression of aIPSCs was that it only occurred in a subset of cells. A similar heterogeneous response to AMPAR activation was also seen at the axon terminals of basket cells, where activation of AMPARs suppressed Ca\(^{2+}\)-entry in 50-60% of terminals (Rusakov et al, 2005). In the present study, postnatal day 12-20 mice were used. During this period there was a decrease in the probability of GABA release (Puozat and Hestrin, 1997; Liu and Lachamp, 2006), although the underlying mechanism is not clear. One possibility is that it is due to an increase in the expression of parvalbumin in stellate cells, leading to a change in presynaptic Ca signaling (Collin et al, 2005). I found that the terminals of stellate cells that have a low release probability cannot undergo a further reduction in GABA release (since a correlation between the AMPA-induced reduction in transmitter secretion and the initial release probability was observed). This suggests that release probability is one of factors that determines whether or not AMPAR activation is effective. However the possibility that AMPARs are absent at terminals with a low release probability cannot be ruled out.

The developmental switch in AMPAR-mediated regulation of evoked GABA release is consistent with the results of Bureau and Mulle (1998). In their study AMPAR activation was shown to enhance mIPSC frequency only in immature stellate cells, but not in mature cells. However in contrast to the AMPA-induced suppression of evoked GABA release, AMPA application potentiated the spontaneous release of GABA from stellate cells. This difference between the regulation of spontaneous and evoked release is not due to the activation of distinct subtypes of AMPARs because both responses involve activation of Ca-impermeable AMPARs (but not Ca-permeable AMPARs; Satake et al, 2006). While the mechanism underlying the potentiation of spontaneous release is not known, AMPAR activation can reduce Ca entry through voltage-gated Ca channels via a G-protein-coupled signaling pathway, leading to the suppression of evoked GABA release (Satake et al, 2004; Rusakov et al, 2005). Similar contrasting actions of presynaptic AMPARs on evoked and spontaneous release of inhibitory transmitters has been observed in the spinal cord dorsal horn (Engelman et al, 2006).

Glutamate can presynaptically activate AMPARs and NMDARs, producing opposing effects on the release of GABA from stellate/basket cells with distinct temporal dynamics. In basket cells, stimulation of climbing fibres activates AMPARs and transiently suppresses GABA release (Satake et al, 2000). Burst activity of climbing fibres also activates NMDARs and enhances GABA release for tens of minutes (Duguid and Smart, 2004). By contrast presynaptic glutamate receptors on stellate cells can be activated by burst stimulation of parallel fibres. Activation of NMDARs induces a **long-lasting enhancement** of GABA release (Liu and Lachamp, 2006), whereas activation of AMPARs **transiently suppresses** the presynaptic release of GABA. How glutamate
modulates GABA release appears to depend, in part, on the initial release probability of the particular synapse. The presynaptic release of GABA can be either transiently suppressed by the activation of AMPARs at synapses that have a high release probability, or persistently enhanced by the activation of NMDARs if the initial release probability is low (Liu and Lachamp. 2006). Thus the glutamatergic modulation of GABA release from stellate cells switches from a transient suppression to a lasting potentiation as the GABAergic release probability decreases during development (Pouzat and Hestrin, 1997).
FIGURE LEGENDS

Figure 1: Stimulation of parallel fibres (PF) transiently suppresses the amplitude of autaptic/autoreceptor current (aIPSC) at 36°C. (A). Average control aIPSCs prior to PF stimulation (trace 1), and aIPSCs recorded at 100 ms (trace 2) and 1 minute (trace 3) following a burst of 4 depolarizations of PFs at 100Hz. (B). Left: aIPSC amplitude of individual cells without NBQX and in the presence of 10 μM NBQX. Right: time course of PF-stimulation induced suppression of aIPSCs. (C) Burst stimulation-induced change in aIPSC amplitude (=100*(aIPSC2-aIPSCcontrol)/aIPSCcontrol, where aIPSCcontrol = (aIPSC1+aIPSC3)/2). Each open symbol represents a separate experiment and filled symbols are average values (*, p<0.03; **, p<0.005).

Figure 2. Activation of AMPARs suppresses GABA release from immature stellate cells. (A) Application of 0.3 μM AMPA reduced the amplitude of aIPSCs in P12-15 stellate cells. aIPSCs prior to and during the application of AMPA. (i) AMPA application reduced the amplitude of aIPSCs in a P12-15 stellate cell; (ii) AMPA suppressed the aIPSC amplitude in the presence of 10 μM CPP. (iii) AMPA application failed to suppress the amplitude of autaptic/autoreceptor currents in a P17-19 stellate cell. (B) Group data of aIPSC amplitude prior, during and after AMPA application (left; P12-15, n=16; P17-19; n = 6; **, p<0.005, paired t-test) and percentage change in the current amplitude (right; *, p < 0.03). (C) AMPA-induced suppression of aIPSCs is associated with a change in the paired pulse ratio. Top panels: examples of aIPSCs evoked by two depolarizations separated by 20 ms. Bottom panel: mean paired pulse ratio (PPR=amplitude1/amplitude2; *: p<0.03, paired t-test). (D) Plot of 1/(coefficient of variation)² vs current amplitude (I). Values of 1/CV² and I were normalized to their control values before the application of AMPA. (R² = 0.43, n = 26, slope = 1.04 ± 0.24; p<0.0004). Each symbol represents a separate cell from different slices. Data in E include those shown in (B) and 4 additional P16 cells. (E). Average mIPSC frequency, prior to, during and after AMPA application in P12-13 stellate cells (*: p< 0.05; n = 6).

Figure 3. Modulation of GABA release depends on developmental age and the initial synaptic release probability. (A) Correlation between PPR and developmental stage (R² = 0.53, n=37, slope = 0.12±0.2, p<0.0001). (B) Correlation between initial PPR and AMPA-induced change in aIPSC amplitude (R² = 0.34, n=27, slope = 33.5 ± 9.5, p=0.002). Filled symbols are experiments in which AM-251 and SCH50911 was included during AMPA application. Each symbol represents a separate cell from different slices. Data in B include those shown in Fig 2B and 4 additional P16 cells. (C) Plot of average % change in aIPSC amplitude during AMPA application vs PPR from P12-13 cells in extracellular solutions that contained 2 mM and 1 mM Ca (n=6). Comparisons between the two groups: PPR, p< 0.001; % AMPA-induced change, p < 0.02. (D) Plot of the change in aIPSC amplitude during AMPA application (the transient change) and 15-30 minutes after AMPA application (the lasting change) in individual cells (R² = 0.66, n = 11, slope = 1.55±0.38, n = 11, p<0.003; dark circles are data re-analyzed from previous experiments reported in Liu and Lachamp, 2006). (E) Plot of average change in the aIPSC amplitude during AMPA application vs the lasting potentiation from cells with a PPR greater than 0.6 (n=5; in one P18 cell PPR was not determined) and less than 0.5.
(n=5). Comparisons between the two groups: PPR, p<0.006; % lasting change, p<0.03; % transient change, p<0.02. (F). Plot of average change in the aIPSC amplitude during transient suppression and the lasting potentiation from P13-16 (n=6) and P17-19 cells (n=5). Comparisons between the two groups: % lasting change, p<0.03; % transient change, p<0.05.
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Figure 2  Liu

![Graphs and charts showing data comparisons and statistical analyses related to AMPA application.](Image)

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